Daniel Konziella

Glial-neuronal interactions in experimental brain disorders

Dr. philos thesis
Norwegian University of Science and Technology

I. Preface and acknowledgments

This thesis is submitted for the degree of “Doctor of Philosophy” at the Norwegian University of Science and Technology, Trondheim. The work was carried out from august 2000 to mars 2005. In 2000 I had a five months' research stay at the Department of Toxicology and Pharmacology, NTNU, with a stipend from the European Union's Leonardo foundation. In the past years I have been to Trondheim and the department, which later changed its name to Department of Clinical Neuroscience, on many occasions. These visits were unfortunately never longer than a couple of days or weeks, since I finished medical studies in Germany and started my clinical work in Sweden and Denmark.

However, without the help and support of my supervisor and friend Prof. Dr. Ursula Sonnewald this work would have never been accomplished. Ursula's enthusiasm makes research a most interesting and driving experience. Her friendly advice in both private and professional questions is always precise, empathic and valuable. She has a great talent to turn young researchers from all over the world into a real team, making it an honor to be a part of it. I feel deep gratitude for all the fruitful discussions we had and have, for her introduction into the fields of neuroscience in general and of Nuclear Magnetic Spectroscopy in special, and last, but not least, for all her encouragement and patience.

Moreover, all these persons turned my time in Trondheim into an unforgettable experience: Eiliv Brenner is thanked warmly for all the discussion and friendly chats in the laboratory and pubs and of course for the preparation of brain tissue extracts and analysis of NMR spectra in Papers IV and V. Hong Qu analyzed spectra in Paper II and is a joyful and pleasant person. The excellent technical help of Bente Urfjell with HPLC in all papers is greatly acknowledged as are her friendliness and laughter. The help of Elvar M. Evjolfsson with statistics in Paper IV and V is highly appreciated. Prof. Olav Sletvold is thanked among others for an exciting trip to a conference to Tromsø. Dr. Maria Carlsson and Katarina Rydén Markinhuhta, from Carlsson Research AB in Gothenburg, Sweden, are thanked for monoamine analyzation in Paper V and their friendly welcome in Sweden.

I thank Abdel Bidar, Janniche Hammer and Asta Håberg for pleasant discussion of results in, respectively, Paper I, II and IV. Prof. Dr. med. Thomas Brinker, Department of Neurosurgery, Klinikum Hannover, and Dr.med. Wolf Lüdemann, International Neuroscience Institute, both Hanover, Germany, are thanked among others for teaching me the basic principles of scientific research and the kaolin-model of hydrocephalus. Henrik Eikås, Asbjørn Nielsen, Elisabeth Qvigstad and Haytham Eloqayli did not contribute directly to this work, but are all thanked for their friendship. Last, not least, I feel deep gratitude for all the support and love my parents, my brothers and my sister, my girl friend and all my friends gave and give me.

II Abbreviations

ADP; adenosine diphosphate

AMPA; α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid

ATP; adenosine triphosphate

CBF; cerebral bloof flow

CNS; central nervous system

CRFC; cortical, retrosplenial and frontal cortex

CSF; cerebrospinal fluid

fMRT; functional magnetic resonance tomography

GABA; γ-aminobutyric acid

GAD; glutamate dehydrogenase

GLUT; glucose transporter proteins

GS; glutamine synthetase

HPLC; high pressure liquid chromatography

ICP; intracranial pressure

ip; intraperitoneal

MK801; Dizocilpine

MR; magnetic resonance

MRI; Magnet Resonance Imaging

NMDA; N-methyl-D-aspartate

NMRS; nuclear magnetic resonance spectroscopy

NPH; normal pressure hydrocephalus

PDH; pyruvate dehydrogenase

PAG; phosphate activated glutaminase

PC; pyruvate carboxylase

PCP; phencyclidine

PTZ; pentylenetetrazole

SAMP8; senescence-accelerated mice P8

sc; subcutant

TCA; tricarboxylic acid cycle

TE; temporal lobe

III List of Papers

This thesis is based on the following papers:

1. D. Kondziella, A. Bidar, B. Urfjell, O. Sletvold, U. Sonnewald: The pentylentetrazole-kindling model of epilepsy in old and young SAMP8 mice: Behavior and metabolism. Neurochemistry International, 2002 (40): 413-418

2. D. Kondziella, J. Hammer, O. Sletvold, U. Sonnewald: The pentylenetetrazole-kindling model of epilepsy in SAMP8 mice: Glial neuronal metabolic interactions. Neurochemistry International, 2003 (43): 629-637

3. D. Kondziella, Q. Hong, W. Luedemann, T. Brinker, O. Sletvold, U. Sonnewald: Astrocyte metabolism is disturbed in the early development of experimental hydrocephalus. Journal of Neurochemistry, 2003 (85) 274-281

4. E. Brenner, D. Kondziella, A. Håberg, U. Sonnewald: Impaired glutamine metabolism in NMDA receptor hypofunction induced by MK801. Journal of Neurochemistry. In press.

5. D. Kondziella, E. Brenner, E.M. Evjolfsson, K.R. Markinhuhta., M.L. Carlsson, U. Sonnewald: Glial-neuronal interactions are impaired in the schizophrenia model of repeated MK801 exposure. Submitted.

These papers are not included in this thesis:

6. D. Kondziella, W. Luedemann, T. Brinker, O. Sletvold, U. Sonnewald: Alterations in brain metabolism, CNS morphology and CSF dynamics in adult rats with kaolin-induced hydrocephalus. Brain Research, 2002 (927): 35-41

7. U. Sonnewald and D. Kondziella: Neuronal glial interaction in different neurological diseases studied by ex vivo ^{13}C NMR spectroscopy. NMR in Biomedicine, 2003 (16): 424-429

8. W. Luedemann, D. Kondziella, K. Tienken, P. Klinge, T. Brinker, D. Berens von Rautenfeld: Spinal cerebrospinal fluid pathways and their significance for the compensation of kaolin-hydrocephalus. Acta Neurochirurgica (Suppl.), 2002 (81): 271-273

9. W. Lüdemann, Y. Nonaka, D. Kondziella, K. Tienken, D. Berens von Rautenfel: CSF spinal outflow pathways under physiological conditions and in hydrocephalus. Neurological Surgery (No Shinkei Geka). In press.

10. D. Kondziella, H. Maetzel: The Sting in the Tail: Palinopsia and Syncope. Journal of Neurology. In press.

11. D. Kondziella, P. Brodersen, K. Hansen: Cavernous haemangioma of the spinal cord – conservative or operative treatment? Submitted.

12. K. Tienken, D. Kondziella, D. Berens von Rautenfeld, T. Brinker, W. Ludemann: A ferritin tracer study of compensatory spinal CSF outflow pathways in kaolin-induced hydrocephalus. Submitted.

IV Summary

When [1-^{13}C]glucose and [1,2- ^{13}C]acetate are given simultaneously, information of astrocytic and neuronal metabolism can be obtained in the same animal. This is because acetate is exclusively taken up by astrocytes, while the major part of acetyl-CoA derived from glucose is metabolized in neurons. In the present thesis glial-neuronal interactions in experimental models of epilepsy, hydrocephalus and schizophrenia were examined by ^{13}C NMR spectroscopy and other techniques.

Pentylenetetrazole (PTZ)-kindling mainly altered metabolism of astrocytes in 2 months old and of glutamatergic neurons in 8 months old senescence accelerated mice P8 (SAMP8), a genetic model of aging. In the presence of PTZ, phenobarbital decreased labeling of most metabolites from both [1-^{13}C]glucose and [1,2-^{13}C]acetate in young SAMP8, although in older animals only GABAergic neurons were affected. Phenobarbital normalized glutamate labeling from [1-^{13}C]glucose in old PTZ animals. Aging of SAMP8 lead to decreased mitochondrial activity in glutamatergic neurons, as shown by decreased glutamate labeling from [1-^{13}C]glucose in old control animals compared to young controls. Additionally, old SAMP8 mice had disturbed astrocytic metabolism indicated by lowered glutamine synthesis from [1,2-^{13}C]acetate. In the early development of rat kaolin-hydrocephalus astrocyte metabolism was impaired only. First later, at the chronic stage, neuronal metabolism became affected as well. A decrease in [4,5-^{13}C]glutamate and unchanged [4,5-^{13}C]glutamine indicated impaired transport of astrocytic glutamine to glutamatergic neurons four weeks after hydrocephalus induction. While a single dose of the NMDA antagonist MK801 mainly disturbed metabolism in the rat temporal lobe (TE), repeated administration lead mostly to metabolic impairment in the cingulate, retrosplenial and frontal cortex (CRFC). Evidence was found for decreased neurotransmitter release from synaptic vesicles and impaired conversion of glutamine to glutamate in neurons. Moreover, MK801 lead to compartmentation of glutamine metabolism, where glutamine labeled from neuronal glutamate was handled differently than

glutamine from astrocytic glutamate. Repeated MK801 administration provoked predominantly altered neuronal metabolism, while metabolism of astrocytes seemed relatively unaffected. This might add to the disturbances of the cortico-striato-thalamo-cortical loop caused by NDMA receptor blockade and hence to the sensory gating deficits provoking cortical sensory overstimulation and psychosis.

In contrast to the hydrocephalus experiment, the studies of epilepsy in SAMP8 and of NMDA receptor hypofunction did not show primary astrocytic impairment. Thus, astrocytic function differs from disorder to disorder. Although there is no general pattern of glial-neuronal interactions, the so-called glutamate-glutamine cycle is frequently disturbed.

Table of contents

I. Preface and acknowledgments ... 1
II Abbreviations ... 3
III List of Papers ... 5
IV Summary ... 7
1.Introduction ... 10
1.1. Neurons, astrocytes and other glia cells ... 10
1.2. The Tricarboxylic Acid Cycle and brain energy metabolism ... 12
1.3. Neurotransmitters ... 13
1.4. Glial-neuronal interactions and the metabolism of amino acid neurotransmitters ... 13
1.4.1. Glutamate ... 14
1.4.2. GABA ... 16
1.5. General criteria for animal models of brain disorders ... 18
1.6. Epilepsy ... 19
1.6.1. Animal models of epileptic disorders ... 20
1.7. Hydrocephalus ... 23
1.7.1. Animal models of hydrocephalus ... 24
1.8. Glutamate Excitotoxicity and Schizophrenia ... 26
1.8.1. Animal models of Schizophrenia and NMDA-toxicity ... 29
1.9. NMR Spectroscopy ... 30
1.9.1. ^{13}C NMR Spectroscopy application to neuroscience ... 31
1.9.1.1. Labeling from [1-^{13}C]glucose ... 35
1.9.1.2. Labeling from [1,2-^{13}C]acetate ... 36
2.Materials and Methods ... 37
2.1. Experimental Procedures ... 37
2.1.1. The PTZ-Model of Epilepsy and SAMP8 ... 37
2.1.2. The Kaolin-Model of Hydrocephalus ... 37
2.1.3. The NMDA receptor antagonist MK801 and schizophrenia ... 38
2.2. High Pressure Liquid Chromatography ... 39
2.3 ^{13}C NMR spectroscopy ... 40
2.4. ^{1}H NMR spectroscopy ... 40
2.5. Statistics ... 40
3.Aims of Study ... 41
4.Synopsis of Papers ... 42
Paper I ... 42
Paper II ... 43
Paper III ... 43
Paper IV ... 44
Paper V ... 45
5.Discussion ... 47
5.1. Glial-neuronal interactions in the PTZ model of epilepsy in SAMP8 ... 47
5.2. Glial-neuronal interactions in adult rats with kaolin-induced hydrocephalus ... 49
5.3. Glial-neuronal interactions in MK801 induced NMDA receptor hypofunction and experimental schizophrenia ... 52
5.4. Is there a common pattern of glial-neuronal interactions in different brain disorders? 56
5.5. Validation of Methods ... 57
6.Conclusions ... 60
7.References ... 62
8.Papers I – V ... 76

1. Introduction

1.1. Neurons, astrocytes and other glia cells

The brain consists of brain cells, blood and blood vessels, cerebrospinal fluid and the cerebral meninges. The brain cells can be divided into neurons and glial cells. Neurons are chemically and electrically excitable cells, which are critical to signaling of information over shorter or longer distances. GABAergic interneurons in the basal ganglia for example have very short axons, while the axons of the first motorneurons stretch from the motor cortex to the frontal column of the spinal cord and thus can be up to one meter long.

The term "glia " was originally introduced by Virchow. He characterized glia cells as "Nervenkitt", literally "nerve glue", and thought that these cells mainly had a static function (Virchow, 1846). However, more than hundred years later, we know that glial cells do not only act as a scaffolding for neurons. There are macroglia, which include oligodendrocytes and astrocytes; microglia and ependymal cells. While the latter stand for the main part of cerebrospinal fluid production and line the brain ventricles and the spinal central canal, microglia are residential macrophages having immunological and phagocytic properties. Oligodendrocytes provide the myeline sheath around the neuronal axons, thus guaranteeing quick and undisturbed electrical signaling.

Astrocytes (literally "star-formed cells") probably have the most complex role of all glial cells. Although far from being completely understood, astrocytes are thought to offer metabolic and structural support to neurons and in recent years evidence has arisen that astrocytes might even be intimately involved in neuronal information processing (Hansson and Rönnebäck, 2003). Astrocytic end feet, together with the gap junctions between the capillary endothelial cells, are part of the blood brain barrier (Abbott et al., 1992; Ballabh et al., 2004). Due to their location between capillaries and neurons, astrocytes link neuronal activity to energy metabolism and cerebral blood flow. Thus, astrocytes are thought to feed "hungry neurons ...[and]...deliver food for thoughts" as

Meeks and Mennerick put it (2003). It has been suggested that end-feet of activated astrocytes might even feed back onto capillaries leading to local vasodilation and therewith to increased oxygen and glucose delivery (Paulson and Newman, 1987). Indeed, neuron-induced Ca^{2+} elevations in astrocytes can lead to release of vasodilatory substances from astrocyte end-feet, thereby increasing local blood flow (Zonta et al., 2003; Anderson et al., 2003). Moreover, astrocytes supply neurons with neurotransmitter precursors and their uptake of extracellular glutamate, K^+, H^+ and many other ions and molecules from the synaptical cleft is crucial to brain cell homeostasis. It is noteworthy in this context, that astrocytes can express aquaporin-4, which is the predominant water channel in the neuropil of the CNS (Nagelhus et al., 2004; Simard and Nedergaard, 2004). Although not electrically excitable, astrocytes probably can communicate with each other by e.g. Ca^{2+} waves (Bennett et al., 2003; Giaume et al., 2003; Hansson and Rönnebäck, 2003). By enwraping pre- and postsynaptic terminals they can modulate neurotransmission (Nedergaard et al., 2003; Newman, 2003) and evidence has been found that they even might release glutamate under some circumstances in a Ca^{2+}-dependant manner (Nedergaard et al., 2002; Liu et al., 2004). Several studies in the last decade have demonstrated intense communication between neurons and astrocytes (Rouach et al., 2004). As outlined above, astrocytic Ca^{2+} levels become elevated in response to neuronal input, which in turn influences synaptic activity. It has even been hypothesized that astrocytes might form a gliovascular network consisting of microdomaines and higher-order gliovascular units matching local neuronal activity and blood flow (Nedergaard et al., 2003). At the same time they might regulate neuronal firing thresholds through coordinative glial signaling and thereby establishing not only the structural, but also the functional architecture of the brain (Nedergaard et al., 2003). The intimate function of astrocytes in the so-called “glutamine-glutamate-GABA-cycle” (Hertz et al., 1979) is referred to later in the text. Thus, although astrocytes have been relatively overlooked during many decades, they have gained unexpected attention in recent years. They are “stars at last” (Ransom et al., 2003). Today it is well known that altered glial neuronal interaction plays a crucial role in many neurological and neuropsychiatric conditions.

1.2. The Tricarboxylic Acid Cycle and brain energy metabolism

The main energy source for the mammalian brain is glucose, which is transported from the blood across the blood brain barrier to neurons and glia. Lactate, fatty acids, ketone bodies, acetate and other substrates cannot compensate for insufficient glucose supply to the brain as is evident from cerebral dysfunction in hypoglycemia. The uptake of glucose is maintained by special glucose transporter proteins (GLUT) in endothelial (the 55-kDA isoform of GLUT1) and glial cells (the 45-kDA isoform of GLUT1; Choeiri et al., 2005; Wong et al., 2005), neurons (GLUT3; Maher, 1995) and microglia (GLUT5; Horikoshi et al., 2003). Inside the cell, glucose is phosphorylated to glucose-6-phosphate, which has a central position since from here glycolysis, the pentose phosphate pathway or glycogen formation originate. The cytosolic glycolysis transforms one molecule of glucose into two molecules of pyruvate and adenosine triphosphate (ATP) is formed from adenosine diphosphate (ADP). Pyruvate might enter the mitochondrial tricarboxylic acid (TCA) cycle after oxidative decarboxylation to acetyl coenzyme A. This step is catalyzed by pyruvate dehydrogenase (PDH). Then acetyl-CoA derived from glucose or other energy metabolites, can be completely metabolized to CO2 and H2O. Taking into account the net results from glycolysis, TCA cycle and the electron transport chain, one molecule of glucose can generate 36 molecules of ATP. However, it is interesting to note that in the *immature* rat brain, ketone bodies can represent about 30-70% of the total energy metabolism (Nehlig, 2004).

However, glycolysis and TCA cycle do not serve as energy producing cataplerotic pathways only, but they also provide carbon skeletons for the synthesis of metabolites such as glutamate, GABA, glutamine and many others as is outlined below. The main anaplerotic enzyme in the brain is pyruvate carboxylase (PC) present in glia only (Patel, 1974; Shank et al., 1985; Wiesinger et al., 1997). PC converts pyruvate to oxalacetate, which then condenses with acetyl CoA to provide net synthesis of a molecule of α-ketoglutarate, thereby replenishing the TCA cycle intermediates. Thus, the TCA cycle can be described as a biochemical turntable which is also intimately involved in neurotransmitter synthesis in both astrocytes and neurons.

1.3. Neurotransmitters

Neurotransmitters are chemical substances interacting at synapses between nerve cells and their targets in the central or peripheral nervous system. The action potential causes neurotransmitter release from presynaptic vesicles mediated by Ca^{2+}. Simplified, chemical synapses can be divided into direct and indirect synapses. At the former, the transmitters bind to ionotropic receptors in the membrane of the postsynaptic cell leading to the opening of ion channels and membrane potential changes by influx of extracellular ions. At the latter, metabotropic receptors and second messenger systems are involved. Transmitters are then taken up into the postsynaptic neuron or astrocytes by specific reuptake mechanisms or, alternatively, they are degraded by enzymatic activity in the synaptic cleft such as acetylcholine.

Serotonin, acetylcholine, the amino acids glutamate, γ-aminobutyric-acid (GABA) and glycine and the catecholamines adrenaline, noradrenaline and dopamine are termed classical neurotransmitters, while non-classical transmitters include peptides, NO, CO and many others. In contrast to old doctrines, more than one type of neurotransmitter may be released by a single neuron, although the specificity of neurotransmitter release allows the distinction between for example glutamatergic, GABAergic or noradrenergic neurons.

1.4. Glial-neuronal interactions and the metabolism of amino acid neurotransmitters

Glial and neuronal metabolism are intimately connected. Figure 1 gives a simplified illustration of glial neuronal metabolic interaction. Neurons lack the main anaplerotic enzyme in the brain, namely pyruvate carboxylase, and are therefore depending on astrocytic supply of TCA cycle intermediates since every drain of neuronal amino acids would otherwise lead to a shortage of neurotransmitter precursors. Moreover, astrocytes take up neuronal glutamate which will lead to further depletion of transmitters in neurons. Thus, net synthesis of neuronal TCA cycle metabolites and compounds like glutamate and GABA require the entry of a four carbon unit. Pyruvate carboxylase in astrocytes transforms pyruvate to oxalacetate resulting after condensation with acetyl CoA in the formation of

the TCA cycle intermediate citrate, which can be further converted to α-ketoglutarate. From α-ketoglutarate glutamate can be formed with help of glutamate dehydrogenase or different transaminases (see Westergaard et al., 1995, for a review), but more important, glutamate can emerge from glutamine after hydrolyzation by phosphate-activated glutaminase (Kvamme et al., 2000). The latter pathway is part of the so-called "glutamine-glutamate-cycle", which was first introduced in the late 1960ies (Berl and Clarke, 1969; Van den Berg and Garfinkel, 1971) and then later extended to the "glutamine-glutamate-GABA-cycle" (Hertz, 1979). Shortly, astrocytes release glutamine into the extracellular space. From there glutamine is taken up by neurons converting it to glutamate and GABA. After release upon depolarization the transmitters are cleared from the synaptic cleft by astrocytes transforming glutamate to glutamine again and the cycle is closed. Glutamate from glutamine can be converted to α-ketoglutarate and, as can be seen in Figure 1, can enter the TCA cycle both in neurons and astrocytes. Note that GABA is predominately taken up into neurons (Schousboe et al., 2000 and 2003).

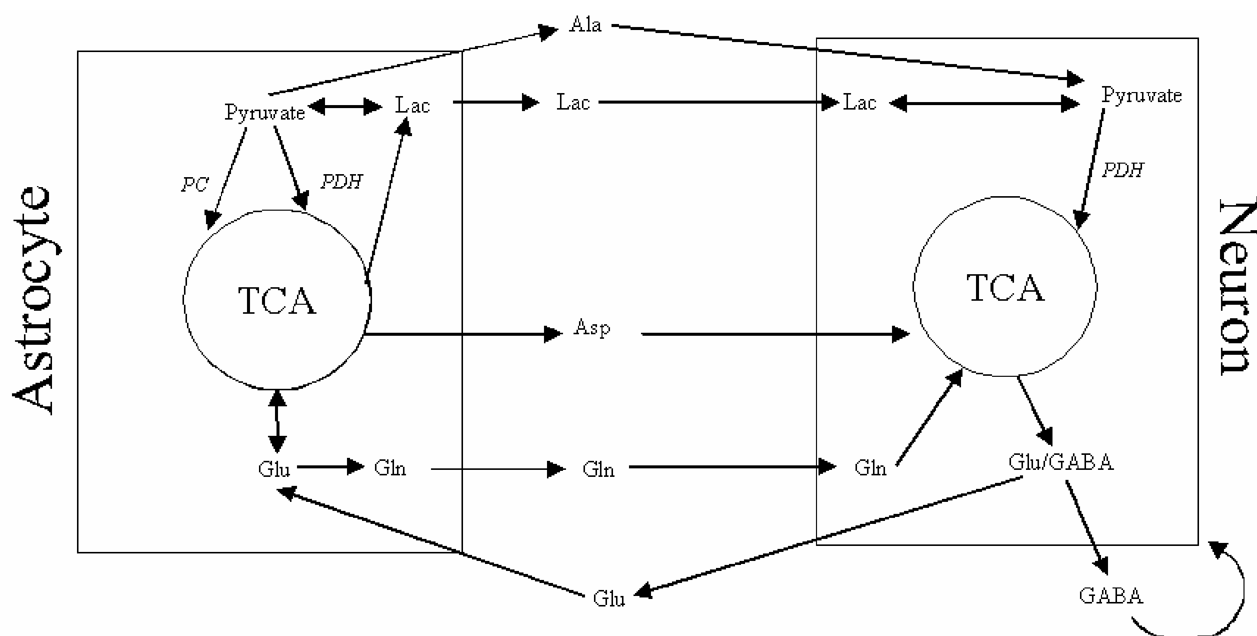


Figure 1. Simplified presentation of glial-neuronal interactions. See *Introduction* for details.

1.4.1. Glutamate

Glutamate is the most important excitatory neurotransmitter in the mammalian CNS. Beside the

neurotransmitter pool, there is a metabolic pool of glutamate, which in astrocytes after conversion to glutamine by glial specific glutamine synthetase can be metabolized in the TCA cycle for energy production as outlined above. Alternatively, it is incorporated into numerous proteins or peptides such as glutathione. Since glutamate usually does not cross the blood brain barrier (Hawkins et al., 1995), it is mainly produced within the CNS itself and most of the neurotransmitter pool is stored in the synaptical vesicles of glutamatergic neurons. Thus, under physiological circumstances intracellular concentration of glutamate exceeds extracellular concentration by a factor of 10^3-10^5 (Hamberger et al., 1983; Lehmann et al., 1983; Schousboe, 2003). Glutamate homeostasis is crucial to brain function due to two reasons. First, fast removal of glutamate from the synaptical cleft guarantees short glutamate action on the postsynaptic target cell and thereby precise information signaling. Second, high extracellular concentration of glutamate is cell damaging and the neurotoxicity of excessive glutamate release is of paramount importance in many neurological disorders (see section 1.4.). Indeed, reverse transport of glutamate from glial cytosol into the extracellular space induced by release of glutamate and potassium by damaged nerve cells (Billups and Attwell, 1996) exacerbates the deleterious effects of brain injury. However, evidence has also been found that vesicular release of glutamate from astrocytes in a Ca^{2+}-dependant manner might under more physiological circumstances contribute to neuronal-astrocytic information signaling as explained earlier (Nedergaard et al., 2002; Liu et al., 2004).

Five distinct glutamate transporters have been cloned so far, namely GLAST (EAAT1), GLT (EAAT2), EAAC (EAAT3), EAAT4 and EAAT5 (see Danbolt, 2001, for an extensive review). The significance of astrocytic clearing of glutamate as part of the “glutamate-glutamine-cycle” is illustrated by the fact that GLAST and GLT, which account for most of the glutamate transport, are restricted to astroglial cells. In contrast, EAAT3-5, transporters of minor importance for glutamate clearance from the synaptic cleft, are expressed on both glia and neurons and have a less ubiquitous localization with EAAT4 being mainly restricted to cerebellar purkinje cells and EAAT5 to retinal cells (Arriza et al., 1997; Eliasof et al., 1998). There are two different types of glutamate receptors: G-protein-coupled, second messenger activating metabotropic receptors (mGluRs) and ligand gated

ionotropic receptors (iGluRs). The latter consist of cation channels and are distinguished according to their different sensitivities to glutamate analogues: N-methyl-D-asparte (NMDA), kainate and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. Thus, as outlined in 1.4. NMDA, kainic acid and AMPA are widely used experimental substances. However, it should be remembered that, although many substances modify the NMDA receptor, the natural transmitter for the receptors is none of these analogues, but glutamate.

Both in neurons and astrocytes the acetyl CoA used for glutamate synthesis is mainly produced from glucose after glycolysis. However, in contrast to neurons, astrocytes can have a netsynthesis of glutamate since they can convert pyruvate to oxalacetate. As mentioned above, glutamate can be transformed to glutamine by glutamine synthetase, which is exclusively localized in astroglia (Norenberg et al., 1979). Glutamine can then be released by astrocytes as part of the "glutamine-glutamate-cycle". Neurons convert glutamine to glutamate via phophate activated glutaminase (PAG), which is situated in the inner mitochondrial membrane (Kvamme et al., 2000), and depend on astrocytic glutamine supply.

1.4.2. GABA

Being the most important inhibitory neurotransmitter in the mammalian CNS, GABA plays a significant role both in normal and pathological conditions. It is mainly found in GABAergic neurons, e.g. in the basal ganglia it is released by GABAergic interneurons to inhibit excitatory neurons projecting to the motor cortex (Carlsson et al., 2001). Its depletion and the blockade of GABA receptors by substances such as picrotoxin, pentylenetetrazole or penicillin lead to increased excitatory brain function as seen in convulsions. GABA is synthesized both in the cell body and the nerve terminal directly from glutamate by glutamate decarboxylase, which consist in two isoforms, GAD_{65} and GAD_{67} (Martin and Rinmvall, 1993). The latter has been associated with the production of cytoplasmic GABA, while GAD_{65} probably stands for the major part of vesicular GABA (Kaufmann et al., 1991; Waagepetersen et al., 1999). In the so-called GABA-shunt GABA can be converted by GABA aminotransferase to form succinic semialdehyde, which afterwards is oxidized

to succinate permitting four of five C-atoms from α-ketoglutarate to re-join the TCA cycle (Balazs et al., 1970).

However, upon release into the synaptical cleft the major part of GABA is taken up again by the same GABAergic neurons and only to a lesser extent by astrocytes (Schousboe, 2000 and 2003). Therefore it remains controversial how much of GABA synthesis is depending on astrocytic glutamine supply (Cerdan et al., 1990; Hertz et al., 1992; Preece and Cerdan, 1996). Moreover, conflicting results have been found concerning the conversion from glutamine to GABA. In vitro data point towards initial glutamine incorporation, after conversion to glutamate and α-ketoglutarate, into the TCA cycle of GABAergic neurons (Westergaard et al., 1995; Waagepetersen et al., 1999), whereas in vivo data suggest immediate conversion to GABA via glutamate (Hassel et al., 1995 and 1998; Sonnwald et al., 1996).

Two ionotropic GABA receptors, $GABA_A$ and $GABA_C$, and one metabotropic receptor, $GABA_B$, have been identified so far. As a distinct feature, $GABA_A$ receptors are regulated by allosteric modulation (Costa, 1991). They have four subunits, α, β, γ an δ (Johnston, 1996; Vafa and Schofield, 1998), each with multiple variants, and exhibit binding sites for among others benzodiazepines and barbiturates. GABA transporters exist as four subtypes: GAT-1, predominantly present on GABAergic neurons and to a lesser extend on astrocytes, GAT-2, GAT-3 and low-affinity subtype BGT-1. Note that the amount of GABA transferred from neurons to astrocytes is significantly smaller than the corresponding transport of glutamate and most of the released GABA is cleared from the synaptic cleft by re-uptake into GABAergic neurons (Schousboe et al., 2000 and 2003) as previously outlined.

1.5. General criteria for animal models of brain disorders

Four major criteria are important when establishing experimental models of neurological and psychiatric disorders.

1. Face validity: How well is e.g. depressive behaviour copied by the animal model? Face validity may be difficult to achieve due to the fact that animals simply are not human beings. Thus, only some of the traits seen in depression can be mimicked. For example, fatigue and decreased sexual behaviour can be imitated, but not the feeling of guilt.

2. Causative validity: How well does the factor, that induces the modelled behaviour, correspond to the current theories of what causes the illness?

3. Construct validity : How well does the neurobiological correlates of the observed behaviour correspond to the theoretical model of what underlies human diseases? Causative and construct validity are more or less the same. They are difficult to achieve as well, since our knowledge about many of the underlying causes of brain disorders are still very limited. However, this is why animal models are established.

4. Predictive validity: How well does the model predict the therapeutic activity of drugs used to treat the disorder? Predictive validity may be the most important point, because animal models with high predictive validity are the ones used in pharmacological research. A model of epilepsy or affective disorder that corresponds to the same pharmacological treatment used in humans but not to other drugs, has high predictive validity.

All animal procedures in the present thesis were approved by the Norwegian Animal Research Authority.

1.6. Epilepsy

Epilepsy can be defined as a group of chronic brain diseases leading to repetitive, most often spontaneous epileptic seizures. While about 5% of all the world's population experiences at least one single epileptic seizure in life, 0.5-1% develop epilepsy, which therefore is one of the most common severe neurological diseases (Sander and Shorvon, 1996). In Europe, the prevalence is ca. 650 cases in 100,000 inhabitants. The incidence is highest in childhood, lowest in adolescence, early and middle adulthood and rises again from age 65 (Brodie and Dichter, 1997).

In short, abnormal paroxysmal depolarization of individual neurons due to disturbances of ion channels, neurotransmission or electrolytes spreads out by multi-cellular pathological synchronization. Thus, epileptic seizures can be evoked by structural brain lesions, but repetitive seizures can on their own cause selective cell damage in the cerebral cortex, hippocampus, brain stem and other areas (Gutula et al., 2003). Strong genetic disposition, occurrence at a certain age and lack of a known lesion are all characteristic for idiopathic or genuine epilepsy, while symptomatic epilepsy is provoked by a distinctive cause such as perinatal complications, cerebral deformities, brain trauma and tumor, vascular lesions, alcoholism, encephalitis and many more. The clinical manifestations are traditionally divided into partial seizures, which start in only one cerebral hemisphere, and generalized seizures, in which both hemispheres are involved. The International Classification of Epileptic Seizures, introduced 1981 by the International League against Epilepsy, distinguishes simple partial seizures without impaired consciousness, complex partial seizures with disturbed consciousness and partial seizures with secondary generalization. Primary generalized seizures include absences (petit mal), tonic, clonic, tonic-clonic (grand mal), myoclonic and atonic convulsions. Moreover, there are unclassified seizures such as neonatal seizures and neonatal spasms. To the differential diagnosis of epilepsy belong syncope, psychogenic fits, narcolepsy, drop attacks due to insufficiency of the vertebrobasilar blood circulation, global transitory amnesia, hyperventilation tetany and certain psychiatric states such as katatonic and dissociative syndromes.

1.6.1. Animal models of epileptic disorders

Concordant to the great variety of disorders which constitute epilepsy, many different animal models have been introduced. Status epilepticus can be induced by lithium and pilocarpine (Nehlig et al., 2002). Generalized seizures are moreover mimicked by e.g. different genetic models such as genetically epilepsy-prone rats (Hjeresen et al., 1987), electrically induced seizures (Castellion et al., 1965) or by one of the kindling models such as the well-established Pentylentetrazole (PTZ)-kindling model. Epileptic kindling refers to the regular application of chemical or electrical stimuli in sub-threshold doses, which due to the additive effect consequently lead to epileptic seizures probably by altered concentrations of extra-and intracellular ions and impairment of specific membrane functions (Bradford, 1995). For a review on kindling models see Löscher and Schmidt (1988). Pentylentetrazole is a chemical convulsant exerting its epileptogenic action by binding to the picrotoxin-binding site of the post-synaptic $GABA_A$ receptor (Macdonald and Barker, 1978; Ramamjaneyulu and Ticku, 1984), thereby decreasing GABA release and consequently enhancing neuronal depolarization (De Deyn and Macdonald, 1989). PTZ-kindling was used in the present studies (papers I and II). By increasing GABA inhibition Phenobarbital is able to prevent epileptic seizures and, since it is a barbiturate, its efficacy and toxicity are greatest in later life (Macdonal and Barker, 1977; Kitani et al., 1988). However, kindling behavior can be induced by many other chemical convulsants, including NMDA antagonists such as MK801, CGP 37849 and CGP 39551 (Loscher and Honack, 1991).

Moreover, there are a number of animal models, both genetic (Nehlig and Boehrer, 2003) and chemically induced by γ-hydroxybutyrate (GHB) (Snead et al., 1999), for generalized absence seizures with appropriate spike-wave patterns similar to human epilepsy and response to clinically used therapeutic agents.

While simple partial seizures may be induced by focal micro-application of e.g. penicillin (Meldrum and Naquet, 1970) or picrotoxin (Usunoff et al., 1969) on the cerebral cortex, direct injection of tetanus toxin into the hippocampus (Jefferys et al., 1995) or systemically (Sperk, 1994; Watanabe et al., 1999) or intrahippocampal administered kainate (de Vasconcelos et al., 2005) lead to complex partial seizures. Kainate, a glutamate receptor antagonist widely used to induce temporal lobe seizures, evokes hippocampal sclerosis and disturebd glial-neuronal interactions (Mueller et al., 2000; Qu et al., 2003). Another model of limbic seizures is the pilocarpine model (Garzillo and Mello, 2002).

Epilepsy due to post-traumatic brain injury is evoked by the model of the chronic isolated cortex (Echlin and Battista, 1963), focal iron-induced epilepsy (Willmore et al., 1978) and fluid perkussion (Lowenstein et al., 1992). Moreover, there are also models for neonatal hyperthermic (Dube et al., 2000), other febrile seizures (Bender et al., 2004), epilepsy in cortical dysplasia (Schwartzkroin et al., 2004) and hypoxia-induced seizures (Jensen et al., 1995). Even models for delicate epileptic disorders such as reflex epilepsy exist. The Fayoumi strain of chickens (Fepi) carries a recessive autosomal gene mutation leading to photogenic and audiogenic reflex epilepsy in homozygotes (Batini et al., 2004). Seizures are characterized by stimulus-locked motor symptoms followed by generalized self-sustained convulsions. Spikes and spike and waves patterns at rest are seen on EEG recordings and are suppressed during seizures and replaced by a desynchronized pattern of activity (Batini et al., 2004).

Unfortunately, at least models of generalized seizures are somewhat limited by the relatively low and sporadic incidence of truly spontaneous seizures. Thus, most of the epileptic events occur more or less directly after manipulation such as the intraperitoneal administration of Pentylentetrazole or electrical stimulation. All epileptic models, however, have lead to significant insight into the etiology of epilepsy and consequently to therapeutic options such as antiepileptic brain surgery, valgus nerve stimulation and most important medical therapy including carbamazepine, valproate, phenytoin, benzodiazepines, barbiturates, oxcarbazepine, lamotrigen, gabapentin, tiagabine,

levetiracetam, zonisamide, topiramate and others. Please see Fisher (1989), Kupferberg (2001) and Jefferys (2003) for general reviews on models of epilepsy and resulting pathophysiological findings. For the role of glutamate transporters in experimental epilepsy please consider Maragakis and Rothstein (2004).

However, as with other brain diseases, it should be noted that glutamate plays a pivotal role in epilepsy. Alteration of glutamate transporter expression and function may be an essential part of epileptogenesis (see Maragakis and Rothstein, 2004, for a review). Tanaka et al. (1997) found neuronal loss in the hippocampus of GLT1 knock-out mice resulting in seizures and increased mortality. Compared to wild-type mice GLAST knock-out mice responded to Pentylentetrazole with increased seizure activity (Watanabe et al., 1999). Others described a down-regulation of the neuronal EAAT3 transporter in some hippocampal areas of kainate-kindled rats (Simantov et al., 1999). Also decreased GABA activity is related to changes in glutamate transporter as was shown by Sekuty and co-workers, who demonstrated a 50% loss of hippocampal GABA amounts associated with knock-down of EAAC1 (Sepkuty et al., 2002).

1.7. Hydrocephalus

The healthy brain produces as much cerebrospinal fluid (CSF) as it resorbs. If production surpasses elimination, hydrocephalus develops and the amount of CSF in the intracranial space is increased to the expense of brain tissue and blood volume. Different forms of hydrocephalus are distinguished: In non-communicative hydrocephalus an obstruction in the ventricular system above the spinal cord due to tumors, blood clots, meningeal inflammation and many more causes ventricle enlargement and increased intracranial pressure (ICP). In contrast, the communicative hydrocephalus is characterized by open CSF pathways, but impaired CSF absorption. Underlying conditions are e.g. sinus venous thrombosis, subarachnoidal bleedings and meningeal inflammation involving the Granulationes arachnoidales, all of them leading to disturbed CSF elimination into the venous system. CSF overproduction due to carcinoma or papilloma of the plexus choroideus is a very seldom cause for hydrocephalus. This is also true for primary brain tissue loss leading to hydrocephalus ex vacuo, which refers to the compensative filling of the empty space by CSF.

The so-called “Normal Pressure Hydrocephalus” (NPH) is a form of communicative hydrocephalus with ventriculomegaly and impaired CSF absorption but without elevated ICP. Clinically, NPH is characterized by the trias of dementia, urine incontinence and gait disturbances. Hydrocephalus and other conditions with raised ICP lead to several symptoms depending on the time of development and the patient's age. In infants with open fontanelles acute hydrocephalus can cause massive head enlargement and the so-called sunset phenomenon. In older children or adults increased ICP provokes headache, malaise and vomiting. Papilloedema develop after several days and the sunset-phenomenon may occur due to ocular palsy of the third cranial nerve. If not treated correctly, a further increase of ICP compromises brain stem function accompanied by loss of brain stem reflexes, coma and death.

Treatment of hydrocephalus may either be operative or conservative depending on the underlying causes and progression of the disease. To date the exact time for surgical intervention or even the

very indication for ventricular shunt therapy still remain problematic and in most cases invasive diagnostic procedures are required. In future non-invasive monitoring of brain metabolism might help to select the hydrocephalic patients who benefit from surgery. However, to achieve this goal profound knowledge about the metabolic alterations in hydrocephalus is necessary, but not yet available. Since a lumbar puncture is a relatively safe and uncomplicated technique, most studies on brain metabolism in humans have evaluated levels of neurotransmitters, neuropeptides and amino acids in CSF and not brain tissue (Inagawa et. al, 1980; Engelsen et al., 1985; Barecca et al., 1991; Zeman et al., 1991; Malm et al., 1991 and 1994; Raftopopulus et al., 1996; Yamamoto et al., 1999). Although this methodological disadvantage is not relevant for animal studies, the few studies published on brain metabolism of hydrocephalic rats or other animals are quite contradictory as outlined below.

1.7.1. Animal models of hydrocephalus

While Higashi et al. reported increased amounts of dopamine, noradrenaline and homovanilic acid in rat cerebrum (Higashi et al., 1986), others found decreased activity of noradrenergic and dopaminergic neurons in rats with one and four weeks old hydrocephalus (Miyake et al., 1992). In rabbit brain with hydrocephalus dopamine release was enhanced in cerebellum, hypothalamus, mesencephalon and pons including medulla oblongata, but lowered in cortex and nucleus caudatus (Miwa et al., 1982). Alterations in cholinergic, GABAergic and dopaminergic neuronal metabolism were found in rat basal ganglia (Tashiro et al., 1997) and in hippocampus levels of acetylcholine and noradrenaline were decreased (Egawata et al., 2002). A decline of dopaminergic neurons in the substantia nigra was shown in rats with acute hydrocephalus, but not subacute hydrocephalus (Ishizaka et al., 2000). In neonatal hydrocephalus decreased amounts of glutamine, glutamate, aspartate, N-acetyl-aspartate and alanine were detected by using ^{1}H-Magnetic-Resonance-Spectroscopy (Harris et al., 1997; Jones et al., 1997), while there were significantly higher levels of aspartate, glutamate and glutamine in rats with infantile hydrocephalus (Del Bigio and Vriend, 1998).

However, today we know that hydrocephalus is a process involving the whole brain and is not, as formerly believed, restricted to the periventricular area: Changes in metabolism have been described in the basalganglia (Tashiro et al., 1997; Ishizaka et al., 2000), in the cerebellum, hypothalamus, mesencephalon, pons, medulla oblongata and nucleus caudatus (Miwa et al., 1982), hippocampus (Egawata et al., 2002) and last, not least, in the cortex (Miwa et al., 1982, Harris et al., 1997; Jones et al., 1997; Del Bigio and Vriend, 1998, Egawata et al., 2002).

Experimental studies on hydrocephalus have been conducted since the beginning of the twentieth century. The great neurosurgeon W.E. Dandy together with K.D. Blackfan published the very first study in 1914 (Dandy and Blackfan, 1914). Although already described in 1932 by Dixon et al., the kaolin-model is still one of the most studied hydrocephalus models and was used in paper III. The instillation of Kaolin into the cisterna magna causes aseptic inflammation of the basal meninges, which leads to the closure of the foramina Magendi and Luschka of the fourth ventricle and consequently to the development of non-communicative hydrocephalus. In the acute phase of rat kaolin-hydrocephalus four weeks after treatment ICP and CSF outflow resistance are highest, while the chronic phase is defined by normal basal pressure, declining outflow resistance and progression of ventriculomegaly (Kondziella et al., 2002). In the same study only moderate metabolic changes were detected by HPLC in the acute period with increased glutamine and decreased taurine in the cerebrum and increased alanine in the brain stem. However, distinct biochemical changes occurred during the chronic period such as a remarkable decrease of glutamate, glutamine and taurine in the cerebellum and other changes in cerebrum and brain stem (Kondziella et al., 2002).

1.8. Glutamate Excitotoxicity and Schizophrenia

Glutamate is centrally involved in brain diseases ranging from Alzheimer's to schizophrenia and has many sites of action both intra- and extracellular. However, one pathophysiological feature of glutamate is relevant for nearly every brain disorder leading to impaired cell functioning or cell death: This is the neurotoxicity of excessive glutamate release, which causes extreme calcium influx into the cells.

The dual role of glutamate, both vitally important and highly toxic, has been known for a long time (Olney, 1969). In order to establish new treatments, NMDA receptor (and other glutamate) antagonists have been tested clinically in many CNS disorders including riluzole in amyotrophic lateral sclerosis (Bensimon et al., 1994), memantine in Alzheimer's disease (Fleischhacker et al., 1986), selfotel in stroke (Davis et al., 2000), CP-101,606 in brain trauma (Bullock et al., 1999) and many more. Unfortunately, results of these studies have been quite disappointing so far. In amyotrophic lateral sclerosis, riluzole, the only available treatment at the moment, prolongs survival of the average patient by two months – mean duration of the disease is three to five years (Festoff et al., 2003; Miller et al., 2003). In focal cerebral ischaemia there was even a trend toward increased mortality with selfotel treatment (Muir and Lees, 2003).

Extensive research is now performed on the formerly relatively overlooked AMPA-receptor (Furukawa et al., 2003; Rego et al., 2003; Van Damme et al., 2003). However, there is also increasing knowledge about the previously underestimated complexity of the NMDA receptor. While earlier experimental studies reported mainly a neuroprotective potential of glutamate antagonists (Park et al., 1988; Ozyurt et al., 1988; Faden et al., 1989; Foster et al., 1988; Choi et al., 1988; Shapira, 1990), soon the additional neurotoxic properties of glutamate/NMDA antagonism were recognized (Olney et Farber, 1994; Farber et al., 1995, 1996 and 1998; Kim et al., 1999). Also recent papers report both positiv (Aono et al., 2002; Calzada et al., 2002; Miguel-Hidalgo et al., 2002; Williams et al., 2002; Brandt et al., 2003; Calabresi et al., 2003; Zieminska et al., 2003; Lee et al., 2004) and negative influence on neurons (Dave et al., 2001; Farber 2002a and b; Ogita et al.,

2003; Olney et al., 2003).

How can results concerning glutamate antagonism be so conflicting? The answer probably lies in the diversity of neurons and glutamate receptor subtypes and -units. Thus, the blockade of glutamate release of glutamatergic neurons acting on GABAergic interneurons might be neurotoxic by increasing indirectly glutamate release at a second glutamatergic neuron behind the interneuron (Farber et al., 1995). On the contrary, the blockade of NMDA receptors at the second glutamatergic neuron at the end of the chain might be neuroprotective. Moreover, it is now well-understood that there are different NMDA receptors and even many different subunits on each receptor (Danbolt, 2001; Bleich 2003).

In the past years glutamatergic hypofunction has been recognized to play a significant role in schizophrenia, which is as an endogenous psychosis and a major psychiatric disorder. Prodrome, active and residual symptoms include delusions, hallucinations, disorganized speech, psychomotor disturbances such as grossly disorganized, catatonic or stereotyped behavior, and negative symptoms such as affective flattening. Men and women are equally often affected, although schizophrenia develops earlier in male patients, who on average become symptomatic in their early or mid twenties, approximately five years prior to females (Häfner et al., 1992). Prevalence worldwide is 0.5-1.0% and incidence 0.05%, largely independent from socio-cultural aspects (Häfner, 1993).

According to the dopamine hypothesis of schizophrenia the clinical symptoms of this disorder are the consequence of central dopaminergic hyperactivity. Of the three major dopamingergic pathways, namely the nigrostriatal, the tuberoinfundibular and the mesolimbic-mesocortical systems, only the latter is believed to be relevant to schizophrenia, which might explain the fact, why Parkinson's disease caused by dopaminergic hypofunction in the nigrostriatal system can coexist with schizophrenia. The dopamine hypothesis arose from psychopharmacological findings showing that dopamine-antagonists have antipsychotic effects in schizophrenic patients and moreover, that the effectiveness of these drugs is positive correlated to their ability to block dopamine receptors, especially the Dopamine-D_2-receptor. Furthermore, L-dopa, the precursor of

dopamine and still the therapeutic cornerstone in Parkinson's disease, can produce psychotic symptoms. Recently the hyperdopaminergic hypothesis of schizophrenia has received more direct support from neuroimaging studies (Breier et al., 1997; Abi-Dargham et al., 1998 and 2000). However, there are significant arguments against the believe that increased dopamine activity might be the single cause to psychotic conditions. For instance, studies of the CSF homovanillic acid, the main dopamine metabolite, have in general shown no altered concentrations in schizophrenic patients compared to controls (Bowers, 1970; Berger at al., 1980). Even more relevant might be the fact that glutamate-receptor antagonists, such as phencyclidine, ketamine or MK801 acting on NMDA-receptors, have strong psychomimetic effects with hallucinations and psychomotor signs. Moreover, lysergic acid diethylamide, abbreviated LSD, produces psychotic symptoms also without interfering with dopamine receptors, but by binding to the serotonin-5HT-2α-receptor. Thus, in recent years evidence has arisen that the hypothesis of primary dopaminergic hyperfunction has to be revised or, at least, extended, and a more differentiated approach including dopaminergic, glutamtergic and serotonergic neurotransmitter systems is favored (for a review see Carlsson et al., 2001). The intimate connection of dopamine and glutamate metabolism has lead to the hypothesis of glutamate hypofunction causing increased dopamine activity and schizophrenic characteristics (Carlsson et al., 1999; Jentsch and Roth, 1999), but also a NMDA recepetor hypofunction model of glutamate induced neurotoxicity without direct involvement of dopamine metabolism has been proposed (Olney, 1989).

However, with a deeper understanding of the NMDA receptor not only new knowledge about the origin of different brain diseases like schizophrenia will arise. Even a second generation of NMDA receptor antagonists acting more specifically on different subunits of the receptor and thus new potential therapies might be developed. Future aspects for epidemiology and socio-economy look huge in the light of an increasingly aging population in the western world (Kilpatrick and Tilbrook, 2002). For a review on the “enormous potential of NMDA recpetor antagonists” see Smith, 2003.

1.8.1. Animal models of Schizophrenia and NMDA-toxicity

In the late 1950s Phencyclidine (PCP) was introduced as a general dissociative anesthetic (Johnstone et al., 1958; Collins et al., 1960; Corssen and Domino, 1966). NMDA glutamate-receptor antagonists, such as PCP, ketamine or dizocilpine-maleate (MK801; 5R, 10S]-[+]-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine), have been shown to cause strong psychomimetic effects with hallucinations and psychomotor signs and have been used extensively in schizophrenia research. In contrast to dopaminergic agonists, which only mimic the positive symptoms of schizophrenia, NMDA antagonists produce the whole spectrum including negative and cognitive symptoms. They are therefore considered appropriate schizophrenia models.

One of the best characterized non-competitive antagonists of the PCP binding site of the NMDA receptor is MK801, which is even more selective than PCP (Carlsson et al., 2001). MK801 causes behavioral changes in rodents such as hypermobility, head weaving and ataxia (Loscher et al., 1991), altered cerebral metabolism such as excessive cerebral glucose supply (Loubinoux et al., 1994) and is able to minimize cell damage in the hippocampus during ischaemia, but also to induce neuronal degeneration in the cingulate cortex (Wozniak et al., 1998). Thus, it is a widely studied substance in experimental research of both schizophrenia and glutamate neurotoxicity.

1.9. NMR Spectroscopy

Nuclear Magnetic Resonance Spectroscopy (NMRS) allows the noninvasive study of biochemical processes in vivo, ex vivo and in vitro. Thus, cell cultures, tissue, animals and humans can be examined. Like Nuclear Magnet Resonance Imaging (MRI) and functional Imaging (fMRI), NMRS does not depend on x-rays or the application of radioactive material. The main principle is that atoms with uneven mass numbers and/or odd numbers of protons have a nuclear spin or so-called magnetic moments. A large number of nuclei with magnetic moments exist, but in practice less than ten have made an impact on biochemical research: ^{13}C is mainly used to examine metabolic pathways as is outlined below. High energy phosphates and phospholipids can be studied using ^{31}P NMRS and levels of amino acids, glucose, lactate and NAA using ^{1}H NMRS, while studies on drug distribution mostly involve ^{19}F NMRS. Less often used nuclei are ^{7}Li, ^{14}N and ^{23}Na.

When a strong external magnetic field (B_0) is applied to a sample, the earlier randomly oriented atoms with a magnetic moment align themselves with respect to B_0 either with or against its direction. The distribution of these spins therefore has different energy states and the difference can be described as $\Delta E = \gamma h B_0 /2\pi$, γ being the gyromagnetic ratio. The distribution of the spins is characterized by the *Bolzman formula*: $N\beta/N\alpha = e^{-\Delta E/K} B^{T}$, where $N\beta$ equals the number of spins in the higher energy state, $N\alpha$ the number of spins in the lower energy state, K_B is the Bolzman's constant and T the absolute temperature. When applying a second magnetic field B_1 in form of a radio frequency pulse vertically on B_0, the spins will change their distribution and absorb the energy from the RF pulse if the frequency of the pulse is the same with which the spins resonate, namely the so-called *Larmor frequency*: $\nu = \gamma B_0 /2\pi$. At the time B_1 is removed the spins will return to their former distribution, inducing a current in the MR coil. This current is recorded as the MR signal and is called the free induction decay (FID). Since the FID is usually received from a homogenous sample containing nuclei with different resonance frequencies, these different compounds have to be resolved by converting amplitude against time to amplitude against frequency, which can be achieved using the mathematical operation known as *Fourier*

transformation. A scan refers to one cycle of pulsing and data acquisition. Since the signal to noise ratio increases with the square root of the number of scans, a reasonable high number of scans should be performed to obtain reliable results, depending on concentration of the analyzed metabolites.

Both MR spectroscopy and imaging exploit the magnetic features of nuclei with nuclear spin ($I \neq 0$) when placed in a uniform magnetic field. The nuclear spins are then oriented in different energy levels ($n=2I+I$) by equilibrium processes. A radio frequency energy is applied to induce transmission between the different energy states. The excited nuclei return to equilibrium due to longitudinal and transversal relaxation processes. This time-dependent decay is acuired and Fourrier transformed into a frequency-dependent spectrum. Depending on the molecular environment of a certain nucleus a specific peak is registered.

Spectral analysis: Tissue, blood, urine and other fluids comprise a tremendous amount of MR detectable compounds. Therefore NMR spectra are often very complex and different approaches are used to investigate them. Peak areas and intensities can be compared to an internal reference such as ethylene glycol, which was used in the present studies. To correct for partial saturation factors for relaxation and *nuclear Overhauser effects (NOE)* were applied to all the ^{13}C spectra.

NOE is due to proton decoupling of the ^{13}C NMR spectra and the intensities of the ^{13}C signals are usually increased up to 200%. The NOE depend on conditions influencing relaxation such as the number of protons covalently bound and will therefore vary from nucleus to nucleus. In the present ^{13}C NMRS experiments some spectra were taken with a long inter scan delay and broad-band decoupling during acquisition to obtain heteronuclear decoupling, but to avoid NOE.

For information on ^{1}H NMR spectroscopy see Materials and Methods.

1.9.1. ^{13}C NMR Spectroscopy application to neuroscience

^{13}C NMRS is an excellent tool to obtain information about metabolic pathways and glial-neuronal metabolic interactions, especially since not only cell cultures and animal models can be studied, but

also patients. See Sonnewald et al. (1996) for an comprehensive review for the application of ^{13}C NMRS on cell cultures, Sonnewald and Kondziella (2003) on animal models for neurological diseases and Taylor et al. (1996) for glial neuronal interaction. The natural abundance of ^{13}C is only 1.1%, thus ^{13}C labeled precursors and products are easily detected. However, it is important to observe that the occurrence of two neighboring ^{13}C atoms, causing homonuclear spin-spin-coupling, leads to splitting and displacement of the resonance from the central singlet resonance, which is present if the ^{13}C is alone. ^{13}C - ^{13}C spin coupling makes the detection of label particularly specific, because the likeliness of two naturally adjacent ^{13}C atoms in the same molecule is very small, which is why precursors such as [1,2-^{13}C]acetate can be used. For an exhaustive description of homonuclear splitting patterns in biological molecules see Cerdan et al., (1990) and Bachelard and Badar-Goffer for quantification of spectra (1993). ^{13}C- 1H coupling is called heteronuclear coupling. Barany et al. (1985) have established a detailed allocation of resonances in rat brain extracts and the application of ^{13}C NMRS to the study of metabolic pathways in guina pig brain slices has been performed for the first time by Bachelard and Badar-Goffer, who used [1-^{13}C]glucose both in extracts and during superfusion. Since then glial neuronal metabolic interaction has been studied both in rat brain extracts (Shank et al., 1993), cultures of cortical astrocytes, neurons and co-cultures thereof both in cerebrum and cerebellum (Sonnewald et al., 1997) and in different animal models such as the stroke model of temporary occlusion of the middle cerebral artery (Haaberg et al., 1998).

Neurons metabolize the major part of acetyl-CoA derived from glucose, while acetate is selectively taken up by astrocytes due to a specialized transport system being absent or less active in neurons (Minchin and Beart, 1975; Sonnewald et al., 1991; Waniewski and Martin, 1998). Thus, by simultaneous administration of [1-^{13}C]glucose and [1,2-^{13}C]acetate neuronal and astrocytic metabolism can be studied in the same animal (Taylor et al., 1996), which constitutes the main principle of glial neuronal metabolic studies using ^{13}C NMRS. Injection of ^{13}C-labeled glucose and acetate leads to efficient labeling of many metabolites, as is shown in the brain extract spectrum in Figure 2. Label from [1-^{13}C]glucose can be quantified by analyzing the singlet peaks in the different

compounds. In contrast, the doublets seen in the spectrum are mostly derived from [1,2-^{13}C]acetate and thus astrocytic metabolism. By comparing the doublets with the singlets in Figure 2, it is obvious that glutamine is labeled more from [1,2-^{13}C]acetate (doublet) than [1-^{13}C]glucose (singlet); the opposite is the case for glutamate and GABA. Alanine, lactate, C6-N-acetylaspartate (NAA) and succinate are mainly labeled from glucose. Creatine and taurine are not labeled, the naturally abundant ^{13}C gives rise to the observed singlets.

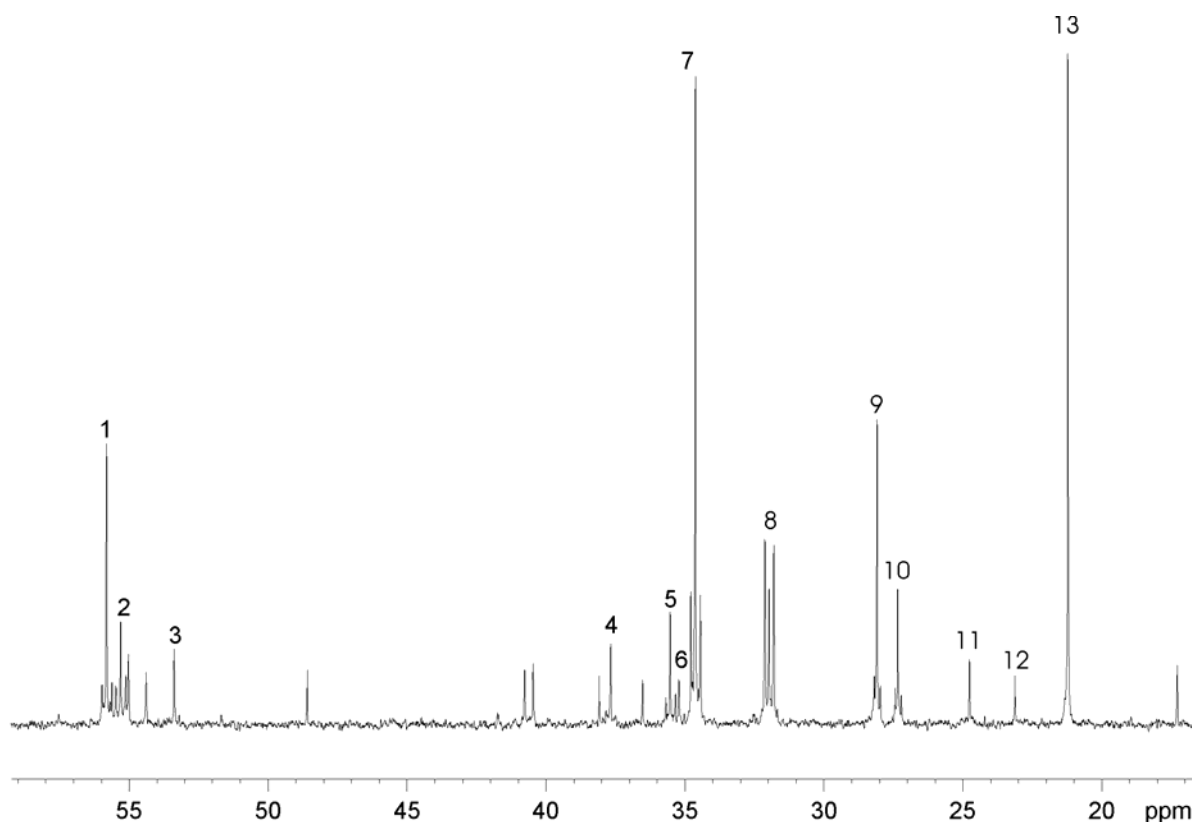


Figure 2. ^{13}C NMR spectrum of temporal lobe extracts from rats injected with MK801 together with [1,2-^{13}C]acetate and [1-^{13}C]glucose. Peak assignments; 1: glutamate C-2; 2: glutamine C-2; 3: aspartate C-2; 4: aspartate C-3; 5: GABA C-2; 6: succinate C-2/C-3; 7: glutamate C-4; 8: glutamine C-4; 9: glutamate C-3; 10: glutamine C-4; 11: GABA C-3; 12: N-acetyl-aspartate C-3; 13: lactate C-3.

Since both acetyl-CoA and oxalacetate can be labeled or unlabeled, the number of possible isotopomers of the TCA metabolites is large and only compounds derived from the firsts two turns are represented in the Figures (Figures 3-4). However, conclusions about the predominant metabolic pathways can be drawn from the acetate/glucose utilization ratios of metabolites such as glutamate, glutamine and GABA.

1.9.1.1. Labeling from [1-^{13}C]glucose

As described for glucose in general in section 1.1.2. and as can be seen in Figure 3 [1-^{13}C]glucose is converted to pyruvate via glycolysis and can form [3-^{13}C]alanine and [3-^{13}C]lactate. Pyruvate may enter the TCA cycle via [2-^{13}C]acetyl-CoA, which will lead to the formation of [4-^{13}C]glutamate and glutamine or [2-^{13}C]GABA. Alternatively, pyruvate can be carboxylated by pyruvate carboxylase (PC) to oxalacetate, which will lead to the synthesis of [2-^{13}C]glutamate and glutamine or [4-^{13}C]GABA.

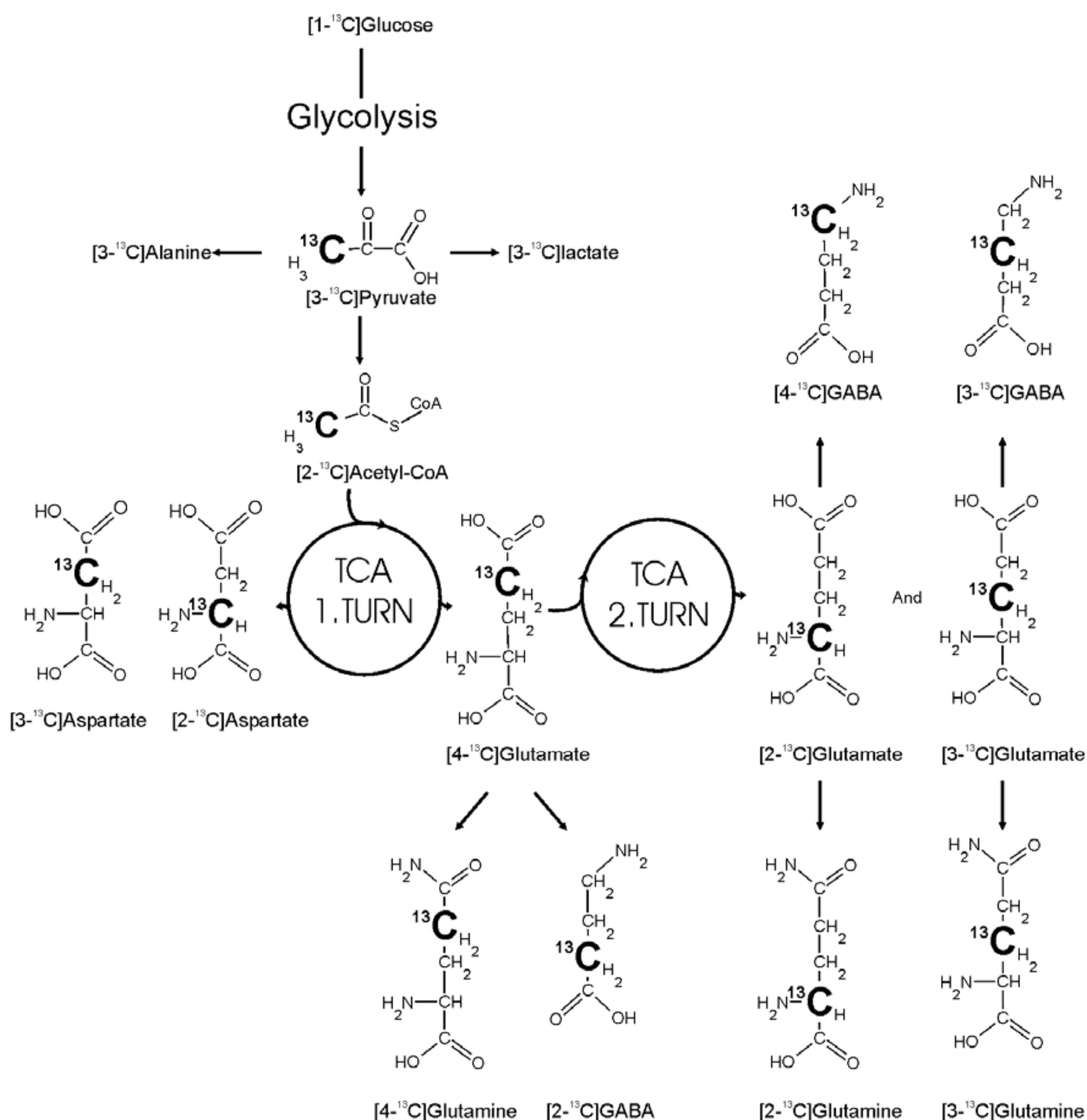


Figure 3. Schematic presentation of isotopomers of glutamate, glutamine and GABA derived from [1-^{13}C]glucose after the first and second turn of the tricarboxylic acid (TCA) cycle.

1.9.1.2. Labeling from [1,2-^{13}C]acetate

[1,2-^{13}C]acetate can also be converted to acetyl-CoA, however, the product, [1,2-^{13}C]acetyl-CoA, will have two ^{13}C atoms (Figure 4), resulting in doublet formation. Thus, [4,5-^{13}C]glutamate and glutamine or [1,2-^{13}C]GABA are formed.

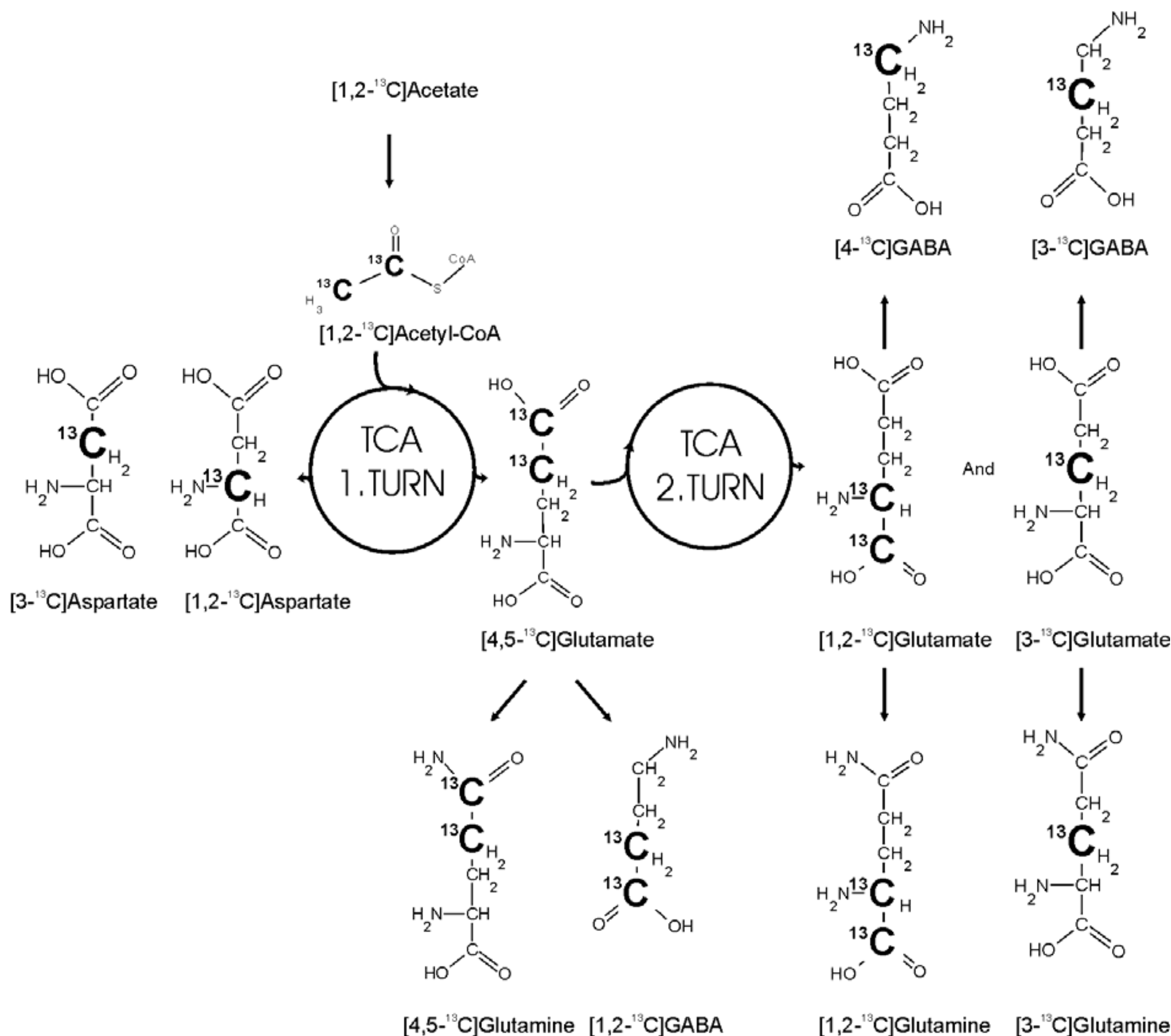


Figure 4. Schematic presentation of isotopomers of glutamate, glutamine and GABA derived from [1,2-^{13}C]acetate after the first and second turn of the tricarboxylic acid (TCA) cycle.

2. Materials and Methods

2.1. Experimental Procedures

2.1.1. The PTZ-Model of Epilepsy and SAMP8

As outlined earlier, the prevalence and incidence of epilepsy is high in the elderly (Sander and Shorvon, 1996). However, most animal models of epilepsy use young adult rodents. Senescence-accelerated mice P8 (SAMP8) represent a model of aging and are, although growing normally, characterized by a life span that is only about half as long as in controls (Abe et al., 1994). Genetically induced learning and memory deficits in SMAP8 (Fujibayashi et al., 1994) might be evoked by substantially increased glioses in hippocampus and cerebral cortex (Nomura et al., 1996). Thus, the PTZ-model of epilepsy was combined with a model of aging: 2 and 8 months old SAMP8 mice received intraperitoneal applications of regular sub-threshold doses of PTZ (35mg/kg) or respectively PTZ and phenobarbital (10mg/kg) every other day for 40 days, while controls were treated with saline. During a period of 30 minutes the behavior was monitored according to a score system: 0=normal behavior, 1= myoclonic jerks, 2=minimal seizures without Straub-tail, 3= minimal seizures with Straub-tail, 4=generalized tonic-clonic seizures with loss of consciousness and postictal phase, 5=like 4 but with rotation on their axis, 6=like 5 and death. On day 40, fifteen minutes after intraperitoneal administration of [1-^{13}C]glucose and [1,2-^{13}C] acetate, the mice were sacrificed, cerebrum, cerebellum and brain stem prepared followed by HPLC and NMR spectroscopy.

2.1.2. The Kaolin-Model of Hydrocephalus

Rats were deeply anaethesized with ketamine (100mg/kg body weight) and medotomidinhydrochlorid (0,5mg/kg) and the cisterna magna was prepared microsurgically by exposing the atlanto-occipital membrane. Following a medial incision a venflon catheter was inserted and 0.1ml kaolin solution carefully injected. The membrane incision was covered using a

3x3mm piece of resorbable gelatin sponge to prevent leakage of Kaolin. After wound closure the rats were allowed to recover and hydrocephalus developed spontaneously. Two, four and six weeks after kaolin injection rats were decapitated and NMR spectroscopy performed on cerebrum, cerebellum and brain stem.

2.1.3. The NMDA receptor antagonist MK801 and schizophrenia

Two different studies, single and repeated administration of MK801, were performed.

First, saline or MK801 (0.5mg/kg body weight) were injected once intraperitoneally in Sprague Dawley rats. Immediately afterwards all subjects received [1-C^{13}]glucose and [1,2- C^{13}]acetate intraperitoneally in the contralateral side of the abdomen. Twenty minutes later animals were sacrificed by decapitation and cingulate, retrosplenial and parts of the frontal cortex (CRFC) were dissected. Moreover, the temporal lobe (TE) was prepared by a 3mm long horizontal cut from the most lateral point of the hemisphere and another cut reaching ventrally through the whole brain. Thus, TE included temporal and piriform cortex, amygdala and parts of the hippocampus. CRFC and TE were then analyzed using HPLC, ^{1}H and ^{13}C NMR spectroscopy.

Second, saline or MK801 (0.5mg/kg body weight) were administered intraperitoneally on any other day for twelve days. Ataxia was assessed by observing rats for 30 minutes after MK801 administration and documenting the number of falls or other sudden drops in posture. Hyperlocomotion was measured during the same period by counting how often rats crossed over from one corner of the cage to the other and passed completely one of the two imaginary lines dividing the cage in four equal quarters. The last MK801 injection was given together with [1-^{13}C]glucose and [1,2- ^{13}C]acetate and twenty minutes afterwards animals were decapitated. TE and CRFC were studied by HPLC, ^{1}H and ^{13}C NMR spectroscopy.

Three MK801 treated rats and five controls did not receive [1-^{13}C]glucose and [1,2-^{13}C]acetate in their last injection. They were deeply anesthetized with ketamine (100mg/kg) and medotomidinehydrochloride (0.5mg/kg) and underwent cardiac perfusion with 20ml heparinized 0.9% saline and a modified Karnovsky solution (2% formaldehyd, 2.5% glutaraldehyd, 0.1M

natriumcacodylate buffer, 0.025% $CaCl_2$; pH 7.4). Brains were removed immediately after perfusion and stored in the same fixative until slicing. All slices were processed by sequential alcohol dehydration, cleared, infiltrated, embedded in paraffin and stained with Hematoxilin-Eosin or Nissl-stained. Using the atlas of Paxinos and Watson (1998) CRFC, TE and hippocampus were identified and examined by light microscopy for signs of neuronal degeneration such as intracytoplasmatic vacuoles, remnant nuclear debris, brightly eosinophilic cytoplasm and local glial reaction, which have been described earlier (Olney et al., 1989).

2.2. High Pressure Liquid Chromatography

Absolute levels of amino acids such as glutamate, GABA, glutamine, alanine, aspartate and taurine were analyzed by High Pressure Liquid Chromatography (HPLC) on a Hewlett Packard system (Agilent, USA) with fluorescence detection after derivatization with o-phthaldialdehyde. In the study of repeated MK801 administration (paper V) also dopamine, noradrenaline and serotonin were measured. As quantification standard a standard solution of amino acids was examined in between all samples, which were normally highly diluted before analysis (Geddes and Wood, 1984). For paper V the monoamine transmitter substances (NA, Da, 5-HT) and acid (DOPAC, 5-HIAA, HVA) metabolites were quantified in brain tissue homogenates by HPLC separations and electrochemical detection. The analytical method is based on two chromatographic separations for amines and acids. Both systems are equipped with a reverse phase coloumn (Luna C18(2), dp 3µm, 50 x 2mm i.d., Phenomex), and electrochemical detection is accomplished at two potentials on glassy carbon electrodes (MF-1000, Bioanalytical Systems, Inc.). The aqueous mobile phase (0.4ml/min) for the acid system contains citric acid 14mM, sodium citrate 10mM, MeOH 15% (v/v) and EDTA 0.1mM. Detection potentials relative to Ag/AgCl reference are 0.45 and 0.6V. The aqueous ion pairing mobile phase (0.5ml/min) for the amine system contains citric acid 5mM, sodium citrate 10mM, MeOH 9%(v/v), decane sulfonic acid 0.45mM and EDTA 0.1 mM. Detection potentials relative to Ag/AgCl reference are 0.45 and 0.65V.

2.3 ^{13}C NMR spectroscopy

Proton decoupled 150.92 MHz ^{13}C NMR spectra were obtained using a Bruker DRX-600 spectrometer after the samples had been re-dissolved in 200 µL D_2O containing ethylene glycol 0.1% as an internal standard. Scans were accumulated with a 30º pulse angle and 30 kHz spectral width with 64K data points. The number of scans was typically 10,000. The acquisition time was 1.08 s, the relaxation delay 0.5 s. Factors for nuclear Overhauser and relaxation effects were applied to all spectra.

2.4. ^{1}H NMR spectroscopy

^{1}H is the natural form and easily detected by NMR spectroscopy (for reviews see Bachelard and Badar-Goffer, 1993; Sonnewald et al., 1994). The acquisition time is short. However, the disadvantage is that the chemical shift range is very narrow and peaks may overlap. ^{1}H NMR spectroscopy is used to quantify concentrations of amino acids, lactate, glucose and other small molecules. For the experiments in papers IV and V a DRX-600 spectrometer was used to obtain ^{1}H NMR spectra with a sweep width of 12 kHz with 32K data points. The pulse angle was 90°, the acquisition time 1.36 s and the relaxation delay was 10 s. The number of scans was 400. Water suppression was set at the residual H_2O resonance.

2.5. Statistics

The statistical differences between groups were analyzed by several methods such as ANOVA and student's t-test, depending on the number of groups. Please see the different papers for more information. $p<0.05$ was considered significant.

3. Aims of Study

To answer the following questions:

1. What are the glial-neuronal interactions in experimental epilepsy in SAMP8 and how are they influenced by phenobarbital? Are there differences in behavior in old SAMP8 compared to young SAMP8? What impact has aging on brain metabolism?

2. What are the glial-neuronal interactions in experimental hydrocephalus? How do these interactions change with the time course of hydrocephalus development?

3. What are the glial-neuronal interactions in experimental schizophrenia, respectively in NMDA antagonism? What are the differences in brain metabolism between a single acute and repeated MK801 administration?

4. Is there evidence for neuronal degeneration in the cortex of rats treated repeatedly with MK801? Is there a different pattern of impairment in different cortical localizations? How might cortical metabolism of monoamines such as dopamine, noradrenaline and serotonin be connected with glial-neuronal glutamatergic interactions?

5. Is there a common pattern of glial-neuronal interactions in the different brain disorders? Do astrocytes preserve and protect neuronal metabolism? Does disturbance of glial-neuronal interactions start with astrocytic impairment?

6. What additional information of disordered brain biochemistry does ^{13}C NMRS reveal compared to HPLC?

4. Synopsis of Papers

Paper I

D. Kondziella, A. Bidar, B. Urfjell, O. Sletvold, U. Sonnewald: The pentylentetrazole-kindling model of epilepsy in old and young SAMP8 mice: Behaviour and metabolism. Neurochemistry International, 2002 (40): 413C-418.

Pentylentetrazole (PTZ)-kindling was induced in senescence-accelerated mice P8 (SAMP8), a model for aging. 2 months and 8 months old SAMP8 received either PTZ (35mg/kg), PTZ (35mg/kg) and Phenobarbital (10mg/kg) or saline intraperitoneally every second day for 40 days and their behavior was monitored using the following score list: (0) normal behavior, (1) myoclonic jerks, (2) minimal seizures without Straub-tail, (3) minimal seizures with Straub-tail, (4) generalized tonic-clonic seizures with loss of consciousness and postictal phase, (5) like (4) but with rotation on their axis. On day 40, the animals were decapitated and amounts of glutathione, glutamine, glutamate, GABA, aspartete, alanine plus taurine were measured by HPLC. Although each mouse had an individual seizure pattern, no statistical difference were found between young and old SAMP8. PTZ evoked seizures leading to death occurred in two of the six 8 months old animals, but not in any 2 months old animal. Atypical absence seizures were restricted to old animals and occurred in three animals. Significantly lower amounts of GABA, glutamine and glutathione were noted in 8 months old SAMP8 controls compared to 2 months old. However, concentrations of metabolites were the same in all groups of 2 months old animals, whereas 8 months old SAMP8 had raised GABA, glutamine and glutathione levels when treated with either PTZ alone or together with Phenobarbital. Thus, it was concluded that, in terms of absolute metabolite concentrations, brain metabolism of old SAMP8 is more susceptible to PTZ and Phenobarbital compared to young SAMP8.

Moreover, MR imaging was performed on a 2.3T DRX 100 Biospec from Bruker (Germany) on one 2 months old PTZ animal and one young control, which later were excluded from the metabolic

studies, however, no differences were found.

Paper II

D. Kondziella, J. Hammer, O. Sletvold, U. Sonnewald: The pentylenetetrazole-kindling model of epilepsy in SAMP8 mice: Glial neuronal metabolic interactions. Neurochemistry International, 2003 (43): 629-637.

Pentylentetrazole(PTZ)-kindling was combined with the aging model of senescence-accelerated mice P8 (SAMP8) as described in Paper I. On day 40, the animals were injected with intraperitoneal [1-^{13}C]glucose (0.3M, 543mg/kg) plus [1,2-^{13}C]acetate (0.6M, 504mg/kg) and ^{13}C NMRS was performed on the whole brains. In 2 months old SAMP8 PTZ-kindling decreased labeling of glutamine C-4 both from [1-^{13}C]glucose and [1,2-^{13}C]acetate, while it lowered labeling in glutamate C-4 from [1-^{13}C]glucose only, indicating that PTZ-kindling affected mainly astrocytes in younger and glutamatergic neurons in older animals. Surprisingly, in the presence of PTZ, phenobarbital decreased labeling of most metabolites from both [1-^{13}C]glucose and [1,2-^{13}C]acetate in young SAMP8, although in older animals only GABAergic neurons were affected as suggested by an increase in GABA labeling. In addition, phenobarbital normalized glutamate labeling from [1-^{13}C]glucose in the old PTZ/phenobarbital animals.

Paper III

D. Kondziella, Q. Hong, W. Luedemann, T. Brinker, O. Sletvold, U. Sonnewald: Astrocyte metabolism is disturbed in the early development of experimental hydrocephalus. Journal of Neurochemistry,2003 (85) 274-281.

Two, four and six weeks after the Kaolin injection into the cisterna magna of adult Sprague Dawley rats, [1-^{13}C]glucose (0.3M, 543mg/kg) plus [1,2-^{13}C]acetate (0.6M, 504mg/kg) were administered intraperitoneally and cerebrum, cerebellum and brainstem dissected. Interestingly, ^{13}C NMRS revealed that labeling of most amino acids derived from [1-^{13}C]glucose remained unaffected, while labeling from [1,2-^{13}C]acetate was significantly altered in various metabolites during the acute

period of hydrocephalus. Cerebral [1,2-^{13}C]lactate and brainstem [1,2-^{13}C] GABA were increased, while taurine was decreased in cerebrum two weeks after hydrocephalus induction. Additional two weeks later labeling from [1,2-^{13}C]acetate was statistically decreased in cerebral [4,5-^{13}C]glutamate, [1,2-^{13}C]glutamate and [1,2-^{13}C]GABA. N-acetylaspartate (NAA), a neuronal marker, remained unchanged in week four, and first in the chronic period of hydrocephalus labeling of NAA-C6 was lowered in cerebellum and brainstem, but not in cerebrum. Thus, it was concluded that astrocyte metabolism is disturbed in the early development of kaolin-hydrocephalus and only later, at the chronic stage, neuronal metabolism becomes affected as well.

Paper IV

E. Brenner, D. Kondziella, A. Håberg, U. Sonnewald: Impaired glutamine metabolism in NMDA receptor hypofunction induced by MK801. Submitted.

Sprague-Dawley rats received either a single application of MK801 (0.5mg/kg) or saline together with [1-^{13}C]glucose (543mg/kg) and [1,2-^{13}C]acetate (504mg/kg). After decapitation the temporal lobe and the cingulate, retrosplenial and frontal cortices (CRFC) were prepared and examined by HPLC, ^{1}H NMRS and ^{13}C NMRS. Hypofunction of the NMDA receptor induced similar changes in both brain areas investigated. However, the changes were most pronounced in the temporal lobe. Generally, only labeling form [1-^{13}C]glucose was affected by MK801. The only change in labeling from [1,2-^{13}C]acetate was in an isotopomer of glutamine derived from the second turn of the TCA cycle. In CRFC and temporal lobe amounts of both labeled and unlabeled glutamine were increased, whereas those of aspartate were decreased. The decrease in labeling of aspartate in the CRFC was more pronounced than the decrease in concentration, leading to decreased ^{13}C enrichment. The amount of lactate formed from unlabeled glucose or other unlabeled substrates was also increased in both areas. In temporal lobe, not in CRFC, increased concentrations of glutamate, GABA, succinate, glutathione and inositol were detected together with increased labeling of GABA and succinate from [1-^{13}C]glucose and glutamine from [1,2-^{13}C]acetate. ^{13}C enrichment was increased in succinate and decreased in glutamate. Whereas labeled and unlabeled glutamine was increased,

this was only the case for unlabeled glutamate. The results point towards a disturbance in glutamate-glutamine cycling and thus interaction between neurons and glia, since labeling of these two amino acids from glucose was affected differently.

Paper V

D. Kondziella, E. Brenner, E.M. Evjolfsson, K.R. Markinhuhta., M.L. Carlsson, U. Sonnewald: Glial-neuronal interactions are impaired in the schizophrenia model of repeated MK801 exposure. Submitted.

NMDA receptors such as Phencyclidine and MK801 provoke psychotic and other neuropsychiatric symptoms. Thus, repeated MK801 administration can serve as an animal model of schizophrenia. Saline or MK801 (0.5mg/kg) were injected every other day for twelve days. Hyperlocomotion and ataxia were measured semi-quantitavely for half an hour after each injection. The last dose was given together with [1-^{13}C]glucose (543mg/kg) and [1,2-^{13}C]acetate (504mg/kg) followed by decapitation 20 minutes later. Temporal lobe (TE) and retrosplenial, cingulate and parts of frontal cortex (CRFC) extracts were studied by HPLC, ^{13}C and 1H NMR spectroscopy. Five controls and three MK801 animals underwent cardiac perfusion and Hematoxilin-Eosin- and Nissl-stained histological slices from RSC and TE were examined by light microscopy, but no morphological changes were found. Behavioral alterations such as head waving, hyperlocomotion, abducted hindlimbs and ataxia were found and were characterized by considerable inter- and intravariability.

MK801 affected the CRFC to a much greater extent than the temporal lobe with significant increases in the levels of glutathione, glutamate and taurine, but unchanged cortical amounts of dopamine, noradrenaline and serotonin. [4,5-^{13}C]glutamate and [4,5-1^{13}C]glutamine, derived from [1,2-^{13}C]acetate, were significantly decreased in CRFC. Label from [1-^{13}C]glucose was affected in the same brain region with decreases of [4-^{13}C]glutamate, [2-^{13}C]GABA and [4-^{13}C]GABA. Glutamate cycling from [1-^{13}C]glucose was changed in both the investigated brain areas and an increase of the cycling ratio for ^{13}C from [1,2-^{13}C]acetate was found for glutamine in the CRFC. Acetate/glucose ratios of glutamate and glutamine were decreased in the CRFC. It was

concluded that the present findings might add to the disturbances of the cortico-striato-thalamo-cortical loop caused by NDMA receptor blockade and hence to the sensory gating deficits provoking sensory overstimulation of the cortex and psychosis. Moreover, astrocytic function and the glutamine-glutamate cycle are probably of greater importance for schizophrenia pathophysology than hitherto recognized.

5. Discussion

5.1. Glial-neuronal interactions in the PTZ model of epilepsy in SAMP8

Each mouse had an individual seizure pattern and besides the fact that deaths and atypical absence seizure were restricted to old SAMP8, no statistical differences were found between young and old PTZ -kindled mice. Although statistically significant, the results concerning the old animals, which received either PTZ alone or together with Phenobarbital, should be interpreted cautiously due to the reduced number of animals in the 8 months groups. However, the observed deaths in these groups are in line with former results showing that the lethal dose for PTZ (Nokubo and Kitani, 1988) and Phenobarbital (Kitani et al., 1988) is reduced in mice with increasing age.

In the HPLC study no biochemical changes were observed between the groups of 2 months old, while in the 8 months old both treatment groups had significantly higher amounts of GABA, glutamine, glutathione and alanine plus taurine compared to 8 months old controls. Since in the 8 months old treatment groups glutamate concentration was unchanged, while an increase was seen in GABA, glutamine and glutathione, it appeared likely that an increased glutamate release lead to raised production of glutamate related products as a possible neuroprotective adaption. SAMP8 mice receiving both PTZ and Phenobarbital had the same glutamate concentration as the PTZ-kindled mice, thus it is probable that glutamate levels are influenced by PTZ rather than by the seizures themselves.

According to the HPLC results, cerebral metabolism of 8 months old animals seemed more sensitive to PTZ: In terms of absolute metabolite concentrations, brain metabolism of old SAMP8 was more susceptible to PTZ and Phenobarbital compared to young SAMP8. Moreover, it was suggested that glutamate metabolism was altered in old SAMP8 and that excessive glutamate might be transformed into glutamate related metabolites, possibly in astrocytes.

However, this interpretation was only partly confirmed by NMRS results, indicating that PTZ-kindling affected astrocytes in younger animals (decreased labeling in glutamine C4 from [1-C13C]glucose and [1,2-^{13}C]acetate) and glutamatergic neurons in older animals (decreased labeling

in glutamate C4 from [1- ^{13}C]glucose). Surprisingly, PTZ-kindling did not enhance glutamate in 2 or 8 months old SAMP8. On the contrary, glutamate labeling was significantly decreased in the 8 months old PTZ animals. A possible explanation might be that PTZ-kindling enhances the density of glutamate-binding sites on excitatory neurons, which has indeed been shown earlier (Schroeder et al., 1999).

Being in line with HPLC results, aging alone lead to decreased mitochondrial activity in glutamatergic, not GABAergic, neurons, as shown by decreased glutamate labeling from [1-^{13}C]glucose in old control animals compared to young controls. Moreover, old SAMP8 mice had impaired astrocytic metabolism, since glutamine synthesis from [1,2-^{13}C]acetate was lowered. Phenobarbital in the presence of PTZ decreased labeling of most metabolites from both [1-^{13}C]glucose and [1,2-^{13}C]acetate in younger animals with the exception of GABAergic neurons, however, in older animals only GABAergic neurons were affected as indicated by an increase in GABA labeling. Thus, in contrast to the HPLC results, NMRS revealed significant metabolic alterations provoked by Phenobarbital, which were most pronounced in the 2 months old animals. Together with the unchanged amino acid levels the decrease of labeling of most metabolites from both precursors points towards decreased turnover of metabolites in the 2 months old animals treated with both PTZ and Phenobarbital. Interestingly, co-administration of PTZ and Phenobarbital lead to considerably greater changes than PTZ alone. Since PTZ probably acts to a greater extent by increasing the number of glutamate-binding sites than by interfering with metabolism, as was outlined above, the observed metabolic changes are presumably caused by Phenobarbital alone and not by the combined effect of PTZ and Phenobarbital.

Furthermore Phenobarbital increased GABA-labeling in young and decreased it in old animals, underlining again the significant role of GABA for the growing efficacy and neurotoxicity of barbiturates in elderly subjects (Macdonald and Barker, 1977; Kitani et al., 1988). Howerever, it is interesting that in the old PTZ/phenobarbital animals phenobarbital normalized glutamate labeling from [1-^{13}C]glucose compared to the old PTZ group.

Additionally, alanine labeling from [1-^{13}C]glucose and glutamate C4 from repeated [1,2-^{13}C]acetate

labeling were decreased in the young animals receiving both Phenobarbital and PTZ, indicating impaired astrocytic metabolism, since alanine labeling in the cortex is presumably an astrocytic process (Sonnewald et al., 1991; Westergaard et al., 1993). Transfer of labeled glutamine from astrocytes to neurons was probably reduced as well.

Thus, HPLC results showed only metabolic changes in the 8 months old, and not in the 2 months old mice. However, NMRS results indicated changes both in the young and the old animals, namely impaired metabolism of glutamatergic neurons in older and of astrocytes in younger rodents.

5.2. Glial-neuronal interactions in adult rats with kaolin-induced hydrocephalus

Interestingly, while labeling of most metabolites derived from [1-^{13}C]glucose remained without alterations, labeling from [1,2-^{13}C]acetate was affected. Two weeks after kaolin-treatment labeling from [1,2-^{13}C]GABA was increased in brain stem and from [1,2-^{13}C]lactate in cerebrum. Four weeks after hydrocephalus-induction labeling of [4,5-^{13}C]glutamate, [1,2-^{13}C]glutamate and [1,2-^{13}C]GABA in cerebrum were decreased. Labeling of CH_3 in N-acetyl-aspartate, being the only altered neuronal marker, was decreased in cerebellum and brainstem six weeks after kaolin-injection into the cisterna magna.

Thus, brain metabolism during the first two weeks of hydrocephalus is not severely damaged, since apart from an increase in cerebrum [1,2-^{13}C]lactate and brain stem [1,2-^{13}C]GABA no other changes were observed. Astrocyte metabolism in cerebrum is, however, distinctly disturbed from week 4 as shown by decreased labeling of metabolites as [4,5-^{13}C]glutamate, [1,2-^{13}C]glutamate and [1,2-^{13}C]GABA from [1,2-^{13}C]acetate. In addition [4,5-^{13}C]glutamate was decreased in cerebellum. The decreased acetate/glucose utilization ratios for glutamate in cerebrum and cerebellum and for GABA in cerebrum reflects altered astrocytic biochemistry as well. Astrocytes release [4,5-^{13}C]glutamine from [1,2-^{13}C]acetate for neuronal use. Neurons convert this glutamine to [4,5-^{13}C]glutamate, which is mostly stored in glutamatergic neurons (Ottersen and Storm-Mathisen, 1984). Decreased transport of astrocytic glutamine to glutamatergic neurons in cerebrum was clearly shown by the decrease in [4,5-^{13}C]glutamate from 4 weeks after kaolin installation,

since [4,5-^{13}C]glutamine was unchanged. The same was apparently true for the cerebellum. This decrease in neurotransmitter precursor could not remain without consequences for the glutamatergic neurons because glutamate released from neurons is mainly taken up by astrocytes. Thus, it needs to be replenished by glutamine from astrocytes and this constitutes the so-called "glutamine-glutamate cycle" (Hertz 1979). Since no changes in glutamate concentrations were found in an earlier study using HPLC (Kondziella et al., 2002), glutamatergic neurons probably either released less glutamate to decrease the need for replenishment or glutamate from neurons was consumed to a lesser extent in the astrocytic TCA cycle. Impairment of these cycles is, however, not likely at the early stages of hydrocephalus as labeling from [1-^{13}C]glucose remained unaffected at all time points after kaolin injection. Alternatively, increased synthesis of neuronal glutamate from unlabeled precursors might compensate for the decrease in [4,5-^{13}C]glutamate, however, this would eventually lead to depletion of metabolites as neurons lack an anaplerotic pathway (Shank et al., 1985) and, consequently, to loss of cell function.

Several metabolites from [1,2-^{13}C]acetate were altered, but labeling from [1-^{13}C]glucose was only changed in the acetyl group of NAA. NAA is localized to the cytosol of neurons and has been proposed as a neuronal marker (Tsai and Coyle, 1995). The amount of label in NAA was decreased in cerebellum and brainstem from 6 weeks, but remained unchanged in cerebrum, indicating a slight impairment of mitochondrial function in these two brain regions in the chronic period of hydrocephalus. Since ventricular enlargement in experimental hydrocephalus does not stop until 8 weeks after kaolin instillation (Braun et al., 1999), these neuronal disturbances might be related to mechanical distortion of the brainstem and cerebellum due to continuing expansion of the third and fourth ventricle.

[1,2-^{13}C]lactate concentration was slightly increased in cerebrum 2 and 6 weeks after hydrocephalus onset, while a decrease was noticed in the brainstem in week 6. Via the TCA cycle, [1,2-^{13}C]lactate is obtained from [1,2-^{13}C]acetate, as was shown by Hassel and Sonnewald (1995). Note that lactate can be labeled via the TCA cycle in cultured astrocytes from [U-^{13}C]glutamate, which was not the case in cultured neurons (Sonnewald et al., 1996). Therefore the increase in [1,2-^{13}C]lactate levels

observed in the present study probably occurred in astrocytes. However, the decrease in [4,5-^{13}C]glutamate and [1,2-^{13}C]GABA took place in neurons, confirming impaired glial-neuronal interaction.

In an earlier study it was shown that the acute phase of hydrocephalus in the first four weeks after kaolin-injection is characterized by ICP, increased CSF resistance, beginning ventriculomegaly, enhanced brain water content and moderate alterations in brain biochemistry as measured by HPLC (Kondziella et al., 2002). Glutamine concentration is increased and taurine concentration decreased in the cerebrum, while alanine is increased in the brain stem. However, the chronic phase is defined by normal ICP, declining CSF outflow resistance, continuing ventricular enlargement and distinct changes in the biochemical parameters such as a remarkable decrease of glutamate, glutamine and taurine in the cerebellum, a decrease of taurine and alanine plus an increase in glutamine in the cerebrum and an increase of alanine in the brain stem (Kondziella et al., 2002).

While in the same study the amount of taurine remained decreased in the cerebrum, the amount of glutamine increased, indicating reactive gliosis, since glutamine synthesis in brain is an exclusively astrocytic process. Indeed, the largest effect of hydrocephalus in the cerebrum was seen in taurine, an osmolyte (Pasantes-Morales et al., 2002), which was decreased during all time points. It was hypothesized that the enhancing ICP and CSF outflow resistance evoke cell oedema which is compensated for by release of taurine. This protective mechanism might become insufficient during the fourth week, as indicated by increasing brain water content (Kondziella et al., 2002).

However, this hypothesis could not be confirmed in the present NMRS study, since the concentration of inositol, which like taurine has a role as an osmotic regulator and is thought to be mostly of astrocytic origin (Wolfson et al., 2000), was not altered. Therefore swelling of astrocytes is probably not a prominent element in kaolin-hydrocephalus and might instead be true for oligodendrocytes in white matter. In this context it is interesting that evidence for an earlier disturbance of cerebral blood flow in white matter than in gray matter has been reported (Da Silva et al., 1995; Kristensen et al., 1996).

Thus, in the acute stage of experimental hydrocephalus astrocytic metabolism is clearly affected and

only later, during the chronic period six weeks after induction of hydrocephalus, neuronal metabolism appears to be moderately impaired.

5.3. Glial-neuronal interactions in MK801 induced NMDA receptor hypofunction and experimental schizophrenia

A single dose of MK801 provoked similar metabolic changes in CRFC and TE, however, the changes were more pronounced in the latter. The amount of glutamine formed from [1,2-^{13}C]acetate derived from the first turn of the TCA cycle, [4,5-^{13}C]glutamine, was unchanged, whereas [1,2-^{13}C]glutamine was increased in the temporal lobe. The latter form of glutamine is derived from [1,2-^{13}C]acetate as well, however, 2-oxoglutarate for synthesis of [1,2-^{13}C]glutamine via [1,2-^{13}C]glutamate has stayed in the TCA cycle for an additional turn. Thus, in the temporal lobe, mitochondrial function was altered in the astrocytes. No changes were observed in [4,5-^{13}C]glutamine, [4,5-^{13}C]glutamate and [1,2-^{13}C]GABA from [1,2-^{13}C]acetate demonstrating unperturbed metabolic flux from astrocytes to neurons in both areas of the brain. These observations point towards compartmentation of glutamine metabolism, where glutamine labeled from neuronal glutamate ([4-^{13}C]glutamate) is handled in a different compartment than glutamine from astrocytic glutamate.

While astrocytic metabolism seemed relatively preserved, decreased neurotransmitter release from synaptic vesicles and impaired conversion of glutamine to glutamate in neurons were noticed. However, mitochondrial metabolism in neurons appeared largely preserved, since N-acetylaspartate levels remained unchanged. Glutamate synthesis from ^{13}C labeled precursors was not influenced by a single dose of MK801. Only unlabeled glutamate was enhanced in the temporal lobe, implying that vesicular and initially unlabeled neurotransmitters are not in rapid balance with the cytosolic amino acid pool of [4-^{13}C]glutamate. In contrast, GABA synthesis from [1-^{13}C]glucose was affected by acute NMDA receptor antagonism, since [3-^{13}C]GABA from the second turn of the TCA cycle was increased in the temporal lobe.

Thus, a single dose of MK801 severely disturbs the “glutamine-glutamate-GABA-cycle” between

neurons and astrocytes as was seen by the significant increase in labeled and unlabeled glutamine in both TE and CRFC. Presumably, NMDA receptor blockade reduces Ca^{2+} influx into the cell and consequently increases activity of glutamine synthetase. Phosphate activated glutaminase, however, appears not capable of transforming the increased amount of glutamine into glutamate, possibly due to impaired activation secondary to the low intracellular Ca^{2+} concentration. As a consequence, glutamine probably accumulates in astrocytes. See Figures 5a and 5b for a schematic presentation.

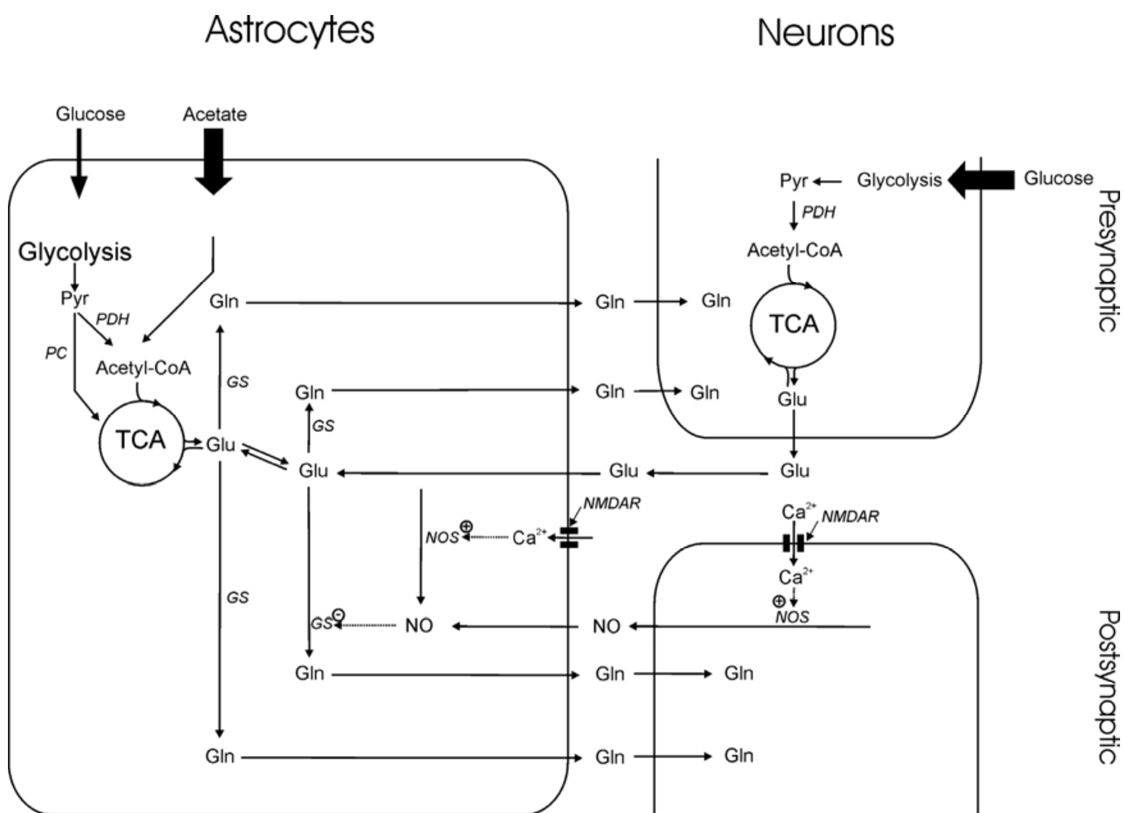


Figure 5a. Interactions of an astrocyte with pre- and post-synaptic neurons. Glutamate synthesized by neurons is released into the synaptic cleft, activating NMDA receptors on astrocytes and neurons and is deactivated by uptake mostly into astrocytes. In these glutamate is converted to glutamine by glutamine synthetase, an enzyme modulated by nitric oxide. For abbreviations see Figure 5b.

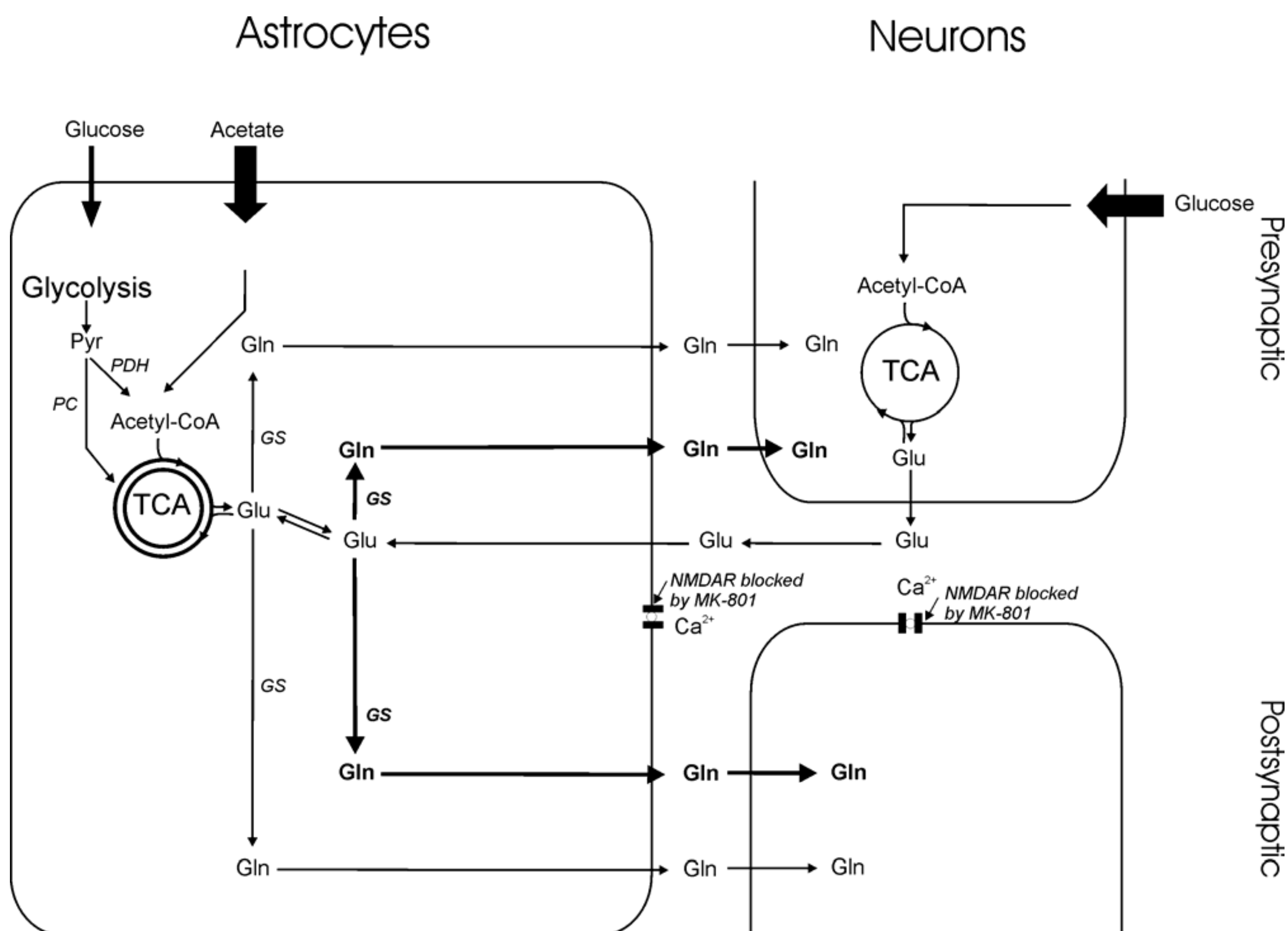


Figure 5b. Schematic presentation of the interactions of an astrocyte with pre- and post-synaptic neurons after a single administration of MK801. Reduced NO production due to reduced activation of the NMDA receptor will lead to increased activity of GS. This will be most pronounced in the synaptic region and will affect the glutamine-glutamate cycle between neurons and astrocytes. *, Glutamine in the non-synaptic region mostly labeled from [1,2-^{13}C]acetate; ⊕, increased enzyme activity; ⊖, decreased enzyme activity; stippled lines, modulation of enzyme activity. Abbreviations: GS, glutamine synthetase; NMDA receptor, N-methyl-D-aspartate receptor; NO, nitric oxide; NOS, nitric oxide synthase; PAG, phosphate activated glutaminase; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase.

No morphological changes were found after repetitive of MK801. Since repeated MK801 administration did not induce any morphological changes detectable by light microscopy, neuronal changes were probably functional, not structural, underlining earlier findings (Brosnan-Watters et al., 1999). While absolute levels and turnover of noradrenaline, dopamine and serotonin were not changed, significant increases in the concentrations of glutamate, glutathione and taurine were found. Glutamate and glutamine, derived from [1,2-^{13}C]acetate and thus astrocytes, were significantly decreased in CRFC. Labeling from [1-^{13}C]glucose and thus mostly neuronal

metabolism was disturbed in the same brain region with lowered labeling of glutamate and GABA. The decreased amount of [4,5-^{13}C]glutamate might have its cause in impaired efflux of [4,5-^{13}C]glutamine from astrocytes to neurons, suggesting that the glutamine-glutamate cycle is disturbed during repeated NMDA-antagonist administration and possibly schizophrenia. The decreased acetate-versus-glucose utilization ratio for glutamate, pointing towards lowered astrocytic contribution to glutamate formation, is in line with this assumption. An intriguing interpretation might be that glutamate, released from neurons, accumulates in astrocytes. The present model of repeated MK801 administration mimics the increased glutamate/glutamine activity found in drug naive patients with first episode schizophrenia (Theberge et al., 2002; Hashimoto et al., 2005). Additionally, the decreased labeling both in astrocytes and neurons may mirror the transition to lower glutamatergic function seen in chronic schizophrenia patients. The decreases in astrocytic function and the glutamine-glutamate-GABA cycle might add to the disturbances of the cortico-striato-thalamo-cortical loop caused by NDMA receptor blockade. The impaired thalamic filter probably evokes sensory gating deficits with a decreased signal-to-noise-ratio, which has its clinical correlate in the characteristic difficulties of psychotic patients to differentiate between relevant and irrelevant information. Consequently, sensory overstimulation of the cortex and psychosis develop (Figure 6).

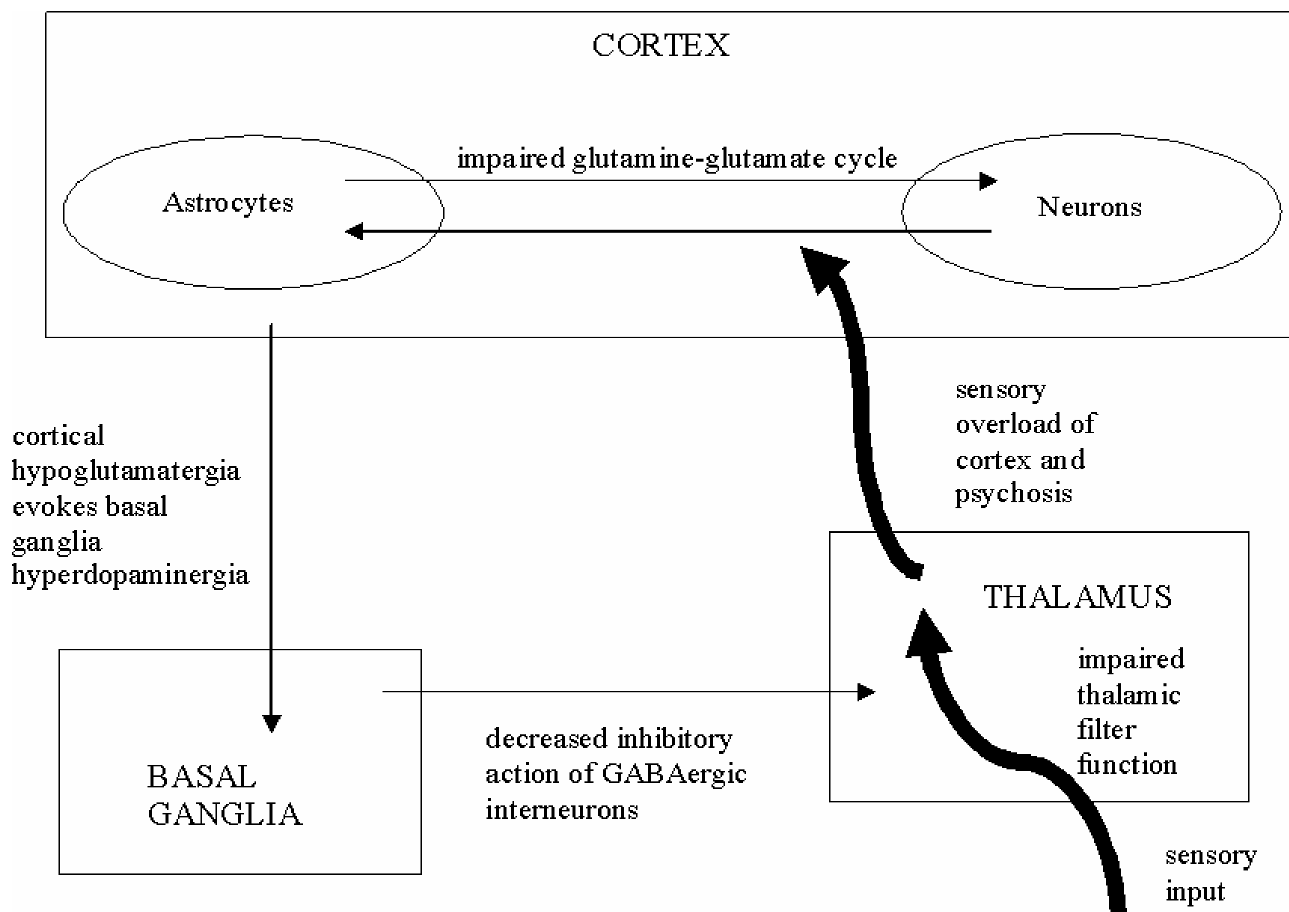


Figure 6. A presentation of the so-called cortico-striato-thalamo-cortical loop, which is believed to be impaired in schizophrenia and in NMDA hypofunction induced by glutamate antagonists such as MK801, PCP or ketamine. Basically, the thalamic filter function appears disturbed, which is clinically mirrored by the well-known fact that psychotic patients have great difficulties to separate important from irrelevant information. Altered glial-neuronal interactions in the cortex might contribute to this.

5.4. Is there a common pattern of glial-neuronal interactions in different brain disorders?

Do astrocytes preserve or protect neuronal metabolism? In the study on hydrocephalus astrocyte metabolism was impaired first. In the kainate model of temporal lobe epilepsy early astrocyte hyperactivity lead to delayed changes in neuronal neurotransmitter metabolism (Müller et al., 2000; Qu et al., 2003). These findings might indicate that glial cells either protect neurons initially or, alternatively, damaged glial cells cause delayed neuronal hypermetabolism. Bear in mind, however, that in this thesis the term "metabolism" mainly refers to glial-neuronal interactions involving glutamate and related metabolites, but does not include other substances such as the catecholamines dopamine, noradrenaline and adrenaline, which of course might have been significantly affected in

the present experiments. At least in the experiment of repeated MK801 administration, cortical dopamine was not decreased.

In contrast to the hydrocephalus and temporal lobe epilepsy experiments, the studies of epilepsy in SAMP8 and of NMDA receptor hypofunction did not show primary astrocytic impairment, thus indicating that there is no general pattern of glial-neuronal interactions, but that astrocytic function differs from disorder to disorder. After a single MK801 dose, astrocytic metabolism remained relatively unaffected, but evidence was found for a decreased neurotransmitter release from synaptic vesicles and impaired conversion of glutamine to glutamate in neurons. Repeated MK801 administration lead primarily to altered neuronal and to a lesser extent impaired astrocytic metabolism. Moreover, neuronal glutamate turnover was decreased in all brain regions after acute generalized seizures induced by a single PTZ-injection, whereas astrocyte metabolism remained unchanged (Eloqayli et al., 2003).

Even neurotoxic properties of astrocytic metabolism have been described: In the stroke model of middle cerebral artery occlusion both astrocytic and neuronal metabolism was impaired early in the core of the ischemic infarct, but in the penumbra glutamine from preserved astrocytes lead to glutamate neurotoxicity (Haaberg et al., 2001). Furthermore, neuronal-glial relationship changes with age, as shown by the SAMP8 study, in which disturbed metabolism was observed in the astrocytes of young and of glutamatergic neurons in old animals.

5.5. Validation of Methods

What additional information of disordered brain biochemistry does ^{13}C NMRS reveal compared to HPLC? HPLC-results in the PTZ-model of epilepsy suggested that glutamate metabolism was altered in old SAMP8 and that excessive glutamate was transformed into glutamate related metabolites, possibly in astrocytes. This was only partly confirmed by NMRS results showing altered glutamate metabolism, but a decrease, not an increase in glutamate labeling. Moreover, although HPLC indicated undisturbed brain metabolism in young SAMP8 treated with Phenobarbital and PTZ or PTZ alone, NMRS revealed profound changes in astrocytic metabolism

in these animals. However, HPLC results were interpreted such that aging in control SAMP8 lead to impaired astrocytic metabolism, which was corroborated by NMRS showing decreased glutamine synthesis from [1,2-^{13}C]acetate.

In the hydrocephalus studies, HPLC and NMRS revealed lower levels of amino acid, respectively labeling, in the brainstem compared to cerebrum, probably reflecting the greater content of gray matter in the cerebrum. Moreover, with both methods evidence was found for impaired neuronal metabolism in the cerebellum during the chronic phase of kaolin-hydrocephalus. In the experiment of acute MK801 induced NMDA hypofunction a significant increase in [4-^{13}C]glutamine and a decrease in [2-^{13}C]aspartate was noted in the CRFC (NMRS) with corresponding alterations for the absolute levels of aspartate and glutamine (HPLC).

Compartmentation, intercellular and intracellular, is well-documented and ^{13}C NMRS studies have also confirmed this concept (Waagepetersen et al., 2003; Sonnewald et al., 2004; Qu et al., 2005). Recently, the first in vivo evidence of the compartmentation of uptake and metabolism of glucose in neurons and astrocytes has been reported (Nehlig et al., 2004). Also in the present experiments evidence for compartmentation was found. For example, in rats treated with a single dose of MK801 glutamine labeled from neuronal glutamate ([4-^{13}C]glutamate) was handled differently than glutamine from astrocytic glutamate ([4,5-^{13}C]glutamate). However, since HPLC only measures absolute metabolite levels, its contribution to the study of intracellular compartmentation is very limited.

In conclusion it can be stated that, although NMR studies are very expensive, ^{13}C NMRS will presumably gain considerable clinical impact in the future. Elegant human in vivo studies are already emerging (for a review see Ross et al., 2003)and also combinations of in vivo and ex vivo studies have been published (Garcia-Espinosa et al., 2004; Martinez-Bisbal et al., 2004). The dynamic picture of metabolic changes obtained by ^{13}C NMRS complements the rather static facts obtained by ^{1}H NMRS and methods such as HPLC. Combination of ^{13}C and ^{1}H NMRS allows the detection of energy metabolism and neurotransmission during functional activation, thereby further strengthening our understanding of the neurochemical basis of brain function (de Graaf et al., 2003).

However, when possible, NMRS and HPLC data should always be interpreted together, since differences in the amounts of unlabeled and labeled metabolites can give valuable information.

6. Conclusions

1. PTZ-kindling alters mainly metabolism of astrocytes in younger and of glutamatergic neurons in older SAMP8.

2. In the presence of PTZ, phenobarbital decreases labeling of most metabolites from both [1-^{13}C]glucose and [1,2-^{13}C]acetate in young SAMP8, although in older animals only GABAergic neurons are concerned. In addition, phenobarbital normalizes glutamate labeling from [1-^{13}C]glucose in the old PTZ/phenobarbital animals.

3. Aging of SAMP8 leads to decreased mitochondrial activity in glutamatergic neurons, as shown by decreased glutamate labeling from [1-^{13}C]glucose in old control animals compared to young controls. Moreover, old SAMP8 mice have disturbed astrocytic metabolism, which is indicated by lowered glutamine synthesis from [1,2-^{13}C]acetate.

4. Astrocyte metabolism is impaired in the early development of kaolin-hydrocephalus and only later, at the chronic stage, neuronal metabolism becomes affected as well.

5. A decrease in [4,5-^{13}C]glutamate and unchanged [4,5-^{13}C]glutamine indicates impaired transport of astrocytic glutamine to glutamatergic neurons 4 weeks after hydrocephalus induction.

6. While a single dose of MK801 mainly disturbs metabolism in the temporal lobe, repeated administration leads mostly to metabolic impairment in the CRFC.

7. A single dose of MK801 profoundly affects the glutamine-glutamate-GABA-cycle between

neurons and astrocytes and evidence has been found for decreased neurotransmitter release from synaptic vesicles and impaired conversion of glutamine to glutamate in neurons.

8. MK801 leads to compartmentation of glutamine metabolism, where glutamine labeled from neuronal glutamate is handled in a different compartment than glutamine from astrocytic glutamate.

9. Repeated MK801 administration provokes primarily altered neuronal metabolism, while astrocytic metabolism appears to be relatively unaffected.

10. Although the glutamate antagonist MK801 induces a state of NMDA hypofunction, it increases glutamate concentration in the TE of rats subjected to a single dose and in the CRFC of rats treated with repeated doses.

11. In contrast to the hydrocephalus experiment, the studies of epilepsy in SAMP8 and of NMDA receptor hypofunction do not show primary astrocytic impairment. Thus, astrocytic function differs from disorder to disorder and there is no general pattern of glial-neuronal interactions.

7. References

Abbott NJ, Revest PA, Romero IA: Astrocyte-endothelial interaction: physiology and pathology. Neuropathol Appl Neurobiol, 1992 (18): 424-433

Abe Y, Yuasa M, Kajwara Y, Hosono M: Defects of immune cells in the senescence-accelerated mouse: a model for learning and memory deficits in the aged. Cell Immunol 1994, (157): 59-69

Abi-Dargham A, Gil R, Krystal J, Baldwin RM, Seibyl JP, Bowers M et al: Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. Am J Psychiatry 1998, (155): 761-767

Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles L et al.: Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. Proc Natl Acad Sci U S A 2000, (97): 8104-8109

Anderson CM, Nedergaard Maiken: Actrocyte-mediated control of cerebral microcirculation. Trends Neurosci 2003 (26): 340-344

Aono M, Lee Y, Grant ER, Zivin RA, Pearlstein RD, Warner DS, Bennet ER, Laskowitz DT: Apolipoprotein E protects against NMDA excitotoxicity. Neurobiol Dis, 2002 (11): 214-220

Arriza JL, Eliasof S, Kavanaugh MP, Amara SG: Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. Proc Natl Acad Sci USA, 1997 (94): 4155-4160

Bachelard H, Badar-Goffer R: NMR spectroscopy in neurochemistry. J Neurochem, 1993 (61): 412-429

Balazs R, Machiyama Y, Hammond BJ, Julian T, Richter D: The operation of the gamma-aminobutyrate bypath the tricarboxylic acid cycle in brain tissue in vitro. Biochem J, 1970 (116): 445-461

Ballabh P, Braun A, Nedergaard M: The blood-brain barrier: an overview. Structure, regulation, and clinical implications. Neurobiol Disease 2003 (26): 1-13

Barany M, Arus C, Chang YC: Natural-abundance ^{13}C NMR of brain. Magn Reson Med, 1985 (2): 289-295

Barreca T, Franceschini R, Sinai C, Cataldi A, Francaviglia N, Silvestro C, Rolandi E: Metoclopramide increases plasma but not cerebrospinal fluid vasopressin levels in man: study in hydrocephalic patients. Horm Res, 1991 (35): 239-241

Batini C, Teillet MA, Naquet R: An avian model of genetic reflex epilepsy. Arch Ital Biol. 2004 (142): 297-312

Bender RA, Dube C, Baram TZ: Febrile seizures and mechanisms of epileptogenesis: insights from an animal model. Adv Exp Med Biol. 2004 (548): 213-225

Bennett MV, Contreras JE, Bukauskas FF, Saez JC: New roles for astrocytes: gap junction

hemichannels have something to communicate. Trends Neurosci, 2003 (26): 610-617

Bensimon G, Lacomblez L, Meininger V: A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. N Engl J Med, 1994 (330): 585-591

Berl S, Clarke DD: Compartmentation of amino acid metabolism. In Handbook of Neurochemistry, Vol. 2, Lajtha A (ed.). Plenum Press: New York, 1969: 447-472

Berger PA, Faull KF, Kilkowski J, Anderson PJ, Kraemer H, Davis KL, Barchas JD: CSF monoamine metabolites in depression and schizophrenia. Am J Psychiatry, 1980 (13C7): 174-80

Billups B, Attwell D: Modulation of non-vesicular glutamate release by pH. Nature, 1996 (379): 171-174

Bleich S, Römer K, Wiltfang J, Kornhuber J: Glutamate and glutamate receptor system: a target for drug action. Int J Ger Psych, 2003 (18): 33-40

Bowers MB Jr: Cerebrospinal fluid 5-hydroxyindoles and behavior after L-tryptophan and pyridoxine administration to psychiatric patients. Neuropsychopharmacology, 1970 (9): 599-604

Bradford HF: Glutamate, GABA and epilepsy. Progr Neurobiol, 1995 (47): 477-511

Brandt C, Potschka H, Loscher W, Ebert U: N-methyl-D-aspartate receptor blockade after status epilepticus protects against limbic brain damage but not against epilepsy in the kainate model of temporal lobe epilepsy. Neuroscience. 2003, (118):727-740

Brandt MR, Cummons TA, Potestio L, Sukoff SJ, Rosenzweig-Lipson S: Effects of the NMDA receptor antagonist perzinfotel (EAA-090) on chemically-induced thermal hypersensitivity. J Pharmacol Exp Ther. 2005 Mar 16; [Epub ahead of print]

Braun KP, van Eijsden P, Vandertop WP, de Graaf RA, Gooskens RH, Tulleken KA, Nicolay K: Cerebral metabolism in experimental hydrocephalus: an in vivo 1H and 31P magnetic resonance spectroscopy study. J Neurosurg, 1999 (91):660-8

Breier A, Su TP, Saunders R, Carson RE, Kolachana BS, de Bartolomeis A et al.: Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. Proc Natl Acad Sci U S A ,1997 (94): 2569-2574

Brodie MJ, Dichter MA: Established antiepileptic drugs. Seizure, 1997 (6):477-511

Brosnan-Watters G, Wozniak DF, Nardi A, Olney JW: Parallel recovery of MK-801-induced spatial learning impairment and neuronal injury in male mice. Pharmacol Biochem Behav. 1999, (62):111-22

Bullock MR, Merchant RE, Carmack CA, Doppenberg E, Shah AK, Wilner KD, Ko G, Williams SA: An open-label study of CP-101,606 in subjects with a severe traumatic head injury or spontaneous intracerebral hemorrhage. Ann N Y Aca Sci, 1999 (890): 51-58

Calabresi P, Centonze D, Cupini LM, Costa C, Pisani F, Bernardi G: Ionotropic glutamate receptors: still a target for neuroprotection in brain ischemia? Insights from in vitro studies. Neurobiol Dis, 2003 (12):82-8

Calzada P, Centonze D, Cupini LM, Costa C, Pisani F, Bernardi G: Ionotropic glutamate receptors: still a target for neuroprotection in brain ischemia? Insights from in vitro studies. Neurobiol Dis, 2003 (12): 82-88

Castellion AW, Swinyard EA, Goodman LS: Effect of maturation on the development and reproducibility of audiogenic and electroshock seizures in mice. Exp Neurol. 1965 (13):206-217

Carlsson A, Hansson LO, Waters N, Carlsson ML: A glutamatergic deficiency model of schizophrenia. Br J Psychiatry Suppl., 1999 (37): 2-6

Carlsson A, Waters N, Holm-Waters S, Tedroff, Nilsson M, Carlsson ML: Interactions between monoamines, glutamate, and GABA in Schizophrenia: New Evidence. Annu Rev Pharmacol Toxicol 2001 (41): 237-260

Cerdan S, Kunnecke B, Seelig J: Cerebral metabolism of [1,2-^{13}C]acetate as detected by in vivo and in vitro ^{13}C NMR. J Biol Chem, 1990 (265): 12916-12926

Choeiri C, Staines W, Miki T, Seino S, Messier C: Glucose transporter plasticity during memory processing. Neurosci 2005 (130): 591-600

Choi DW, Koh JY, Peters S: Pharmacology of glutamate neurotoxicity in cortical cell cultures: attenuation by NMDA antagonists. J Neurosci, 1988 (8): 185-196

Collins VJ, Gottlieb CA, Cohen BD, Rosenbaum EA: Intravenous nonbarbiturate, nonnarcotic analgesics: preliminary studies. I. Cyclohexylamines. Anaesth Anal, 1960 (39): 303-306

Corsson G, Domino EF: Dissociative anesthesia: further pharmacological studies and first clinical experiences with the phencyclidine derivate Cl-581. Anaesth Analg, 1966 (45): 29-40

Costa E: The allosteric modulation of $GABA_A$ receptors. Neuropsychopharmacology, 1991 (4): 225-235

Danbolt NC: Glutamate uptake. Progress Neurobiol, 2001 (65): 1-105

Dandy WE, Blackfan KD: Internal hydrocephalus, an experimental, clinical and pathological study. Am J Dis Chil, 1914 (8): 406-481

da Silva MC, Michowicz S, Drake JM, Chumas PD, Tuor UI: Reduced local cerebral blood flow in periventricular white matter in experimental neonatal hydrocephalus-restoration with CSF shunting. J Cereb Blood Flow Metab, 1995 (15):1057-65

Dave JR, Lin Y, Ved HS, Koenig ML, Clapp L, Hunter J, Tortella FC: RS-100642-198, a novel sodium blocker, provides differential neuroprotection against hypoxia/hypoglycamia, veratridine or glutamate-mediated neurotoxicity in primary cultures of rat cerebellar neurons. Neurotox Res., 2001 (3): 381-395

Davis SM, Lees KR, Albers GW, Diener HC, Markabi S, Karlsson G, Norris J: Selfotel in acute ischemic stroke: possible neurotoxic effects of an NMDA antagonist. Stroke, 2000 (31): 347-354

Del Bigio MR, Vriend JP: Monamine neurotransmitter and amino acids in the cerebrum and stiatum of immature rats with kaolin-induced hydrocephalus. Brain Res, 1998 (798): 119-126

De Deyn PP, Macdonald RL: Effects of antiepileptic drugs on GABA responses and on reduction of

GABA responses by PTZ and DMCM on mouse neurons in cell culture. Epilepsia, 1989 (30): 17-25

De Graaf RA, Mason GF, Patel AB, Behar KL, Rothman DL: In vivo 1H-[13C]-NMR spectroscopy of cerebral metabolism. NMR Biomed 2003, (16)339-357

De Vasconcelos AP, Bouilleret V, Riban V, Wasterlain C, Nehlig A. Role of nitric oxide in cerebral blood flow changes during kainate seizures in mice: genetic and pharmacological approaches. Neurobiol Dis. 2005 Mar;18(2):270-81

Dixon WE, Heller H: Experimentelle Hypertonie durch Erhöhung des intrakraniellen Druckes. Arch Exp Pathol Pharmacol, 1932 (166): 265-275

Dube C, Chen K, Eghbal-Ahmadi M, Brunson K, Soltesz I, Baram TZ: Prolonged febrile seizures in the immature rat model enhance hippocampal excitability long term. Ann Neurol, 2000 (47): 336-344

Echlin FA, Battista A: Epileptiform seizures from chronic isolated cortex. Arch Neurol, 1963: 154-170

Egawata T, Mishima K, Egashira M, Fukuzawa M, Abe K, Yae T, Iwasaki K, Fujowara M: Impairment of spatial memory in kaolin-induced hydrocephalic rats associated with changes in the hippocampal cholinergic and noradrenergic contents. Behavioural Brain Res, 2002 (129): 31-39

Eliasof S, Arriza JL, Leighton BH, Kavanaugh MP, Amara SG: Excitatory amino acid transporters of the salamander retina: identification, localization and function. J Neurosci, 1998 (18): 698-712

Eloqayli H, Dahl CB, Gotestam KG, Unsgard G, Hadidi H, Sonnewald U: Pentylenetetrazole decreases metabolic glutamate turnover in rat brain. J Neurochem, 2003 (85):1200-1207

Engelsen BA, Fosse VM, Myrseth, fonum F: Elevated concentrations of glutamate and aspartate in human venticular cerebrospinal fluid (vCSF) during episodes of increased CSF pressure and clinical signs of impaired brain circulation. Neurosci Lett, 1985 (62): 97-102

Farber NB, Wozniak DF, Price MT, Labrueyere J, Huss J, St.Peter H, Olney JW: Age-specific neurotoxicity in the rat associated with NMDA receptor blockade: potential relevance to schizophrenia? Biol Psych, 1995 (12): 788-796

Farber NB, Foster J, Duhan NL, Olney JW: Olanzapine and fluperlapine mimic clozapine in prevengting MK801 neurotoxicity. Schizophr Res, 1996 (21): 33-37

Farber NB, Hanslick J, Kirkby C, McWilliams L, Olney JW: Serotonergic agents that activate 5HT2A receptors prevent NMDA antagonist neurotoxicity. Neuropsychopharmacology, 1998 (18): 57-62

Farber NB, Kim SH, Dikranian K, Jiang XP, Heinkel C: Receptor mechanisms and circuitry underlying NMDA antagonist neurotoxicity. Mol Psychiatry, 2002 (7): 32-43

Farber NB, Jiang XP, Heinkel C, Nemmers B: Antiepileptic drugs and agents that inhibit voltage-gated sodium channels prevent NMDA antagonist neurotoxicity. Mol Psychiatry, 2002 (7): 726-733

Farber NB. The NMDA receptor hypofunction model of psychosis. Ann N Y Acad Sci. 2003 Nov;1003:119-30.

Faden AI, Demediuk P, Panter SS, Vink R: The role of excitatory amino acids and NMDA receptors in traumatic brain injury. Science, 1989 (244): 798-800

Festoff BW, Suo Z, Citron BA: Prospects for the pharmacology of amyotrophic lateral sclerosis: old strategies and new paradigms for the third millenium. CNS Drugs, 2003 (17): 699-717

Foster AC, Gill R, Woodruff GN: Neuroprotective effects of MK-801 in vivo: selectivity and evidence for delayed degeneration mediated by NMDA receptor activation. J Neurosci, 1988 (8): 4745-4754

Fisher RS: Animal models of the epilepsies. Brain Res Brain Res Rev, 1989 (14): 245-278

Fleischhacker WW, Buchgeber A, Schubert A: Memantine in the treatment of senile dementia of the Alzheimer type. Prog Neurophsychopharmacol Biol Psychiatry, 1986 (10): 87-93

Fujibayashi Y, Waki A, Wada K, Ueno M, Magata Y, Yonekura Y, Konishi J, Takeda T, Yokoyma A: Differential aging pattern of cerebral accumulation of radiolabeled glucose and amino acid in the senescence accelerated mouse (SAM), a new model for the study of memory impairment. Biol Pharm Bull, 1994 (17): 102-105

Furukawa T, Hoshino S, Kobayashi S, Asakura T, Takahashi M, Atsumi T, Teramoto A: The glutamate AMPA receptor antagonist, YM872, attenuates cortical tissue loss, regional cerebral edema and neurological motor deficits after experimental brain injury in rats. J Neurotrauma, 2003 (20): 269-278

Garcia-Espinosa MA, Rodrigues TB, Sierra A, Benito M, Fonseca C, Gray HL, Bartnik BL, Garcia-Martin ML, Ballesteros P, Cerdan S: Cerebral glucose metabolism and the glutamine cycle as detected by in vivo and in vitro 13C NMR spectroscopy. Neurochem Int, 2004 (45):297-303

Garzillo CL, Mello LE: Characterization of reactive astrocytes in the chronic phase of the pilocarpine model of epilepsies. Epilepsia, 2002 (43, Suppl.): 107-109

Geddes JW, Wood JD: Changes in the amino acid content of nerve endings (synaptosomes) induced by drugs that alter the metabolism of glutamate and γ-aminobuttyric acid. J Neurochem 1984 (42): 16-24

Giaume C, Venance L, Hofer T: Calcium waves between glial cells: how astrocytes communictate. Med Sci (Paris), 2003 (23): 6728-6739

Gutula TP, Hagen J, Pitkanen A: Do epileptic seizures damage the brain? Curr Opin Neurol 2003 (16): 189-195

HaabergA, Qu H, Haraldseth O, Unsgaard G, Sonnewald U: In vivo injection of [1-^{13}C]glucose and [1,2-^{13}C]acetate combinded with ex vivo ^{13}C nuclear magnetic resonance spectroscopy: a new approach to the study of the middle cerebral artery occlusion in the rat. J Cereb Blood Flow Metabol, 1998 (18): 1223-1232

Haberg A, Qu H, Saether O, Unsgard G, Haraldseth O, Sonnewald U: Differences in neurotransmitter synthesis and intermediary metabolism between glutamatergic and GABAergic neurons during 4 hours of middle cerebral artery occlusion in the rat: the role of astrocytes in neuronal survival. J Cereb Blood Flow Metab, 2001 (21):1451-63

Häfner H, Riecher-Rösler A, Maurer K, Fätkenheuer B, Löffler W: First onset and early

symptomatology of schizophrenia. A chapter of epidemiological and neurobiological research into age and sex differences. Eur Arch Psychiatry Clin Neurosci, 1992 (242): 109-118

Häfner H: What is Schizophrenia? Neurology, Psychopharmacology and Brain Research, 1993 (2): 36-52

Hamberger A, Berthold CH, Karlsson B, Lehmann A, Nystrom B: Extracellular GABA, glutamate and glutamine in vivo perfusion-dialysis of rabbit hippocampus. Neurol neurobiol, 1983 (7):473-492

Hansson E, Rönnebäck L: Gila neuronal signaling in the central nervous system. FASB J, 2003 (17):341-348

Harris NG, Plant HD, Inglis BA, Briggs RW, Jones HC: Neurochemical changes in the cerebral cortex of treated and untreated hydrocephalic rat pups quantified with in vitro 1H-NMR spectroscopy. J Neurochem, 1997 (68): 305-312

Hashimoto K, Engberg G, Shimizu E, Nordin C, Lindstrom LH, Iyo M: Elevated glutamine/glutamate ratio in cerebrospinal fluid of first episode and drug naive schizophrenic patients. BMC Psychiatry 2005, (5):6.

Hassel B, Sonnewald U, Fonnum F: Glial-neuronal interaction as studied by cerebral metabolism of [2-^{13}C]acetate and [1-^{13}C]glucose: an ex vivo ^{13}C NMR spectroscopy study. J Neurochem, 1995 (20): 413-420

Hassel B, Johannessen CU, Sonnewald U, Fonnum F: Quantification of the GABA shunt and the importance of the GABA shunt versus the 2-oxoglutarate dehydrogenase pathway in GABAergic pathways. J Neurochem, 1998 (71): 1511-1518

Hawkins RA, DeJoseph MR, Hawkins PA: Regional brain glutamate transport in rats at normal and raised concentrations of circulating glutamate. Cell Tissue Res, 1995 (281): 207-214

Hertz L: Functional interactions between neurons and astrocytes. I. Turnover and metabolism of putative amino acid transmitters. Prog Neurobiol, 1979 (13C): 277-323

Hertz L, Peng L, Westergaard N, Yudkoff M, Schousboe A: Neuronal-astrocytic interactions in metabolism of transmitter amino acids of the glutamate family. In: Schousboe A., Diemer NH, Kofod H (eds.): Drug research related neuroactive amino acids. Munksgaard, 1992, Copenhagen, pp. 30-48

Hjeresen DL, Franck JE, Amend DL: Ontogeny of seizure incidence, latency and severity in genetically epilepsy prone rats. Dev Psychobiol, 1987 (20): 355-363

Higashi K, Asahisa H, Ueda N, Kobayashi K, Hara K, Noda Y: Cerebral blood flow and metabolism in experimental hydrocephalus. Neurol Res, 1986 (8): 169-176

Horikoshi Y, Sasaki A, Taguchi N, Maeda M, Tsukagoshi H, Sato K, Yamaguchi H: Human GLUT5 immunolabeling is useful for evaluating microglial status in neuropathological study using paraffin sections. Acta Neuropathol 2003 (105): 157-162

Inagawa T, Ishikawa S, Uozomi T: Homovanillic acid and 5-hydroxindoleacetic acid in ventricular CSF of comatose patients with obstructive hydrocephalus. J Neurosurg, 1980 (52): 635-641

Ishizaka R, Tashiro Y, Inomoto T, Hasimoto N: Acute and subacute hydrocephalus in a rat neonatal model: correlation with functional injury of neurotransmitter systems. Pediatr Neurosurg, 2000 (33): 298-305

Jefferys JG, Borck C, Mellanby J: Chronic focal epilepsy induced by intracerebral tetanus toxin. Ital J Neurol Sci, 1995 (16): 27-32

Jefferys JG: Models and mechanisms of experimental epilepsies. Epilepsia. 2003 (44 Suppl): 44-50

Jensen FE: An animal model of hypoxia-induced perinatal seizures. Ital J Neurol Sci, 1995 (16): 59-68

Jentsch JD, Roth RH: The neuropsychopharmacology of phencyclidine: From NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology, 1999 (20): 201-225

Jones HC, Harris NG, Rocca JR, Andersohn RW: Progressive changes in cortical metabolites at three stages of infantile hydrocephalus studied by in vitro NMR spectroscopy. J Neurotrrauma, 1997 (14): 587-602

Johnston GA: GABAA receptor pharmacology. Pharmacol Ther, 1996 (69): 173-198

Johnstone M, Evans V, Baigel S: Sernyl (Cl-395) in clinical anesthesia. Br J Anaesth, 1958 (31): 433-439

Kaufmann DL, Houser CR, Tobin AJ: Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. J Neurochem, 1991 (56): 720-723

Kilpatrick GJ, Tilbrook GS: Memantine. Merz. Curr Opin Investig Drugs, 2002 (3): 798-806

Kim SK, Price MT, Olney JW, Farber NB: Excessive cerebrocortical release of acetylcholine induced by NMDA antagonists is reduced by GABAergic and alpha2-adrenergic agonists. Mol Psychiatry, 1999 (4): 344-352

Kitani K, Sato Y, Kanai S, Ohta M, Nokubo M, Masuda Y: The neurotoxicity of phenobarbital and its effect in preventing pentylenetetrazole-induced maximal seiyure in ageing mice. Arch Gerontol Geriatr, 1988 (7): 261-271

Kondziella D, Ludemann W, Brinker T, Sletvold O, Sonnewald U: Alterations in brain metabolism, CNS morphology and CSF dynamics in adult rats with kaolin-induced hydrocephalus. Brain Res, 2002 (927): 35-41

Kristensen B, Malm J, Fagerland M, Hietala SO, Johansson B, Ekstedt J, Karlsson T: Regional cerebral blood flow, white matter abnormalities, and cerebrospinal fluid hydrodynamics in patients with idiopathic adult hydrocephalus syndrome. J Neurol Neurosurg Psychiatry, 1996 (60):282-288

Kupferberg H: Animal Models used in the screening of antiepileptic drugs. 2001 (42, Suppl): 7-12

Kvamme E, Roberg B, Torgner IA: Phosphate-activated glutaminase and mitochondrial glutamine transport in the brain. Neurochem Res, 2000 (25): 1407-1419

Lee J, Kim MS, Park C, Jung EB, Choi Dh, Kim TY, Moon SK, Park R: Morphine prevents glutamate-induced death of primary rat neonatal astrocytes through modulation of intracellular redox. Immunopharmacol Immunotoxicol, 2004 (26): 17-28

Lehmann A, Isacsson H, Hamberger A: Effects of in vivo administration of kainic acid on the extracellular amino acid pool in rabbit hippocampus. J Neurochem, 1983: (40) 1314-1320

Liu QS, Xu Q, Arcuino G, Kang J, Nedergaard M: Astrocyte-mediated activation of neuronal kainate receptors. Proc Natl Acad Sci USA 2004 (101): 3172-3177

Loscher W, Honack D: Anticonvulsant and behavioral effects of two novel competitive N-Methyl-D-aspartic acid recpetor antagonists, CGP 37849 and CGP 39551, in the kindling model of epilepsy. Comparison with MK801 and carbamazepine. J Pharmacol Exp Ther, 1991 (256): 432-440

Loscher W, Schmidt D: Which animal models should be used in the search for new antiepileptic drugs? Epilepsy Research, 1988 (2): 145-181

Loubinoux I, Meric P, Borredon J, Correze JL, Gillet B, Beloeil JC, Tiffon B, Mispelter J, Lhoste JM, Jacques S: Cerebral metabolic changes induced by MK801: a 1D (phosphorus and proton) and 2D (proton) in vivo NMR spectroscopy study. Brain Res, 1994 (643): 115-124

Lowenstein DH, Thomas MJ, Smith DH, McIntosh TK: Selective vulnerability of dentate hilar neurons following traumatic brain injury: a potential mechanistic link between head trauma and disordes of the hippocampus. J Neurosci, 1992 (12): 4846-4853

Macdonald RL, Barker JL: Different actions of anticonvulsant and anesthetic barbiturates revealed by use of cultured mammalian neurons. Science, 1978 (200): 775-777

Maher F: Immunolocalization of GLUT1 and GLUT3 glucose transporters in primary cultured neurons and glia. J Neurosci Res, 1995 (42): 459-469

Malm J, Kristensen B, Ekstedt J, Adolfsson R, Weber P: CSF monoamine metabolites, cholinesterases and lactate in the adult hydrocephalus syndrome (normal pressure hydrocephalus) related to CSF hydrodynamic parameters. J Neurol Neurosurg Psychiatry, 1991 (54): 252-259

Malm J, Kristensen B, Ekstedt J, Wester P: CSF concentration gradients of monoamine metabolites in patients with hydrocephalus. J Neurol N eurosurg Psychiatry, 1994 (57): 1026-1033

Maragakis NJ, Rothstein JD: Glutamate transportes: animal models of neurological disease. Neurobiol Dis, 2004 (15): 461-473

Martinez-Bisbal MC, Marti-Bonmati L, Piquer J, Revert A, Ferrer P, Llacer JL, Piotto M, Assemat O, Celda B: 1H and 13C HR-MAS spectroscopy of intact biopsy samples ex vivo and in vivo 1H MRS study of human high grade gliomas. NMR Biomed, 2004 (17):191-205

Martin DL, Rimvall K: Regulation of gamma-aminobutyric acid synthesis in the brain. J Neurochem 1993 (60): 395-407

Meeks JP, Mennerick S: Feeding hungry neurons: astrocytes deliver food for thought. Neuron, 2003 (27):187-189

Meldrum BS, Naquet R: Effects of psilocybin, dimethyltryptamine and various lysergic acid

derivativeson photically-induced epilepsy in the baboon (Papio papio). Br J Pharmacol, 1970 (40):144-152

Miguel-Hidalgo JJ, Alvarez XA, Cacabelos R, Quack G: Neuroprotection by memantine against neurodegeneration induced by beta-amyloid (1-40). Brain Res, 2002 (958): 210-221

Minchin MC, Beart PM: Compartmentation of amino acid metabolism in the rat posterior pituitary. J Neurochem, 1975 (24): 881-884

Miller RG, Mitchell JD, Lyon M, Moore Dh: Riluzole for amyotrophic lateral sclerosis(ALS)/motor neuron disease (MND). Amyotroph Lateral Scler Other Motor Neuron Disord, 2003 (4): 191-206

Miwa S, Inagaki C, Fujiwara M, Takaori S: The activities of noradrenergic and dopaminergic neuron system in experimental hydrocephalus. J Neurosurg, 1982 (57): 67-73

Miyake H, Eghwrudjakpor PO, Sakamoto T, Mori K: Catecholamine alterations in experimental hydrocephalus. Childs Nerv Syst, 1992 (8): 243-246

Muir KW, Lees KR: Excitatory amio acid antagonists for acute stroke. Cochrane Database Syst Rev, 2003 (3): CD001244

Muller B, Qu H, Garseth M, White LR, Aasly J, Sonnewald U: Amino acid neurotransmitter metabolism in neurones and glia following kainate injection in rats. Neurosci Lett, 2000 (279):169-72

Nagelhus EA, Amiry-Moghaddam M, Lehmann A, Ottersen OP. Taurine as an organic osmolyte in the intact brain: immunocytochemical and biochemical studies. Adv Exp Med Biol. 1994 (359):325-334

Nagelhus EA, Mathiisen TM, Ottersen OP: Aquaporin-4 in the central nervous system: cellular and subcellular distribution and coexpression with KIR4.1. Neuroscience 2004 (129): 905-913

Nedergaard M, Ransom B, Goldman SA: New roles for astrocytes: redefining the functional architecture of the brain. Trends Neurosci, 2003 (26): 523-530

Nedergaard M, Takano T, Hansen AJ: Beyond the role of glutamate as a neurotransmitter. Nat rev Neurosci 2002 (3): 748-755

Nehlig A, Dube C, Koning E. Status epilepticus induced by lithium-pilocarpine in the immature rat does not change the long-term susceptibility to seizures. Epilepsy Res. 2002 Sep;51(1-2):189-97.

Nehlig A, Boehrer A. Effects of remacemide in two models of genetically determined generalized epilepsy, the GAERS and the audiogenic Wistar AS. Epilepsy Res. 2003 Jan;52(3):253-61.

Nehlig A, Wittendorp-Rechenmann E, Lam CD. Selective uptake of [14C]2-deoxyglucose by neurons and astrocytes: high-resolution microautoradiographic imaging by cellular 14C-trajectography combined with immunohistochemistry. J Cereb Blood Flow Metab. 2004 Sep;24(9):1004-14

Nehlig A. Brain uptake and metabolism of ketone bodies in animal models. Prostaglandins Leukot Essent Fatty Acids. 2004 Mar;70(3):265-75

Newmann EA: New roles for astrocytes: regulation of synaptic transmission. Trends Neurosci, 2003

(26): 536-542

Nokubo M, Kitani K. Age-dependent decrease in the lethal threshold of pentylenetetrazole in mice. Life Sci. 1988;43(1):41-7.

Nomura Y, Yamanaka Y, Kitamura Y, Arima T, Ohnuki T, Oomura Y, Sasaki K, Nagashima K, Ihara Y: Senescence-accelerated mouse. Neurochemical studies on aging. Ann N Y Acad Sci, 1996 (786): 410-418

Norenberg MD, Martinez-Hernandez A: Fine structural localization of glutamine synthetase in astrocytes of rat brain. Brain Res 1979 (161): 303-310

Ogita K, Okuda H, Yamamoto Y, Nishiyama N, Yoneda y: In vivo neuroprotective role of NMDA receptors against kainate-induced excitotoxicity in murine hippocampal pyrimidal neurons. J Neurochem, 2003 (85): 199-209

Olney JW: Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science, 1969 (164): 719-721

Olney JW: Excitatory amino acids and neuropsychiatric disorders. Biol Psychiatry, 1989 (26): 505-525

Olney JW, Farber NB: Efficacy of clozapine compared with other antipsychotics in preventing NMDA-antagonist neurotoxicity. J Clin Psychiatry, 1994 (Suppl, 55): 43-46

Olney JW, Wozniak DF, Farber NB: Glutamate receptor dysfunction and Alzheimer's disease. Restor Neurol Neurosci, 1994 (13): 75-83

Olney JW, Wozniak DF, Jevtovic-Todorovic V, Farber NB, Bittigau P, Ikonomidou C: Glutamate and GABA receptor dysfunction in the fetal alcohol syndrome. Neurotox Res, 2002 (4):315-325

Ottersen OP, Storm-Mathisen J. Glutamate- and GABA-containing neurons in the mouse and rat brain, as demonstrated with a new immunocytochemical technique. J Comp Neurol, 1984 (229):374-392

Oz G, Henry PG, Seaquist ER, Gruetter R. Direct, noninvasive measurement of brain glycogen metabolism in humans. Neurochem Int, 2003 (43):323-329

Ozyurt E, Graham DI, Woodruff GN, McCulloch J: Protective effect of the glutamate antagonist, Mk-801 in focal cerebral ischemia in the cat. J Cereb Blood Flow Metab, 1988 (8): 138-143

Qu H, Eloqayli H, Mueller B, Aasly J, Sonnwald U: Glial-neuronal interactions following kaninate injections in rats. Neurochemistry International, 2003 (42): 101-106

Qu H, Eloqayli H, Sonnewald U. Pentylenetetrazole affects metabolism of astrocytes in culture. J Neurosci Res, 2005 (79):48-54

Patel AJ, Johnson AL, Balazs R: Metabolic compartmentation of glutamate associated with the formation of gamma-aminobutyrate. J Neurochem, 1974 (23): 1271-1279

Park CK, Nehls DG, Graham DI, Teasdale GM, McGulloch J: The glutamate antagonist MK-801 reduces focal ischemic brain damage in the rat. Ann Neurol, 1988 (24): 543-551

Paulson OB, Newman EA: Does the release of potassium from astrocytic endfeet regulate cerebral blood flow? Science, 1987 (237): 896-898

Pasantes-Morales H, Franco R, Ordaz B, Ochoa LD: Mechanisms counteracting swelling in brain cells during hyponatremia. Arch Med Res, 2002 (33):237-244

Preece NE, Cerdan S: Metabolic precursors and compartmentation of cerebral GABA in vigabatrin-treated rats. J Neurochem, 1996 (67): 1718-1725

Raftopolous C, Dethy S, Laute MA, Goldman S, Naini AB, Przedborski S, Hildebrand J: Slow increase of homovanillic acid in cerebrospinal fluid after levodopa administration. Mov. Disord, 1996 (11): 59-62

Ramanjaneyulu R, Ticku MK: Interactions of pentamethylenetetrazole and tetrazole analogues with the picrotoxinin site of the benzodiazepine-GABA receptor-ionophore complex. Eur J Pharmacol, 1984 (98):337-45

Ransom B, Behar T, Nedergaard M: New roles for astrocytes (stars at last). Trends Neurosci, 2003 (10): 520-522

Rego AC, Monteiro RE, Silva AP, Malva JO, Oliveira CR: Mitochondrial apoptetic cell death and moderate superoxide generation upon selective activation of non-desensitizing AMPA receptors in hippocampal cultures. J Neurochem, 2003 (86): 792-804

Rouach N, Koulakoff A, Giaume C: Neurons set the tone of gap junctional communication in astrocytic networks. Neurochem Int, 2004 (45): 264-272

Ross B, Lin A, Harris K, Bhattacharya P, Schweinsburg B: Clinical experience with 13C MRS in vivo. NMR Biomed, 2003 (16):358-369

Sander JW, Shorvon SD: Epidemiology of the epilepsies. J Neurol Neurosurg Psychiatry, 1996 (61): 433-443

Schousboe A, Westergaard N, Sonnewald U, Petersen SB, Huang R, Peng L, Hertz L: Glutamate and glutamine metabolism and compartmentation in astrocytes. Dev Neurosci, 1993 (15):359-366

Schousboe A: Pharmacological and functional characterization of astrocytic GABA transport: a short review. Neurochem Res, 2000 (25): 1241-1244

Schousboe A: Role of astrocytes in the maintenance and modulation of glutamatergic and GABAergic neurotransmission. Neurochem Res, 2003 (28):347-352

Schroeder H, Becker A, Schroeder U, Hoellt V: 3H-L-glutamate binding and 3H-D-aspartate release from hippocampal tissue during the development of pentylenetetrazole kindling in rats. Pharmacol Biochem Behav, 1999 (62):349-352

Schwartzkroin PA, Roper SN, Wenzel HJ: Cortical dysplasia and epilepsy: animal models. Adv Exp Med Biol. 2004 (548):145-74

Sepkuty JP, Cohen AS, Eccles C, Rafiq A, Behar K, Ganel R, Coulter DA, Rothstein JD:
A neuronal glutamate transporter contributes to neurotransmitter GABA synthesis and epilepsy. J Neurosci, 2002 (22): 6372-6379

Shank RP, Bennett GS, Freytag SO, Campbell GL: Pyruvate carboxylase: an astrocytie-specific enzyme implicated in the replenishment of amino acids neurotransmitter pool. Brain Res, 1985 (329): 362-367

Shank RP, Leo GC, Zielke HR: Cerebral metabolic compartmentation as revealed by nuclear magnetic resonance analysis of D-[1-13C]glucose metabolism. J Neurochem, 1993 (61):315-323

Shapira Y, Yadid G, Cotev S, Niska A, Shohami E: Protective effect of MK-801 in experimental brain injury. J Neurotrauma, 1990 (7): 131-139

Simard M, Nedergaard M: The neurobiology of glia in the context of water and ion homeostasis. Neuroscience 2004 (129): 877-896

Simantov R, Crispino M, Hoe W, Broutman G, Tocco G, Rothstein JD, Baudry M: Changes in expression of neuronal and glial glutamate transporters in rat hippocampus following kainate-induced seizure activity. Brain Res Mol Brain Res, 1999 (65):112-123

Smith PF. Therapeutic N-methyl-D-aspartate receptor antagonists: will reality meet expectation? Curr Opin Investig Drugs, 2003 (4):826-832

Snead OC III, Depaulis A, Vergnes M: Absence epilepsy: advances in experimental animal models. In: Delgado-Escueta AV, Wilson WA, Olsen RW eds. Jasper's basic mechanisms of the epilepsies. Philadelphia: Lippincott Williams & Wilkins, 1999: 253-278.

Sonnewald U, Westergaard N, Krane J, Unsgard G, Petersen SB, Schousboe A: First direct demonstration of preferential release of citrate from astrocytes using [13C]NMR spectroscopy of cultured neurons and astrocytes. Neurosci Lett, 1991 (128):235-239

Sonnewald U, Gribbestad IS, Westergaard N, Nilsen G, Unsgard G, Schousboe A, Petersen SB: Nuclear magnetic resonance spectroscopy: biochemical evaluation of brain function in vivo and in vitro. Neurotoxicol, 1994 (15):579-590

Sonnewald U, White LR, Odegard W, Westergaard N, Bakken IJ, Aasly J, Unsgard G, Schousboe A: MRS study of glutamate metabolism in cultured neurons/glia. Neurochem Res, 1996 (21): 987-993

Sonnewald U, Schousboe A, Westergaard N: ^{13}C and ^{1}H MRS of cultured neurons and glia. In Magnetic resonance and imaging in neurochemistry, Nachelard (ed). Plenum Press New York, 1997: 10-41

Sonnewald U, Kondziella D: Neuronal glial interaction in different neurological diseases studied by ex vivo ^{13}C NMR spectroscopy. NMR Biomed, 2003 (16): 424-429

Sonnewald U, Schousboe A, Qu H, Waagepetersen HS: Intracellular metabolic compartmentation assessed by 13C magnetic resonance spectroscopy.Neurochem Int, 2004 (45):305-310

Sperk G: Kainic acid seizures in the rat. Prog Neurobiol, 1994 (42):1-32

Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Kikuchi T, Okuyama S, Kawashima N, Hori S, Takimoto M, Wada K: Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. Science, 1997 (276):1699-1702

Tashiro Y, Drake JM, Chakrabortty S, Hattori T: Functional injury of cholinergic, GABAergic and dopaminergic systems in the basal ganglia of adult rats with kaolin-induced hydrocephalus. Brain Res, 1997 (770): 45-52

Taylor A, McLean M, Morris P, Bachelard H: Approaches to studies on neuronal/glial relationships by ^{13}C-MRS Analysis. Dev Neurosci, 1996 (18): 434-442

Theberge J, Bartha R, Drost DJ, Menon RS, Malla A, Takhar J, Neufeld RW, Rogers J, Pavlosky W, Schaefer B, Densmore M, Al-Semaan Y, Williamson PC: Glutamate and glutamine measured with 4.0 T proton MRS in never-treated patients with schizophrenia and healthy volunteers. Am J Psychiatry, 2002 (159):1944-1946

Tsai G, Coyle JT: N-acetylaspartate in neuropsychiatric disorders. Prog Neurobiol, 1995 (46):531-540

Usunoff G, Atsev E, Tchavdarov D: On the mechanisms of picrotoxin epileptic seizure (macro- and micro-electrode investigations): Electroencephalogr Clin Neurophysiol. 1969 (27):444

Vafa B, Schofield PR: Heritable mutations in the glycine, $GABA_A$, and nicotinic acetycholine receptors provide new insights into the ligand-gated ion channel superfamily. Int Rev Neurobiol, 1998 (42): 285-332

Van Damme P, Leysson M, Callewart G, Robbrecht W, Van den Bosch L: The AMPA receptor antagonist NBQX prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis. Neurosci Lett, 2003 (343): 81-84

Van den Berg CJ, Garfinkel D: A simulation study of brain compartments. Metabolism of glutamate and related substances in mouse brain. Biochem J, 1971 (123): 211-218

Virchow R: Über das granulierte Ansehen der Wandungen der Gehirnventrikel. Allg Z Psychiatr, 1846 (3): 242

Waagepetersen HS, Sonnewald U, Schousboe A: The GABA paradox: multiple roles as metabolite, neurotransmitter, and neurodifferentiative agent. J Neurochem, 1999 (73): 471-479

Waagepetersen HS, Sonnewald U, Schousboe A: Compartmentation of glutamine, glutamate, and GABA metabolism in neurons and astrocytes: functional implications. Neuroscientist, 2003 (9):398-403

Waniewski RA, Martin DL: Preferential utilization of acetate by astrocytes is attributable to transport. J Neurosci, 1998 (18): 5225-5233

Watanabe T, Morimoto K, Hirao T, Suwaki H, Watase, Tanake K: amygdala-kindled and pentylenetetrazole-induced seizures in glutamate transporter GLAST-deficient mice. Brain Res, 1999 (845): 92-96

Westergaard N, Sonnewald U, Schousboe A: Metabolic trafficking between neurons and astrocytes: the glutamate/glutamine cycle revisited. Dev Neurosci, 1995 (17): 203-211

Wiesinger H, Hambrecht B, Dringen R: Metabolic pathways for glucose in astrocytes. GLIA 1997 (21): 22-34

Williams AJ, Ling G, McCabe RT, Tortella FC: Intrathecal CGX-1007 is neuroprotective in a rat

model of focal cerebral ischemia. Neuroreport, 2002 (13):821-824

Wolfson M, Bersudsky Y, Hertz E, Berkin V, Zinger E, Hertz L: A model of inositol compartmentation in astrocytes based upon efflux kinetics and slow inositol depletion after uptake inhibition. Neurochem Res, 2000 (25):977-982

Wong HY, Chu TS, Chan YW, Fok TF, Fung LW, Fung KP, Ho YY: The effects of phenytoin and its metabolite 5-(4-hydroxyphenyl)-5-phenylhydantoin on cellular glucose transport. Life Scie, 2005 (76): 1859-1872

Wozniak DF, Dikranian K, Ishimaru MJ, Nardi A, Corso TD, Tenkova T, Olney JW, Fix AS: Disseminated corticolimbic neuronal degeneration induced in rat brain by MK801: Potential relevance to Alzheimer's Disease. Neurobiol Disease, 1998 (5): 305-322

Yamamoto T, Rossi S, Stiefel M, Doppenberg E, Zauner A, Bullock R, Marmarou A: CSF and ECF glutamate concentrations in head injured patients. Acta Neurochir Suppl, 1999 (75): 17-19

Zeman J, Broswart J: The effect of amino acids on neurotransmitter metabolites in the cerebrospinal fluid. Cas Lek Cesk, 1991 (13C0): 425-427

Zieminska E, Stafiej A, Lazarewicz JW: Role of group I metabotropic glutamate receptors and NMDA receptors in homocysteine-evoked acute neurodegeneration of cultured cerebellar granule neurons. Neurochem Int, 2003 (43): 481-492

Papers I – V

Paper I

ELSEVIER

Neurochemistry International 40 (2002) 413–418

NEUROCHEMISTRY
International

www.elsevier.com/locate/neuint

The pentylenetetrazole-kindling model of epilepsy in SAMP8 mice: behavior and metabolism

Daniel Kondziella[a,b], Abdel Bidar[c], Bente Urfjell[a], Olav Sletvold[a,d], Ursula Sonnewald[a,*]

[a] Department of Clinical Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway
[b] Medizinische Hochschule Hannover, Hannover, Germany
[c] Department of Imaging and Anesthesia, Norwegian University of Science and Technology, Trondheim, Norway
[d] Department of Geriatrics, Norwegian University of Science and Technology, Trondheim, Norway

Received 18 June 2001; received in revised form 17 August 2001; accepted 27 August 2001

Abstract

This work describes a novel epilepsy model, combining pentylenetetrazole (PTZ) kindling with the senescence-accelerated mouse P8 (SAMP8) a model for aging. The 2- and 8-month-old SAMP8 mice were treated with PTZ, phenobarbital plus PTZ or saline every 48 h during a period of 40 days. Both 2- and 8-month-old PTZ-kindled mice showed a behavioral pattern that was very similar to severe chronic epilepsy with secondary generalized seizures. Two out of six 8-month-old animals died in the PTZ group. Interestingly, atypical absence seizures were limited to the 8-month-old PTZ group. Furthermore, 8-month-old mice were more sensitive to the sedative effect of phenobarbital. The concentrations of several amino acids were examined by HPLC. Lower levels of amino acids were found in the 8-month-old compared to the 2-month-old control animals. No biochemical changes were observed between the groups of 2-month-old animals, while in the 8-month-old animals both treatment groups showed significantly higher concentrations of GABA, glutamine and glutathione. Thus, it could be shown that cerebral metabolism of 8-month-old SAMP8 mice was more sensitive to PTZ and phenobarbital than metabolism of 2-month-old mice. Furthermore, it is suggested that glutamate metabolism in brains of 8-month-old SAMP8 mice is altered and that excessive glutamate is transformed, in considerable amounts, into glutamate related metabolites, possibly in astrocytes.
© 2002 Elsevier Science Ltd. All rights reserved.

Keywords: PTZ-kindling; Glutamate; GABA; Glutamine; SAMP8; Aging

1. Introduction

The prevalence and incidence of epilepsy are highest in later life with around 25% of new cases occurring in elderly people, many of whom will have concomitant neurodegenerative, cerebrovascular, or neoplastic diseases (Stephen and Brodie, 2000). Thus, the aim of the present study was to combine an animal model of epilepsy with one of aging, senescence-accelerated mouse P8 (SAMP8), with spontaneously occurring age-related deficits in learning and memory a genetic model for gerontological studies (Fujibayashi et al., 1994). They are characterized by a short life span (ca. half a normal mouse life) and normal growth (Abe et al., 1994). In the present study this model was combined with the pentylenetetrazole (PTZ) kindling model for chronic epilepsy.

Epilepsy can be described as a group of neurological disorders characterized by recurrent episodes of convulsive seizures, loss of consciousness, sensory disturbances, abnormal behavior or all of these. Excessive excitatory activity and/or low inhibitory activity are thought to lead to seizures due to disturbances of specific membrane functions and disturbed amounts of extra- and intracellular ions (Bradford, 1989). Common to all types of epilepsy are uncontrolled electrical discharges of neurons with excessive high frequency and synchronicity.

A well-established model in epilepsy research is the PTZ-kindling of mice and rats. PTZ is a substance that is thought to suppress the inhibitory effects of some neurotransmitters, especially GABA (Bradford, 1989; De Deyn and Macdonald, 1995; De Boer et al., 1982). It was found that concentrations as low as 10^{-5} M increased both the release of GABA and glutamate in rat cortical slices by about 25% (De Boer et al., 1982), and lead to an easier depolarization of neurons and thus possibly to epileptic seizures. The enhanced GABA release appears contradictory at first, but may be explained by a desensitization of GABA receptors due to the long-lasting enhancement (Kamphuis et al., 1990). PTZ-kindling is the regular appliance of sub-threshold doses of PTZ. After several injections treated

* Corresponding author. Tel.: +47-73-590492; fax: +47-73-598655.
E-mail address: ursula.sonnewald@medisin.ntnu.no (U. Sonnewald).

0197-0186/02/$ – see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0197-0186(01)00104-8

animals develop a behavioral pattern that is very similar to chronic epilepsy with secondary generalized seizures.

The kindling model can be combined with the study of anticonvulsant drugs such as phenobarbital. Although the use of phenobarbital has decreased over the last few decades, it is still a very common clinical drug with a high anti-epileptic potential. Phenobarbital prevents seizures by supporting the inhibitory effects of GABA and both its neurotoxicity and the efficacy are supposed to increase with age of the animals (Macdonald and Barker, 1977; Kitani et al., 1988).

The aim of the present study was to examine the behavioral pattern of SAMP8 mice and the amount of glutamate, GABA, glutathione, glutamine, aspartate, alanine and taurine in 2- and 8-month-old mice kindled with PTZ, and PTZ plus phenobarbital.

2. Experimental procedures

2.1. Materials

All animal procedures were approved by the local ethics committee. SAMP8 mice were kindly provided by the council of SAM research, Kyoto, Japan. Details of this mouse strain are given in Abe et al. (1994).

Seventeen 2-month-old and seventeen 8-month-old SAMP8 mice of both sexes were divided into three groups each: one group received PTZ (35 mg/kg), a second group received PTZ (35 mg/kg) and phenobarbital (10 mg/kg), one group served as control (0.3 ml saline). The weight of the 2- and 8-month-old animals was slightly different (2-month-old: 27.8 ± 2.05 g; 8-month-old 31.5 ± 3.1 body weight). However, it was decided to administer the drug relative to body weight since the distribution volume will be larger in heavier animals. The solutions were given intraperitoneal every second day between 09.00 h and 15.00 h for 40 days. Mice treated also with phenobarbital received the barbiturate 30 min before the PTZ, as the anticonvulsive effect of phenobarbital is highest after 30 min (Loscher et al., 1991). Animals were kept at 22 °C, 60% humidity, one per cage at a light/dark shift of 12 h and had water and food ad libitum. All mice were observed for 30 min after each injection and their behavior was judged with a score using the score list given the footnote of Table 1.

After 40 days the mice were decapitated, and the heads dropped into liquid nitrogen. The brains were homogenized in 7% perchloric acid and extraction was performed as described earlier (Håberg et al., 1998). The amounts of glutathione, glutamine, glutamate, GABA, aspartate, alanine plus taurine were measured by HPLC (HP 1100 System with fluorescence detection, Agilent, CA, USA) using a hypercoil AA-ODS column (5 μm, 2.1 mm × 200 mm). The results were analyzed using the ANOVA test followed by the independent samples t-test and $P > 0.05$ was considered as significant.

MR imaging was performed on a 2.3T DRX 100 Biospec from Bruker (Germany) essentially as described by Bouilleret et al. (2000). Two mice were imaged: one young PTZ animal that had several severe seizures and one young control. The animals were anesthetized with a subcutaneous injection of Hypnorm/Dormicum/sterile water (1:1:2), 1 ml/kg and imaged before and 30 min after administration via a tail vein of the contrast agent gadodiamide (Omniscan; Nycommed Amersham, Oslo, Norway) at a dose of 0.5 mmol/kg.

3. Results

Both 8- and 2-month-old animals treated with PTZ alone developed a behavioral pattern very similar to chronic epilepsy with focal onset and secondary generalized

Table 1
Individual scores of 2-month-old (A) and 8-month-old (B) SAMP8 mice receiving PTZ (35 mg/kg) intraperitoneally every second day for 40 days[a]

	Injection no.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
(A) 2-month-old SAMP8 mice																				
Mouse A	0	0	0	0	0	0	0	3	0	2	2	2	4	3	3	5	4	4	4	4
Mouse B	0	0	0	0	0	0	0	5	3	5	4	4	4	3	4	3	5	5	3	1
Mouse C	0	0	0	0	0	0	1	1	2	3	5	4	1	3	3	3	4	3	3	3
Mouse D	0	0	0	0	1	4	3	0	3	5	4	5	5	4	5	3	5	5	3	2
Mouse E	0	0	0	0	1	1	0	0	1	5	0	4	4	3	1	1	4	3	3	0
(B) 8-month-old SAMP8 mice																				
Mouse A	4	0	0	0	0	0	3	3	4	3	3	3	5	3	3	4	3	3	3	4
Mouse B	0	0	0	0	0	1	0	1	1	4	3	4	2	6						
Mouse C	0	0	0	0	0	1	0	2	5	5	4	4	3	3	4	4	4	4	4	3
Mouse D	0	0	0	0	1	2	1	1	4	3	4	3	4	4	2	4	4	4	3	2
Mouse E	0	0	0	0	0	2	3	3	5	5	3	3	6							
Mouse F	0	0	0	0	0	0	0	0	2	0	4	0	3	3	4	4	3	3	3	3

[a] All mice were observed for 30 min after each injection and their behavior was judged with a score out of the score list below; 0 = normal behavior, 1 = myoclonic jerks, 2 = minimal seizures without Straub-tail, 3 = minimal seizures with Straub-tail, 4 = generalized tonic-clonic seizures with loss of consciousness and postictal phase, 5 = like 4 with rotation on their axis, 6 = like 5 and death, underlining = absence-seizure.

Table 2
Concentrations of amino acids (μmol/g brain weight) in 2-month-old and 8-month-old SAMP8 mice treated every 48 h over a period of 40 days with PTZ or with PTZ and phenobarbital (Phb)[a]

	2 months			8 months		
	Control	PTZ	PTZ/Phb	Control	PTZ	PTZ/Phb
Glutathione	1.25 ± 0.26*	1.34 ± 0.14*	1.50 ± 0.41*	0.90 ± 0.25	1.25 ± 0.14*	1.21 ± 0.17
Aspartate	2.63 ± 0.45	2.32 ± 0.38	2.31 ± 0.13	2.12 ± 0.26	2.484 ± 0.13	2.32 ± 0.10
Glutamate	9.06 ± 3.47	9.14 ± 0.68	9.30 ± 0.93	8.03 ± 0.91	9.01 ± 0.68	9.01 ± 0.46
Glutamine	4.95 ± 0.43*	4.72 ± 0.39*	4.74 ± 0.47*	4.03 ± 0.24	4.76 ± 0.21*	4.65 ± 0.25*
Alanine + taurine[b]	9.14 ± 0.80*	9.71 ± 0.89*	9.75 ± 1.22*	7.61 ± 1.27	9.66 ± 0.67*	9.52 ± 0.32*
GABA	2.47 ± 0.50*	2.46 ± 0.25*	2.75 ± 0.38*	1.89 ± 0.47	2.57 ± 0.25*	2.53 ± 0.14*

[a] The control mice received NaCl-solution. The metabolites were measured by HPLC, for details see Section 2. The results were analyzed using the ANOVA test followed by the independent samples *t*-test.
[b] These two compounds could not be separated.
* Significantly different from the 8-month-old control mice ($P < 0.05$).

seizures. All animals with one exception needed 4–8 injections to show the first symptoms and 9–13 injections to show severe secondary generalized seizures, as shown in Table 1. The seizures appeared to reach a plateau within only two to six injections after onset of seizures. There was no statistical difference between the scores of the tow groups of animals. However, it should be noted that each mouse had an individual behavioral pattern. Thus, all mice had generalized seizures from only three to nine times out of a possible 20. A mouse that had reacted with severe generalized seizures at one time, could show only minor symptoms or even a total lack of symptoms the next time. This can be clearly seen in the first animal in the 8-month-old PTZ group (Table 1B) which received a score of four after the first injection, but showed no symptoms at all after the next five injections.

Seizures leading to death were observed in the 8-month-old PTZ group only, where two out of six animals died, while no deaths were found in the 2-month-old PTZ group. The 8-month-old PTZ animals also showed impaired consciousness before the onset of generalized tonic-clonic seizures. In these three cases (numbers underlined in Table 1B) 1–2 min after PTZ application the mice did not react either to acoustic, sensory or tactile stimulation. They all regained consciousness before having the typical generalized seizures. Two out of three animals died shortly afterwards due to severe tonic-clonic seizures. Theses phases of impaired consciousness could clearly be differentiated from the normal postictal unconsciousness phase after generalized seizures and can be best described as atypical absence seizures.

Furthermore it is worth mentioning that it is possible to evoke seizures in mice by vestibular stimulation. When held up in the air and turned on their axis (tail-twisted) mice showed tonic seizures lasting for up to 3 s, involving all of the body musculature including the facial muscles. In the present experiments this was the case for the control mice and especially for the PTZ treated mice. Mice treated with PTZ and phenobarbital showed this pattern only within half an hour after the PTZ application. No seizures were observed in the presence of phenobarbital with the single exception of a 2-month-old animal that received a score of three after the application of phenobarbital followed by PTZ. In contrast to the 2-month-old, the 8-month-old animals were more sensitive to the sedative effect of the first three phenobarbital injections. Three out of six animals died in the group of 8-month-old animals receiving both PTZ and phenobarbital.

The average weight of the mouse brains was 0.42±0.03 g, and there were no differences between groups. The concentrations of GABA, glutamate, glutamine, aspartate, glutathione and alanine plus taurine were measured using HPLC (Table 2). The 8-month-old control group showed lower values than all other groups for GABA, glutamine, glutathione and alanine plus taurine. No significant changes in the metabolite levels of 2-month-old mice were found, but significant alterations were found indeed between the different groups of 8-month-old animals. Compared to the controls the concentrations of glutathione, glutamine, GABA, alanine and taurine were increased both in the old mice treated with PTZ alone or together with phenobarbital.

Although it was possible to differentiate between several brain structures like cortex and the basal ganglia using MRI, no differences between the images of a PTZ-kindled mouse and a control were found.

4. Discussion

The kindling model of epilepsy is currently the most used animal model for the study of epilepsy. The regular systemical application of PTZ in rats and mice is, together with electrical kindling, one of the most common kindling models (Bradford, 1989). The PTZ-kindling model mimics, in a very reliable way, the development of complex-partial epilepsy with secondary generalization due to its relatively slow onset and offers the opportunity to study the epileptogenic process in a detailed manner. Once maximally kindled, the PTZ-kindling model allows the induction of maximum seizures at will and thus makes it possible to examine seizure-related events (Bradford, 1989).

4.1. Aging

SAMP8 mice are known as a murine model of accelerated aging and memory dysfunction (Fujibayashi et al., 1994). It has been shown that gliosis markedly increased with aging in the cerebral cortex and hippocampus of SAMP8 (Nomura et al., 1996). Furthermore, a generally increased metabolism of glucose has been described for this mouse strain (Fujibayashi et al., 1994; Sato et al., 1994). In the present study, striking differences were found between 8- and 2-month-old SAMP8 in the concentrations of GABA, glutamine, glutathione and alanine plus taurine. These metabolites all decreased in the 8-month-old animals compared to the 2-month controls, while the glutamate and aspartate levels remained unchanged. These findings are in line with former published values for aspartate in C57/B1/6J mice, but differ in the concentrations of other examined metabolites (Kirzinger and Fonda, 1978). Kirzinger and Fonda (1978) found unchanged values for glutathione, GABA, alanine and glutamate, but an increase in glutamine with age. However, Fonda et al. (1973) reported unchanged values for glutamate, an increase in aspartate and a decrease in GABA in extremely old mice (36 months) of the same mouse strain. These differences might reflect the fact that different mouse strains were evaluated.

4.2. The effects of PTZ

It has been shown that age has an effect on the kindling phenomenon. Different electrical stimuli are necessary to produce kindling in suckling rats as compared to adult rats (Moshe, 1981). Fanelli and McNamara (1986) showed that development of kindling required greater numbers of stimulations in middle-aged than in young–adult animals. In the present study no statistically significant difference was found between the scores of the 8- and 2-month-old kindled animals. However, an age-dependent decrease in the lethal threshold of PTZ in “old age” mice (27 months for males and 30 months for females) has been shown by Nokubo and Kitani (1988). This is in agreement with the present findings, where two out of six animals died in the 8-month-old group. Also, changes in metabolism were only detected in the 8-month-old animals. Thus, cerebral metabolism in young SAMP8 mice is less sensitive to PTZ than metabolism in old SAMP8 mice. No statistical differences were found in glutamate and aspartate concentrations in all groups, although the amount of glutamate was characteristically lower in the old control mice but did not reach statistical significance. It is well known that high extracellular concentrations of glutamate, the major excitatory neurotransmitter (Fonnum, 1984), are severely neurotoxic (Schousboe et al., 1992). Removal of glutamate from the extracellular space is mediated by Na^+/K^+-dependent high-affinity glutamate transporters, termed GLAST and GLT1 in astrocytes and EAAC1 in neurons (for review see Gegelashvili and Schousboe, 1998). Astrocytes take up glutamate and form glutamine as part of the so called “glutamate–glutamine cycle” (Van den Berg and Garfinkel, 1971), and also glutathione and other products. Whereas in GABAergic neurons glutamate can be converted to GABA, glutathione and other products. Since in the 8-month-old animals the glutamate concentration is unchanged while an increase is seen in GABA, glutamine and glutathione in the kindled animals (Table 2), it appears likely that an increased glutamate release lead to increased production of glutamate related products as a possible neuroprotective adaptation. It has been shown that PTZ-kindling in rats enhanced the release of glutamate into the extracellular space and lead to a distinct decrease of its removal into the cells (Li et al., 2000; Schunzel et al., 1992). Furthermore, Muller et al. (2000) showed that neuronal metabolism was enhanced in rats receiving kainic acid, another model of epilepsy. Whether these phenomena are a causative factor for the kindling process or a result of the seizures remains unclear. However, SAMP8 mice receiving both PTZ and phenobarbital had the same glutamate concentration as the PTZ-kindled mice, thus it is more likely that the glutamate levels are influenced by PTZ rather than seizures. Other published findings are in line with this hypothesis (Bradford, 1989; Meldrum et al., 1999; Schroeder et al., 1998). It is interesting to note that GABA levels were increased in the 8-month-old PTZ treated animals. An increased GABA concentration was also observed in brain biopsies from patients with intractable epilepsy (Aasly et al., 1999). This is paradoxical since most of the anti-epileptic treatment aims at increasing the GABA concentration.

Furthermore, it has been shown that PTZ leads to an increased regional cerebral metabolic glucose rate (rCMGlcR) (Pereira de Vasconcelos et al., 1990). The increase in glycolysis is connected to a higher production of pyruvate. This pyruvate can be converted into lactate or alanine. Indeed, increased levels of alanine plus taurine were found in the present study. Furthermore, the concentration of glutathione, the major antioxidant in brain (Dringen et al., 2000), was increased and can thus have had an enhanced protective effect.

It is worth noticing that the concentrations of the measured metabolites in the 8-month-old mice treated with PTZ increased in such a way that they became similar to the concentrations in the 2-month-old mice. The treated mice became so to speak “biochemically younger”. This observation was also reflected somewhat in the behavior of the treated animals who appeared livelier than control. It has earlier been reported that different doses of PTZ had either facilitating, disruptive, or no effect on avoidance learning (Krivanek, 1971a,b). However, it has been shown that PTZ-kindling has a negative effect on shuttle-box learning, as it might contribute to impaired memory storage (Becker et al., 1994). Furthermore it has been shown that kindling related impairment effects on cognitive functions increase with age (Grecksch et al., 1997).

4.3. The effects of phenobarbital plus PTZ

Phenobarbital is believed to exert its effect by supporting the GABAergic inhibition by binding at the GABA receptor–ionophore complex and by altering the conductance at the chloride channel associated with the GABA receptor (Ito et al., 1996) and showed a good anti-epileptic profile. Epileptic symptoms were shown neither by young nor old animals receiving both PTZ and phenobarbital, with the single exception by a 2-month-old animal that received a score of three after the application of phenobarbital followed by PTZ. In contrast to the 2-month-old, the 8-month-old animals were more sensitive to the sedative effect of the first phenobarbital injection. This phenomenon vanished, however, after two more applications. Three out of six animals died in the group of 8-month-old animals receiving both PTZ and phenobarbital, even though they had never shown any kind of epileptic symptoms or other possible reactions to PTZ. This is in agreement with a study by Kitani et al. (1988) showing that both the neurotoxicity and efficacy of phenobarbital increased with age.

Interestingly, the glutamate conversion described above seemed to be enhanced also in those animals that due to the phenobarbital showed no seizures. This may seem contradictory since, in contrast to PTZ, phenobarbital is known to suppress the rCMGlcR (Pereira de Vasconcelos et al., 1990). However, to our knowledge, no study has yet examined glucose metabolism, when PTZ and phenobarbital were co-administered. The present results suggest that the PTZ effect dominates the phenobarbital effect on the concentrations of the measured metabolites, even though phenobarbital is able to completely antagonize the PTZ induced seizures. This assumption is supported by the finding that Methotrexat also suppresses the rCMGlcR, but does not increase the phenobarbital effect when given simultaneously (Hoffman and Alfon, 1992).

4.4. Magnetic resonance imaging

Although it was possible to differentiate between several brain structures like cortex and the basal ganglia using MRI, no differences between the images of a PTZ-kindled mouse and a control were found. In man MRI is able to demonstrate brain abnormalities in some epilepsy patients, sometimes due to the scar tissue which replaces the lost neuronal cells. However, no MRI study yet was able to demonstrate these changes in PTZ-kindled rodents. Pohle et al. (1997) showed by microscopic analysis that PTZ induced seizures lead to neuronal cell loss in the rat hippocampus, while other studies did not find any histological alterations (De Feo et al., 1986; Laroia et al., 1997). Pohle et al. (1997) examined PTZ-kindled rats, while all the other studies were carried out on rodents receiving only a single large PTZ dose. Thus, it appears likely that only chronic seizures lead to demonstrable histological changes. However, these changes are too small to be detected by MRI at present. Interestingly, Bouilleret et al. (2000) could show morphological changes in MR-images using the kainic acid model of mesial temporal lobe epilepsy (mTLE). In this model a hippocampal sclerosis is induced in mice by direct intrahippocampal injection of kainic acid. A unilateral increased T2 signal could be detected in the hippocampus 120 days after the injection which was interpreted as a sign of gliosis. The PTZ-kindling model does not lead to mTLE but to generalized seizures with focal onset, and the convulsive drug is not injected directly into the brain, but was given intraperitoneal. Thus, it is likely that the PTZ-kindling model in mice does not cause histological alterations in the brain demonstrable with present MR techniques.

5. Conclusion

It can be stated that the present animal model is well suited to examine epilepsy related changes of behavior and cerebral metabolism in the aged.

Acknowledgements

This research was supported by the SINTEF Unimed and Blix Foundations. The generous gift of the SAMP8 mice from "The Council of SAM Research", Kyoto, Japan, is gratefully acknowledged.

References

Aasly, J., Silfvenius, H., Aas, T.C., Sonnewald, U., Olivecrona, M., Juul, R., White, L.R., 1999. Proton magnetic resonance spectroscopy of brain biopsies from patients with intractable epilepsy. Epilepsy Res. 35, 211–217.

Abe, Y., Yuasa, M., Kajiwara, Y., Hosono, M., 1994. Defects of immune cells in the senescence-accelerated mouse: a model for learning and memory deficits in the aged. Cell Immunol. 157, 59–69.

Becker, A., Greksch, G., Matthies, H., 1994. The influence of diazepam on learning processes impaired by pentylenetetrazole kindling. Naunyn-Schmiedebergs Arch. Pharmacol. 349, 492–496.

Bouilleret, V., Nehlig, A., Marescaux, C., Namer, I.J., 2000. Magnetic resonance imaging follow-up of progressive hippocampal changes in a mouse model of mesial temporal lobe epilepsy. Epilepsia 41, 642–650.

Bradford, H.F., 1989. Glutamate, GABA and epilepsy. Epilepsia 30, 17–25.

De Boer, T., Stoof, J.C., van Duijn, H., 1982. The effects of convulsant and anticonvulsant drugs on the release of radiolabeled GABA, glutamate, noradrenaline, serotonine and acetylcholine from rat cortical slices. Brain Res. 253, 153–160.

De Deyn, P.P., Macdonald, R.L., 1995. Effects of antiepileptic drugs on GABA responses and on reduction of GABA responses by PTZ and DMCM on mouse neurons in cell culture. Prog. Neurobiol. 47, 477–511.

De Feo, M.R., Mecarelli, O., Palladini, G., Ricci, G.F., 1986. Long-term effects of early status epilepticus on the acquisition of conditioned avoidance behavior in rats. Epilepsia 27, 476–482.

Dringen, R., Gutterer, J.M., Hirrlinger, J., 2000. Glutathione metabolism in brain metabolic interaction between astrocytes and neurons in the defense against reactive oxygen species. Eur. J. Biochem. 267, 4912–4916.

Fanelli, R.J., McNamara, J.O., 1986. Effects of age on kindling and kindled seizure-induced increase of benzodiazepine receptor binding. Brain Res. 362, 17–22.

Fonda, M.L., Acree, D., Auerbach, S., 1973. The relationship of γ-aminobutyrate levels and its to age in brains of mice. Arch. Biochem. Biophys. 159, 622–628.

Fonnum, F., 1984. Glutamate: a neurotransmitter in mammalian brain. J. Neurochem. 42, 1–11.

Fujibayashi, Y., Waki, A., Wada, K., Ueno, M., Magata, Y., Yonekura, Y., Konishi, J., Takeda, T., Yokoyama, A., 1994. Differential aging pattern of cerebral accumulation of radiolabeled glucose and amino acid in the senescence accelerated mouse (SAM), a new model for the study of memory impairment. Biol. Pharm. Bull. 17, 102–105.

Gegelashvili, G., Schousboe, A., 1998. Cellular distribution and kinetic properties of high-affinity glutamate transporters. Brain Res. Bull. 45, 233–238.

Grecksch, G., Becker, A., Rauca, C., 1997. Effect of age on pentylenetetrazol-kindling and kindling-induced impairments of learning performance. Pharmacol. Biochem. Behav. 56, 595–601.

Håberg, A., Qu, H., Haraldseth, O., Unsgård, G., Sonnewald, U., 1998. In vivo injection of [1-^{13}C]glucose and [1,2-^{13}C]acetate combined with ex vivo ^{13}C nuclear magnetic resonance spectroscopy: a novel approach to the study of middle cerebral artery occlusion in the rat. J. Cereb. Blood Flow Metabol. 18, 1223–1232.

Hoffman, A., Alfon, J., 1992. High-dose methotrexate does not affect the pharmacodynamics of phenobarbital hypnotic action but decreases the central nervous system (CNS) sensitivity to pentylenetetrazole-induced maximal seizures in rats. Pharm. Res. 9, 1295–1298.

Ito, T., Suzuki, T., Wellman, S.E., Ho, I.K., 1996. Pharmacology of barbiturate tolerance/dependence: $GABA_A$ receptors and molecular aspects. Life Sci. 59, 169–195.

Kamphuis, W., Huisman, E., Dreijer, A.M., Ghijsen, W.E., Verhage, M., Lopes da Silvia, F.H., 1990. Kindling increases the K (+)-evoked Ca2 (+)-dependent release of endogenous GABA in area CA1 of rat hippocampus. Brain Res. 511, 63–70.

Kirzinger, S., Fonda, M., 1978. Glutamine and ammonia metabolism in the brains of senescent mice. Exp. Geront. 13, 255–261.

Kitani, K., Sato, Y., Kanai, S., Ohta, M., Nokubo, M., Masuda, Y., 1988. The neurotoxicity of phenobarbital and its effect in preventing pentylentetrazole-induced maximal seizures in aging mice. Arch. Gerontol. Geriatr. 7, 261–271.

Krivanek, J.A., 1971a. Facilitation of avoidance learning by pentylenetetrazol as a function of task difficulty, deprivation and shock level. Psychopharmacologia 20, 213–219.

Krivanek, J.A., 1971b. Facilitation of avoidance learning by pentylenetetrazol as a function of task difficulty, deprivation and shock level. Psychopharmacologia 20, 213–219.

Laroia, N., McBridge, L., Baggs, R., Guillet, R., 1997. Dextromethorphan ameliorates effects of neonatal hypoxia on brain morphology and seizure threshold in rats. Brain Res. Dev. Brain Res. 100, 29–34.

Li, Z., Yamamoto, Y., Moromoto, T., Ono, J., Okada, S., Yamatodani, A., 2000. The effect of pentylenetetrazole-kindling on the extracellular glutamate and taurine levels in the frontal cortex of rats. Neurosci. Lett. 282, 117–119.

Loscher, W., Honack, D., Fassbender, C.P., Nolting, B., 1991. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentylenetetrazole seizure models. Epilepsy Res. 8, 171–189.

Macdonald, R.L., Barker, J.L., 1977. Phenobarbital enhances GABA-mediated postsynaptic inhibition in cultured mammalian neurons. Trans. Am. Neurol. Assoc. 102, 139–140.

Meldrum, B.S., Akbar, M.T., Chapman, A.G., 1999. Glutamate receptors and transporters in genetic and acquired models of epilepsy. Epilepsy Res. 36, 189–204.

Moshe, S.L., 1981. The effects of age on the kindling phenomenon. Dev. Psychobiol. 14, 75–81.

Muller, B., Qu, H., Garseth, M., White, L.R., Aasly, J., Sonnewald, U., 2000. Amino acid neurotransmitter metabolism in neurones and glia following kainate injection in rats. Neurosci. Lett. 279, 169–172.

Nokubo, M., Kitani, K., 1988. Age-dependent decrease in the lethal threshold of pentylenetetrazole in mice. Life Sci. 43, 41–47.

Nomura, Y., Yamanaka, Y., Kitamura, Y., Arima, T., Ohnuki, T., Sasaki, K., Nagashima, K., Ihara, Y., 1996. Senescence-accelerated mouse. Neurochemical studies on aging. Ann. New York Acad. Sci. 786, 410–418.

Pereira de Vasconcelos, A., Bøyet, S., Nehlig, A., 1990. Consequences of chronic phenobarbital treatment on local cerebral glucose utilization in the developing rat. Brain Res. Dev. Brain Res. 53, 168–178.

Pohle, W., Becker, A., Grecksch, G., Juhre, A., Willenberg, A., 1997. Piracetam prevents pentylentetrazole kindling-induced neuronal loss and learning deficits. Seizure 6, 467–474.

Sato, E., Inoue, A., Kurokawa, T., Ishibashi, S., 1994. Early changes in glucose metabolism in the cerebrum of senescence accelerated mouse: involvement of glucose transporter. Brain Res. 637, 133–138.

Schousboe, A., Frandsen, A., Krogsgaard-Larsen, P., 1992. Pharmacological and functional characterization of excitatory amino acid mediated cytotoxicity in cerebral cortical neurons. Cell Biol. Toxicol. 8, 93–100.

Schroeder, H., Becker, A., Hoellt, V., 1998. Sensitivity and density of glutamate receptor subtypes in the hippocampal formation are altered in pentylenetetrazole-kindled rats. Exp. Brain Res. 120, 345–348.

Schunzel, G., Wolf, G., Pomrenke, U., Pomrenke, C., Schmidt, W., 1992. Pentylenetetrazole kindling and factors of glutamate transmitter metabolism in rat hippocampus. Neuroscience 49, 527–530.

Stephen, L.J., Brodie, M.J., 2000. Epilepsy in elderly people. Lancet 355, 1441–1446.

Van den Berg, C.J., Garfinkel, D., 1971. A simulation study of brain compartments. Metabolism of glutamate and related substances in mouse brain. Biochem. J. 123, 211–218.

Paper II

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Neurochemistry International 43 (2003) 629–637

NEUROCHEMISTRY International

www.elsevier.com/locate/neuint

The pentylenetetrazole-kindling model of epilepsy in SAMP8 mice: glial–neuronal metabolic interactions

Daniel Kondziella[a,1], Janniche Hammer[a], Olav Sletvold[a,b], Ursula Sonnewald[a,*]

[a] *Department of Neuroscience and Locomotion, Norwegian University of Science and Technology, Trondheim, Norway*

[b] *Department of Geriatrics, Norwegian University of Science and Technology, Trondheim, Norway*

Received 24 October 2002; received in revised form 3 February 2003; accepted 4 February 2003

Abstract

Recently, a new experimental model of epilepsy was introduced by the authors [Neurochem. Int. 40 (2002) 413]. This model combines pentylenetetrazole (PTZ)-kindling in senescence-accelerated mice P8 (SAMP8), a genetic model of aging. Since imbalance of glutamate and GABA is a major cause of seizures, the study of glial–neuronal interactions is of primary importance. Nuclear magnetic resonance spectroscopy (NMRS) is an excellent tool for metabolic studies. Thus, we examined whether NMRS when combined with administration of [1-^{13}C]glucose and [1,2-^{13}C]acetate might give valuable insights into neurotransmitter metabolism in this new model of epilepsy and aging. The 2- and 8-month-old SAMP8 were kindled with PTZ alone, received PTZ and phenobarbital (PB), or served as controls. In older animals, PTZ-kindling decreased labeling in glutamate C-4 from [1-^{13}C]glucose, whereas, in the younger mice, labeling in glutamine C-4 was decreased both from [1-^{13}C]glucose and [1,2-^{13}C]acetate. It could be concluded that PTZ-kindling affected astrocytes in younger and glutamatergic neurons in older animals. In the presence of PTZ, phenobarbital decreased labeling of most metabolites in all cell types, except GABAergic neurons, from both labeled precursors in the younger animals. However, in older animals only GABAergic neurons were affected by phenobarbital as indicated by an increase in GABA labeling.
© 2003 Elsevier Science Ltd. All rights reserved.

Keywords: ^{13}C NMRS; PTZ-kindling; SAMP8; Amino acids; Metabolism; Aging

1. Introduction

Most animal models involve young adult rodents, which are not comparable in age to the majority of patients. Senescence-accelerated mice P8 (SAMP8) are an excellent genetic model for studying age-related diseases (Abe et al., 1994; Sato et al., 1994) and have been used e.g. to examine the glucose metabolism in the older brain (Fujibayashi et al., 1994; Sato et al., 1994). SAMP8 mice live only half the normal life of a mouse, but show normal growth (Abe et al., 1994). Additionally, they are characterized by spontaneous age-related impairment in higher functions such as learning and memory (Fujibayashi et al., 1994). Furthermore, gliosis is markedly increased with aging in the cerebral cortex and hippocampus of SAMP8 (Nomura et al., 1996) which makes it very interesting to analyze glial–neuronal interaction by ^{13}C magnetic resonance spectroscopy (see later).

Epilepsy is the tendency to epileptic seizures, associated with paroxysmal discharge of cerebral neurons. Traditionally it is divided into generalized or partial epilepsy and characterized by a large variety of symptoms including disturbances in sensorimotor systems and alterations of behavior and consciousness. However, epilepsy is one of the most common serious neurological disorders affecting man (Sander and Shorvon, 1996). The prevalence and incidence of epilepsy is high in later life (Stephen and Brodie, 2000) and medical therapy is often complicated by cardiovascular, renal and hepatic disorders. Furthermore, associated neoplastic, degenerative, ischemic and hemorrhagic brain diseases worsen the condition in many geriatric patients. Presently, very little is known about the biochemical alterations in the brain of elderly patients with epilepsy. In order to develop appropriate treatment strategies relevant animal models have to be developed.

Animal models of epilepsy often involve inhibition of the synthesis of GABA. Such inhibition has been shown to promote seizures, as does the administration of GABA antagonists and glutamate agonists (Hosford, 1995). Pentylenetetrazole (PTZ) is a chemical convulsant frequently used in the study of seizures. It should be noted that the mechanism of action of PTZ is only partially understood. It is generally believed that PTZ exerts its effects

* Corresponding author. Tel.: +47-73-590492; fax: +47-73-598655.
E-mail address: ursula.sonnewald@medisin.ntnu.no (U. Sonnewald).
[1] On leave from Hanover Medical School, Hanover, Germany.

0197-0186/$ – see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0197-0186(03)00093-7

by binding to the picrotoxin-binding site of the postsynaptic $GABA_A$ receptor (Macdonald and Barker, 1977, 1978). PTZ is known to decrease the effects of GABA and other inhibitory neurotransmitters, thus leading to an easier depolarization of neurons (Bradford, 1989; De Deyn and Macdonald, 1995; De Boer et al., 1982). The regular application of sub-threshold doses of PTZ is called PTZ-kindling and provokes a behavior very similar to chronic secondary generalized seizures. These are probably due to disturbances of specific membrane functions and disturbed amounts of extra- and intracellular ions caused by excessive excitatory and/or low inhibitory activity (Bradford, 1989). The barbiturate phenobarbital (PB) can prevent these seizures (Kondziella et al., 2002, and references therein) by supporting the inhibitory effect of GABA. Like other barbiturates, phenobarbital is more potent and toxic with increasing age of the individual (Macdonald and Barker, 1977; Kitani et al., 1988).

Nuclear magnetic resonance spectroscopy (NMRS) is a useful tool for the study of alterations in neuronal–glial interactions (Lapidot and Gopher, 1994; Hassel et al., 1997; Håberg et al., 1998; Chapa et al., 2000) and has also been used to study glutamate–glutamine cycling in the epileptic human hippocampus (Petroff et al., 2002). Acetate is selectively taken up by astrocytes by a specialized transport system, which is absent or less active in neurons (Waniewski and Martin, 1998), whereas glucose is thought to be metabolized more in the neuronal tricarboxylic acid (TCA) cycle (Minchin and Beart, 1975; Sonnewald et al., 1991). Using ^{13}C NMRS it has been calculated that acetyl CoA derived from glucose is predominantly metabolized in the neuronal tricarboxylic acid cycle in rats (Qu et al., 2000). Thus, by simultaneous injection of [1-^{13}C]glucose and [1,2-^{13}C]acetate and NMRS analysis of brain extracts information about neuronal and astrocytic metabolism can be obtained in the same animal (Taylor et al., 1996).

Recently, PTZ-kindling in SAMP8 mice was performed in our laboratory and it could be concluded that significant information concerning behavior and metabolism can be obtained using this new model for epilepsy in the elderly (Kondziella et al., 2002). As described previously, glutamate metabolism and the so-called glutamine–glutamate–GABA cycle (Berl and Frigyesi, 1969; Van den Berg and Garfinkel, 1971; Hertz, 1979) as an expression of glial–neuronal interaction is of particular interest in the pathophysiology of epilepsy. Consequently, NMRS might be useful in the examination of this. The central question in the present study was whether NMRS could provide new information about neurons, astrocytes and their metabolic interaction in our new model of PTZ-kindling in SAMP8 mice. Thus, 2- or 8-month-old SAMP8 were kindled with PTZ, received PTZ together with phenobarbital or served as controls. Before decapitation, mice were injected with [1-^{13}C]glucose and [1,2-^{13}C]acetate and metabolic changes associated with aging, seizures and phenobarbital were analyzed using ^{13}C NMRS.

2. Materials and methods

2.1. Materials

SAMP8 mice were a generous gift from The Council of SAM Research, Kyoto, Japan. For details of this mouse strain, see Abe et al. (1994). The [1-^{13}C]glucose and [1,2-^{13}C]acetate (99% enriched) and D_2O (99.9%) were from Cambridge Isotopes Laboratories (Woburn, MA, USA), ethylene glycol from Merck (Darmstadt, Germany). PTZ from Sigma (St. Louis, MO, USA). All other chemicals were of the purest grade available from regular commercial sources.

2.2. Animal procedures

All animal procedures were approved by the local ethics committee. The experiments were performed on seventeen 2-month-old and seventeen 8-month-old SAMP8 of both sexes. The 2-month-old animals weighed 27.8 ± 2.05 g, the 8-month-old animals weighed 31.5 ± 3.1 g. Because the volume of distribution increases with increasing weight of the animal, we injected the drugs relative to body weight. The mice were separated into six groups receiving the following solutions intraperitoneally every second day between 9 a.m. and 3 p.m. for 40 days: PTZ (35 mg/kg), PTZ (35 mg/kg) + phenobarbital (10 mg/kg), 0.3 ml saline. Since the anticonvulsive effect is maximal after 30 min (Loscher et al., 1991), phenobarbital was always given 30 min before administration of PTZ. For 30 min after each injection all animals were monitored and drug effects were judged with a score as explained earlier (Kondziella et al., 2002).

Animals were kept one per cage at a light/dark cycle of 12 h and had free access to water and food. Temperature was 22 °C and humidity 60%.

On day 40, the mice received intraperitoneal injections of [1-^{13}C]glucose (0.3 M solution, 543 mg/kg) plus [1,2-^{13}C]acetate (0.6 M solution, 504 mg/kg). Fifteen minutes after ^{13}C injection, animals were sacrificed by decapitation and the heads were snap frozen in liquid nitrogen. The whole brain was dissected and extracted as described earlier by Håberg et al. (1998). The amounts of some unlabelled metabolites in the brain specimen were measured by high pressure liquid chromatography (HPLC) and these results together with the findings in behavior, judged by the score list mentioned previously, have been published separately in this journal (Kondziella et al., 2002).

2.3. NMR spectroscopy

Proton decoupled 125.5 MHz ^{13}C NMR spectra were obtained on a Bruker DRX-500 spectrometer. Samples were re-dissolved in D_2O containing ethylene glycol 0.1% as an internal standard. Spectra were accumulated using a 35° (pulse angle), 25 kHz spectral width with 64K data points. The acquisition time was 1.3 s, and a 2.5 s relaxation delay

was used. The number of scans was typically 10,000. Factors for nuclear Overhauser effects were applied to all spectra.

2.4. Data analysis

Relevant peaks from NMR spectra were obtained, and the amounts of ^{13}C were quantified from the integrals of the peak areas using ethylene glycol as an internal standard. Results are presented as mean ± standard deviation. Differences between groups were analyzed statistically with ANOVA followed by independent samples *t*-tests and $P < 0.05$ was considered significant.

3. Results

Without exception and regardless of the animals' age, PTZ-kindling induced a behavioral pattern virtually identical to chronic epilepsy with focal onset and secondary generalized seizures. The latter occurred after a mean of 10 injections, started between 2 and 5 min after PTZ administrations and lasted up to 30 s with a postictal period of a few minutes. PTZ evoked seizures leading to death occurred in two of the six 8-month-old animals, but not in any 2-month-old animal. An overview of these results is given in Fig. 1. Phenobarbital efficiently prevented seizures, however, three of the six 8-month-old animals treated with phenobarbital and PTZ died. For more details see Kondziella et al. (2002).

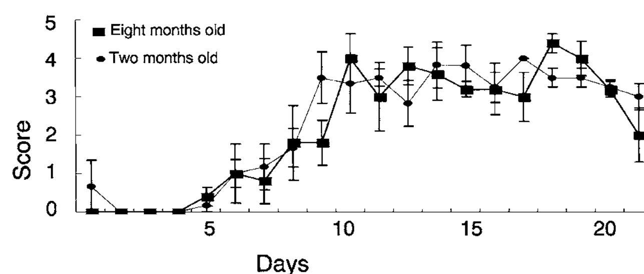


Fig. 1. Scores of 2-month-old (●) and 8-month-old (■) SAMP8 mice receiving pentylenetetrazole (35 mg/kg) intraperitoneally every second day for 40 days. All mice were observed for 30 min after each injection and their behavior was judged with a score from the following list: (0) normal behavior, (1) myoclonic jerks, (2) minimal seizures without Straub-tail, (3) minimal seizures with Straub-tail, (4) generalized tonic–clonic seizures with loss of consciousness and postictal phase, (5) like (4) but with rotation on their axis. Figures based on numbers from Kondziella et al. (2002).

Injection of ^{13}C-labeled glucose and acetate led to efficient labeling of many metabolites as can be seen in Fig. 2. Label from [1-^{13}C]glucose is thought to be mainly metabolized in the neuronal compartment and can be quantified by analyzing the singlet peaks in the different metabolites. The doublets seen in the spectrum were derived from [1,2-^{13}C]acetate and thus astrocytic metabolism. Simplified schemes of the metabolic pathways of these two substrates are shown in Figs. 3 and 4. [1-^{13}C]glucose is converted to pyruvate via glycolysis and can form alanine and lactate. Pyruvate can enter the TCA cycle via [2-^{13}C]acetyl CoA

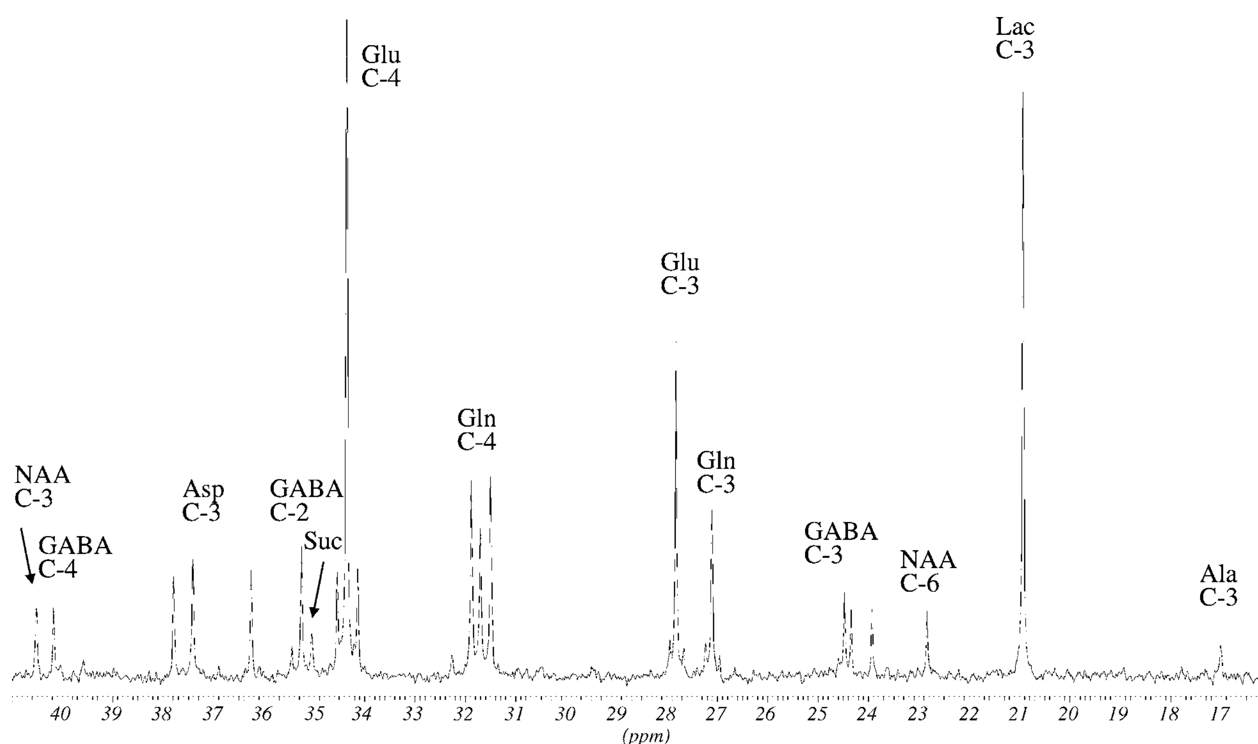


Fig. 2. ^{13}C NMR spectrum of mouse brain extract (2-month-old PTZ group). SAMP8 were injected with [1-^{13}C]glucose and [1,2-^{13}C]acetate and brains were extracted. Ala: alanine; Asp: aspartate; Gln: glutamine; Glu: glutamate; Lac: lactate; NAA: *N*-acetyl aspartate; Suc: succinate.

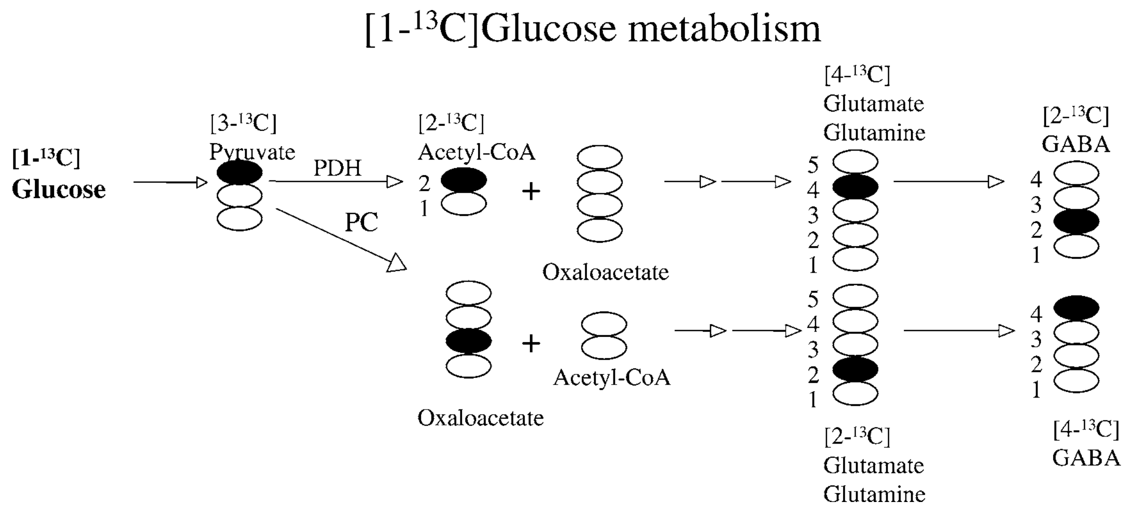


Fig. 3. Schematic representation of isotopomers arising from [1-^{13}C]glucose after the first turn of the tricarboxylic acid cycle and via pyruvate carboxylation, represents ^{13}C. PC: pyruvate carboxylase; PDH: pyruvate dehydrogenase.

or after carboxylation by pyruvate carboxylase to oxaloacetate (Fig. 3). Pyruvate carboxylase is localized in astrocytes, not neurons (Shank et al., 1985). [1,2-^{13}C]acetate can also be converted to acetyl CoA, however, the product [1,2-^{13}C]acetyl CoA will be having two ^{13}C atoms (Fig. 4), resulting in doublet formation. Since both acetyl CoA and oxaloacetate can be labeled in the present experiments, the number of possible isotopomers of the metabolites derived from the TCA cycle is large and not all compounds presented in Tables 1 and 2 are represented in Figs. 3 and 4. By comparing the doublets with singlets, it can be seen in Fig. 2 that glutamine was labeled more from [1,2-^{13}C]acetate (doublet) than [1-^{13}C]glucose (singlet), the opposite was the case for glutamate, GABA and lactate. Alanine, *N*-acetyl aspartate (NAA) and succinate are only labeled from glucose. Creatine and taurine are not labeled, the naturally abundant ^{13}C is gives rise to the observed singlets.

In Table 1, values are given for the amounts of ^{13}C in the different metabolites in the 2-month-old mice. PTZ only affected labeling in glutamine C-4 both from [1-^{13}C]glucose and [1,2-^{13}C]acetate as seen in the singlet and doublet. Phenobarbital, however, had a profound effect on labeling from both precursors. Glutamine and glutamate C-4 both singlet and doublet were decreased as compared to control, glutamine C-4 singlet was also different from that of the PTZ group. Furthermore, the doublet of doublets in glutamate C-4 (Fig. 2) obtained from repetitive cycling (Cerdan et al., 1990) was also decreased compared to control. NAA C-6 (data not shown) and aspartate C-3 were decreased compared to control and alanine C-3 and succinate C-2 + C-3 were decreased compared to the PTZ group.

In the control groups, differences were also observed between the 2- and 8-month-old animals. In the 8-month-old group values for glutamine C-4 doublet and glutamate C-4 singlet and doublet were decreased (Table 2). Fewer changes were observed in label incorporation between the different groups in the 8-month-old animals. PTZ-kindling lead to a decreased label incorporation into glutamate C-4 and C-2. Compared to the controls and the PTZ group the only changes in the group receiving PTZ and phenobarbital were an increase in GABA and a decrease in alanine labeling (Table 2). Cycling ratios and pyruvate carboxylation compared to dehydrogenation ratios were also calculated and statistically evaluated. No differences were found between the groups (results not shown).

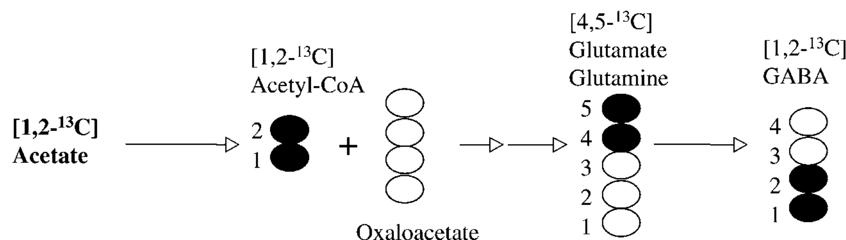


Fig. 4. Schematic representation of isotopomers arising from [1,2-^{13}C]acetate after the first turn of the tricarboxylic acid cycle, represents ^{13}C. For more details, see Cerdan et al. (1990).

Table 1
Amounts of ^{13}C in 10^{-9} mol/g of tissue in brain extracts from 2-month-old SAMP8

	Amounts of ^{13}C		
	Control ($n = 5$)	PTZ ($n = 6$)	PTZ + PB ($n = 6$)
[1,2-^{13}C]acetate			
Glutamine C-4	336.1 ± 88.9	274.5 ± 70.20 a	210.9 ± 14.5 a
Glutamine C-2	53.0 ± 16.7	56.5 ± 17.4	34.3 ± 2.5 a,b
Glutamate C-4	167.5 ± 50.9	132.7 ± 42.3	96.4 ± 6.9 a
Glutamate C-4	90.3 ± 25.2	77.2 ± 16.6	48.7 ± 16.1 a
Glutamate C-2	64.6 ± 22.9	44.9 ± 12.7	34.3 ± 3.9 a
Lactate C-2	28.0 ± 2.6	23.6 ± 8.2	13.0 ± 3.1 a,b
[1-^{13}C]glucose			
Glutamine C-4	134.5 ± 42.2	121.5 ± 21.2 a	86.0 ± 3.7 a,b
Glutamine C-2	100.1 ± 24.7	94.9 ± 13.1	76.9 ± 3.3 a
Glutamate C-4	462.9 ± 150.8	381.6 ± 98.5	325.8 ± 10.9 a
Glutamate C-2	242.7 ± 84.4	197.0 ± 43.0	156.8 ± 12.2 a
GABA C-2	83.1 ± 22.8	79.1 ± 26.0	63.3 ± 4.8
GABA C-4	55.7 ± 17.0	50.0 ± 11.7	38.7 ± 5.4 a
Aspartate C-3	76.3 ± 26.0	67.4 ± 15.7	54.1 ± 3.5 a
Alanine C-3	22.8 ± 8.8	28.6 ± 12.6	15.2 ± 3.5 b
Lactate C-3	359.2 ± 137.7	358.0 ± 134.2	303.1 ± 25.2
Succinate C-2 + C-3	23.3 ± 7.3	22.7 ± 6.1	10.0 ± 2.5 b
Taurine C-2	62.3 ± 11.6	72.4 ± 17.1	63.2 ± 7.2

Mice were treated every 48 h over a period of 40 days with pentylenetetrazole (PTZ) or with PTZ and phenobarbital (PB) and were thereafter injected with [1-^{13}C]glucose and [1,2-^{13}C]acetate (for details see Section 2). The results are expressed as mean ± standard deviation and were analyzed using ANOVA followed by the independent samples *t*-test. PTZ: pentylenetetrazole; PB: phenobarbital. The letter 'a' implies statistically different from control, and the letter 'b' implies statistically different from PTZ.

3.1. Discussion

The accelerated senescence-prone mouse strain, SAMP8, with spontaneously occurring age-related deficits in learning and memory may help provide new information on the metabolic changes in aging. Cerebral glucose transport and metabolism has been investigated by several authors to study abnormalities in glucose handling in relation to

Table 2
Amounts of ^{13}C in 10^{-9} mol/g of tissue in brain extracts from 8-month-old SAMP8

	Amounts of ^{13}C		
	Control ($n = 5$)	PTZ ($n = 4$)	PTZ + PB ($n = 3$)
[1,2-^{13}C]acetate			
Glutamine C-4	200.6 ± 60.1 c	202.8 ± 41.3	244.5 ± 46.4
Glutamine C-2	45.1 ± 12.8	38.5 ± 7.2	53.0 ± 2.3
Glutamate C-4	89.2 ± 21.9 c	91.4 ± 21.8	91.6 ± 16.0
Glutamate C-4	62.2 ± 20.2 c	44.2 ± 21.2	64.9 ± 3.1 b
Glutamate C-2	43.5 ± 12.1 c	32.2 ± 13.6	46.1 ± 11.9
GABA C-3	12.0 ± 4.0	17.7 ± 2.5	26.0 ± 12.3 a
[1-^{13}C]glucose			
Glutamine C-4	117.7 ± 13.1	102.5 ± 5.8	114.7 ± 13.7
Glutamine C-2	89.5 ± 5.7	81.7 ± 6.4	99.4 ± 12.9
Glutamate C-4	350.6 ± 33.8 c	262.2 ± 27.8 a	384.9 ± 25.7
Glutamate C-2	179.0 ± 7.2 c	151.2 ± 22.3 a	185.9 4.7
GABA C-2	79.4 ± 11.1	70.8 ± 4.3	99.7 ± 16.0 b
GABA C-4	47.8 ± 7.1	37.4 ± 5.1	48.6 ± 0.5
Aspartate C-3	68.3 ± 6.1	54.1 ± 14.4	73.7 ± 3.1
Alanine C-3	21.9 ± 4.8	32.0 ± 10.7	16.4 ± 0.4 b
Lactate C-3	378.1 ± 129.5	366.1 ± 100.8	343.7 ± 23.4
Succinate C-2 + C-3	18.5 ± 5.8	14.7 ± 2.0	17.6 ± 2.5
Taurine C-2	62.0 ± 8.4	66.5 ± 2.7	64.4 ± 2.4

Mice were treated every 48 h over a period of 40 days with pentylenetetrazole (PTZ) or with PTZ and phenobarbital (PB) and were thereafter injected with [1-^{13}C]glucose and [1,2-^{13}C]acetate (for details see Section 2). The results are expressed as mean ± standard deviation and were analyzed using ANOVA followed by the independent samples *t*-test. The letter 'a' implies statistically different from control, the letter 'b' implies statistically different from PTZ, and the letter 'c' implies statistically different from 2-month-old SAMP8.

age. Fattoretti et al. (2001) documented an age-dependent decrease in glucose transporter Glut3 expression in discrete areas of rat hippocampus. Glut3 is the predominant glucose transporter in neurons and is found abundantly in regions with high synaptic density characterized by frequent bursts of functionally adequate metabolic activity. These findings lend support to the critical role of an impaired metabolism in age-related brain dysfunction and disease. Fujibayashi et al. (1994) studied 2-[^{14}C]deoxyglucose accumulation in the SAMP8 brain and found that it was not different from control at 1 month of age, but decreased from 2–3 months of age, corresponding with the impairment of memory in the SAMP8 at 2–3 months. In contrast to this, Sato et al. (1994) found increased glucose metabolism in 4–8-week-old SAMP8 and correlated this with a transient overproduction of the glucose transporter protein in the cerebral cortex. In the present study glucose concentration could not be measured. However, lactate production from glucose was unchanged in the two groups and was not affected by PTZ or PTZ plus phenobarbital. Thus it seems unlikely that glycolysis was affected by aging. This is possibly because the transient change in transporters was already finished by 2 months.

Glutamate, which is the major excitatory neurotransmitter (Fonnum, 1984), is a potential neurotoxin (Olney and Ho, 1970; Schousboe et al., 1992). Disruption in glutamate homeostasis is thought to be a factor in the pathogenesis of a number of neurological and psychiatric disorders including epilepsy. Although both neurons and astrocytes take up glutamate, it has been shown that astrocytes are responsible for the major part of this uptake (Gegelashvili and Schousboe, 1998). Astrocytes take up glutamate and metabolize it to form glutamine as part of the so-called glutamate–glutamine cycle (Berl and Frigyesi, 1969; Van den Berg and Garfinkel, 1971; Hertz, 1979). Moreover, part of the exogenous glutamate enters the TCA cycle after conversion to 2-oxoglutarate (Yu et al., 1982; Sonnewald et al., 1993). Analysis of labeling patterns and amounts of ^{13}C in metabolites derived from the TCA gives information about mitochondrial activity. Thus, the decrease in glutamate labeling from [1-^{13}C]glucose in the 8-month-old animals compared to the 2-month-old indicates that glutamatergic neurons displayed decreased mitochondrial activity, whereas GABAergic neurons were not affected. Decreased mitochondrial activity with aging was also observed by Omata et al. (2001) using incubation of fresh brain slices from SAMP8 with [^{18}F]2-fluoro-2-deoxy-D-glucose and positron autoradiography. In the present study it was shown that astrocytic metabolism was also impaired. As mentioned earlier, acetate is only metabolized by astrocytes and thus acetate labeling can be used to monitor astrocytic processes. In addition, glutamine synthesis is also an astrocytic event since glutamine synthetase, the enzyme responsible for glutamine synthesis, is only present in glia (Martinez-Hernandez et al., 1977). Synthesis of glutamine from [1,2-^{13}C]acetate was decreased in the 8-month-old SAMP8 indicating impaired astrocytic metabolism. Furthermore, compared to the values for the 2-month-old group, glutamate synthesis from [1,2-^{13}C]acetate was also decreased in the 8-month-old animals. This indicates that transport of glutamine to glutamatergic neurons was also impaired. Decreased astrocytic function was also reported in our previous study using HPLC, showing decreased glutamine concentration in brain extracts from of 8-month-old SAMP8 (Kondziella et al., 2002).

3.2. Pentylenetetrazole

PTZ activates excitatory mechanisms in brain cells and glutamate is thought to play a central role in this context. Li et al. (2000) studied the extracellular concentration of glutamate and taurine in the frontal cortex of freely-moving PTZ-kindled rats using in vivo microdialysis. A significant and sustained increase in glutamate was observed, whereas, no significant changes were found in taurine in the kindled rats (Li et al., 2000). However, our previous results showed that the total amount of glutamate was unchanged (Kondziella et al., 2002). In agreement with the study by Li et al. (2000), no changes were detected in the taurine concentration in the present study in both age groups treated with PTZ. Surprisingly, PTZ-kindling did not enhance glutamate labeling in 2- or 8-month-old SAMP8. On the contrary, glutamate labeling was significantly decreased in the 8-month-old animals, receiving PTZ. A possible explanation could be that PTZ-kindling, in addition to decreasing glutamate turnover in the 8-month-old animals enhances the density of glutamate-binding sites on excitatory neurons. In this way PTZ-kindling could lead to epileptic seizures without interfering greatly with metabolism. This hypothesis is supported by the results of Schroeder et al. (1999) and Silva Brum and Elisabetsky (2000). Schroeder et al. (1999) showed that amino acid release is increased in the early phase of PTZ-kindling development, whereas after completion of kindling, the density of excitatory amino acid-binding sites is enhanced. In the 2-month-old animals receiving PTZ only glutamine labeling from both [1-^{13}C]glucose and [1,2-^{13}C]acetate was decreased. No differences were observed in glutamate labeling, indicating again that an increase in excitatory receptors could be the mechanism of action for PTZ.

Our previous evaluations showed only metabolic changes in the 8-month-old mice compared to controls, but not in the 2-month-old animals (Kondziella et al., 2002). However, the results of the present study using NNMR spectroscopy point not only towards a more impaired metabolism in glutamatergic neurons in older animals but also towards altered astrocytic metabolism in younger animals.

Although no deaths occurred in the group of younger animals, in the 8-month-old receiving PTZ, two of the six animals died during seizures. As explained earlier (Kondziella et al., 2002), this is in line with findings of Nokubo and

Kitani (1988) demonstrating that the necessary lethal amount of PTZ in old mice is significantly lower than that for the respective younger groups.

3.3. Phenobarbital and pentylenetetrazole

As stated previously, it has been shown that in aged animals both the potency and the neurotoxicity of phenobarbital are enhanced (Kitani et al., 1988). Phenobarbital probably acts by increasing the effect of inhibitory GABAergic neurons, as it binds at the GABA receptor–ionophore complex and, as a consequence, alters the conductance at the chloride channel related to the GABAA receptor (Ito et al., 1996). Phenobarbital showed a good anti-epileptic profile in the present study. However, three of the six animals died in the group of 8-month-old animals receiving both PTZ and phenobarbital, even though they had never shown any kind of epileptic symptoms or other possible reactions to PTZ. The lethal complications might be caused by the well-known respiratory depression of phenobarbital. Although the cause of death remains unclear, this is in agreement with the study by Kitani et al. (1988) mentioned before. The results obtained in this group were statistically significant. However, caution should be used when generalizing these results, because of the reduced number of animals in this group.

Amino acid levels were not affected in SAMP8 mice receiving phenobarbital together with PTZ as compared to animals only receiving PTZ, as was shown by our previous study (Kondziella et al., 2002). In the present study, labeling of most metabolites from both [1-^{13}C]glucose and [1,2-^{13}C]acetate in the phenobarbital plus PTZ group was significantly decreased in the 2-month-old but not in the 8-month-old animals. This, together with the unchanged concentrations of the amino acids, indicated that turnover of metabolites was decreased in the 2-month-old group receiving PTZ and phenobarbital. It should be noted that the co-administration of PTZ and phenobarbital lead to considerably more significant alterations in the measured metabolites than PTZ alone. There are two possible explanations for this finding. Either these changes are due to the combined effect of phenobarbital and PTZ, or they are due to phenobarbital alone. Since PTZ probably acts more by enhancing the glutamate-bindings sites than by interfering with metabolism, as was explained previously, the latter explanation is most likely. Indeed, phenobarbital depressed cerebral metabolism in rat brain as shown by Shank et al. (1993).

It should be noted that intraperitoneal administration of phenobarbital produces dose-dependent decreases in cerebral blood flow with no significant difference between young and aged rats (Baughman et al., 1986). However, the same authors found that the difference in cerebral oxygen consumption between young versus aged rats suggested that high-dose phenobarbital may depress cerebral metabolic processes more in aged rats. The opposite was observed in the present study. Most metabolic processes measured were decreased in the 2-month-old animals, only lactate, taurine and GABA C-2 labeling were unchanged in the presence of phenobarbital and PTZ. This indicated that glycolysis was unchanged and that GABAergic neurons were less sensitive to the effects of phenobarbital in the 2-month-old mice. Interestingly, the GABAergic neurons responded most to phenobarbital in the 8-month-old animals. GABA labeling was increased both from [1,2-^{13}C]acetate and [1-^{13}C]glucose compared to control and PTZ groups respectively. Thus, 2-month-old animals showed a decreased GABA labeling, whereas 8-month-old animals showed an increased labeling. What mechanisms induced this changed sensitivity to phenobarbital is presently unclear, but the findings presented here underline the important role of GABA for efficacy and neurotoxicity of barbiturates and changes with increasing age (Macdonald and Barker, 1977; Kitani et al., 1988).

Alanine labeling from [1-^{13}C]glucose and glutamate C-4 from repeated [1,2-^{13}C]acetate labeling were decreased in the PTZ plus phenobarbital group compared to the PTZ group in the 2-month-old animals. Alanine labeling in the cortex is presumably an astrocytic process (Sonnewald et al., 1991; Westergaard et al., 1993). Thus, the results indicate impaired astrocytic metabolism and transfer of labeled glutamine from astrocytes to neurons in the 2-month-old animals.

4. Conclusions

In conclusion, the present results demonstrate that the current practice of using young rodents as models for neurological diseases in adult and older humans may be unsuitable and findings should therefore be interpreted with caution, since it is not possible to extrapolate results obtained in young experimental animals to old ones. Fundamental changes in the biochemical interactions between neurons and astrocytes as well as in the amounts of excitatory and inhibitory neurotransmitters occur during aging in response to pharmacological agents. For example PTZ-kindling seems to affect astrocytes predominately in younger and glutamatergic neurons mainly in older animals. In addition, we showed that ^{13}C NNMR spectroscopy is a valuable tool for the study of neuronal–glial interaction in the PTZ-kindling model of epilepsy in SAMP8 mice.

Acknowledgements

This research was supported by the SINTEF Unimed and Blix Foundations. The generous gift of the SAMP8 mice from The Council of SAM Research, Kyoto, Japan, as well as the excellent technical assistance of Bente Urfjell are gratefully acknowledged. The European Union is thanked for a Leonardo stipend to D. Kondziella.

References

Abe, Y., Yuasa, M., Kajiwara, Y., Hosono, M., 1994. Defects of immune cells in the senescence-accelerated mouse: a model for learning and memory deficits in the aged. Cell Immunol. 157, 59–69.

Baughman, V.L., Hoffman, W.E., Miletich, D.J., Albrecht, R.F., 1986. Effects of phenobarbital on cerebral blood flow and metabolism in young and aged rats. Anesthesiology 65, 500–505.

Berl, S., Frigyesi, T.L., 1969. The turnover of glutamate, glutamine, aspartate and GABA labeled with [1-^{14}C]acetate in caudate nucleus, thalamus and motor cortex (cat). Brain Res. 12, 444–455.

Bradford, H.F., 1989. Glutamate, GABA and epilepsy. Epilepsia 30, 17–25.

Cerdan, S., Kunnecke, B., Seelig, J., 1990. Cerebral metabolism of [1,2-^{13}C_2]acetate as detected by in vivo and in vitro ^{13}C NMR. J. Biol. Chem. 265, 12916–12926.

Chapa, F., Cruz, F., Garcia-Martin, M.L., Garcia-Espinosa, M.A., Cerdan, S., 2000. Metabolism of [1-^{13}C]glucose and [2-^{13}C, 2-^{2}H$_3$]acetate in the neuronal and glial compartments of the adult rat brain as detected by [^{13}C, ^{2}H]NMR spectroscopy. Neurochem. Int. 37, 217–228.

De Boer, T., Stoof, J.C., van Duijn, H., 1982. The effects of convulsant and anticonvulsant drugs on the release of radiolabeled GABA, glutamate, noradrenaline, serotonine and acetylcholine from rat cortical slices. Brain Res. 253, 153–160.

De Deyn, P.P., Macdonald, R.L., 1995. Effects of antiepileptic drugs on GABA responses and on reduction of GABA responses by PTZ and DMCM on mouse neurons in cell culture. Prog. Neurobiol. 47, 477–511.

Fattoretti, P., Bertoni-Freddari, C., Di Stefano, G., Casoli, T., Gracciotti, N., Solazzi, M., Pompei, P., 2001. Quantitative immunohistochemistry of glucose transport protein (Glut3) expression in the rat hippocampus during aging. J. Histochem. Cytochem. 49, 671–672.

Fonnum, F., 1984. Glutamate: a neurotransmitter in mammalian brain. J. Neurochem. 42, 1–11.

Fujibayashi, Y., Waki, A., Wada, K., Ueno, M., Magata, Y., Yonekura, Y., Konishi, J., Takeda, T., Yokoyama, A., 1994. Differential aging pattern of cerebral accumulation of radiolabeled glucose and amino acid in the senescence accelerated mouse (SAM), a new model for the study of memory impairment. Biol. Pharm. Bull. 17, 102–105.

Gegelashvili, G., Schousboe, A., 1998. Cellular distribution and kinetic properties of high-affinity glutamate transporters. Brain Res. Bull. 45, 233–238.

Håberg, A., Qu, H., Haraldseth, O., Unsgård, G., Sonnewald, U., 1998. In vivo injection of [1-^{13}C]glucose and [12-^{13}C]acetate combined with ex vivo ^{13}C NMR spectroscopy: a novel approach to the study of MCA occlusion in rat. J. Cereb. Blood Flow Metab. 18, 1223–1232.

Hassel, B., Bachelard, H., Fonnum, F., Sonnewald, U., 1997. Trafficking of amino acids between neurons and glia in vivo. Effects of inhibition of glial metabolism by fluoroacetate. J. Cereb. Blood Flow Metab. 17, 1230–1238.

Hertz, L., 1979. Functional interactions between neurons and astrocytes. I. Turnover and metabolism of putative amino acid transmitters. Prog. Neurobiol. 13, 277–323.

Hosford, D.A., 1995. Models of primary generalized epilepsy. Curr. Opin. Neurol. 8, 121–125.

Ito, T., Suzuki, T., Wellman, S.E., Ho, I.K., 1996. Pharmacology of barbiturate tolerance/dependence: GABAA receptors and molecular aspects. Life Sci. 59, 169–195.

Kitani, K., Sato, Y., Kanai, S., Ohta, M., Nokubo, M., Masuda, Y., 1988. The neurotoxicity of phenobarbital and its effect in preventing pentylenetetrazole-induced maximal seizure in aging mice. Arch. Gerontol. Geriatr. 7, 261–271.

Kondziella, D., Bidar, A., Urfjell, B., Sletvold, O., Sonnewald, U., 2002. The pentylenetetrazole kindling model of epilepsy in old and young SAMP8 mice: behavior and metabolism. Neurochem. Int. 40, 413–418.

Lapidot, A., Gopher, A., 1994. Cerebral metabolic compartmentation. Estimation of glucose flux via pyruvate carboxylase/pyruvate dehydrogenase by ^{13}C NMR isotopomer analysis of D-[U-^{13}C]glucose metabolites. J. Biol. Chem. 269, 27198–27208.

Li, Z., Yamamoto, Y., Morimoto, T., Ono, J., Okadam, S., Yamatodani, A., 2000. The effect of pentylenetetrazole-kindling on the extracellular glutamate and taurine levels in the frontal cortex of rats. Neurosci. Lett. 282, 117–119.

Loscher, W., Honack, D., Fassbender, C.P., Nolting, B., 1991. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentylenetetrazole seizure models. Epilepsy Res. 8, 171–189.

Macdonald, R.L., Barker, J.L., 1977. Phenobarbital enhances GABA-mediated postsynaptic inhibition in cultured mammalian neurons. Trans. Am. Neurol. Assoc. 102, 139–140.

Macdonald, R.L., Barker, J.L., 1978. Different actions of anticonvulsant and anesthetic barbiturates revealed by use of cultured mammalian neurons. Science 200, 775–777.

Martinez-Hernandez, A., Bell, K.P., Norenberg, M.D., 1977. Glutamine synthetase: glial localization in brain. Science 195, 1356–1358.

Minchin, M.C., Beart, P.M., 1975. Compartmentation of amino acid metabolism in the rat dorsal root ganglion a metabolic and autoradiographic study. Brain Res. 83, 437–449.

Nokubo, M., Kitani, K., 1988. Age-dependent decrease in the lethal threshold of pentylenetetrazole in mice. Life Sci. 43, 41–47.

Nomura, Y., Yamanaka, Y., Kitamura, Y., Arima, T., Ohnuki, T., Oomura, Y., Sasaki, K., Nagashima, K., Ihara, Y., 1996. Senescence-accelerated mouse. Neurochemical studies on aging. Ann. N. Y. Acad. Sci. 786, 410–418.

Olney, J.W., Ho, O.L., 1970. Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine. Nature 227, 609–611.

Omata, N., Murata, T., Fujibayashi, Y., Waki, A., Sadato, N., Yoshimoto, M., Wada, Y., Yonekura, Y., 2001. Age-related changes in energy production in fresh senescence-accelerated mouse brain slices as revealed by positron autoradiography. Dement. Geriatr. Cogn. Disord. 12, 78–84.

Petroff, O.A., Errante, L.D., Rothman, D.L., Kim, J.H., Spencer, D.D., 2002. Glutamate–glutamine cycling in the epileptic human hippocampus. Epilepsia 43, 703–710.

Qu, H., Håberg, A., Haraldseth, O., Unsgård, G., Sonnewald, U., 2000. ^{13}C MR spectroscopy study of lactate as substrate for rat brain. Dev. Neurosci. 22, 429–436.

Sander, J.W., Shorvon, S.D., 1996. Epidemiology of the epilepsies. J. Neurol. Neurosurg. Psychiatr. 61, 433–443.

Sato, E., Inoue, A., Kurokawa, T., Ishibashi, S., 1994. Early changes in glucose metabolism in the cerebrum of senescence accelerated mouse: involvement of glucose transporter. Brain Res. 637, 133–138.

Schousboe, A., Frandsen, A., Krogsgaard-Larsen, P., 1992. Pharmacological and functional characterization of excitatory amino acid mediated cytotoxicity in cerebral cortical neurons. Cell Biol. Toxicol. 8, 93–100.

Schroeder, H., Becker, A., Schroeder, U., Hoellt, V., 1999. ^{3}H-L-glutamate binding and ^{3}H-D-aspartate release from hippocampal tissue during the development of pentylenetetrazole kindling in rats. Pharmacol. Biochem. Behav. 62, 349–352.

Shank, R.P., Bennett, G.S., Freytag, S.O., Campbell, G.L., 1985. Pyruvate carboxylase: an astrocyte-specific enzyme implicated in the replenishment of amino acid neurotransmitter pools. Brain Res. 329, 364–367.

Shank, R.P., Leo, G.C., Zielke, H.R., 1993. Cerebral metabolic compartmentation as revealed by nuclear magnetic resonance analysis of D-[1-^{13}C]glucose metabolism. J. Neurochem. 61, 315–323.

Silva Brum, L.F., Elisabetsky, E., 2000. Antiepileptogenic properties of phenobarbital: behavior and neurochemical analysis. Pharmacol. Biochem. Behav. 67, 411–416.

Sonnewald, U., Westergaard, N., Krane, J., Unsgård, G., Petersen, B., Schousboe, A., 1991. First direct demonstration of preferential release

of citrate from astrocytes using [^{13}C] NMR spectroscopy of cultured neurons and astrocytes. Neurosci. Lett. 128, 235–239.

Sonnewald, U., Westergaard, N., Petersen, S.B., Unsgård, G., Schousboe, A., 1993. Metabolism of [U-^{13}C]glutamate in astrocytes studied by ^{13}C MR spectroscopy: incorporation of more label into lactate than into glutamine demonstrates the importance of tricarboxylic acid cycle. J. Neurochem. 61, 1179–1182.

Stephen, L.J., Brodie, J., 2000. Epilepsy in elderly people. Lancet 355, 1441–1446.

Taylor, A., McLean, M., Morris, P., Bachelard, H., 1996. Approaches to studies of neuronal/glial relationships by ^{13}C MRS analysis. Dev. Neurosci. 18, 434–442.

Van den Berg, C.J., Garfinkel, D., 1971. A stimulation study of brain compartments. Metabolism of glutamate and related substances in mouse brain. Biochem. J. 123, 211–218.

Waniewski, R.A., Martin, D.L., 1998. Preferential utilization of acetate by astrocytes is attributable to transport. J. Neurosci. 18, 5225–5233.

Westergaard, N., Varming, T., Peng, L., Sonnewald, U., Hertz, L., Schousboe, A., 1993. Uptake, release, and metabolism of alanine in neurons and astrocytes in primary cultures. J. Neurosci. Res. 35, 540–545.

Yu, A.C.H., Schousboe, A., Hertz, L., 1982. Metabolic fate of ^{14}C-labeled glutamate in astrocytes in primary cultures. J. Neurochem. 39, 954–960.

Paper III

Paper III is not included due to copyright.

Paper IV

Impaired glutamine metabolism in NMDA receptor hypofunction induced by MK801

Eiliv Brenner[1], Daniel Kondziella[1,3], Asta Håberg[2], Ursula Sonnewald[1*]

1. Department of Neuroscience, Norwegian University of Science and Technology (NTNU), N-7489 Trondheim, Norway.
2. Department of Circulation and Medical Imaging, Norwegian University of Science and Technology (NTNU), N-7489 Trondheim, Norway.
3. Department of Neurology and Neurobiology Research Unit, Rigshospitalet, DK-2100 Copenhagen, Denmark

*Address for correspondence: Prof. U. Sonnewald
Dept. of Neuroscience
Faculty of Medicine, NTNU
Olav Kyrres gate 3
N-7489 Trondheim
Norway

Tel: 47-73590492
Fax: 47-73598655
E-mail: Ursula.Sonnewald@ntnu.no

Abbreviations used: CRFC, cingulate, retrosplenial and middle frontal cortices; GS, glutamine synthetase; GSH, glutathione; MK801, Dizocilpine [5R, 10S]-[+]-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine; NMDA, N-methyl-D-aspartate; NAA, N-acetylaspartate; NMRS, nuclear magnetic resonance spectroscopy; NO, nitric oxide; PC, pyruvate carboxylase; PCP, phencyclidine; PDH, pyruvate dehydrogenase; TCA, tricarboxylic acid.

Abstract

Paradoxically, glutamate receptor antagonists have neurotoxic and psychotogenic properties in addition to their neuroprotective potential during excessive glutamate release. In the present study the noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist MK801 was used to examine glial-neuronal interactions in NMDA receptor hypofunction. Rats were given a subanesthetic dose of MK801 together with [1-^{13}C]glucose and [1,2-^{13}C]acetate, and brains were removed 20 min later. Analyses of extracts from cingulate, retrosplenial plus middle frontal cortices (CRFC) and temporal lobe were performed using HPLC and ^{13}C and ^{1}H nuclear magnetic resonance spectroscopy. Hypofunction of the NMDA receptor induced similar changes in both brain areas investigated, however, the changes were most pronounced in the temporal lobe. Generally, only labeling from [1-^{13}C]glucose was affected by MK801. In CRFC and temporal lobe amounts of both labeled and unlabeled glutamine were increased, whereas those of aspartate were decreased. In the CRFC the decrease in labeling of aspartate was greater than the decrease in concentration, leading to decreased ^{13}C enrichment. In temporal lobe, not in CRFC, increased concentrations of glutamate, GABA, succinate, glutathione and inositol were detected together with increased labeling of GABA and succinate from [1-^{13}C]glucose. ^{13}C Enrichment was decreased in glutamate and increased in succinate. The results point towards a disturbance in glutamate-glutamine cycling and thus interaction between neurons and glia, since labeling of glutamate and glutamine from glucose was affected differently.

Running title: Effects of MK801 on metabolism

Key words: GABA, glutamine, NMR spectroscopy, rats, neuroprotection, neurotoxicity.

Introduction

The dual potential of glutamate, both vitally important and highly toxic, has been known for a long time (Blood *et al.* 1969). Altered glutamate homeostasis is implicated in a variety of neurological and psychiatric disorders ranging from Alzheimer's disease and epilepsy to schizophrenia. N-methyl-D-aspartate (NMDA) and other glutamate receptor antagonists have been developed in order to establish new treatments. Several have been in clinical trials such as Riluzole against amyotrophic lateral sclerosis (Bensimon *et al.* 1994), memantine in Alzheimer's disease (Fleischhacker *et al.* 1986), selfotel in stroke (Davis *et al.* 2000) and CP-101,606 in brain trauma (Bullock *et al.* 1999). These agents are thought to reduce neurotoxicity of excessive glutamate release mediated by increased Ca^{2+} influx into neurons. Unfortunately, results from clinical studies have been quite disappointing (Festoff *et al.* 2003; Miller *et al.* 2003; Muir and Lees 2003). While earlier experimental studies reported mainly a neuroprotective potential of glutamate antagonists (Park *et al.* 1988; Ozyurt *et al.* 1988; Faden *et al.* 1989; Foster *et al.* 1988; Choi *et al.* 1988; Shapira *et al.* 1990), soon the additional neurotoxic potential of NMDA antagonism was recognized (Farber *et al.* 1995; Olney and Farber 1994; Farber *et al.* 1996; Farber *et al.* 1998; Kim *et al.* 1999). Another problematic feature of NMDA antagonists such as phencyclidine (PCP) and ketamine is that they elicit psychotic and other neuropsychiatric symptoms such as psychomotor, spatial and memory impairment.(Javitt and Zukin 1991; Krystal *et al.* 1994).

One of the best-studied NMDA receptor antagonists is the noncompetetive antagonist MK801 (Dizocilpine; [5R, 10S]-[+]-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine) that binds at the phencyclidine (PCP) binding site inside the ion channel. MK801 was first studied due to its ability to minimize cell damage in models of ischemic attacks (Gill *et al.* 1987) and epilepsy (Collins and Olney 1982; Sloviter 1983). This compound is a more selective and potent NMDA receptor antagonist than both PCP and ketamine (Carlsson *et al.* 2001), which is

why MK801 is preferentially used in studies of NMDA receptors in psychosis and schizophrenia (Olney and Farber 1995). Systemic injection of MK801 in rats has been shown to induce concentration and time dependent pathomorphological changes in specific brain regions. Toxic effects were most pronounced and first observed in the retrosplenial cortex (Olney *et al.* 1989). With increasing antagonist concentration, changes were also seen in other brain areas including piriform and enthorinal cortex, dentate gyrus and amygdale (Horvath *et al.* 1997).

A major function of NMDA receptors in polysynaptic circuitry is to increase the signal to inhibitory GABAergic interneurons. It is hypothesized that the neurotoxic potential of NMDA receptor antagonists results from blockade of NMDA receptors on GABAergic interneurons alleviating the GABAergic inhibition on the downstream glutamatergic neurons. Thus, glutamate release is indirectly increased (Farber *et al.* 1995). However, blockade of NMDA receptors at the glutamatergic neurons at the end of the chain might be neuroprotective. This circuitry, which is thought to underlie both the neurotoxic and psychotogenic properties of NMDA receptor antagonism, is well studied (Carlsson *et al.* 2001; Farber *et al.* 2002), but few studies have focused on astrocyte-neuronal interaction during treatment with NMDA receptor antagonists. The major role of astrocytes in the adult brain is to support neurons metabolically, whereas under pathological conditions their role can both be deleterious and neuroprotective (Aschner *et al.* 2002). The glutamate-glutamine cycle, linking glutamatergic neurons and astrocytes, is an important part in the supportive role of astrocytes (van den Berg and Garfinkel 1971). Glutamate released from neurons in glutamatergic neurotransmission is mainly taken up by astrocytes (Gegelashvili and Schousboe 1997; Gegelashvili and Schousboe 1998) and this is compensated for by a flow of glutamine from astrocytes to neurons. Since astrocytes are intensely involved in glutamate homeostasis (Sonnewald *et al.* 1997), it is important to examine glial-neuronal interactions in a state of NMDA receptor hypofunction produced by MK801. An excellent tool for the study of these interactions is ^{13}C nuclear magnetic resonance (NMR) spectroscopy, which

enables studies of the fate of glutamate, glutamine, GABA and other metabolites in both astrocytes and neurons in the same animal (Taylor *et al.* 1996). It is possible to distinguish between neuronal and glial pathways by using specifically labeled precursors such as [1-^{13}C]glucose and [1,2-^{13}C]acetate. Acetate is selectively taken up by astrocytes since they contain a specialized transport system, which is absent or less active in neurons (Waniewski and Martin 1998), whereas acetyl CoA derived from glucose has been calculated to be metabolized more in the neuronal tricarboxylic acid (TCA) cycle in rats (Qu *et al.* 2000). NMR spectroscopy provides a dynamic picture of brain metabolism in particular when combined with proton (^{1}H) NMR spectroscopy and HPLC (Sonnewald and Kondziella 2003). These methods were used to examine alterations in glutamate-glutamine-GABA metabolism and glial-neuronal interaction in the cingulate and the retrosplenial cortices and part of the temporal lobe, the brain areas that are most affected by NMDA receptor antagonists, after a single injection of MK801.

Materials and Methods

Materials

Fifteen male Sprague Dawley rats with an average weight of 250 g were obtained from Möllegaard Breeding Centre, Copenhagen, Denmark and used for the experiment. [1-^{13}C]Glucose, [1,2-^{13}C]acetate and D_2O (99,9%) were purchased from Cambridge Isotopes Laboratories (Woburn, MA, USA), ethylene glycol from Merck (Darmstadt, Germany). *o*-phthaldialdehyde and MK801 (Dizocilpine; [5R, 10S]-[+]-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine) were from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of the purest grade available from local commercial sources.

Methods

All animal procedures were approved by the Norwegian Animal Research Authority. Prior to experiments the animals had free access to food and water and were kept five per cage at a light/dark cycle of 12 h, humidity 60%, temperature 22ºC. During the experiment each animal was placed in its own cage and allowed 30 min for acclimatization. The animals in the treatment group (8 rats) received a solution consisting of MK801 (0.5 mg/kg), [1-^{13}C]glucose (543 mg/kg) and [1,2-^{13}C]acetate (504 mg/kg), the control animals (7 rats) were given a solution with the same concentration of [1-^{13}C]glucose and [1,2-^{13}C]acetate in sterile water, but without MK801. Animals in both groups were injected intraperitoneally with 10 ml/kg of the respective solutions. Twenty minutes after the injection the animals were killed by decapitation and the head snap frozen in liquid nitrogen, and later stored at -80ºC. Brains were removed, and two different areas of each hemisphere were dissected. The first area included the cingulate, the retrosplenial and the medial parts of the frontal cortices (CRFC). The second area, which in the present study will be referred to as the temporal lobe, was dissected using a horizontal cut from the most lateral point of the hemisphere extending approximately 3 mm medially and a second sagittal cut extending ventrally through the whole brain. This resulted in a sample including temporal cortex, piriform cortex, entorhinal cortex, amygdala and parts of the hippocampus. The dissection lasted max. 3 min and was performed on ice with the brains still frozen. After dissection, brain tissue was homogenized in 7% (w/v) perchloric acid and centrifuged at 4,000 g for 5 min. The procedure was repeated with dH_2O, the supernatants pooled and neutralized with 1 M KOH followed by lyophilization.

High Pressure Liquid Chromatography (HPLC)

HPLC analyses to determine the total amounts of amino acids were carried out using the HP 1100 System from Agilent (Palo Alto, CA, USA) with fluorescence detection, after

derivatization with *o*-phthaldialdehyde (Geddes and Wood 1984). Amino acids were separated on a ZORBAX SB-C18 (4.6×250 mm, 5 µm) column from Agilent using 50 mM sodium acetate buffer (pH 7.0) and methanol as eluents.

^{13}C NMR spectroscopy

A Bruker DRX-600 spectrometer (Fälladen, Germany) was used to obtain proton decoupled 150.92 MHz ^{13}C NMR spectra. For this procedure the samples were re-dissolved in 400 µL D_2O containing ethylene glycol 0.1% as an internal standard. Scans were accumulated with a 30º pulse angle and 30 kHz spectral width with 64K data points. The acquisition time was 1.08 s, the relaxation delay 2.5 s and the number of scans was typically 10,000. To all spectra factors to correct for nuclear Overhauser and relaxation effects were applied.

^{1}H NMR spectroscopy

A Bruker DRX-600 spectrometer was used to obtain ^{1}H NMR spectra with a sweep width of 12 kHz with 32K data points. The pulse angle was 90°, the acquisition time 1.36 s and the relaxation delay was 10 s. The number of scans was 400. Water suppression was set at the residual H_2O resonance.

Data analysis

To interpret the results it is necessary to analyze the metabolism of [1-^{13}C]glucose and [1,2-^{13}C]acetate. Via glycolysis [1-^{13}C]glucose can be transformed to [3-^{13}C]pyruvate, which can be metabolized to [3-^{13}C]alanine or [3-^{13}C]lactate. [3-^{13}C]pyruvate can also enter the TCA cycle via [2-^{13}C]acetyl-CoA and then [4-^{13}C]glutamate can be formed, which in turn can be converted to [4-^{13}C]glutamine in astrocytes or [2-^{13}C]GABA in GABAergic neurons (Figure 2). However, if the label stays in the TCA cycle for an additional turn, [2-^{13}C] or [3-^{13}C]glutamate and glutamine

and [4-^{13}C] or [3-^{13}C]GABA can be formed (Figure 2). All these compounds are represented in the spectra by singlets (Figure 1). [1,2-^{13}C]Acetate labeling is very similar to labeling from [1-^{13}C]glucose, however, not only one, but two adjacent carbon atoms are labeled resulting mainly in doublet formation in the NMR detectable products (Figure 1). [1,2-^{13}C]acetate can be converted to [1,2-^{13}C]acetyl-CoA, which can, after several steps, give rise to [4,5-^{13}C]glutamine in astrocytes (Figure 3). This glutamine can be converted to [4,5-^{13}C]glutamate or [1,2-^{13}C]GABA in the appropriate neurons. [1,2-^{13}C]glutamine is formed from [1,2-^{13}C]glutamate, if 2-[4,5-^{13}C]oxoglutarate does not leave the cycle after the first turn but stays for an additional turn (Figure 3). In fact, many more isotopomers arise from the TCA cycle, since both acetyl-CoA and oxaloacetate can be labeled or unlabeled (Figure 2).

Metabolic ratios were calculated as follows: The acetate versus glucose utilization ratio is an approximation for the relative contribution from astrocytes and neurons to glutamate, glutamine and GABA formation (Taylor *et al.* 1996). Incorporation of [1,2-^{13}C]acetate yields [4,5-^{13}C]glutamate, [4,5-^{13}C]glutamine and [1,2-^{13}C]GABA, whereas [1-^{13}C]glucose gives rise to [4-^{13}C]glutamate, [4-^{13}C]glutamine and [2-^{13}C]GABA. The acetate versus glucose utilization ratios are expressed as [4,5-^{13}C]glutamate (glutamine)/[4-^{13}C]glutamate (glutamine), and [1,2-^{13}C]GABA/[2-^{13}C]GABA. The ^{13}C cycling ratio gives an indication of how long label stays in the TCA cycle before incorporation into glutamate and glutamine. The cycling ratio for ^{13}C from [1,2-^{13}C]acetate was calculated as follows: 2×[1,2-^{13}C]glutamate (glutamine) /[4,5-^{13}C]glutamate (glutamine).

The amounts of ^{13}C and ^{1}H in the different metabolites were quantified from integrals of the relevant peaks obtained from NMR spectra. Ethylene glycol served as an internal standard, and was set to 157.79 nmol for the analyses of ^{13}C spectra and to 43.035 µmol for ^{1}H spectra. ^{13}C Enrichment was calculated by subtracting the naturally abundant ^{13}C obtained from HPLC or ^{1}H spectroscopy from the amount of ^{13}C in the singlet of a particular peak, dividing the difference

by the amount of metabolite and multiplying by 100. All results are given as mean ± standard deviation. Statistics were performed using the unpaired two-tailed Student's t-test and $p < 0.05$ was regarded as significant.

Results

Intraperitoneal injection of MK-801 led to hyperlocomotion, ataxia, abducted hindlimbs, flat body posture and stereotyped behavior such as head weaving in all rats, as described by others (Loscher and Honack 1991). Metabolites were efficiently labeled by [1-^{13}C]glucose and [1,2-^{13}C]acetate as can be seen in Figure 1. It is important to notice that most of the singlet peaks are derived from [1-^{13}C]glucose and thus predominantly neuronal metabolism, whereas the doublets in the spectrum (Figure 1) mainly originate from astrocytic metabolism of [1,2-^{13}C]acetate. Figures 2 and 3 are simplified schemes of the metabolic conversions of [1-^{13}C]glucose and [1,2-^{13}C]acetate respectively. It becomes apparent, when comparing the doublets with the singlets in Figure 1, that glutamate and GABA are labeled more from [1-^{13}C]glucose (singlet) than from [1,2-^{13}C]acetate (doublet) in contrast to glutamine, which is labeled predominantly from [1,2-^{13}C]acetate (Figure 1). Lactate, alanine and aspartate are mostly labeled from glucose. MK801 affected the CRFC much less than the temporal lobe. As can be seen in Table 1, there was a significant increase in the amount of [4-^{13}C]glutamine in the CRFC of animals treated with MK801, while [2-^{13}C]aspartate was decreased compared to controls. In the temporal lobe, however, amounts of [4-^{13}C]glutamine, [2-^{13}C]glutamine, [1,2-^{13}C]glutamine, [3-^{13}C]GABA and [2-^{13}C] plus [3-^{13}C]succinate were significantly increased, whereas the amount of [2-^{13}C]aspartate was significantly reduced. In the CRFC cycling in the TCA cycle of precursors for glutamine derived from [1,2-^{13}C]acetate increased significantly from 0.49±0.05 to 0.56±0.04. Cycling of ^{13}C from [1,2-^{13}C]acetate in glutamate and GABA was unaltered. Pyruvate carboxylation was not affected by MK801 as (C-2-C3)/C-4 ratios in glutamate and glutamine and (C-4-C3)/C-2 in GABA were unchanged (results not shown). Not only labeling but also amounts of metabolites were affected by MK801 (Table 2). Again, changes were most pronounced in temporal lobe where levels of glutathione, glutamate, glutamine, GABA, lactate,

succinate and inositol were significantly increased. In CRFC the concentration of aspartate was decreased, whereas that of glutamine was significantly increased. Serine, taurine, alanine (measured by HPLC), NAD and ADP plus ATP (measured by ^{1}H NMRS) were not affected by MK801 (results not shown). The changes in the metabolic pathways leading to the results presented above have been incorporated into a model shown in Figure 4 a,b. From the amounts of metabolite and the ^{13}C contents in the singlets it is possible to calculate % enrichment. For calculation see Materials and Methods. Only singlets are relevant in this context since the doublets are 100% labeled in most cases since naturally abundant doublets appear only if compounds are present in extremely high concentration. In most cases labeling and amount were either increased or decreased to the same extent and thus enrichment was unchanged. However, in the CRFC enrichment in aspartate C-2 decreased from 5.76±0.60 to 4.91±0.60, $p<0.03$. In temporal lobe glutamate C-4 enrichment decreased from 5.86±1.16 to 4.31±1.03, $p<0.02$, also glutamate C-2 and 3 had similar decreases, whereas succinate increased from 2.21±1.07 to 3.67±0.62, $p<0.02$.

Discussion

Energy metabolism

Duncan et al. (1999) showed that injection of subanesthetic doses of ketamine (25 mg/kg) or MK801 (1 mg/kg) caused, 35 min later, pronounced increase in [^{14}C]-2-deoxyglucose (2-DG) uptake in the structures in the temporal lobe and also in cingulate and retrosplenial cortex. In light of this we investigated the effects of MK801 on [1-^{13}C]glucose metabolism in cingulate plus retrosplenial cortex and temporal lobe. No differences were found in [1-^{13}C]glucose concentration in brain 20 min after injection of MK801 (0.5 mg/kg), confirming earlier reports (Loubinoux *et al.* 1994). The glycolysis product lactate was, however, increased in MK801 treated animals in the present study in both areas. This is in line with enhanced 2-DG uptake, followed by increased glycolysis. Loubinoux et al. (1994) showed an overall increase in lactate production in rat brain 20 min after MK801 (0.5 mg/kg) injection using *in vivo* ^{1}H NMRS. These authors did not detect any changes in N-acetylaspartate (NAA) levels. NAA is the most abundant amino acid in the brain and is located in neurons (Baslow 2003). It is synthesized from aspartate and acetyl CoA through the reaction catalyzed by acetyl-CoA-aspartate N-acetyltransferase, an enzyme localized in neuronal mitochondria. Loss of NAA can indicate both mitochondrial dysfunction and/or cell death. In agreement with Loubinoux et al. (1994) no differences were found in the NAA concentration in the present study indicating that mitochondrial metabolism in neurons was not affected by MK801. Our findings that ATP, ADP levels were not altered by MK801 lends further support to this interpretation, and is in agreement with previous reports of unchanged ATP levels ATP levels.

Glutamine

Activation of NMDA receptors by the natural agonist glutamate opens the ion channel allowing entry of Ca^{2+} and Na^{+} into the postsynaptic neuron. Recent evidence suggests that NMDA

receptors are also present on astrocytes (Krebs *et al.* 2003). Metabolic interaction between astrocytes and neurons is essential, since neurons lack the anaplerotic enzyme pyruvate carboxylase (Yu *et al.* 1983). Glutamine synthetase (GS), a glial specific enzyme (Norenberg and Martinez-Hernandez 1979), catalyses the reaction between ammonia and glutamate. In order to synthesize glutamate and GABA for release, neurons depend on glutamine supply from astrocytes. In the present study glutamine concentrations were increased in response to MK801. This is in agreement with a study by Kosenko et al. (2003), that showed evidence of a nitric oxide (NO) mediated control of GS. These authors showed that in the presence of 2 mg/kg MK801 the decreased entry of Ca^{2+} through the NMDA receptors caused reduced production of calmoduline, leading to impaired activity of nitric oxide synthase and subsequently decreased NO production. Glutamine synthetase is inhibited by NO and thus more glutamine is produced at low NO levels (Kosenko *et al.* 2003; 1994). Loscher and Honack (1991) also reported increase of glutamine in the frontal cortex, 3 h after injection of MK801 (0.1 mg/kg). Increased glutamine concentrations were also detected in both brain areas investigated in the present study. Glutamine concentration and also [4-^{13}C]glutamine formed from [1-^{13}C]glucose were increased. Glutamate was also increased, but [4-^{13}C]glutamate was unchanged, resulting in decreased ^{13}C enrichment. These results indicate a disruption in the glutamate–glutamine cycle caused by MK801. Interestingly, the amount of glutamine formed from [1,2-^{13}C]acetate derived from the first turn of the TCA cycle, [4,5-^{13}C]glutamine, was unchanged, whereas [1,2-^{13}C]glutamine was increased in the temporal lobe. The latter form of glutamine is derived from [1,2-^{13}C]acetate as well, however, 2-oxoglutarate for synthesis of [1,2-^{13}C]glutamine via [1,2-^{13}C]glutamate has stayed in the TCA cycle for an additional turn. Thus, in the temporal lobe, mitochondrial function was altered in the astrocytes. No changes were observed in [4,5-^{13}C]glutamine, [4,5-^{13}C]glutamate and [1,2-^{13}C]GABA from [1,2-^{13}C]acetate demonstrating unperturbed metabolic flux from astrocytes to neurons in both areas of the brain. These observations indicate that not all GS was

affected by MK801, and point towards compartmentation of glutamine metabolism, where glutamine labeled from neuronal glutamate ([4-^{13}C]glutamate) is handled in a different compartment than glutamine from astrocytic glutamate. A possible mechanism is that Ca^{2+} was decreased at the dendritic part of the cell body, near the synapses, and only there the NO production was reduced. Less NO would then diffuse over the astrocytic membrane leading to increased GS activity in the synaptic region of the astrocyte. The glutamate taken up by astrocytes in the synaptic region is mostly glutamate released from neuronal vesicles and therefore labeled from [1-^{13}C]glucose. In parts of astrocytes bordering synapses there could be an increased conversion of [4-^{13}C]glutamate to [4-^{13}C]glutamine. Since NO is a very reactive species, it cannot diffuse far and reduced NO production near the synapses will not influence GS activity in other parts of the astrocytes, where glutamate is predominantly derived from astrocytic metabolism (i.e. [4,5-^{13}C]glutamate) and levels of [4,5-^{13}C]glutamine will not be changed.

The increased amounts of both labeled and unlabeled glutamine have to be compensated for by anaplerosis via pyruvate carboxylase or decrease in metabolites that can be converted to oxaloacetate, such as glutamate. In the present study the pyruvate carboxylase to dehydrogenase ratio was not affected by MK801. Furthermore glutamate, GABA, succinate and glutathione concentrations were increased, and only the amount of aspartate was decreased. However, this decrease was not sufficient to account for the increase in the other metabolites. Another possibility is a decrease in pyruvate recycling, but this pathway is not very prominent (Waagepetersen *et al.* 2002; Lapidot and Gopher 1994; Kunnecke *et al.* 1993) and there were no indications of increased pyruvate recycling in the present study. Altogether the results indicate that MK801 disrupted the metabolic balance in the synaptic region of the astrocytes and thus the glutamate-glutamine cycle. In additional support of astrocytic dysfunction is the finding that

inositol, an osmoregulator, was increased in the temporal lobe of rats treated with MK801, which can be considered to indicate glial cell swelling (Brand *et al.* 1993).

Glutamate

In the present study increased concentration of glutamate was detected 20 min after injection of MK801 in the temporal lobe. In previous studies elevated glutamate levels have been observed 3 h after injection of MK801 in the piriform cortex, which is a part of the temporal lobe. Löscher et al. (1991) suggested that the increased glutamate levels resulted from feedback elevation of glutamate synthesis induced by MK801 blockade of postsynaptic NMDA receptors. However, results in the present study challenge this hypothesis since ^{13}C enrichment in glutamate was decreased.

GABA

Glutamine released by astrocytes can also function as precursor for the inhibitory neurotransmitter GABA via glutamate (Sonnewald *et al.* 1993). Thus the concept of the glutamate-glutamine cycle was extended to a glutamate–glutamine-GABA cycle. Löscher et al. (1991) reported increased concentrations of GABA in the piriform and frontal cortices after injection of MK801. .The same was observed in the present study in temporal lobe but not in the CRFC. GABA is converted to succinate via the GABA shunt, which accounts for approximately 10% of the TCA cycle flux (Martin and Rimvall 1993). GABA amount and labeling from the TCA cycle were increased to the same extend in temporal lobe whereas succinate labeling increased more than the amount leading to increased ^{13}C enrichment. Thus the glutamate–glutamine-GABA cycle was altered in addition to the glutamate–glutamine cycle. However, the drain of GABA from neurons to astrocytes is relatively modest (Schousboe and Waagepetersen 2004; Peng *et al.* 1993), and glutamine transport has been shown to be more intense in

glutamatergic neurons than cortical neurons and astrocytes (Su *et al.* 1997; Varoqui *et al.* 2000). Disturbances in the glutamate–glutamine-GABA cycle might therefore not be as deleterious as in the glutamate–glutamine cycle.

The fact that effects on GABA were only observed in temporal lobe might be due to higher GABAergic activity in the temporal lobe as reflected by the larger amount of GABA in the temporal lobe compared to CRFC.

Glutathione

Being the major antioxidant in the brain (Dringen and Hirrlinger 2003), glutathione (GSH) plays a fundamental role in protecting cells from damage by reactive oxygen species generated among others from dopamine metabolism. A deficit in GSH might lead to degenerative processes in the surrounding of dopaminergic terminals resulting in loss of connectivity (Schulz *et al.* 2000). GSH also potentiates the NMDA receptor's response to glutamate (Janaky *et al.* 1999). The increased level of GSH in rats in the present study could point to a decreased use of antioxidants, possibly caused by decrease in NO production due to decrease Ca^{2+} influx. It should be noted that GSH could increase the effect of glutamate on the NMDA receptors.

Conclusion

Hypofunction of NMDA receptors induces similar changes in glutamate-glutamine cycling in both areas investigated, however, the changes were most pronounced in the temporal lobe, where also the glutamate-glutamine-GABA cycle was impaired. It can be postulated that increase of glutamine synthetase activity is the cause of the neurotoxic effects of MK801.

Acknowledgments: This research was supported by the Epilepsy Foundation. The excellent technical assistance of Bente Urfjell is greatly appreciated.

References

Aschner M., Sonnewald U. and Tan K. H. (2002) Astrocyte modulation of neurotoxic injury. *Brain Pathol.* **12,** 475-481.

Baslow M. H. (2003) N-acetylaspartate in the vertebrate brain: metabolism and function. *Neurochem. Res.* **28,** 941-953.

Bensimon G., Lacomblez L. and Meininger V. (1994) A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. *N. Engl. J. Med.* **330,** 585-591.

Blood F. R., Oser B. L., White P. L. and Olney J. W. (1969) Monosodium glutamate. *Science* **165,** 1028-1029.

Brand A., Richter-Landsberg C. and Leibfritz D. (1993) Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. *Dev. Neurosci.* **15,** 289-298.

Bullock M. R., Merchant R. E., Carmack C. A., Doppenberg E., Shah A. K., Wilner K. D., Ko G. and Williams S. A. (1999) An open-label study of CP-101,606 in subjects with a severe traumatic head injury or spontaneous intracerebral hemorrhage. *Ann. N. Y. Acad. Sci.* **890,** 51-58.

Carlsson A., Waters N., Holm-Waters S., Tedroff J., Nilsson M. and Carlsson M. L. (2001) Interactions between monoamines, glutamate, and GABA in schizophrenia: new evidence. *Annu. Rev. Pharmacol. Toxicol.* **41,** 237-260.

Choi D. W., Koh J. Y. and Peters S. (1988) Pharmacology of glutamate neurotoxicity in cortical cell culture: attenuation by NMDA antagonists. *J. Neurosci.* **8,** 185-196.

Collins R. C. and Olney J. W. (1982) Focal cortical seizures cause distant thalamic lesions. *Science* **218,** 177-179.

Davis S. M., Lees K. R., Albers G. W., Diener H. C., Markabi S., Karlsson G. and Norris J. (2000) Selfotel in acute ischemic stroke : possible neurotoxic effects of an NMDA antagonist. *Stroke* **31,** 347-354.

Dringen R. and Hirrlinger J. (2003) Glutathione pathways in the brain. *Biol. Chem.* **384,** 505-516.

Duncan G. E., Miyamoto S., Leipzig J. N. and Lieberman J. A. (1999) Comparison of brain metabolic activity patterns induced by ketamine, MK-801 and amphetamine in rats: support for NMDA receptor involvement in responses to subanesthetic dose of ketamine. *Brain Res.* **843,** 171-183.

Faden A. I., Demediuk P., Panter S. S. and Vink R. (1989) The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science* **244,** 798-800.

Farber N. B., Foster J., Duhan N. L. and Olney J. W. (1996) Olanzapine and fluperlapine mimic clozapine in preventing MK-801 neurotoxicity. *Schizophr. Res.* **21,** 33-37.

Farber N. B., Hanslick J., Kirby C., McWilliams L. and Olney J. W. (1998) Serotonergic agents that activate 5HT2A receptors prevent NMDA antagonist neurotoxicity. *Neuropsychopharmacology* **18,** 57-62.

Farber N. B., Kim S. H., Dikranian K., Jiang X. P. and Heinkel C. (2002) Receptor mechanisms and circuitry underlying NMDA antagonist neurotoxicity. *Mol. Psychiatry* **7,** 32-43.

Farber N. B., Wozniak D. F., Price M. T., Labruyere J., Huss J., St P. H. and Olney J. W. (1995) Age-specific neurotoxicity in the rat associated with NMDA receptor blockade: potential relevance to schizophrenia? *Biol. Psychiatry* **38,** 788-796.

Festoff B. W., Suo Z. and Citron B. A. (2003) Prospects for the pharmacotherapy of amyotrophic lateral sclerosis : old strategies and new paradigms for the third millennium. *CNS. Drugs* **17,** 699-717.

Fleischhacker W. W., Buchgeher A. and Schubert H. (1986) Memantine in the treatment of senile dementia of the Alzheimer type. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **10,** 87-93.

Foster A. C., Gill R. and Woodruff G. N. (1988) Neuroprotective effects of MK-801 in vivo: selectivity and evidence for delayed degeneration mediated by NMDA receptor activation. *J. Neurosci.* **8,** 4745-4754.

Geddes J. W. and Wood J. D. (1984) Changes in the amino acid content of nerve endings (synaptosomes) induced by drugs that alter the metabolism of glutamate and gamma-aminobutyric acid. *J. Neurochem.* **42,** 16-24.

Gegelashvili G. and Schousboe A. (1997) High affinity glutamate transporters: regulation of expression and activity. *Mol. Pharmacol.* **52,** 6-15.

Gegelashvili G. and Schousboe A. (1998) Cellular distribution and kinetic properties of high-affinity glutamate transporters. *Brain Res. Bull.* **45,** 233-238.

Gill R., Foster A. C. and Woodruff G. N. (1987) Systemic administration of MK-801 protects against ischemia-induced hippocampal neurodegeneration in the gerbil. *J. Neurosci.* **7,** 3343-3349.

Haberg A. and Sonnewald U. (2004) Contributions of astrocytes to ischemia-induced neuronal dysfuntion in vivo. *Adv. Mol. Cell Biol.* 837-856.

Horvath Z. C., Czopf J. and Buzsaki G. (1997) MK-801-induced neuronal damage in rats. *Brain Res.* **753,** 181-195.

Janaky R., Ogita K., Pasqualotto B. A., Bains J. S., Oja S. S., Yoneda Y. and Shaw C. A. (1999) Glutathione and signal transduction in the mammalian CNS. *J. Neurochem.* **73,** 889-902.

Javitt D. C. and Zukin S. R. (1991) Recent advances in the phencyclidine model of schizophrenia. *Am. J. Psychiatry* **148,** 1301-1308.

Kim S. H., Price M. T., Olney J. W. and Farber N. B. (1999) Excessive cerebrocortical release of acetylcholine induced by NMDA antagonists is reduced by GABAergic and alpha2-adrenergic agonists. *Mol. Psychiatry* **4,** 344-352.

Kosenko E., Llansola M., Montoliu C., Monfort P., Rodrigo R., Hernandez-Viadel M., Erceg S., Sanchez-Perez A. M. and Felipo V. (2003) Glutamine synthetase activity and glutamine content in brain: modulation by NMDA receptors and nitric oxide. *Neurochem. Int.* **43,** 493-499.

Kosenko E., Kaminsky Y, Grau E, Minana MD, Marcaida G, Grisolia S, Felipo V. (1994) Brain ATP depletion induced by acute ammonia intoxication in rats is mediated by activation of the NMDA receptor and Na+,K(+)-ATPase. *J. Neurochem.* **63,** 2172-2178.

Krebs C., Fernandes H. B., Sheldon C., Raymond L. A. and Baimbridge K. G. (2003) Functional NMDA receptor subtype 2B is expressed in astrocytes after ischemia in vivo and anoxia in vitro. *J. Neurosci.* **23,** 3364-3372.

Krystal J. H., Karper L. P., Seibyl J. P., Freeman G. K., Delaney R., Bremner J. D., Heninger G. R., Bowers M. B., Jr. and Charney D. S. (1994) Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch. Gen. Psychiatry* **51,** 199-214.

Kunnecke B., Cerdan S. and Seelig J. (1993) Cerebral metabolism of [1,2-$^{13}C_2$]glucose and [U-$^{13}C_4$]3-hydroxybutyrate in rat brain as detected by ^{13}C NMR spectroscopy. *NMR Biomed.* **6,** 264-277.

Lapidot A. and Gopher A. (1994) Cerebral metabolic compartmentation. Estimation of glucose flux via pyruvate carboxylase/pyruvate dehydrogenase by ^{13}C NMR isotopomer analysis of D-[U-^{13}C]glucose metabolites. *J. Biol. Chem.* **269,** 27198-27208.

Loscher W. and Honack D. (1991) Anticonvulsant and behavioral effects of two novel competitive N-methyl-D-aspartic acid receptor antagonists, CGP 37849 and CGP 39551, in the kindling model of epilepsy. Comparison with MK-801 and carbamazepine. *J. Pharmacol. Exp. Ther.* **256,** 432-440.

Loscher W., Honack D. and Fassbender C. P. (1991) Regional alterations in brain amino acids after administration of the N-methyl-D-aspartate receptor antagonists MK-801 and CGP 39551 in rats. *Neurosci. Lett.* **124,** 115-118.

Loubinoux I., Meric P., Borredon J., Correze J. L., Gillet B., Beloeil J. C., Tiffon B., Mispelter J., Lhoste J. M. and Jacques S. (1994) Cerebral metabolic changes induced by MK-801: a 1D (phosphorus and proton) and 2D (proton) in vivo NMR spectroscopy study. *Brain Res.* **643,** 115-124.

Martin D. L. and Rimvall K. (1993) Regulation of gamma-aminobutyric acid synthesis in the brain. *J. Neurochem.* **60,** 395-407.

Miller R. G., Mitchell J. D., Lyon M. and Moore D. H. (2003) Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Amyotroph. Lateral. Scler. Other Motor Neuron Disord.* **4,** 191-206.

Muir K. W. and Lees K. R. (2003) Excitatory amino acid antagonists for acute stroke. *Cochrane. Database. Syst. Rev.* CD001244.

Norenberg M. D. and Martinez-Hernandez A. (1979) Fine structural localization of glutamine synthetase in astrocytes of rat brain. *Brain Res.* **161,** 303-310.

Olney J. W. and Farber N. B. (1995) NMDA antagonists as neurotherapeutic drugs, psychotogens, neurotoxins, and research tools for studying schizophrenia. *Neuropsychopharmacology* **13,** 335-345.

Olney J. W. and Farber N. B. (1994) Efficacy of clozapine compared with other antipsychotics in preventing NMDA-antagonist neurotoxicity. *J. Clin. Psychiatry* **55 Suppl B,** 43-46.

Olney J. W., Labruyere J. and Price M. T. (1989) Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Science* **244,** 1360-1362.

Ozyurt E., Graham D. I., Woodruff G. N. and McCulloch J. (1988) Protective effect of the glutamate antagonist, MK-801 in focal cerebral ischemia in the cat. *J. Cereb. Blood Flow Metab* **8,** 138-143.

Park C. K., Nehls D. G., Graham D. I., Teasdale G. M. and McCulloch J. (1988) The glutamate antagonist MK-801 reduces focal ischemic brain damage in the rat. *Ann. Neurol.* **24,** 543-551.

Peng L., Hertz L., Huang R., Sonnewald U., Petersen S. B., Westergaard N., Larsson O. and Schousboe A. (1993) Utilization of glutamine and of TCA cycle constituents as precursors for transmitter glutamate and GABA. *Dev. Neurosci.* **15,** 367-377.

Qu H., Haberg A., Haraldseth O., Unsgard G. and Sonnewald U. (2000) ^{13}C MR spectroscopy study of lactate as substrate for rat brain. *Dev. Neurosci.* **22,** 429-436.

Schousboe A. and Waagepetersen H. S. (2004) Role of astrocytes in homeostasis of glutamate and GABA during physiological and pathophysiological conditions. *Adv. Mol. Cell Biol.* **31,** 461-474.

Schulz J. B., Lindenau J., Seyfried J. and Dichgans J. (2000) Glutathione, oxidative stress and neurodegeneration. *Eur. J. Biochem.* **267,** 4904-4911.

Shapira Y., Yadid G., Cotev S., Niska A. and Shohami E. (1990) Protective effect of MK801 in experimental brain injury. *J. Neurotrauma* **7,** 131-139.

Sloviter R. S. (1983) "Epileptic" brain damage in rats induced by sustained electrical stimulation of the perforant path. I. Acute electrophysiological and light microscopic studies. *Brain Res. Bull.* **10,** 675-697.

Sonnewald U. and Kondziella D. (2003) Neuronal glial interaction in different neurological diseases studied by ex vivo ^{13}C NMR spectroscopy. *NMR Biomed.* **16,** 424-429.

Sonnewald U., Westergaard N. and Schousboe A. (1997) Glutamate transport and metabolism in astrocytes. *Glia* **21,** 56-63.

Sonnewald U., Westergaard N., Schousboe A., Svendsen J. S., Unsgard G. and Petersen S. B. (1993) Direct demonstration by [^{13}C]NMR spectroscopy that glutamine from astrocytes is a precursor for GABA synthesis in neurons. *Neurochem. Int.* **22,** 19-29.

Su T. Z., Campbell G. W. and Oxender D. L. (1997) Glutamine transport in cerebellar granule cells in culture. *Brain Res.* **757,** 69-78.

Taylor A., McLean M., Morris P. and Bachelard H. (1996) Approaches to studies on neuronal/glial relationships by ^{13}C-MRS analysis. *Dev. Neurosci.* **18,** 434-442.

van den Berg C. J. and Garfinkel D. (1971) A stimulation study of brain compartments. Metabolism of glutamate and related substances in mouse brain. *Biochem. J.* **123,** 211-218.

Varoqui H., Zhu H., Yao D., Ming H. and Erickson J. D. (2000) Cloning and functional identification of a neuronal glutamine transporter. *J. Biol. Chem.* **275,** 4049-4054.

Waagepetersen H. S., Qu H., Hertz L., Sonnewald U. and Schousboe A. (2002) Demonstration of pyruvate recycling in primary cultures of neocortical astrocytes but not in neurons. *Neurochem. Res.* **27,** 1431-1437.

Waniewski R. A. and Martin D. L. (1998) Preferential utilization of acetate by astrocytes is attributable to transport. *J. Neurosci.* **18,** 5225-5233.

Yu A. C., Drejer J., Hertz L. and Schousboe A. (1983) Pyruvate carboxylase activity in primary cultures of astrocytes and neurons. *J. Neurochem.* **41,** 1484-1487.

Table 1 Amounts of ^{13}C in nmol/g of tissue in brain extracts of control and MK-801-treated rats

	Cingulate/Retrosplenial/Frontal Cortex		Temporal Lobe	
	Control (n=6)	MK801 (n=6)	Control (n=6)	MK801 (n=7)
From [1,2-^{13}C]acetate				
[4,5-^{13}C]glutamate	258.4 ± 40.7	235.1 ± 32.1	212.2 ± 31.6	211.1 ± 31.4
[4,5-^{13}C]glutamine	343.7 ± 51.2	386.7 ± 59.9	342.0 ± 53.0	393.0 ± 47.6
[1,2-^{13}C]glutamine	85.5 ± 19.8	109.0 ± 17.8	79.6 ± 8.1	105.6 ± 9.3*
From [1-^{13}C]glucose				
[4-^{13}C]glutamate	641.2 ± 86.1	641.0 ± 77.6	567.8 ± 36.3	536.3 ± 75.8
[2-^{13}C]glutamate	337.0 ± 52.2	354.6 ± 36.1	279.5 ± 23.9	275.2 ± 41.6
[4-^{13}C]glutamine	137.3 ± 13.3	161.1 ± 19.5*	125.4 ± 10.0	150.8 ± 21.9*
[2-^{13}C]glutamine	104.3 ± 17.4	121.5 ± 12.2	92.5 ± 13.2	114.3 ± 11.5*
[2-^{13}C]aspartate	133.7 ± 16.2	105.2 ± 12.3*	105.2 ± 13.6	80.6 ± 16.9*
[3-^{13}C]GABA[a]	51.2 ± 5.8	47.5 ± 10.3	47.5 ± 9.5	56.9 ± 3.8*
[2-^{13}C] or [3-^{13}C]succinate	32.0 ± 7.6	32.4 ± 4.4	22.6 ± 6.5	39.2 ± 4.3*
[1-^{13}C]glucose	76.6 ± 25.5	94.7 ± 25.5	53.5 ± 16.9	44.5 ± 18.6

Rats were treated with a single intraperitoneal injection of MK801 (0.5mg/kg) or saline together with [1,2-^{13}C]acetate and [1-^{13}C]glucose. Twenty minutes later they were sacrificed (for details see Materials and Methods). The results are expressed as mean ± SD and were analyzed with the Student's *t*-test. *, significantly different from control; a, [3-^{13}C]GABA can also be derived from [1,2-^{13}C]acetate after the second turn of the TCA cycle.

Table 2. Amounts of metabolites in μmol/g of tissue in brain extracts of control and MK-801-treated rats.

	Cingulate/Retrosplenial/Frontal Cortex		Temporal Lobe	
	Control (n=7)	MK801 (n=7)	Control (n=8)	MK801 (n=8)
Glutathione[a]	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.5 ± 0.1*
Aspartate[a]	2.0 ± 0.3	1.7 ± 0.1*	1.8 ± 0.2	1.6 ± 0.2
Glutamate[a]	9.4 ± 1.2	10.3 ± 1.1	8.6 ± 0.9	10.1 ± 1.4*
Glutamine[a]	3.2 ± 0.2	3.5 ± 0.2*	3.3 ± 0.3	4.2 ± 0.2*
GABA[a]	1.3 ± 0.1	1.3 ± 0.1	2.0 ± 0.3	2.5 ± 0.6*
Lactate[c]	4.1 ± 0.6	5.7 ± 0.7*	5.4 ± 0.8	6.2 ± 0.6*
Succinate[b]	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1*
NAA[b]	9.2 ± 0.8	9.6 ± 0.9	8.7 ± 0.8	8.5 ± 0.7
Inositol[b]	6.5 ± 1.7	6.0 ± 1.2	6.2 ± 0.6	8.8 ± 1.5*

Rats were treated with a single intraperitoneal injection of MK801 (0.5mg/kg) or saline and were decapitated twenty minutes later (for details see Materials and Methods). The results are expressed as mean ± SD and were analyzed with the Student's *t*-test. NAA, N-acetyl-apartate; a, results from HPLC analysis; b, results from ^{1}H-NMR analysis; c, results from ^{13}C-NMR analysis; *, significantly different from control.

Legends

Figure 1. ^{13}C NMR spectrum of temporal lobe extracts from rats injected with MK801 together with [1,2-^{13}C]acetate and [1-^{13}C]glucose. Peak assignments; 1: glutamate C-2; 2: glutamine C-2; 3: aspartate C-2; 4: aspartate C-3; 5: GABA C-2; 6: succinate C-2/C-3; 7: glutamate C-4; 8: glutamine C-4; 9: glutamate C-3; 10: glutamine C-4; 11: GABA C-3; 12: N-acetyl-aspartate C-3; 13: lactate C-3. The singlets are mostly derived from [1-^{13}C]glucose and the doublets in the spectrum from [1,2-^{13}C]acetate.

Figure 2. Schematic presentation of isotopomers of glutamate, glutamine and GABA derived from [1-^{13}C]glucose after the first and second turn of the tricarboxylic acid (TCA) cycle.

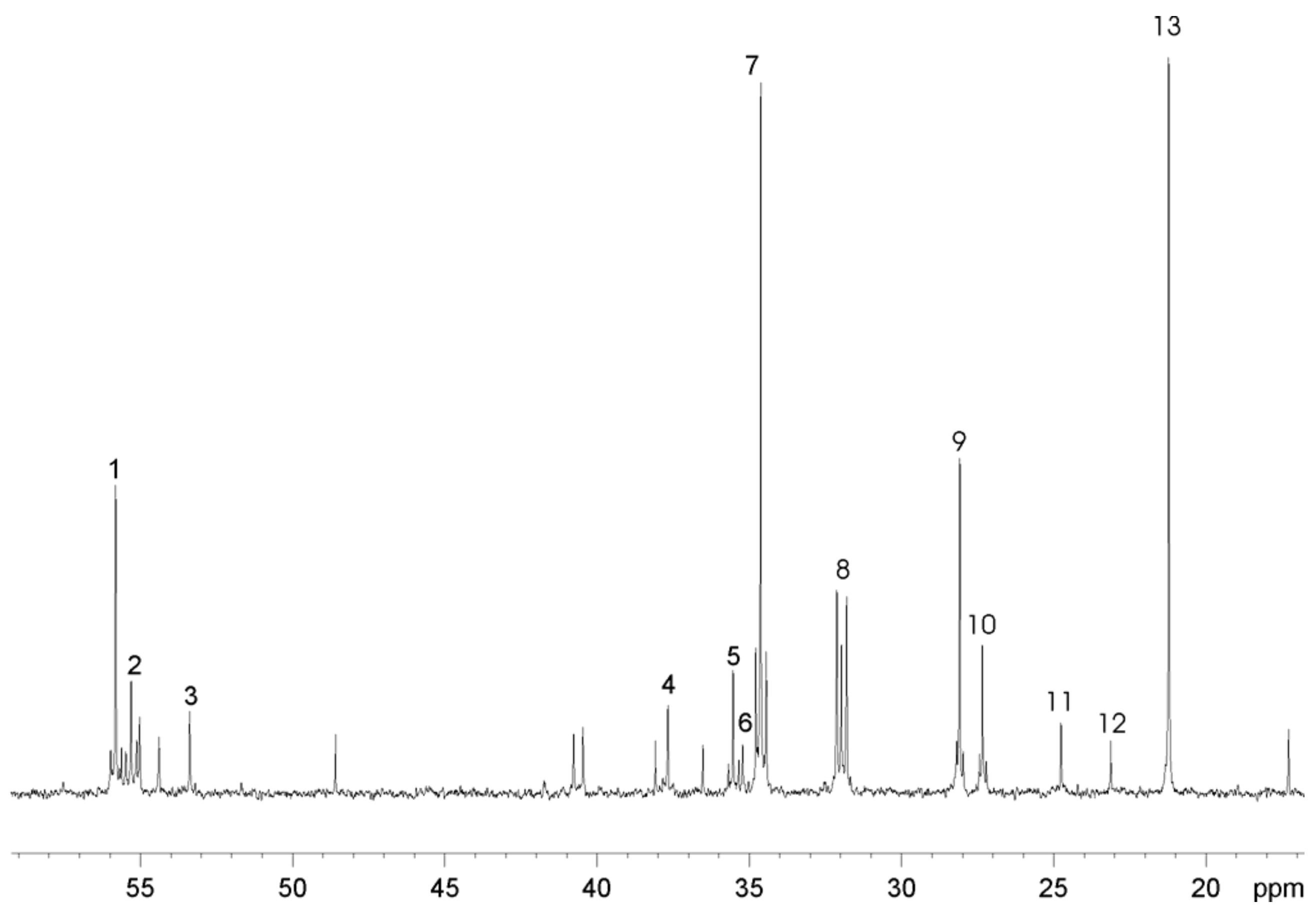
Figure 3. Schematic presentation of isotopomers of glutamate, glutamine and GABA derived from [1,2-^{13}C]acetate after the first and second turn of the tricarboxylic acid (TCA) cycle.

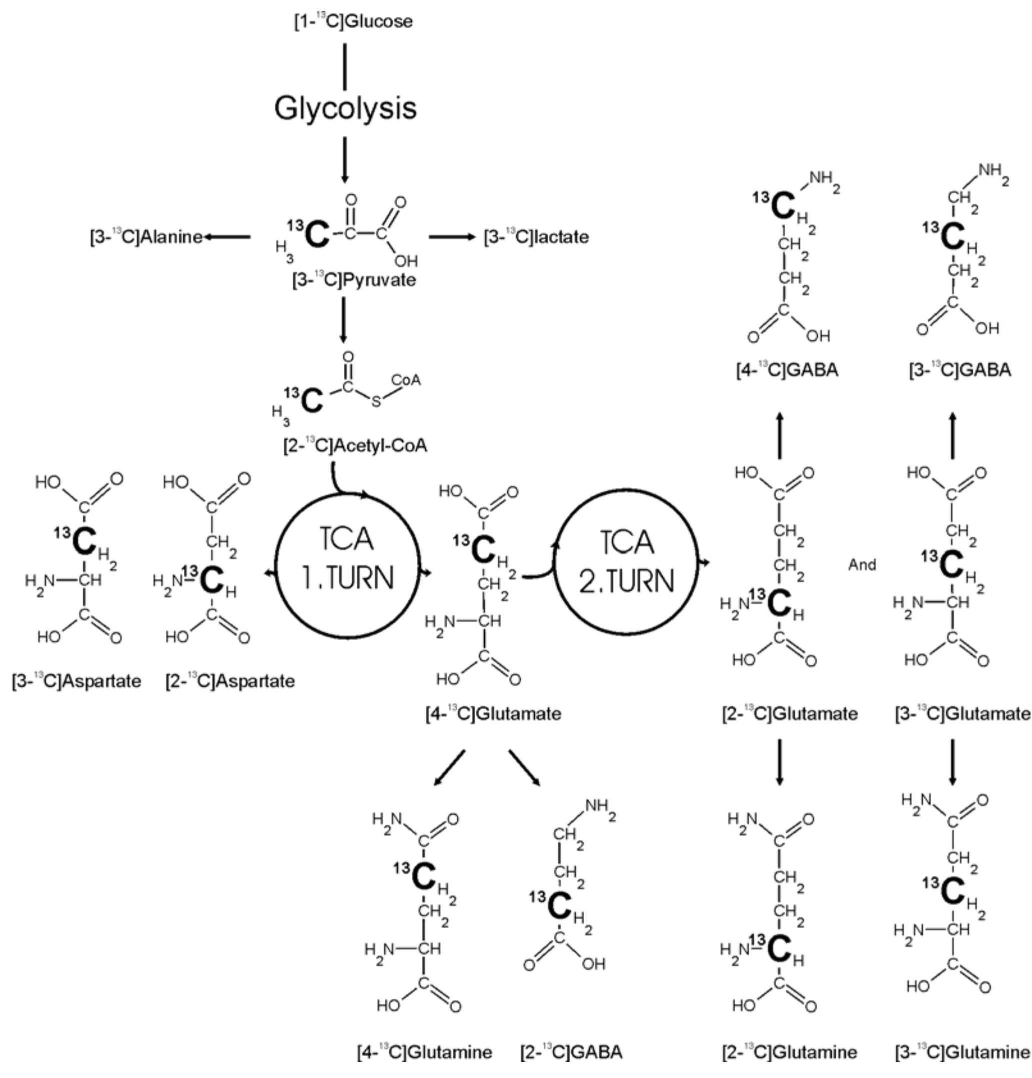
Figure 4

a. Schematic presentation of the interactions of an astrocyte with pre- and post-synaptic neurons. Glutamate synthesized by neurons is released into the synaptic cleft, activating NMDA receptors on both astrocytes and neurons and is deactivated by uptake mostly into astrocytes. In astrocytes glutamate is converted to glutamine by glutamine synthetase, an enzyme modulated by nitric oxide.

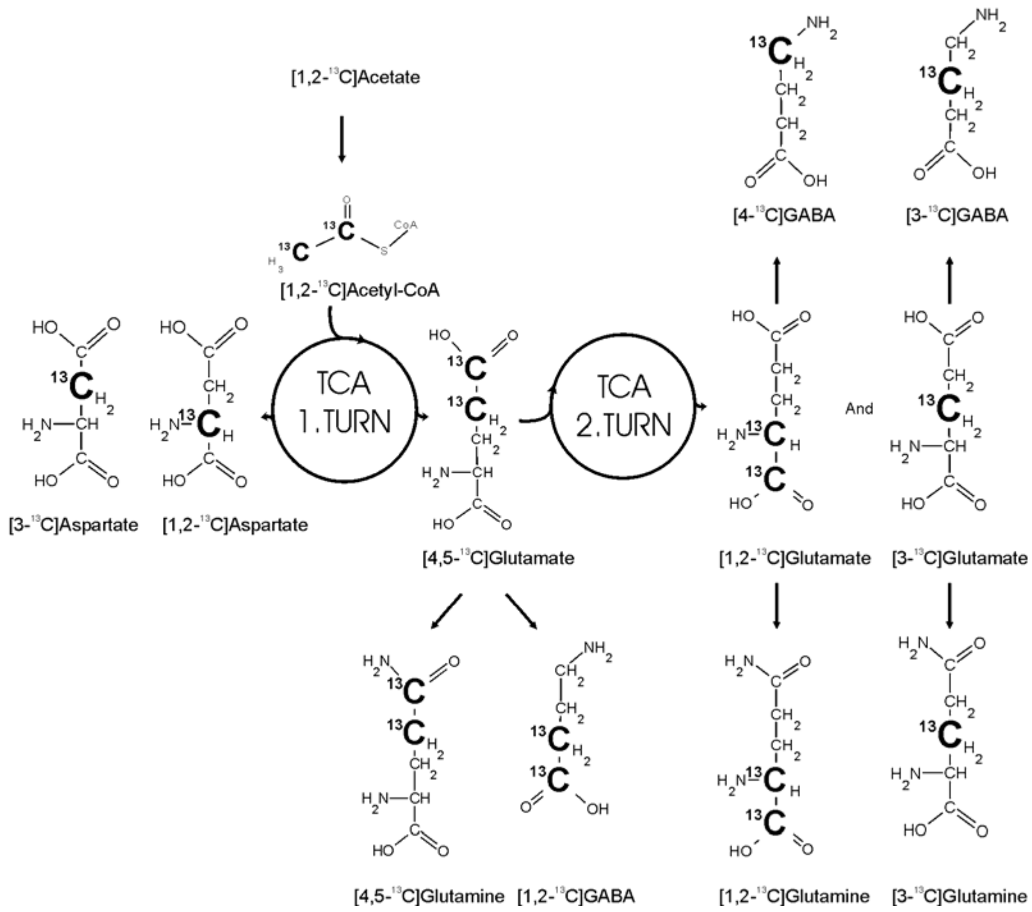
b. Schematic presentation of the interactions of an astrocyte with pre- and post-synaptic neurons in the presence of MK801. Reduced NO production due to reduced activation of the NMDA receptor will lead to increased activity of GS. This will be most pronounced in the synaptic region and will affect the glutamine-glutamate cycle between neurons and astrocytes. *, Glutamine in the non-synaptic region mostly labeled from [1,2-^{13}C]acetate; ⊕, increased enzyme

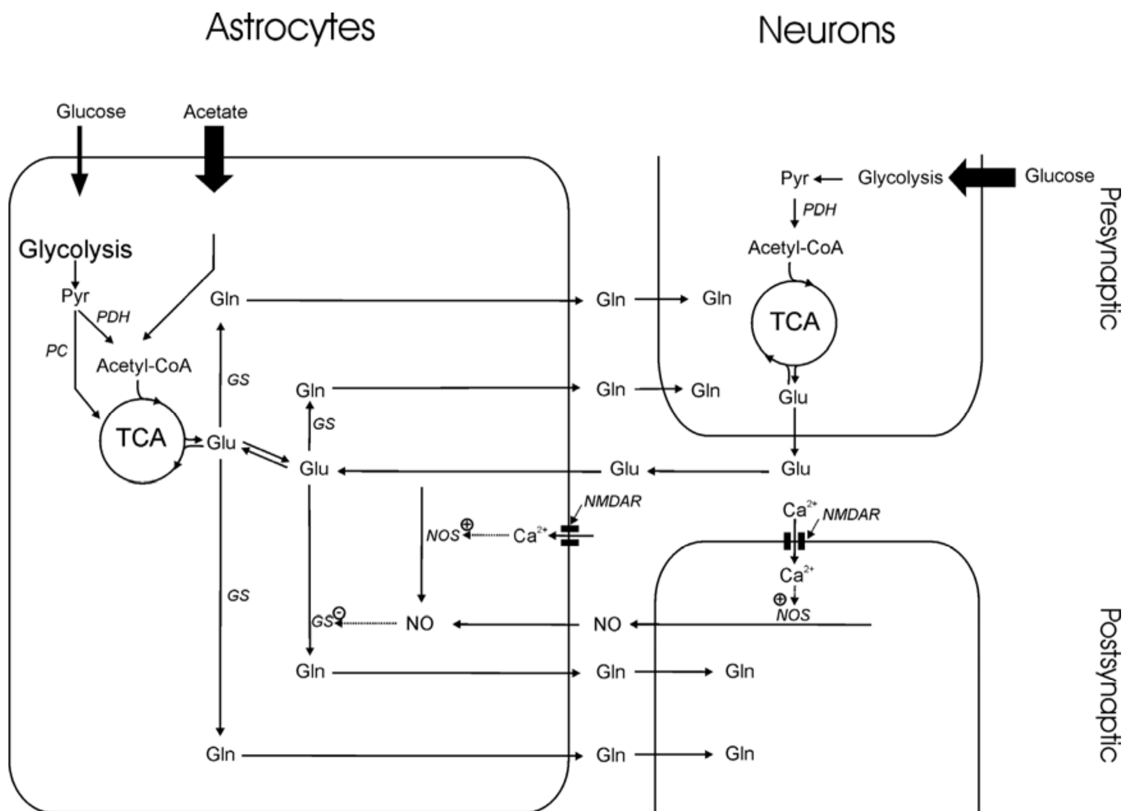
activity; ⊖, decreased enzyme activity; stippled lines, modulation of enzyme activity. Abbreviations: GS, glutamine synthetase; NMDA receptor, N-methyl-D-aspartate receptor; NO, nitric oxide; NOS, nitric oxide synthase; PAG, phosphate activated glutaminase; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase.

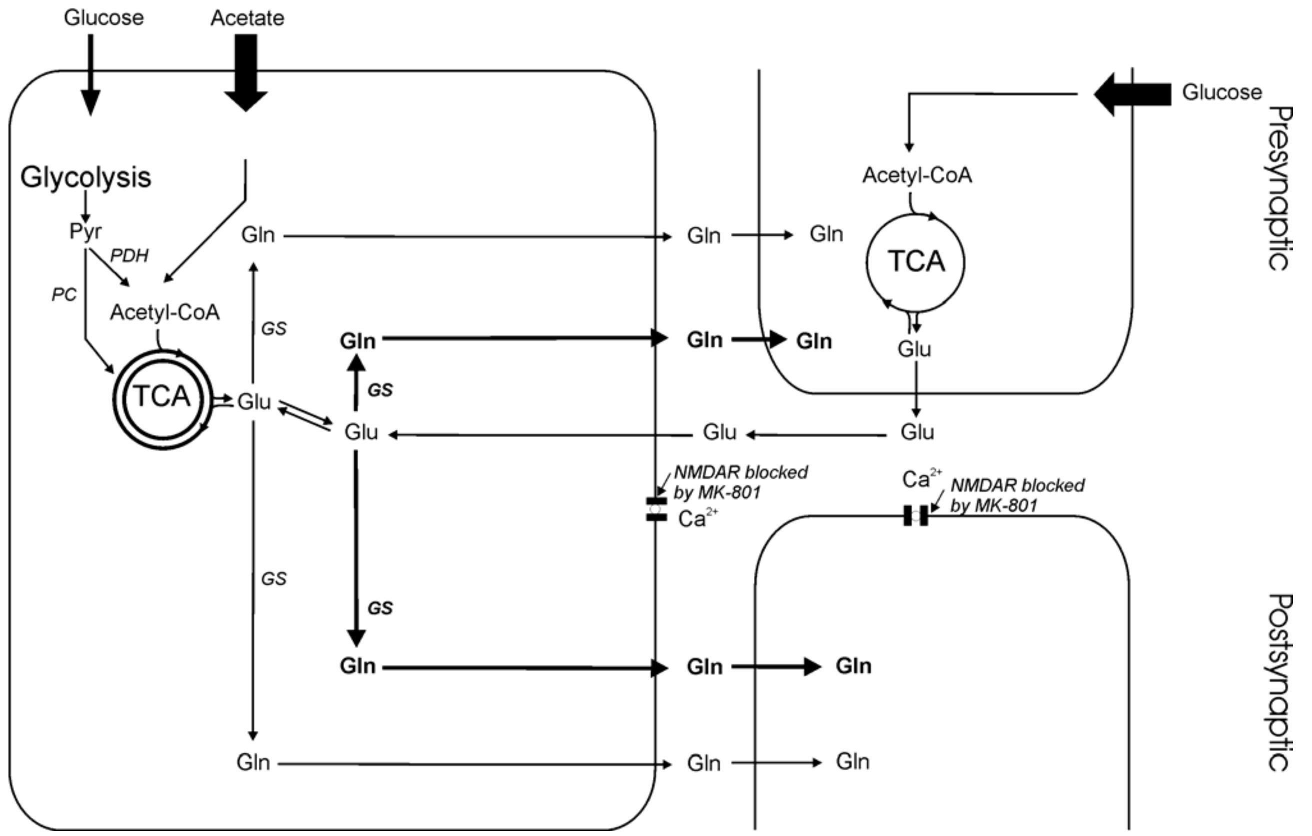

1
2
3
4
5
6
7
8
9
10
11
12
13
55
50
45
40
35
30
25
20
ppm



[1-13C]Glucose
Glycolysis
[3-13C]Alanine
[3-13C]lactate
[3-13C]Pyruvate
[2-13C]Acetyl-CoA
TCA
1.TURN
TCA
2.TURN
[3-13C]Aspartate
[2-13C]Aspartate
[4-13C]Glutamate
[2-13C]Glutamate
And
[3-13C]Glutamate
[4-13C]GABA
[3-13C]GABA
[4-13C]Glutamine
[2-13C]GABA
[2-13C]Glutamine
[3-13C]Glutamine


[1,2-13C]Acetate
[1,2-13C]Acetyl-CoA
TCA 1.TURN
TCA 2.TURN
[3-13C]Aspartate
[1,2-13C]Aspartate
[4,5-13C]Glutamate
[4-13C]GABA
[3-13C]GABA
And
[1,2-13C]Glutamate
[3-13C]Glutamate
[4,5-13C]Glutamine
[1,2-13C]GABA
[1,2-13C]Glutamine
[3-13C]Glutamine


Astrocytes
Neurons
Glucose
Acetate
Glycolysis
Pyr
PDH
PC
Acetyl-CoA
TCA
Glu
Gln
GS
Glu
NOS
Ca2+
NMDAR
NO
Presynaptic
Pyr
Glycolysis
Glucose
PDH
Acetyl-CoA
TCA
Glu
Ca2+
NMDAR
NOS
Postsynaptic



Astrocytes
Neurons
Glucose
Acetate
Glycolysis
Pyr
PDH
PC
Acetyl-CoA
TCA
Gln
GS
Glu
NMDAR blocked by MK-801
Ca2+
Glucose
Presynaptic
Postsynaptic

Paper V

Glial-Neuronal Interactions are Impaired in the Schizophrenia Model of Repeated MK801 Exposure

Daniel Kondziella[1,2], Eiliv Brenner[1], Elvar M. Evjolfsson[1], Katarina R. Markinhuhta[3], Maria L. Carlsson[3,4], Ursula Sonnewald[1]

1. Department of Neuroscience, Norwegian University of Science and Technology (NTNU), N-7489 Trondheim, Norway
2. Department of Neurology and Neurobiology Research Unit, Rigshospitalet, DK-2100 Copenhagen, Denmark
3. Carlsson Research AB, Biotech Center, Arvid Wallgrens Backe 20, SE-41346 Göteborg, Sweden
4. Arvid Carlsson Institute, Institute of Clinical Neuroscience, Göteborg University, Medicinaregatan 11, Box 432, SE-405 30 Göteborg, Sweden

*Address for correspondence: Prof. U. Sonnewald
Dept. of Neuroscience
Faculty of Medicine, NTNU
Olav Kyrres gate. 3
N-7489 Trondheim
Norway

Tel: 47-73590492
Fax: 47-73598655
E-mail: Ursula.Sonnewald@ntnu.no

Running title: Effects of repeated MK801 on metabolism

Key words: Glutamate, GABA, glutamine, dopamine, NMDA glutamate antagonists, rats, NMR spectroscopy, schizophrenia.

Abstract

Schizophrenia-mimicking compounds such as phencyclidine (PCP) and MK801 are antagonists at the N-methyl-D-aspartate (NMDA) receptor and produce the whole spectrum of positive and negative symptoms, including cognitive disturbances. This is one of the most important pillars of the hypoglutamatergic hypothesis of schizophrenia. Since the synthesis of glutamate and GABA in neurons is closely connected to astrocyte metabolism, the study of astrocytic function is essential in this context. In the present study, we induced a state of NMDA receptor hypofunction by administering MK801 every other day for twelve days. The last dose was given together with [1-^{13}C]glucose and [1,2-^{13}C]acetate. Frontal, retrosplenial and cingulate cortices (CRFC) and temporal lobes were examined by ^{13}C and ^{1}H nuclear magnetic resonance spectroscopy, HPLC and light microscopy. Significant increases in the levels of glutamate, glutathione and taurine were seen, whereas amounts and turnover of noradrenaline, dopamine and serotonin were not changed. Glutamate and glutamine, derived from [1,2-^{13}C]acetate and thus astrocytes, were significantly decreased in CRFC as compared to controls. Labeling from [1-^{13}C]glucose and thus mostly neuronal metabolism was affected in the same brain region with decreased labeling of glutamate and GABA. The present model mimics the increased glutamate/glutamine activity found in drug naive patients with first episode schizophrenia. Moreover, the decreased labeling both in astrocytes and neurons shows the transition to lower glutamatergic function seen in chronic schizophrenia patients. The decreases in astrocytic function and the glutamine-glutamate-GABA cycle are of significant importance and might add to the disturbances of the cortico-striato-thalamo-cortical loop caused by NDMA receptor blockade and hence to the sensory gating deficits provoking sensory overstimulation of the cortex and psychosis.

Introduction

The hyperdopaminergic hypothesis of schizophrenia was for a long time based upon indirect pharmacological evidence, but has recently received more direct support from neuroimaging studies.[1,2,3,4] Recently, a modified dopamine hypothesis of schizophrenia has been proposed, postulating dysfunctional dopaminergic synapses leading to secondary alterations in dopaminergic neuronal activity.[5] In the past years glutamatergic hypofunction has been recognized to play a significant role as well. N-methyl-D-aspartate (NMDA) glutamate-receptor antagonists, such as phencyclidine (PCP), ketamine or dizocilpine-maleate (MK801), have been shown to cause strong psychomimetic effects with hallucinations and psychomotor signs and have been used extensively in schizophrenia research. In contrast to dopaminergic agonists, which only mimic the positive symptoms of schizophrenia, NMDA antagonists produce the whole spectrum including negative and cognitive symptoms. Another mechanism, which has been suggested many years ago, involves the serotonin-5HT-2α-receptor, since the 5-HT2 agonists such as lysergic acid diethylamid (LSD) produce psychotic symptoms.[6] Thus, evidence is accumulating showing that the hypothesis of exclusive dopaminergic hyperfunction causing schizophrenia has to be revised or at least, extended. A more differentiated approach includes, in addition to the dopaminergic, also glutamatergic and serotonergic neurotransmitter systems. [7]

It has been suggested that the dysregulation of dopamine transmission in schizophrenia might be secondary to alterations in glutamatergic NMDA receptor mediated transmission. [8,9] A direct linkage between the two hypothesis has recently been shown in an experiment performed in healthy volunteers. It was observed that the amplitude of amphetamine induced dopamine release was significantly enhanced compared to control conditions, when NMDA receptor-mediated transmission was decreased by ketamine administration. [10] Thus, the

elevated dopamine release seen in schizophrenic patients after amphetamine administration might very well be secondary to a failure in glutamatergic control of dopamine neurons. Analogous studies in rats support this interpretation. [11] NMDA receptor hypofunction models not involving dopamine have also been proposed. [12,13,14]

The synthesis of glutamate and GABA in neurons is closely connected to astrocyte metabolism. Being the most important excitatory neurotransmitter in the mammalian CNS, glutamate and its homeostasis are crucial to brain function for several reasons. Firstly, fast removal of glutamate from the synaptic cleft by astrocytes guarantees short glutamate action on the postsynaptic target cell and thereby precise information signaling. Secondly, high extracellular concentration of glutamate is neurotoxic and it has been shown that excessive glutamate release plays a part in the pathophysiology of many brain disorders. Thirdly, since neurons lack the main anaplerotic enzyme in the brain, pyruvate carboxylase,[15] they depend on astrocytic supply of tricarboxylic acid (TCA) cycle intermediates because drain of amino acid neurotransmitters would otherwise lead to a shortage of neurotransmitter precursors. [16] Astrocytes release glutamine into the extracellular space, from where it is taken up by neurons and converted to glutamate and GABA or channeled into the TCA cycle via 2-oxoglutarate to provide carbon skeletons for the synthesis of other metabolites. As mentioned earlier, after release from neurons glutamate is cleared from the synapses by astrocytes, which transform glutamate to glutamine again, and the so-called “glutamine-glutamate- cycle” is closed. [17]

An excellent tool to obtain information about metabolic pathways and glial-neuronal metabolic interaction is ^{13}C nuclear magnetic resonance spectroscopy (NMRS) - see Sonnewald and Kondziella[18] for a review. When [1,2-^{13}C] acetate and [1-^{13}C]glucose are given simultaneously, it is possible to study astrocytic and neuronal metabolism in the same animal. [19] MK801 is one of the best characterized non-competitive antagonists of the PCP binding site of

the NMDA receptor which is also present on astrocytes. [27] Since it is even more selective than PCP[7], MK801 is a widely studied substance in experimental research of both schizophrenia and glutamate neurotoxicity. It causes behavioral changes in rodents such as hyperlocomotion, stereotyped behavior, head weaving and ataxia. [20] Altered cerebral metabolism with excessive cerebral glucose supply has been reported by Loubinoux et al. [21] MK801 is able to minimize cell damage in the rodent hippocampus during ischaemia, but also to induce neuronal degeneration. Toxic effects of MK801 were first observed and are most pronounced in the retrosplenial and cingulate cortex. [22,23] With increasing MK801 concentrations, alterations are also seen in other brain areas including entorhinal and piriform cortices, dentate gyrus and amygdala. [24]

In the present study, we induced a state of NMDA receptor hypofunction in rats by repeated administration of MK801. Then astrocytic-neuronal interactions in frontal, retrosplenial and cingulate cortices and temporal lobe were examined by means of NMRS, HPLC and light microscopy to test the hypothesis that glial-neuronal metabolism is disturbed during experimental schizophrenia provoked by repeated MK801 treatment.

Materials and Methods

Materials

Twenty-six male Sprague Dawley rats with an average weight of 250 g were obtained from Möllegaard Breeding Centre, Copenhagen, Denmark. [1-^{13}C]glucose, [1,2-^{13}C]acetate (both 99% ^{13}C enriched) and D_2O (99,9%) were purchased from Cambridge Isotopes Laboratories (Woburn, MA, USA), ethylene glycol from Merck (Darmstadt, Germany). o-phthaldialdehyde and MK801 (Dizocilpine; [5R, 10S]-[+]-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine) were from Sigma-Aldrich, St. Louis, MO, USA, ketamine (ketalar) from Werner Lambert, Nordic AB, Solna, Sweden and medotomidinehydrochloride from Jansen, Cilag Ltd., Southerton, UK. All other chemicals were of the purest grade available from local commercial sources.

Methods

All animal procedures were approved by the Norwegian Animal Research Authority. Prior to experiments the animals received food and water ad libitum and were kept at a light/dark cycle of 12 h, humidity 60%, temperature 22ºC. During the experiment the animals were housed in individual cages. Saline (13 animals) or MK801 (13 animals; 0.5mg/kg body weight) were administered intraperitoneally every other day for twelve days. The last dose was given together with [1-^{13}C]glucose (543mg/kg, 0.3 M solution) and [1,2-^{13}C]acetate (504mg/kg, 0.6 M solution) followed by decapitation twenty minutes later. The heads were snap frozen in liquid nitrogen and stored at -80ºC. Brains were removed, and two different areas of each hemisphere were dissected. The first area included the cingulate, the retrosplenial and the frontal cortices (CRFC). The

second area, which in the present study will be referred to as the temporal lobe, was dissected by a horizontal cut from the most lateral point of the hemisphere extending approximately 3 mm medially and a second sagittal cut extending ventrally through the whole brain. The resulting sample consisted of the temporal cortex, piriform cortex, entorhinal cortex, amygdala and parts of the hippocampus. The dissection was performed on ice while the brains were still frozen and lasted max. 3 min. After dissection, brain tissue was homogenized in 7% (w/v) perchloric acid and centrifuged at 4.000 g for 5 min. The procedure was repeated, the supernatants pooled and neutralized with 1 M KOH followed by lyophilization.

Histology

Three MK801 treated rats and five controls did not receive [1-^{13}C]glucose and [1,2-^{13}C]acetate in their last injection. They were deeply anesthetized with ketamine (100mg/kg) and medotomidinehydrochloride (0.5mg/kg) and underwent cardiac perfusion with 20ml heparinized 0.9% saline and a modified Karnovsky solution (2% formaldehyd, 2.5% glutaraldehyd, 0.1M natriumcacodylate buffer, 0.025% $CaCl_2$; pH 7.4). Brains were removed immediately after perfusion and stored in the same fixative until slicing. All slices were processed by sequential alcohol dehydration, cleared, infiltrated, embedded in paraffin and stained with Hematoxilin-Eosin or Nissl-stained. Using the atlas of Paxinos and Watson[25] CRFC, TE and hippocampus were identified and examined by light microscopy for signs of neuronal degeneration such as intracytoplasmatic vacuoles, remnant nuclear debris, brightly eosinophilic cytoplasm and local glial reaction, which have been described earlier. [14]

High Pressure Liquid Chromatography (HPLC)

Total amounts of amino acids were determinated by HPLC using the HP 1100 System from Agilent (Palo Alto, CA, USA) with fluorescence detection, after derivatization with o-phthaldialdehyde. Amino acids were separated on a ZORBAX SB-C18 (4.6×250 mm, 5mm) column from Agilent with 50 mM sodium acetate buffer (pH 7.0) and methanol as eluents. The monoamine transmitter substances (NA, DA, 5-HT) and acid (DOPAC, 5-HIAA, HVA) metabolites were quantified in brain tissue homogenates by HPLC separations and electrochemical detection. The analytical method is based on two chromatographic separations for amines and acids. Both systems are equipped with a reverse phase coloumn (Luna C18(2), dp 3µm, 50 x 2mm i.d., Phenomex), and electrochemical detection is accomplished at two potentials on glassy carbon electrodes (MF-1000, Bioanalytical Systems, Inc.). The aqueous mobile phase (0.4 ml/min) for the acid system contains citric acid 14mM, sodium citrate 10mM, MeOH 15% (v/v) and EDTA 0.1mM. Detection potentials relative to Ag/AgCl reference are 0.45 and 0.6V. The aqueous ion pairing mobile phase (0.5 ml/min) for the amine system contains citric acid 5mM, sodium citrate 10mM, MeOH 9%(v/v), decane sulfonic acid 0.45mM and EDTA 0.1 mM. Detection potentials relative to Ag/AgCl reference are 0.45 and 0.65V.

^{13}C NMR spectroscopy

Proton decoupled 150.92 MHz ^{13}C NMR spectra were obtained using a Bruker DRX-600 spectrometer after the samples had been re-dissolved in 200 µL D_2O containing ethylene glycol 0.1% as an internal standard. Scans were accumulated with a 30º pulse angle and 30 kHz spectral width with 64K data points. The number of scans was typically 10,000. The acquisition time was

1.08 s, the relaxation delay 0.5 s. Factors for nuclear Overhauser and relaxation effects were applied to all spectra.

^{1}H NMR spectroscopy

A DRX-600 spectrometer was used to obtain ^{1}H NMR spectra with a sweep width of 12 kHz with 32K data points. The pulse angle was 90°, the acquisition time 1.36 s and the relaxation delay was 10 s. The number of scans was 400. Water suppression was set at the residual H_2O resonance.

Labeling patterns

Label from [1-^{13}C]glucose can be quantified by analyzing the singlet peaks in the different resonances (Figure 1). [18] In contrast, the doublets seen in the spectrum (Figure 1) are mostly derived from [1,2-^{13}C]acetate and thus astrocytic metabolism. [26] Glutamine is labeled more from [1,2-^{13}C]acetate (doublet) than [1-^{13}C]glucose (singlet); the opposite is the case for glutamate and GABA. Alanine, lactate, N-acetylaspartate (NAA) in the C-6 position and succinate are mainly labeled from glucose. Creatine, taurine and the aspartate group in NAA are not labeled; the naturally abundant ^{13}C gives rise to the observed singlets (Figure 1). Since both acetyl-CoA and oxalacetate can be labeled or unlabeled, the number of possible isotopomers of the TCA cycle derived metabolites is large and only compounds derived from the first and the second turns are presented in Figure 2. In addition to analysis of the single peaks, conclusions about the predominant metabolic pathways can be drawn from metabolic ratios (see below). [1-^{13}C]glucose is converted to pyruvate via glycolysis and can form [3-^{13}C]alanine and [3-^{13}C]lactate. Pyruvate

may enter the TCA cycle via [2-^{13}C]acetyl-CoA, which will lead to the formation of [4-^{13}C]glutamate and glutamine or [2-^{13}C]GABA. After the second turn of the TCA cycle this label will be in the [2-^{13}C] or [3-^{13}C] positions of glutamate and glutamine or in the [3-^{13}C] or [4-^{13}C] positions of GABA. Alternatively, pyruvate can be carboxylated by pyruvate carboxylase (PC) to oxalacetate, which will lead to the synthesis of [2-^{13}C]glutamate and glutamine or [4-^{13}C]GABA. [1,2-^{13}C]acetate can also be converted to acetyl-CoA, however, the product, [1,2-^{13}C]acetyl-CoA, will have two ^{13}C atoms resulting in doublet formation. Thus, [4,5-^{13}C]glutamate and glutamine or [1,2-^{13}C]GABA are formed (Figure 2). After the second turn of the TCA cycle this label will be in the [1,2-^{13}C] or [3-^{13}C] positions of glutamate and glutamine and the [2-^{13}C] or [3-^{13}C] positions of GABA.

Metabolic ratios

The acetate versus glucose utilization ratio is an estimation of the relative contribution from neurons and astrocytes to glutamate, glutamine and GABA formation. [19] Incorporation of [1,2-^{13}C]acetate yields [4,5-^{13}C]glutamate, [4,5-^{13}C]glutamine and [1,2-^{13}C]GABA, whereas [1-^{13}C]glucose gives rise to [4-^{13}C]glutamate, [4-^{13}C]glutamine and [2-^{13}C]GABA. The acetate versus glucose utilization ratios are expressed as [4,5-^{13}C]glutamate (glutamine)/[4-^{13}C]glutamate (glutamine), and [1,2-^{13}C]GABA/[2-^{13}C]GABA.

The ^{13}C cycling ratio gives an indication of how long label stays in the TCA cycle before incorporation into glutamate and glutamine. The cycling ratio for ^{13}C from [1,2-^{13}C]acetate was calculated as follows: [1,2-^{13}C]glutamate (glutamine) / [4,5-^{13}C]glutamate (glutamine). The cycling ratio for ^{13}C from [1-^{13}C]glucose was calculated: {[3-^{13}C]glutamate (glutamine) – [1,2-^{13}C]glutamate (glutamine)} / [4-^{13}C]glutamate (glutamine). Ratios of pyruvate carboxylase and

pyruvate dehydrogenase (PC/PDH ratios) give information about the importance of anaplerosis and can be calculated like this: {[2-^{13}C]glutamate (glutamine) - [3-^{13}C]glutamate (glutamine} / [4-^{13}C]glutamate (glutamine). The labeling of GABA from the second turn is identical for [1-^{13}C]glucose and [1,2- ^{13}C]acetate.

Data analysis

The amounts of ^{13}C in the different metabolites were quantified from integrals of the relevant peaks obtained from NMR spectra with ethylene glycol as an internal standard. All results are given as mean ± standard deviation. Statistics were performed using the two-tailed, unpaired Student's t-test; $p < 0.05$ was regarded as significant.

Results

MK-801 induced hyperlocomotion, ataxia, abducted hindlimbs, flat body posture and stereotyped behavior such as head waving, which were characterized by considerable inter- and intravariability. Injection of [1-^{13}C]glucose and [1,2-^{13}C]acetate lead to efficient labeling of many metabolites (Figure 1). Particularly, labeling of glutamate and glutamine C-3 and C-4, GABA C-2 and C-3, aspartate and lactate C-3 are shown in Figure 1. Labeling patterns from [1-^{13}C]glucose and [1,2-^{13}C]aspartate from the first and second turns of the TCA cycle are shown in Figure 2.

MK801 affected the CRFC to a much greater extent than the temporal lobe. As can be seen in Table 1, there was a significant increase in the levels of glutamate, taurine and glutathione in the CRFC compared to control. Concentrations of serine, aspartate, alanine, NAD^+ and ADP plus ATP (data not shown) and N-acetyl-aspartate (NAA) were not affected by MK801. Levels of noradrenaline, serotonin and dopamine remained unchanged, which was also true for the turnover (data not shown) of dopamine (DOPAC/DA and HVA/DA) and serotonin (5-HIAA/5-HT). In the temporal lobe only inositol was increased. The amounts of [4,5-^{13}C]glutamate and [4,5-^{13}C]glutamine, derived from [1,2-^{13}C]acetate, were significantly decreased in CRFC as compared to controls (Table 2). Label from [1-^{13}C]glucose was affected in the same brain region with decreases of [4-^{13}C]glutamate, [2-^{13}C]GABA and [4-^{13}C]GABA, whereas in the temporal lobe both [1,2-^{13}C]acetate and [1-^{13}C]glucose derived metabolites were not altered. Concentration of [3-^{13}C]lactate was unchanged in both areas (data not shown). In Table 3 it can be seen that the cycling ratios for ^{13}C from [1-^{13}C]glucose was increased for glutamate in both investigated brain areas. Moreover, acetate/glucose ratios for glutamate and glutamine were decreased in the CRFC. However, an increase of the cycling ratio for ^{13}C from

[1,2-^{13}C]acetate was found in the CRFC for glutamine. PC/PDH ratios were not changed (data not shown).

Eosin- and Nissl-stained histological slices from CRFC, hippocampus and TE were examined by light microscopy, but no morphological changes were found between MK801 treated rats and controls.

Discussion

Glutamate - glutamine - GABA cycle and glial-neuronal interactions

Increased levels of glutamate were found in the present study. As pointed out in the introduction glutamate and glutamine are very closely linked metabolically. Using HPLC and ^{13}C it is possible to distinguish these two amino acids. However, ^{1}H NMR at low field strength is not capable of fully discerning these two signals. Using ^{1}H NMR it was suggested that the glutamine signal in spectra of anterior cingulate and thalamus of drug naïve first episode schizophrenia patients was increased compared to age matched healthy controls. [51] Furthermore, in cerebrospinal fluid of this patient group an increased ratio of glutamine/glutamate was detected. [50] The authors of the latter study concluded that a dysfunction of the glutamate-glutamine cycle may play a role in the pathophysiology of schizophrenia. It appears that repeated injection of a low dose of MK801 in rats is a good model for first episode schizophrenia. In contrast to drug naive patients with first episode schizophrenia lower levels of glutamine and glutamate were found in the left anterior cingulate cortex of patients with chronic schizophrenia compared to healthy volunteers. [52] The decrease in labeling of glutamate and glutamine in the present study could indicate that a decrease in the amounts will eventually result when first episode patients develop chronic schizophrenia. Thus it is conceivable that the present model can give insight into the transition mechanisms of first episode to chronic state schizophrenia.

In the present study of repeated MK801 administration, levels of glutamate were increased in the CRFC, whereas after a single dose of MK801 this was the case in the temporal lobe. [28] In contrast to acute administration, where the amounts of labeled glutamate were not altered, repeated MK801 injections lead to decreases of both [4-^{13}C]glutamate and [4,5-^{13}C]glutamate in the

CRFC. The fact that the amount of [4-^{13}C]glutamine was unchanged indicates that transfer of glutamate from neurons to astrocytes was not altered and thus an intriguing explanation might be that glutamate, released from neurons, accumulates in astrocytes. The decreased amount of [4,5-^{13}C]glutamate points towards impaired efflux of [4,5-^{13}C]glutamine from astrocytes to neurons, suggesting that the glutamine-glutamate cycle is disturbed during repeated NMDA-antagonist administration[29] and possibly schizophrenia. In line with this is the decrease of the acetate-versus-glucose utilization ratio for glutamate, which indicates lowered astrocytic contribution to glutamate formation. However, the reason for the increase in glucose cycling of glutamate, implying that label stays longer in the neuronal TCA cycle before incorporation into glutamate, is not clear. That the glutamate - glutamine cycle is disrupted in schizophrenia patients as well, is demonstrated by the finding that activity of glutamine synthetase is decreased in post-mortem studies of brains of schizophrenic patients. [30] This disruption of negative cortico-striato-thalamo-cortical feedback might open the thalamic filter, leading to sensory overstimulation of the cortex and consequently to psychosis and other symptoms of schizophrenia (Figure 3). [7]

Glutamine released by astrocytes serves additionally as precursor for the inhibitory neurotransmitter GABA via glutamate. [16] Therefore the concept of the glutamate - glutamine cycle has been extended to the glutamate - glutamine - GABA cycle. [17] The amount of GABA and [1,2-^{13}C]GABA from [1,2-^{13}C]acetate remained unchanged, whereas, [2-^{13}C]GABA was decreased in the CRFC in the present study. Thus, GABA turnover from [1-^{13}C]glucose was affected, which corroborates recent reports of impaired activity of glutamic acid decarboxylase (GAD) in schizophrenic patients. [31,32] In this context it is noteworthy that a decrease in GAD_{67} has been suggested as a link between the glutamatergic and dopaminergic theories of psychosis. [33]

It is interesting that the changes mentioned above occurred in the CRFC in the

model of repeated MK801 administration in accordance with findings in human schizophrenia patients [34-37] and not in the temporal lobe as seen with acute MK801 administration. [28] This indicates that the repeated administration might generate a better schizophrenia model than acute exposure and emphasizes the importance of the frontal and retrosplenial/cingulate cortex for the pathophysiology of schizophrenia.

Dopamine and the interaction with glutamate metabolism

Surprisingly, in contrast to the consequences of acute PCP exposure, repeated PCP administrations lowered dopamine turnover and utilization in the monkey and rat cortex. [38,39] NMDA antagonism appeared to be at least partly responsible for this effect, since a comparable, though smaller, reduction in dopamine transmission in the cortex of rats was seen after subchronic MK801 administration. [40] In these studies as well as in ours, absolute dopamine levels were unaltered. However, since metabolite ratios were unchanged, dopamine turnover was unaffected as well. Jentsch et al. proposed that the absence of PCP-induced changes in tissue concentrations of dopamine itself implied that no direct neurotoxic insult to dopaminergic neurons occurred during subchronic PCP administration. [41] A loss of dopaminergic neurons would otherwise be accompanied by a loss of dopamine content in the terminal fields of these projection neuron. [41]

Decreased corticostriatal glutamatergic neurotransmission results in decreased negative feedback mediated via the so-called indirect striatothalamic pathway. [42] Consequently, thalamic filter function is impaired, leading to sensory overload of the cortex and decreased signal-to-noise-ratio, which has its clinical correlate in the characteristic difficulties of psychotic patients to differentiate between relevant and irrelevant information. Presuming that glutamate

homeostasis is dysfunctional in schizophrenia, the present and other studies suggest that secondary disturbances in glial-neuronal interactions and the glutamine-glutamate cycle may occur. [50,51,52] This will then contribute to imbalance of the cortico-striato-thalamo-cortical feedback as outlined in Figure 3.

As in the study with acute exposure to MK801, [28] glutathione (GSH) concentration was increased during repeated MK801 injection, however, this time in the CRFC. Being the main antioxidant in the brain [43] GSH protects cells from damage by reactive oxygen species originating partly from dopamine metabolism. Decreased GSH levels might lead to degenerative processes in the surroundings of dopaminergic terminals with loss of connectivity as a result. The increased level of GSH in the present study could point to a decreased use of antioxidants, possibly caused by diminished NO production due to lower Ca^{2+} concentration and NMDA blockade. Interestingly, there is evidence that GSH also enhances the NMDA receptor response to glutamate. [44]

Energy metabolism and histology

On histologic examinations no signs of neuronal degeneration were found, which is in agreement with observations by other authors. [45,46] Reversible changes were seen in rat brain exposed to both chronic and acute NMDA antagonist administration with neuronal vacuolar degeneration, remnant nuclear debris and local glial reaction [45,46] and appeared at the earliest 4h after MK801 administration. [47] Permanent damage was only seen at doses twenty times higher than the one used in the present study. [23] Since MK801 administration at low doses induces only reversible morphological changes, neuronal damage must be considered functional, not structural. Especially in light of the normal NAA concentration observed in the present study, it appears that

NAA synthesis and thus neuronal mitochondrial metabolism [48] is not severely affected by MK801. However, decreased labeling of glutamate, GABA and aspartate from [1-^{13}C]glucose indicated somewhat decreased mitochondrial function in neurons in the CRFC. Glycolysis appeared normal as seen in unaltered alanine and lactate levels.

The increase of inositol in the temporal lobe is surprising since it is the only change in metabolite concentration detected in this brain region and may indicate glial swelling. Increase in inositol in the temporal lobe was also reported earlier. [28] Only a minor change was found in labeling in the temporal lobe in the present study, with cycling of label from [1-^{13}C]glucose being increased in glutamate.

Conclusions

Injection of MK801 and other NMDA antagonists in rodents are considered appropriate schizophrenia models because these compounds induce both positive and negative symptoms in humans in contrast to other psychomimetics such as amphetamine. [49] In the present study, repeated MK801 exposure lead to increased glutamate concentration in the CRFC, which mimics the results seen in first episode schizophrenic patients. The decreased levels of [4-^{13}C]glutamate, [4,5-^{13}C]glutamate and [4,5-^{13}C]glutamine could lead to the decrease in these metabolites seen in chronic patients. The present model appears very well suited to study the cascade of events taking place during the transition from first episode to chronic schizophrenia and might help develop new treatment strategies. Furthermore ^{13}C studies can also be carried out in humans and thus ^{13}C NMR spectroscopy might become a tool in diagnosing and treating monitoring in patients. The imbalance in the glutamate homeostasis and thus impaired glial-neuronal interactions might contribute to disturbances in cortico-striato-thalamo-cortical feedback, resulting in sensory

overstimulation of the cortex and thus psychosis (Figure 3).

Acknowledgements

This research was supported by the Norwegian Epilepsy Foundation. We thank Ivar Nordrum for the HE staining of the tissue. The excellent technical assistance of Bente Urfjell is gratefully acknowledged.

References

1. Breier A, Su TP, Saunders R, Carson RE, Kolachana BS, de Bartolomeis A *et al.* Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. Proc Natl Acad Sci U S A 1997; **94**: 2569-2574.

2. Abi-Dargham A, Gil R, Krystal J, Baldwin RM, Seibyl JP, Bowers M *et al.* Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. Am J Psychiatry 1998; **155**: 761-767.

3. Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles L *et al.* Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. Proc Natl Acad Sci U S A 2000; **97**: 8104-8109.

4. Lindstrom LH, Gefver O, Hagberg G, Lundberg T, Bergstrom M, Hartvig P *et al.* Increased dopamine synthesis rate in medial prefrontal cortex and striatum in schizophrenia indicated by L-(beta-11C) DOPA and PET. Biol Psychiatry 1999; **46**: 681-688.

5. Carlsson A, Carlsson ML. Dopaminergic Stabilisers. Schizophr Clin Psychiatry 2005; *in press.*

6. Aghajanian GK, Marek GJ. Serotonin model of schizophrenia: emerging role of glutamate mechanisms. Brain Res Brain Res Rev 2000; **31**: 302-132.

7. Carlsson A, Waters N, Holm-Waters S, Tedroff J, Nilsson M, Carlsson ML. Interactions between monoamines, glutamate, and GABA in Schizophrenia: New Evidence. Annu Rev Pharmacol Toxicol 2001; **41**: 237-260.

8. Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia.Arch Gen Psychiatry 1995; **52**: 998-1007.

9. Carlsson M, Carlsson A, Nilsson M. Schizophrenia: from dopamine to glutamate and back. Curr Med Chem 2004; **11**: 267-277.

10. Kegeles LS, Abi-Dargham A, Zea-Ponce Y, Rodenhiser-Hill J, Mann JJ, Van Heertum RL *et al.* Modulation of amphetamine-induced striatal dopamine release by ketamine in humans: implications for schizophrenia. Biol Psychiatry 2000; **48**: 627-640.

11. Miller DW, Abercrombie ED. Effects of MK-801 on spontaneous and amphetamine-stimulated dopamine release in striatum measured with in vivo microdialysis in awake rats. Brain Res Bull 1996; **40**: 57-62.

12. Carlsson M, Carlsson A. The NMDA antagonist MK-801 causes marked locomotor stimulation in monoamine-depleted mice. J Neural Transm 1989; **75**: 221-226.

13. Carlsson M, Carlsson A. Interactions between glutamatergic and monoaminergic systems

within the basal ganglia - implications for schizophrenia and Parkinson's disease. Trends Neurosci 1990; **13**: 272-276.

14. Olney JW. Excitatory amino acids and neuropsychiatric disorders. Biol Psychiatry 1989; **26**: 505-525.

15. Shank RP, Bennett GS, Freytag SO, Campbell GL. Pyruvate carboxylase: an astrocytie-specific enzyme implicated in the replenishment of amino acids neurotransmitter pool. Brain Res 1985; **329**: 362-367.

16. Sonnewald U, Westergaard N, Schousboe A, Svendsen JS, Unsgard G, Petersen SB. Direct demonstration by [^{13}C]NMR spectroscopy that glutamine from astrocytes is a precursor for GABA synthesis in neurons. Neurochem Int 1993; **22**: 19-29.

17. Sonnewald U, Schousboe A, Waagepetersen H. Neuronal-Astrocytic interactions (TCA cycling), In: The Role of Glia in Neurotoxicity, Part I, Functional interaction between glia, neurons and endothelium, second edition, eds.: Aschner M, Costa L, CRC Press, Boca Raton, Florida, USA. 2005; 111-118.

18. Sonnewald U, Kondziella D. Neuronal glial interaction in different neurological diseases studied by ex vivo ^{13}C NMR spectroscopy. NMR Biomed 2003; **16**: 424-429.

19. Taylor A, McLean M, Morris P, Bachelard H. Approaches to studies on neuronal/glial relationships by ^{13}CNMRS analysis. Dev Neurosci 1996; **18**: 434-442.

20. Loscher W, Honack D. Anticonvulsant and behavioral effects of two novel competitive N-methyl-D-aspartic acid receptor antagonists, CGP 37849 and CGP 39551, in the kindling model of epilepsy. Comparison with MK-801 and carbamazepine. J Pharmacol Exp Ther 1991; **256**: 432-440.

21. Loubinoux I, Meric P, Borredon J, Correze JL, Gillet B, Beloeil JC *et al.* Cerebral metabolic changes induced by MK-801: a 1D (phosphorus and proton) and 2D (proton) in vivo NMR spectroscopy study. Brain Res 1994; **643**: 115-124.

22. Olney JW, Labruyere J, Price MT. Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 1989; **244**: 1360-1362.

23. Wozniak DF, Dikranian K, Ishimaru MJ, Nardi A, Corso TD, Tenkova T *et al.* Disseminated corticolimbic neuronal degeneration induced in rat brain by MK801: Potential relevance to Alzheimer's Disease. Neurobiol Disease 1998; **5**: 305-322.

24. Horvath ZC, Czopf J, Buzsaki G. MK-801-induced neuronal damage in rats. Brain Res 1997; **753**: 181-195.

25. Paxinos G, Watson C: The rat brain in stereotaxic coordinates. 1998, 4th ed: Academic Press Limited

26. Waniewski RA, Martin DL. Preferential utilization of acetate by astrocytes is attributable to

transport. J Neurosci 1998; **18**: 5225-5233.

27. Krebs C, Fernandes HB, Sheldon C, Raymond LA, Baimbridge KG. Functional NMDA receptor subtype 2B is expressed in astrocytes after ischemia in vivo and anoxia in vitro. J Neurosci 2003; **23**: 3364-3372.

28. Brenner E, Kondziella D, Håberg A, Sonnewald U. Impaired glutamine metabolism in NMDA receptor hypofunction induced by MK801. J Neurochem 2005; *in press.*

29. Kosenko E, Llansola M, Montoliu C, Monfort P, Rodrigo R, Hernandez-Viadel M *et al.* Glutamine synthetase activity and glutamine content in brain: modulation by NMDA receptors and nitric oxide. Neurochem Int 2003; **43**: 493-499.

30. Burbaeva GS, Boksha IS, Turishcheva MS, Vorobyeva EA, Savushkina OK, Tereshkina EB. Glutamine synthetase and glutamate dehydrogenase in the prefrontal cortex of patients with schizophrenia. Progr in Neuro-Psychopharmacol Biol Psychiatry 2003; **27**: 675-680.

31. Dracheva S, Elhakem SL, McGurk SR, Davis KL, Haroutunian V. GAD_{67} and GAD_{65} mRNA and protein expression in cerebrocortical regions of elderly patients with schizophrenia. J Neurosci Res 2004; **76**: 581-592.

32. Hossein Fatemi S, Stary JM, Earle JA, Araghi-Niknam M, Eagan E. GABAergic dysfunction in schizophrenia and mood disorders as reflected by decreased levels of glutamic acid

decarboxylase 65 and 67 kDa and Reelin proteins in cerebellum. Schizophr Res 2005; **72**:109-122.

33. Kalkmann HO, Loetscher E. GAD_{67}: the link between the GABA-deficit hypothesis and the dopaminergic and glutamatergic therories of psychosis. J Neuro Trans 2003; **110**: 803-812.

34. Stanley JA, Williamson PC, Drost DJ, Rylett RJ, Carr TJ, Malla A *et al.* An in vivo proton magnetic resonance spectroscopy study of schizophrenia patients. Schizophr Bull 1996; **22**: 597-609.

35. Theberge J, Al-Semaan Y, Williamson PC, Menon RS, Neufeld RW, Rajakumar N *et al.* Glutamate and glutamine in the anterior cingulate and thalamus of medicated patients with chronic schizophrenia and healthy comparison subjects measured with 4.0-T proton MRS. Am J Psychiatry 2003; **160**: 2231-2233.

36. Ohrmann P, Siegmund A, Suslow T, Spitzberg K, Kersting A, Arolt V *et al.* Evidence for glutamatergic neuronal dysfunction in the prefrontal cortex in chronic but not in first-episode patients with schizophrenia: a proton magnetic resonance spectroscopy study. Scizophr Res 2005; **73**: 153-157.

37. Rowland LM, Bustillo JR, Mullins PG, Jung RE, Lenroot R, Landgraf E *et al.* Effects of

ketamine on anterior cingulate glutamate metabolism in healthy humans: a 4-T proton MRS study. Am J Psychiatry 2005; **162**: 394-396.

38. Jentsch JD, Redmond DE Jr, Elsworth JD, Taylor JR, Youngren KD, Roth RH. Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long-term administration of phencyclidine. Science 1997; **277**: 935-955.

39. Jentsch JD, Tran A, Le D, Youngren KD, Roth RH. Subchronic phencyclidine administration reduces meso-prefrontal dopamine utilization and impairs prefrontal cortical-dependant cognition in the rat. Neuropsychopharmacol 1997; **17**: 92-99.

40. Jentsch JD, Taylor JR, Roth RH. Subchronic phencyclidine administration increases mesolimbic dopamine system responsivity and augments stress- and amphetamine-induced hyperlocomotion. Neuropsychopharmacol 1998; **19**: 105-113.

41. Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: From NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacol 1999; **20**: 201-225.

42. Carlsson M. Hypothesis - Are the disparate pharmacological profiles of competitive and un-competitive NMDA antagonists due to different baseline activities of distinct glutamatergic pathways? J Neural Transm 1993; **94**: 1-10.

43. Dringen R, Hirrlinger J. Glutathione pathways in the brain. Biol Chem 2003; **384**: 505-516.

44. Janaky R, Ogita K, Pasqualotto BA, Bains JS, Oja SS, Yoneda Y *et al.* Glutathione and signal transduction in the mammalian CNS. J Neurochem 1999; **73**: 889-902.

45. Wozniak DF, Brosnan-Watters G, Nardi A, McEwen M, Corso TD, Olney JW et al.: MK-801 neurotoxicity in male mice: histologic effects and chronic impairment in spatial learning. Brain Res 1996; **707**:165-179.

46. Brosnan-Watters G, Wozniak DF, Nardi A, Olney JW. Parallel recovery of MK-801-induced spatial learning impairment and neuronal injury in male mice. Pharmacol Biochem Behav 1999; **62**:111-122.

47. Fix AS, Horn JW, Wightman KA, Johnson CA, Long GG, Storts RW et al. Neuronal vacuolization and necrosis induced by the noncompetitive N-methyl-D-aspartate (NMDA) antagonist MK(+)801 (dizocilpine maleate): a light and electron microscopic evaluation of the rat retrosplenial cortex. Exp Neurol 1993; **123**: 204-215.

48. Baslow MH. N-acetylaspartate in the vertebrate brain: metabolism and function. Neurochem Res 2003; **28**: 941-953.

49. Tamminga CA, Lahti AC, Medoff DR, Gao XM, Holcomb HH. Evaluating glutamatergic

transmission in schizophrenia. Ann NY Acad Sci 2003; **1003**:113-118.

50. Hashimoto K, Engberg G, Shimizu E, Nordin C, Lindstrom LH, Iyo M. Elevated glutamine/glutamate ratio in cerebrospinal fluid of first episode and drug naive schizophrenic patients. BMC Psychiatry 2005; 5:6.

51. Theberge J, Bartha R, Drost DJ, Menon RS, Malla A, Takhar J, Neufeld RW, Rogers J, Pavlosky W, Schaefer B, Densmore M, Al-Semaan Y, Williamson PC.Glutamate and glutamine measured with 4.0 T proton MRS in never-treated patients with schizophrenia and healthy volunteers. Am J Psychiatry 2002; **159**:1944-6.

52. Theberge J, Al-Semaan Y, Williamson PC, Menon RS, Neufeld RW, Rajakumar N, Schaefer B, Densmore M, Drost DJ. Glutamate and glutamine in the anterior cingulate and thalamus of medicated patients with chronic schizophrenia and healthy comparison subjects measured with 4.0-T proton MRS. Am J Psychiatry 2003; **160**:2231-3.

Table 1. Total amounts of metabolites in brain extracts of controls (n=8) and MK801-treated rats (n=10).

	CRFC		Temporal Lobe	
Metabolites	**Control**	**MK801**	**Control**	**MK801**
(µg/g braint)				
Glutamate	7.80±1.9	10.13±2.3 *	8.09±1.4	9.36±1.4
GABA	1.48±0.5	1.89±0.7	2.02±0.5	2.16±0.6
Glutamine	2.59±0.9	3.22±1.1	3.03±0.9	2.90±0.4
Glutathione	1.02±0.2	1.30±0.3 *	1.15±0.2	1.05±0.2
Taurine	4.35±0.8	5.20±0.9 *	4.85±0.7	5.41±0.7
N-acetylaspartate	6.00±0.8	5.40±1.1	5.72±0.8	5.00±0.5
Inositol	7.01±0.7	6.99±0.6	8.26±0.8	9.30±0.6 *
(ng/g brain)				
Noradrenaline	193±11	208±11	256±38	283±27
Dopamine	22±11	32±18	1049±194	933±114
Serotonin	429±81	342±79	200±30	238±20
NADH	137±77	165±66	112±65	172±53

Rats received MK801 (0.5mg/kg) or saline intraperitoneally every 48h for six days. On day six [1,2-^{13}C]acetate and [1-^{13}C]glucose were administered (see *Materials and Methods* for details). All results were obtained by HPLC with the exception for levels of N-acetylaspartate, Inositol (assessed by ^{13}C-NMR) and NADH (^{1}H-NMR). The results are expressed as mean ± SD and were analyzed with the two tailed unpaired Student's *t*-test. CRFC = frontal/cingulate/retrosplenial cortex

* significantly different from control

Table 2. Amounts of ^{13}C in nmol/g of tissue in brain extracts from controls (n=8) and MK801-treated rats (n=10).

Metabolites	CRFC		Temporal Lobe	
[1,2-^{13}C]acetate	**Control**	**MK801**	**Control**	**MK801**
[4,5-^{13}C]Glutamate	260.8±44.6	201.0±23.3 *	198.0±29.4	201.5±24.3
[1,2-^{13}C]GABA	22.6±5.7	20.8±6.1	29.4±8.5	25.2±6.2
[4,5-^{13}C]Glutamine	324.0±59.5	268.4±21.1 *	306.5±50.2	285.6±28.6
[1-^{13}C]glucose				
[4-^{13}C]Glutamate	800.7±137.1	666.7±68.5 *	634.0±80.5	622.9±97.8
[2-^{13}C]GABA	76.2±16.9	61.1±9.3 *	91.0±14.8	76.0±16.7
[4-^{13}C]Glutamine	163.8±22.2	166.0±18.4	146.8±17.5	157.3±26.6

Rats were treated with intraperitoneal injection of MK801 (0.5mg/kg) or saline every 48h for six days. On day six [1,2-^{13}C]acetate and [1-^{13}C]glucose were given (see *Materials and Methods* for details). The results are expressed as mean ± SD and were analyzed with the two tailed unpaired Student's *t*-test.

CRFC = frontal/cingulate/retrosplenial cortex

* significantly different from control

Table 3. Acetate/glucose utilization ratios and cycling ratios from [1,2-^{13}C]acetate and [1-^{13}C]glucose from controls (n=8) and MK801-treated rats (n=10).

	CRFC			Temporal Lobe		
MK801	**Acetate/glucose utilization**	**glucose cycling**	**acetate cycling**	**Acetate/glucose utilization**	**glucose cycling**	**acetate cycling**
glutamate	0.29±0.02*	0.53±0.03*	0.39±0.06	0.31±0.04	0.41±0.03*	0.34±0.07
glutamine	1.62±0.11*	0.83±0.05	0.33±0.05*	2.12±0.53	0.02±0.08	0.35±0.05
GABA	0.35±0.10	0.94±0.16		0.33±0.11	0.83±0.08	
Controls						
glutamate	0.33±0.01	0.46±0.03	0.34±0.06	0.33±0.05	0.33±0.02	0.33±0.02
glutamine	1.97±0.15	0.77±0.05	0.27±0.05	1.85±0.34	0.15±0.06	0.31±0.05
GABA	0.31±0.04	0.80±0.06		0.33±0.06	0.85±0.06	

Rats were treated with intraperitoneal injection of MK801 (0.5mg/kg) or saline every 48h for six days. On day six [1,2-^{13}C]acetate and [1-^{13}C]glucose were given and cycling and utilization ratios assessed by NMR spectroscopy (see *Materials and Methods* for details). The results are expressed as mean ± SD and were analyzed with the two tailed unpaired Student's *t*-test.

CRFC = frontal/cingulate/retrosplenial cortex

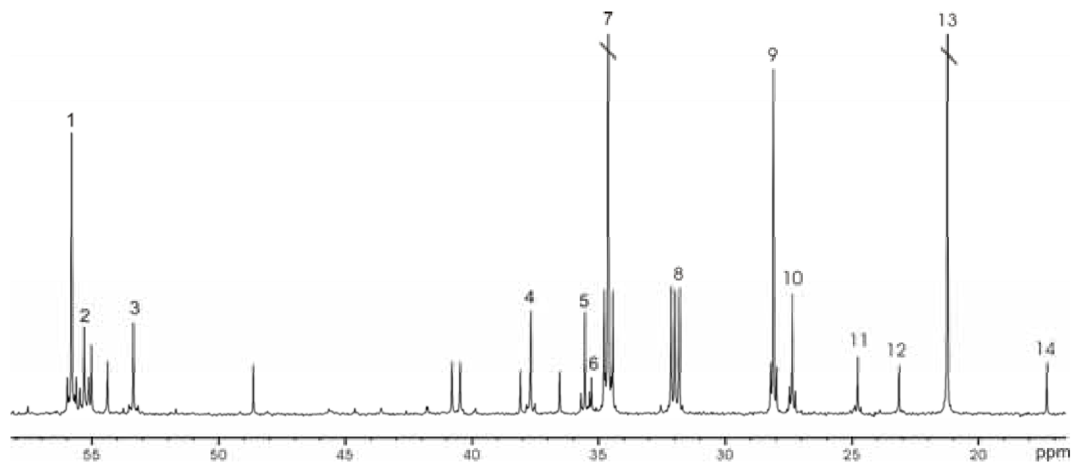
* significantly different from control

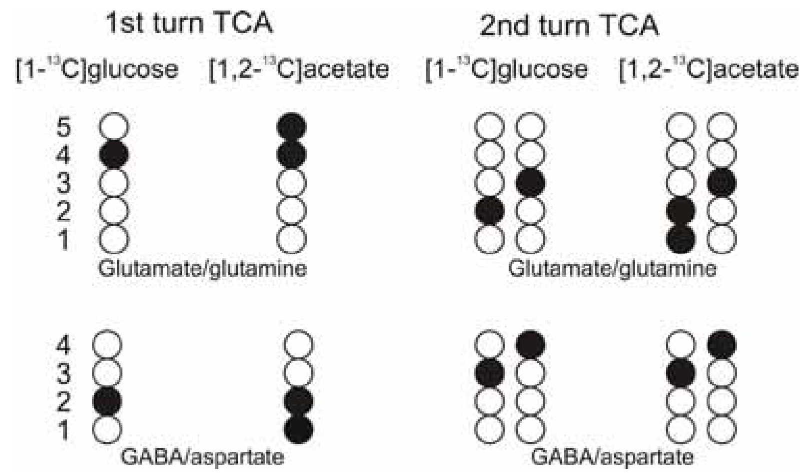
Legends

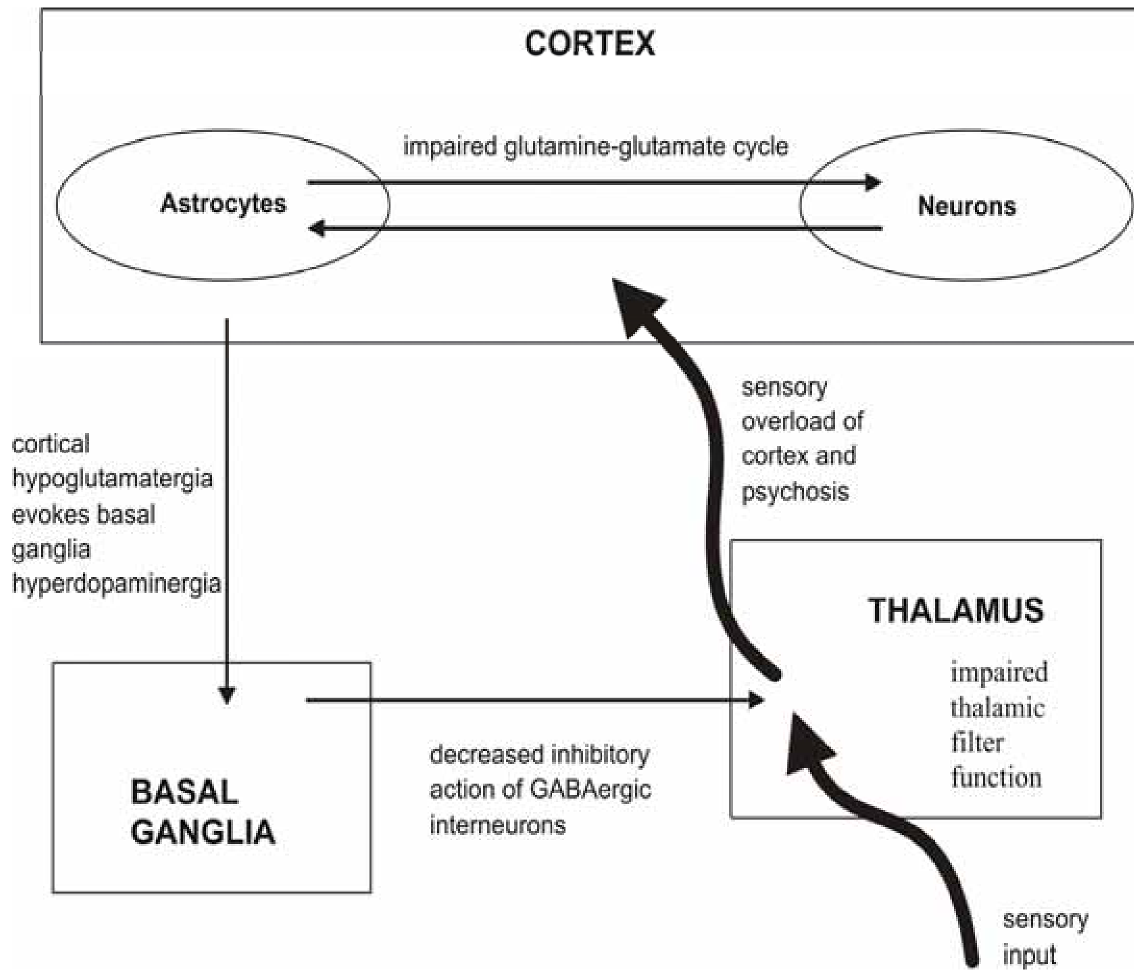
Figure 1. ^{13}C NMR spectrum of temporal lobe extracts from rats injected with MK801 together with [1,2-^{13}C]acetate and [1-^{13}C]glucose. Peak assignments; 1: glutamate C-2; 2: glutamine C-2; 3: aspartate C-2; 4: aspartate C-3; 5: GABA C-2; 6: succinate C-2/C-3; 7: glutamate C-4; 8: glutamine C-4; 9: glutamate C-3; 10: glutamine C-4; 11: GABA C-3; 12: N-acetyl-aspartate C-3; 13: lactate C-3.

Figure 2. ^{13}C labeling patterns in glutamate, glutamine, and GABA from [1-^{13}C]glucose or [1,2-^{13}C]acetate. Full circle represents ^{13}C and empty circle ^{12}C. 1st turn tricarboxylic acid (TCA) cycle: labeled acetyl CoA condensing with unlabelled oxaloacetate; 2nd turn: unlabeled acetyl CoA condensing with labeled oxaloacetate.

Figure 3. Scheme of a possible mechanism for psychosis evoked by impaired cortical glial-neuronal interactions, cortical hypoglutamatergia and basal ganglia hyperdopaminergia. See *Discussion* for details.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
55
50
45
40
35
30
25
20
ppm


1st turn TCA
2nd turn TCA
[1-13C]glucose
[1,2-13C]acetate
[1-13C]glucose
[1,2-13C]acetate
5
4
3
2
1
Glutamate/glutamine
Glutamate/glutamine
4
3
2
1
GABA/aspartate
GABA/aspartate


CORTEX
impaired glutamine-glutamate cycle
Astrocytes
Neurons
cortical
hypoglutamatergia
evokes basal
ganglia
hyperdopaminergia
sensory
overload of
cortex and
psychosis
THALAMUS
impaired
thalamic
filter
function
BASAL
GANGLIA
decreased inhibitory
action of GABAergic
interneurons
sensory
input

Dissertations at the Faculty of Medicine, NTNU

1977
1. Knut Joachim Berg: EFFECT OF ACETYLSALICYLIC ACID ON RENAL FUNCTION
2. Karl Erik Viken and Arne Ødegaard: STUDIES ON HUMAN MONOCYTES CULTURED *IN VITRO*

1978
3. Karel Bjørn Cyvin: CONGENITAL DISLOCATION OF THE HIP JOINT.
4. Alf O. Brubakk: METHODS FOR STUDYING FLOW DYNAMICS IN THE LEFT VENTRICLE AND THE AORTA IN MAN.

1979
5. Geirmund Unsgaard: CYTOSTATIC AND IMMUNOREGULATORY ABILITIES OF HUMAN BLOOD MONOCYTES CULTURED IN VITRO

1980
6. Størker Jørstad: URAEMIC TOXINS
7. Arne Olav Jenssen: SOME RHEOLOGICAL, CHEMICAL AND STRUCTURAL PROPERTIES OF MUCOID SPUTUM FROM PATIENTS WITH CHRONIC OBSTRUCTIVE BRONCHITIS

1981
8. Jens Hammerstrøm: CYTOSTATIC AND CYTOLYTIC ACTIVITY OF HUMAN MONOCYTES AND EFFUSION MACROPHAGES AGAINST TUMOR CELLS *IN VITRO*

1983
9. Tore Syversen: EFFECTS OF METHYLMERCURY ON RAT BRAIN PROTEIN.
10. Torbjørn Iversen: SQUAMOUS CELL CARCINOMA OF THE VULVA.

1984
11. Tor-Erik Widerøe: ASPECTS OF CONTINUOUS AMBULATORY PERITONEAL DIALYSIS.
12. Anton Hole: ALTERATIONS OF MONOCYTE AND LYMPHOCYTE FUNCTIONS IN REALTION TO SURGERY UNDER EPIDURAL OR GENERAL ANAESTHESIA.
13. Terje Terjesen: FRACTURE HEALING AN STRESS-PROTECTION AFTER METAL PLATE FIXATION AND EXTERNAL FIXATION.
14. Carsten Saunte: CLUSTER HEADACHE SYNDROME.
15. Inggard Lereim: TRAFFIC ACCIDENTS AND THEIR CONSEQUENCES.
16. Bjørn Magne Eggen: STUDIES IN CYTOTOXICITY IN HUMAN ADHERENT MONONUCLEAR BLOOD CELLS.
17. Trond Haug: FACTORS REGULATING BEHAVIORAL EFFECTS OG DRUGS.

1985
18. Sven Erik Gisvold: RESUSCITATION AFTER COMPLETE GLOBAL BRAIN ISCHEMIA.
19. Terje Espevik: THE CYTOSKELETON OF HUMAN MONOCYTES.
20. Lars Bevanger: STUDIES OF THE Ibc (c) PROTEIN ANTIGENS OF GROUP B STREPTOCOCCI.
21. Ole-Jan Iversen: RETROVIRUS-LIKE PARTICLES IN THE PATHOGENESIS OF PSORIASIS.
22. Lasse Eriksen: EVALUATION AND TREATMENT OF ALCOHOL DEPENDENT BEHAVIOUR.
23. Per I. Lundmo: ANDROGEN METABOLISM IN THE PROSTATE.

1986
24. Dagfinn Berntzen: ANALYSIS AND MANAGEMENT OF EXPERIMENTAL AND CLINICAL PAIN.
25. Odd Arnold Kildahl-Andersen: PRODUCTION AND CHARACTERIZATION OF MONOCYTE-DERIVED CYTOTOXIN AND ITS ROLE IN MONOCYTE-MEDIATED CYTOTOXICITY.
26. Ola Dale: VOLATILE ANAESTHETICS.

1987
27. Per Martin Kleveland: STUDIES ON GASTRIN.
28. Audun N. Øksendal: THE CALCIUM PARADOX AND THE HEART.
29. Vilhjalmur R. Finsen: HIP FRACTURES

1988
30. Rigmor Austgulen: TUMOR NECROSIS FACTOR: A MONOCYTE-DERIVED REGULATOR OF CELLULAR GROWTH.
31. Tom-Harald Edna: HEAD INJURIES ADMITTED TO HOSPITAL.
32. Joseph D. Borsi: NEW ASPECTS OF THE CLINICAL PHARMACOKINETICS OF METHOTREXATE.

33. Olav F. M. Sellevold: GLUCOCORTICOIDS IN MYOCARDIAL PROTECTION.
34. Terje Skjærpe: NONINVASIVE QUANTITATION OF GLOBAL PARAMETERS ON LEFT VENTRICULAR FUNCTION: THE SYSTOLIC PULMONARY ARTERY PRESSURE AND CARDIAC OUTPUT.
35. Eyvind Rødahl: STUDIES OF IMMUNE COMPLEXES AND RETROVIRUS-LIKE ANTIGENS IN PATIENTS WITH ANKYLOSING SPONDYLITIS.
36. Ketil Thorstensen: STUDIES ON THE MECHANISMS OF CELLULAR UPTAKE OF IRON FROM TRANSFERRIN.
37. Anna Midelfart: STUDIES OF THE MECHANISMS OF ION AND FLUID TRANSPORT IN THE BOVINE CORNEA.
38. Eirik Helseth: GROWTH AND PLASMINOGEN ACTIVATOR ACTIVITY OF HUMAN GLIOMAS AND BRAIN METASTASES - WITH SPECIAL REFERENCE TO TRANSFORMING GROWTH FACTOR BETA AND THE EPIDERMAL GROWTH FACTOR RECEPTOR.
39. Petter C. Borchgrevink: MAGNESIUM AND THE ISCHEMIC HEART.
40. Kjell-Arne Rein: THE EFFECT OF EXTRACORPOREAL CIRCULATION ON SUBCUTANEOUS TRANSCAPILLARY FLUID BALANCE.
41. Arne Kristian Sandvik: RAT GASTRIC HISTAMINE.
42. Carl Bredo Dahl: ANIMAL MODELS IN PSYCHIATRY.

1989

43. Torbjørn A. Fredriksen: CERVICOGENIC HEADACHE.
44. Rolf A. Walstad: CEFTAZIDIME.
45. Rolf Salvesen: THE PUPIL IN CLUSTER HEADACHE.
46. Nils Petter Jørgensen: DRUG EXPOSURE IN EARLY PREGNANCY.
47. Johan C. Ræder: PREMEDICATION AND GENERAL ANAESTHESIA IN OUTPATIENT GYNECOLOGICAL SURGERY.
48. M. R. Shalaby: IMMUNOREGULATORY PROPERTIES OF TNF-α AND THE RELATED CYTOKINES.
49. Anders Waage: THE COMPLEX PATTERN OF CYTOKINES IN SEPTIC SHOCK.
50. Bjarne Christian Eriksen: ELECTROSTIMULATION OF THE PELVIC FLOOR IN FEMALE URINARY INCONTINENCE.
51. Tore B. Halvorsen: PROGNOSTIC FACTORS IN COLORECTAL CANCER.

1990

52. Asbjørn Nordby: CELLULAR TOXICITY OF ROENTGEN CONTRAST MEDIA.
53. Kåre E. Tvedt: X-RAY MICROANALYSIS OF BIOLOGICAL MATERIAL.
54. Tore C. Stiles: COGNITIVE VULNERABILITY FACTORS IN THE DEVELOPMENT AND MAINTENANCE OF DEPRESSION.
55. Eva Hofsli: TUMOR NECROSIS FACTOR AND MULTIDRUG RESISTANCE.
56. Helge S. Haarstad: TROPHIC EFFECTS OF CHOLECYSTOKININ AND SECRETIN ON THE RAT PANCREAS.
57. Lars Engebretsen: TREATMENT OF ACUTE ANTERIOR CRUCIATE LIGAMENT INJURIES.
58. Tarjei Rygnestad: DELIBERATE SELF-POISONING IN TRONDHEIM.
59. Arne Z. Henriksen: STUDIES ON CONSERVED ANTIGENIC DOMAINS ON MAJOR OUTER MEMBRANE PROTEINS FROM ENTEROBACTERIA.
60. Steinar Westin: UNEMPLOYMENT AND HEALTH: Medical and social consequences of a factory closure in a ten-year controlled follow-up study.
61. Ylva Sahlin: INJURY REGISTRATION, a tool for accident preventive work.
62. Helge Bjørnstad Pettersen: BIOSYNTHESIS OF COMPLEMENT BY HUMAN ALVEOLAR MACROPHAGES WITH SPECIAL REFERENCE TO SARCOIDOSIS.
63. Berit Schei: TRAPPED IN PAINFUL LOVE.
64. Lars J. Vatten: PROSPECTIVE STUDIES OF THE RISK OF BREAST CANCER IN A COHORT OF NORWEGIAN WOMAN.

1991

65. Kåre Bergh: APPLICATIONS OF ANTI-C5a SPECIFIC MONOCLONAL ANTIBODIES FOR THE ASSESSMENT OF COMPLEMENT ACTIVATION.
66. Svein Svenningsen: THE CLINICAL SIGNIFICANCE OF INCREASED FEMORAL ANTEVERSION.
67. Olbjørn Klepp: NONSEMINOMATOUS GERM CELL TESTIS CANCER: THERAPEUTIC OUTCOME AND PROGNOSTIC FACTORS.

68. Trond Sand: THE EFFECTS OF CLICK POLARITY ON BRAINSTEM AUDITORY EVOKED POTENTIALS AMPLITUDE, DISPERSION, AND LATENCY VARIABLES.
69. Kjetil B. Åsbakk: STUDIES OF A PROTEIN FROM PSORIATIC SCALE, PSO P27, WITH RESPECT TO ITS POTENTIAL ROLE IN IMMUNE REACTIONS IN PSORIASIS.
70. Arnulf Hestnes: STUDIES ON DOWN´S SYNDROME.
71. Randi Nygaard: LONG-TERM SURVIVAL IN CHILDHOOD LEUKEMIA.
72. Bjørn Hagen: THIO-TEPA.
73. Svein Anda: EVALUATION OF THE HIP JOINT BY COMPUTED TOMOGRAMPHY AND ULTRASONOGRAPHY.

1992

74. Martin Svartberg: AN INVESTIGATION OF PROCESS AND OUTCOME OF SHORT-TERM PSYCHODYNAMIC PSYCHOTHERAPY.
75. Stig Arild Slørdahl: AORTIC REGURGITATION.
76. Harold C Sexton: STUDIES RELATING TO THE TREATMENT OF SYMPTOMATIC NON-PSYCHOTIC PATIENTS.
77. Maurice B. Vincent: VASOACTIVE PEPTIDES IN THE OCULAR/FOREHEAD AREA.
78. Terje Johannessen: CONTROLLED TRIALS IN SINGLE SUBJECTS.
79. Turid Nilsen: PYROPHOSPHATE IN HEPATOCYTE IRON METABOLISM.
80. Olav Haraldseth: NMR SPECTROSCOPY OF CEREBRAL ISCHEMIA AND REPERFUSION IN RAT.
81. Eiliv Brenna: REGULATION OF FUNCTION AND GROWTH OF THE OXYNTIC MUCOSA.

1993

82. Gunnar Bovim: CERVICOGENIC HEADACHE.
83. Jarl Arne Kahn: ASSISTED PROCREATION.
84. Bjørn Naume: IMMUNOREGULATORY EFFECTS OF CYTOKINES ON NK CELLS.
85. Rune Wiseth: AORTIC VALVE REPLACEMENT.
86. Jie Ming Shen: BLOOD FLOW VELOCITY AND RESPIRATORY STUDIES.
87. Piotr Kruszewski: SUNCT SYNDROME WITH SPECIAL REFERENCE TO THE AUTONOMIC NERVOUS SYSTEM.
88. Mette Haase Moen: ENDOMETRIOSIS.
89. Anne Vik: VASCULAR GAS EMBOLISM DURING AIR INFUSION AND AFTER DECOMPRESSION IN PIGS.
90. Lars Jacob Stovner: THE CHIARI TYPE I MALFORMATION.
91. Kjell Å. Salvesen: ROUTINE ULTRASONOGRAPHY IN UTERO AND DEVELOPMENT IN CHILDHOOD.

1994

92. Nina-Beate Liabakk: DEVELOPMENT OF IMMUNOASSAYS FOR TNF AND ITS SOLUBLE RECEPTORS.
93. Sverre Helge Torp: *erb*B ONCOGENES IN HUMAN GLIOMAS AND MENINGIOMAS.
94. Olav M. Linaker: MENTAL RETARDATION AND PSYCHIATRY. Past and present.
95. Per Oscar Feet: INCREASED ANTIDEPRESSANT AND ANTIPANIC EFFECT IN COMBINED TREATMENT WITH DIXYRAZINE AND TRICYCLIC ANTIDEPRESSANTS.
96. Stein Olav Samstad: CROSS SECTIONAL FLOW VELOCITY PROFILES FROM TWO-DIMENSIONAL DOPPLER ULTRASOUND: Studies on early mitral blood flow.
97. Bjørn Backe: STUDIES IN ANTENATAL CARE.
98. Gerd Inger Ringdal: QUALITY OF LIFE IN CANCER PATIENTS.
99. Torvid Kiserud: THE DUCTUS VENOSUS IN THE HUMAN FETUS.
100. Hans E. Fjøsne: HORMONAL REGULATION OF PROSTATIC METABOLISM.
101. Eylert Brodtkorb: CLINICAL ASPECTS OF EPILEPSY IN THE MENTALLY RETARDED.
102. Roar Juul: PEPTIDERGIC MECHANISMS IN HUMAN SUBARACHNOID HEMORRHAGE.
103. Unni Syversen: CHROMOGRANIN A. Phsysiological and Clinical Role.

1995

104. Odd Gunnar Brakstad: THERMOSTABLE NUCLEASE AND THE *nuc* GENE IN THE DIAGNOSIS OF *Staphylococcus aureus* INFECTIONS.
105. Terje Engan: NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY OF PLASMA IN MALIGNANT DISEASE.
106. Kirsten Rasmussen: VIOLENCE IN THE MENTALLY DISORDERED.
107. Finn Egil Skjeldestad: INDUCED ABORTION: Timetrends and Determinants.
108. Roar Stenseth: THORACIC EPIDURAL ANALGESIA IN AORTOCORONARY BYPASS SURGERY.

109. Arild Faxvaag: STUDIES OF IMMUNE CELL FUNCTION *in mice infected with* MURINE RETROVIRUS.

1996

110. Svend Aakhus: NONINVASIVE COMPUTERIZED ASSESSMENT OF LEFT VENTRICULAR FUNCTION AND SYSTEMIC ARTERIAL PROPERTIES. Methodology and some clinical applications.
111. Klaus-Dieter Bolz: INTRAVASCULAR ULTRASONOGRAPHY.
112. Petter Aadahl: CARDIOVASCULAR EFFECTS OF THORACIC AORTIC CROSS-CLAMPING.
113. Sigurd Steinshamn: CYTOKINE MEDIATORS DURING GRANULOCYTOPENIC INFECTIONS.
114. Hans Stifoss-Hanssen: SEEKING MEANING OR HAPPINESS?
115. Anne Kvikstad: LIFE CHANGE EVENTS AND MARITAL STATUS IN RELATION TO RISK AND PROGNOSIS OF CANSER.
116. Torbjørn Grøntvedt: TREATMENT OF ACUTE AND CHRONIC ANTERIOR CRUCIATE LIGAMENT INJURIES. A clinical and biomechanical study.
117. Sigrid Hørven Wigers: CLINICAL STUDIES OF FIBROMYALGIA WITH FOCUS ON ETIOLOGY, TREATMENT AND OUTCOME.
118. Jan Schjøtt: MYOCARDIAL PROTECTION: Functional and Metabolic Characteristics of Two Endogenous Protective Principles.
119. Marit Martinussen: STUDIES OF INTESTINAL BLOOD FLOW AND ITS RELATION TO TRANSITIONAL CIRCULATORY ADAPATION IN NEWBORN INFANTS.
120. Tomm B. Müller: MAGNETIC RESONANCE IMAGING IN FOCAL CEREBRAL ISCHEMIA.
121. Rune Haaverstad: OEDEMA FORMATION OF THE LOWER EXTREMITIES.
122. Magne Børset: THE ROLE OF CYTOKINES IN MULTIPLE MYELOMA, WITH SPECIAL REFERENCE TO HEPATOCYTE GROWTH FACTOR.
123. Geir Smedslund: A THEORETICAL AND EMPIRICAL INVESTIGATION OF SMOKING, STRESS AND DISEASE: RESULTS FROM A POPULATION SURVEY.

1997

124. Torstein Vik: GROWTH, MORBIDITY, AND PSYCHOMOTOR DEVELOPMENT IN INFANTS WHO WERE GROWTH RETARDED *IN UTERO*.
125. Siri Forsmo: ASPECTS AND CONSEQUENCES OF OPPORTUNISTIC SCREENING FOR CERVICAL CANCER. Results based on data from three Norwegian counties.
126. Jon S. Skranes: CEREBRAL MRI AND NEURODEVELOPMENTAL OUTCOME IN VERY LOW BIRTH WEIGHT (VLBW) CHILDREN. A follow-up study of a geographically based year cohort of VLBW children at ages one and six years.
127. Knut Bjørnstad: COMPUTERIZED ECHOCARDIOGRAPHY FOR EVALUTION OF CORONARY ARTERY DISEASE.
128. Grethe Elisabeth Borchgrevink: DIAGNOSIS AND TREATMENT OF WHIPLASH/NECK SPRAIN INJURIES CAUSED BY CAR ACCIDENTS.
129. Tor Elsås: NEUROPEPTIDES AND NITRIC OXIDE SYNTHASE IN OCULAR AUTONOMIC AND SENSORY NERVES.
130. Rolf W. Gråwe: EPIDEMIOLOGICAL AND NEUROPSYCHOLOGICAL PERSPECTIVES ON SCHIZOPHRENIA.
131. Tonje Strømholm: CEREBRAL HAEMODYNAMICS DURING THORACIC AORTIC CROSSCLAMPING. An experimental study in pigs.

1998

132. Martinus Bråten: STUDIES ON SOME PROBLEMS REALTED TO INTRAMEDULLARY NAILING OF FEMORAL FRACTURES.
133. Ståle Nordgård: PROLIFERATIVE ACTIVITY AND DNA CONTENT AS PROGNOSTIC INDICATORS IN ADENOID CYSTIC CARCINOMA OF THE HEAD AND NECK.
134. Egil Lien: SOLUBLE RECEPTORS FOR **TNF** AND **LPS**: RELEASE PATTERN AND POSSIBLE SIGNIFICANCE IN DISEASE.
135. Marit Bjørgaas: HYPOGLYCAEMIA IN CHILDREN WITH DIABETES MELLITUS
136. Frank Skorpen: GENETIC AND FUNCTIONAL ANALYSES OF DNA REPAIR IN HUMAN CELLS.
137. Juan A. Pareja: SUNCT SYNDROME. ON THE CLINICAL PICTURE. ITS DISTINCTION FROM OTHER, SIMILAR HEADACHES.
138. Anders Angelsen: NEUROENDOCRINE CELLS IN HUMAN PROSTATIC CARCINOMAS AND THE PROSTATIC COMPLEX OF RAT, GUINEA PIG, CAT AND DOG.

139. Fabio Antonaci: CHRONIC PAROXYSMAL HEMICRANIA AND HEMICRANIA CONTINUA: TWO DIFFERENT ENTITIES?
140. Sven M. Carlsen: ENDOCRINE AND METABOLIC EFFECTS OF METFORMIN WITH SPECIAL EMPHASIS ON CARDIOVASCULAR RISK FACTORES.

1999

141. Terje A. Murberg: DEPRESSIVE SYMPTOMS AND COPING AMONG PATIENTS WITH CONGESTIVE HEART FAILURE.
142. Harm-Gerd Karl Blaas: THE EMBRYONIC EXAMINATION. Ultrasound studies on the development of the human embryo.
143. Noèmi Becser Andersen:THE CEPHALIC SENSORY NERVES IN UNILATERAL HEADACHES. Anatomical background and neurophysiological evaluation.
144. Eli-Janne Fiskerstrand: LASER TREATMENT OF PORT WINE STAINS. A study of the efficacy and limitations of the pulsed dye laser. Clinical and morfological analyses aimed at improving the therapeutic outcome.
145. Bård Kulseng: A STUDY OF ALGINATE CAPSULE PROPERTIES AND CYTOKINES IN RELATION TO INSULIN DEPENDENT DIABETES MELLITUS.
146. Terje Haug: STRUCTURE AND REGULATION OF THE HUMAN UNG GENE ENCODING URACIL-DNA GLYCOSYLASE.
147. Heidi Brurok: MANGANESE AND THE HEART. A Magic Metal with Diagnostic and Therapeutic Possibilites.
148. Agnes Kathrine Lie: DIAGNOSIS AND PREVALENCE OF HUMAN PAPILLOMAVIRUS INFECTION IN CERVICAL INTRAEPITELIAL NEOPLASIA. Relationship to Cell Cycle Regulatory Proteins and HLA DQBI Genes.
149. Ronald Mårvik: PHARMACOLOGICAL, PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL STUDIES ON ISOLATED STOMACS.
150. Ketil Jarl Holen: THE ROLE OF ULTRASONOGRAPHY IN THE DIAGNOSIS AND TREATMENT OF HIP DYSPLASIA IN NEWBORNS.
151. Irene Hetlevik: THE ROLE OF CLINICAL GUIDELINES IN CARDIOVASCULAR RISK INTERVENTION IN GENERAL PRACTICE.
152. Katarina Tunòn: ULTRASOUND AND PREDICTION OF GESTATIONAL AGE.
153. Johannes Soma: INTERACTION BETWEEN THE LEFT VENTRICLE AND THE SYSTEMIC ARTERIES.
154. Arild Aamodt: DEVELOPMENT AND PRE-CLINICAL EVALUATION OF A CUSTOM-MADE FEMORAL STEM.
155. Agnar Tegnander: DIAGNOSIS AND FOLLOW-UP OF CHILDREN WITH SUSPECTED OR KNOWN HIP DYSPLASIA.
156. Bent Indredavik: STROKE UNIT TREATMENT: SHORT AND LONG-TERM EFFECTS
157. Jolanta Vanagaite Vingen: PHOTOPHOBIA AND PHONOPHOBIA IN PRIMARY HEADACHES

2000

158. Ola Dalsegg Sæther: PATHOPHYSIOLOGY DURING PROXIMAL AORTIC CROSS-CLAMPING
159. CLINICAL AND EXPERIMENTAL STUDIES
160. Christina Vogt Isaksen: PRENATAL ULTRASOUND AND POSTMORTEM FINDINGS – A TEN YEAR CORRELATIVE STUDY OF FETUSES AND INFANTS WITH DEVELOPMENTAL ANOMALIES.
161. Holger Seidel: HIGH-DOSE METHOTREXATE THERAPY IN CHILDREN WITH ACUTE LYMPHOCYTIC LEUKEMIA: DOSE, CONCENTRATION, AND EFFECT CONSIDERATIONS.
162. Stein Hallan: IMPLEMENTATION OF MODERN MEDICAL DECISION ANALYSIS INTO CLINICAL DIAGNOSIS AND TREATMENT.
163. Malcolm Sue-Chu: INVASIVE AND NON-INVASIVE STUDIES IN CROSS-COUNTRY SKIERS WITH ASTHMA-LIKE SYMPTOMS.
164. Ole-Lars Brekke: EFFECTS OF ANTIOXIDANTS AND FATTY ACIDS ON TUMOR NECROSIS FACTOR-INDUCED CYTOTOXICITY.
165. Jan Lundbom: AORTOCORONARY BYPASS SURGERY: CLINICAL ASPECTS, COST CONSIDERATIONS AND WORKING ABILITY.
166. John-Anker Zwart: LUMBAR NERVE ROOT COMPRESSION, BIOCHEMICAL AND NEUROPHYSIOLOGICAL ASPECTS.
167. Geir Falck: HYPEROSMOLALITY AND THE HEART.

168. Eirik Skogvoll: CARDIAC ARREST Incidence, Intervention and Outcome.
169. Dalius Bansevicius: SHOULDER-NECK REGION IN CERTAIN HEADACHES AND CHRONIC PAIN SYNDROMES.
170. Bettina Kinge: REFRACTIVE ERRORS AND BIOMETRIC CHANGES AMONG UNIVERSITY STUDENTS IN NORWAY.
171. Gunnar Qvigstad: CONSEQUENCES OF HYPERGASTRINEMIA IN MAN
172. Hanne Ellekjær: EPIDEMIOLOGICAL STUDIES OF STROKE IN A NORWEGIAN POPULATION. INCIDENCE, RISK FACTORS AND PROGNOSIS
173. Hilde Grimstad: VIOLENCE AGAINST WOMEN AND PREGNANCY OUTCOME.
174. Astrid Hjelde: SURFACE TENSION AND COMPLEMENT ACTIVATION: Factors influencing bubble formation and bubble effects after decompression.
175. Kjell A. Kvistad: MR IN BREAST CANCER – A CLINICAL STUDY.
176. Ivar Rossvoll: ELECTIVE ORTHOPAEDIC SURGERY IN A DEFINED POPULATION. Studies on demand, waiting time for treatment and incapacity for work.
177. Carina Seidel: PROGNOSTIC VALUE AND BIOLOGICAL EFFECTS OF HEPATOCYTE GROWTH FACTOR AND SYNDECAN-1 IN MULTIPLE MYELOMA.

2001

178. Alexander Wahba: THE INFLUENCE OF CARDIOPULMONARY BYPASS ON PLATELET FUNCTION AND BLOOD COAGULATION – DETERMINANTS AND CLINICAL CONSEQUENSES
179. Marcus Schmitt-Egenolf: THE RELEVANCE OF THE MAJOR hISTOCOMPATIBILITY COMPLEX FOR THE GENETICS OF PSORIASIS
180. Odrun Arna Gederaas: BIOLOGICAL MECHANISMS INVOLVED IN 5-AMINOLEVULINIC ACID BASED PHOTODYNAMIC THERAPY
181. Pål Richard Romundstad: CANCER INCIDENCE AMONG NORWEGIAN ALUMINIUM WORKERS
182. Henrik Hjorth-Hansen: NOVEL CYTOKINES IN GROWTH CONTROL AND BONE DISEASE OF MULTIPLE MYELOMA
183. Gunnar Morken: SEASONAL VARIATION OF HUMAN MOOD AND BEHAVIOUR
184. Bjørn Olav Haugen: MEASUREMENT OF CARDIAC OUTPUT AND STUDIES OF VELOCITY PROFILES IN AORTIC AND MITRAL FLOW USING TWO- AND THREE-DIMENSIONAL COLOUR FLOW IMAGING
185. Geir Bråthen: THE CLASSIFICATION AND CLINICAL DIAGNOSIS OF ALCOHOL-RELATED SEIZURES
186. Knut Ivar Aasarød: RENAL INVOLVEMENT IN INFLAMMATORY RHEUMATIC DISEASE. A Study of Renal Disease in Wegener's Granulomatosis and in Primary Sjögren's Syndrome
187. Trude Helen Flo: RESEPTORS INVOLVED IN CELL ACTIVATION BY DEFINED URONIC ACID POLYMERS AND BACTERIAL COMPONENTS
188. Bodil Kavli: HUMAN URACIL-DNA GLYCOSYLASES FROM THE UNG GENE: STRUCTRUAL BASIS FOR SUBSTRATE SPECIFICITY AND REPAIR
189. Liv Thommesen: MOLECULAR MECHANISMS INVOLVED IN TNF- AND GASTRIN-MEDIATED GENE REGULATION
190. Turid Lingaas Holmen: SMOKING AND HEALTH IN ADOLESCENCE; THE NORD-TRØNDELAG HEALTH STUDY, 1995-97
191. Øyvind Hjertner: MULTIPLE MYELOMA: INTERACTIONS BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MICROENVIRONMENT
192. Asbjørn Støylen: STRAIN RATE IMAGING OF THE LEFT VENTRICLE BY ULTRASOUND. FEASIBILITY, CLINICAL VALIDATION AND PHYSIOLOGICAL ASPECTS
193. Kristian Midthjell: DIABETES IN ADULTS IN NORD-TRØNDELAG. PUBLIC HEALTH ASPECTS OF DIABETES MELLITUS IN A LARGE, NON-SELECTED NORWEGIAN POPULATION.
194. Guanglin Cui: FUNCTIONAL ASPECTS OF THE ECL CELL IN RODENTS
195. Ulrik Wisløff: CARDIAC EFFECTS OF AEROBIC ENDURANCE TRAINING: HYPERTROPHY, CONTRACTILITY AND CALCUIM HANDLING IN NORMAL AND FAILING HEART
196. Øyvind Halaas: MECHANISMS OF IMMUNOMODULATION AND CELL-MEDIATED CYTOTOXICITY INDUCED BY BACTERIAL PRODUCTS
197. Tore Amundsen: PERFUSION MR IMAGING IN THE DIAGNOSIS OF PULMONARY EMBOLISM

198. Nanna Kurtze: THE SIGNIFICANCE OF ANXIETY AND DEPRESSION IN FATIQUE AND PATTERNS OF PAIN AMONG INDIVIDUALS DIAGNOSED WITH FIBROMYALGIA: RELATIONS WITH QUALITY OF LIFE, FUNCTIONAL DISABILITY, LIFESTYLE, EMPLOYMENT STATUS, CO-MORBIDITY AND GENDER
199. Tom Ivar Lund Nilsen: PROSPECTIVE STUDIES OF CANCER RISK IN NORD-TRØNDELAG: THE HUNT STUDY. Associations with anthropometric, socioeconomic, and lifestyle risk factors
200. Asta Kristine Håberg: A NEW APPROACH TO THE STUDY OF MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT USING MAGNETIC RESONANCE TECHNIQUES

2002

201. Knut Jørgen Arntzen: PREGNANCY AND CYTOKINES
202. Henrik Døllner: INFLAMMATORY MEDIATORS IN PERINATAL INFECTIONS
203. Asta Bye: LOW FAT, LOW LACTOSE DIET USED AS PROPHYLACTIC TREATMENT OF ACUTE INTESTINAL REACTIONS DURING PELVIC RADIOTHERAPY. A PROSPECTIVE RANDOMISED STUDY.
204. Sylvester Moyo: STUDIES ON STREPTOCOCCUS AGALACTIAE (GROUP B STREPTOCOCCUS) SURFACE-ANCHORED MARKERS WITH EMPHASIS ON STRAINS AND HUMAN SERA FROM ZIMBABWE.
205. Knut Hagen: HEAD-HUNT: THE EPIDEMIOLOGY OF HEADACHE IN NORD-TRØNDELAG
206. Li Lixin: ON THE REGULATION AND ROLE OF UNCOUPLING PROTEIN-2 IN INSULIN PRODUCING ß-CELLS
207. Anne Hildur Henriksen: SYMPTOMS OF ALLERGY AND ASTHMA VERSUS MARKERS OF LOWER AIRWAY INFLAMMATION AMONG ADOLESCENTS
208. Egil Andreas Fors: NON-MALIGNANT PAIN IN RELATION TO PSYCHOLOGICAL AND ENVIRONTENTAL FACTORS. EXPERIENTAL AND CLINICAL STUDES OF PAIN WITH FOCUS ON FIBROMYALGIA
209. Pål Klepstad: MORPHINE FOR CANCER PAIN
210. Ingunn Bakke: MECHANISMS AND CONSEQUENCES OF PEROXISOME PROLIFERATOR-INDUCED HYPERFUNCTION OF THE RAT GASTRIN PRODUCING CELL
211. Ingrid Susann Gribbestad: MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF BREAST CANCER
212. Rønnaug Astri Ødegård: PREECLAMPSIA – MATERNAL RISK FACTORS AND FETAL GROWTH
213. Johan Haux: STUDIES ON CYTOTOXICITY INDUCED BY HUMAN NATURAL KILLER CELLS AND DIGITOXIN
214. Turid Suzanne Berg-Nielsen: PARENTING PRACTICES AND MENTALLY DISORDERED ADOLESCENTS
215. Astrid Rydning: BLOOD FLOW AS A PROTECTIVE FACTOR FOR THE STOMACH MUCOSA. AN EXPERIMENTAL STUDY ON THE ROLE OF MAST CELLS AND SENSORY AFFERENT NEURONS

2003

216. Jan Pål Loennechen: HEART FAILURE AFTER MYOCARDIAL INFARCTION. Regional Differences, Myocyte Function, Gene Expression, and Response to Cariporide, Losartan, and Exercise Training.
217. Elisabeth Qvigstad: EFFECTS OF FATTY ACIDS AND OVER-STIMULATION ON INSULIN SECRETION IN MAN
218. Arne Åsberg: EPIDEMIOLOGICAL STUDIES IN HEREDITARY HEMOCHROMATOSIS: PREVALENCE, MORBIDITY AND BENEFIT OF SCREENING.
219. Johan Fredrik Skomsvoll: REPRODUCTIVE OUTCOME IN WOMEN WITH RHEUMATIC DISEASE. A population registry based study of the effects of inflammatory rheumatic disease and connective tissue disease on reproductive outcome in Norwegian women in 1967-1995.
220. Siv Mørkved: URINARY INCONTINENCE DURING PREGNANCY AND AFTER DELIVERY: EFFECT OF PELVIC FLOOR MUSCLE TRAINING IN PREVENTION AND TREATMENT
221. Marit S. Jordhøy: THE IMPACT OF COMPREHENSIVE PALLIATIVE CARE
222. Tom Christian Martinsen: HYPERGASTRINEMIA AND HYPOACIDITY IN RODENTS – CAUSES AND CONSEQUENCES
223. Solveig Tingulstad: CENTRALIZATION OF PRIMARY SURGERY FOR OVARAIN CANCER. FEASIBILITY AND IMPACT ON SURVIVAL

224. Haytham Eloqayli: METABOLIC CHANGES IN THE BRAIN CAUSED BY EPILEPTIC SEIZURES
225. Torunn Bruland: STUDIES OF EARLY RETROVIRUS-HOST INTERACTIONS – VIRAL DETERMINANTS FOR PATHOGENESIS AND THE INFLUENCE OF SEX ON THE SUSCEPTIBILITY TO FRIEND MURINE LEUKAEMIA VIRUS INFECTION
226. Torstein Hole: DOPPLER ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR FUNCTION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION
227. Vibeke Nossum: THE EFFECT OF VASCULAR BUBBLES ON ENDOTHELIAL FUNCTION
228. Sigurd Fasting: ROUTINE BASED RECORDING OF ADVERSE EVENTS DURING ANAESTHESIA – APPLICATION IN QUALITY IMPROVEMENT AND SAFETY
229. Solfrid Romundstad: EPIDEMIOLOGICAL STUDIES OF MICROALBUMINURIA. THE NORD-TRØNDELAG HEALTH STUDY 1995-97 (HUNT 2)
230. Geir Torheim: PROCESSING OF DYNAMIC DATA SETS IN MAGNETIC RESONANCE IMAGING
231. Catrine Ahlén: SKIN INFECTIONS IN OCCUPATIONAL SATURATION DIVERS IN THE NORTH SEA AND THE IMPACT OF THE ENVIRONMENT
232. Arnulf Langhammer: RESPIRATORY SYMPTOMS, LUNG FUNCTION AND BONE MINERAL DENSITY IN A COMPREHENSIVE POPULATION SURVEY. THE NORD-TRØNDELAG HEALTH STUDY 1995-97. THE BRONCHIAL OBSTRUCTION IN NORD-TRØNDELAG STUDY
233. Einar Kjelsås: EATING DISORDERS AND PHYSICAL ACTIVITY IN NON-CLINICAL SAMPLES
234. Arne Wibe: RECTAL CANCER TREATMENT IN NORWAY – STANDARDISATION OF SURGERY AND QUALITY ASSURANCE

2004

235. Eivind Witsø: BONE GRAFT AS AN ANTIBIOTIC CARRIER
236. Anne Mari Sund: DEVELOPMENT OF DEPRESSIVE SYMPTOMS IN EARLY ADOLESCENCE
237. Hallvard Lærum: EVALUATION OF ELECTRONIC MEDICAL RECORDS – A CLINICAL TASK PERSPECTIVE
238. Gustav Mikkelsen: ACCESSIBILITY OF INFORMATION IN ELECTRONIC PATIENT RECORDS; AN EVALUATION OF THE ROLE OF DATA QUALITY
239. Steinar Krokstad: SOCIOECONOMIC INEQUALITIES IN HEALTH AND DISABILITY. SOCIAL EPIDEMIOLOGY IN THE NORD-TRØNDELAG HEALTH STUDY (HUNT), NORWAY
240. Arne Kristian Myhre: NORMAL VARIATION IN ANOGENITAL ANATOMY AND MICROBIOLOGY IN NON-ABUSED PRESCHOOL CHILDREN
241. Ingunn Dybedal: NEGATIVE REGULATORS OF HEMATOPOIETEC STEM AND PROGENITOR CELLS
242. Beate Sitter: TISSUE CHARACTERIZATION BY HIGH RESOLUTION MAGIC ANGLE SPINNING MR SPECTROSCOPY
243. Per Arne Aas: MACROMOLECULAR MAINTENANCE IN HUMAN CELLS – REPAIR OF URACIL IN DNA AND METHYLATIONS IN DNA AND RNA
244. Anna Bofin: FINE NEEDLE ASPIRATION CYTOLOGY IN THE PRIMARY INVESTIGATION OF BREAST TUMOURS AND IN THE DETERMINATION OF TREATMENT STRATEGIES
245. Jim Aage Nøttestad: DEINSTITUTIONALIZATION AND MENTAL HEALTH CHANGES AMONG PEOPLE WITH MENTAL RETARDATION
246. Reidar Fossmark: GASTRIC CANCER IN JAPANESE COTTON RATS
247. Wibeke Nordhøy: MANGANESE AND THE HEART, INTRACELLULAR MR RELAXATION AND WATER EXCHANGE ACROSS THE CARDIAC CELL MEMBRANE

2005

248. Sturla Molden: QUANTITATIVE ANALYSES OF SINGLE UNITS RECORDED FROM THE HIPPOCAMPUS AND ENTORHINAL CORTEX OF BEHAVING RATS
249. Wenche Brenne Drøyvold: EPIDEMIOLOGICAL STUDIES ON WEIGHT CHANGE AND HEALTH IN A LARGE POPULATION. THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
250. Ragnhild Støen: ENDOTHELIUM-DEPENDENT VASODILATION IN THE FEMORAL ARTERY OF DEVELOPING PIGLETS

251. Aslak Steinsbekk: HOMEOPATHY IN THE PREVENTION OF UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN
252. Hill-Aina Steffenach: MEMORY IN HIPPOCAMPAL AND CORTICO-HIPPOCAMPAL CIRCUITS
253. Eystein Stordal: ASPECTS OF THE EPIDEMIOLOGY OF DEPRESSIONS BASED ON SELF-RATING IN A LARGE GENERAL HEALTH STUDY (THE HUNT-2 STUDY)
254. Viggo Pettersen: FROM MUSCLES TO SINGING: THE ACTIVITY OF ACCESSORY BREATHING MUSCLES AND THORAX MOVEMENT IN CLASSICAL SINGING
255. Marianne Fyhn: SPATIAL MAPS IN THE HIPPOCAMPUS AND ENTORHINAL CORTEX
256. Robert Valderhaug: OBSESSIVE-COMPULSIVE DISORDER AMONG CHILDREN AND ADOLESCENTS: CHARACTERISTICS AND PSYCHOLOGICAL MANAGEMENT OF PATIENTS IN OUTPATIENT PSYCHIATRIC CLINICS
257. Erik Skaaheim Haug: INFRARENAL ABDOMINAL AORTIC ANEURYSMS – COMORBIDITY AND RESULTS FOLLOWING OPEN SURGERY
258. Daniel Kondziella: GLIAL-NEURONAL INTERACTIONS IN EXPERIMENTAL BRAIN DISORDERS