KJ2900: BACHELOR IN CHEMISTRY

The Function of the N-Methyl-D-Aspartate Receptor in Neurodegenerative Diseases

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Abstract

This paper is a literature-review about the treatment of different neurodegenerative diseases, where the N-methyl-D-aspartate (NMDA) receptor is in focus. The function and activation of the receptor will be described, together with the glutamate and glycine binding sites. The crossing of the blood-brain barrier (BBB) to enter the central nervous system (CNS) will be discussed. Some derivatives of the heterocycle-fused quinoline will also be presented.

There are millions of people worldwide who are suffering from neurodegenerative diseases every year. Alzheimer's disease and depression are examples of diseases that are difficult to recover from[1, 2]. The amino acid glutamate is very important in the treatment of these disorders, because glutamate regulates the influx of calcium flow into the cell[3, 4]. The amino acid transports synaptical signals between neurons in the synaptic cleft, and accounts for about 60% of the signals in the brain[5]. The NMDA-receptor is an ionotropic receptor, together with the α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor and the kainate acid (KA) receptor. The NMDA-receptor is a voltage gated ion channel, which is glutamate and glycine dependent[6]. The receptor is a promising target for neurodegenerative diseases, but it is desirable to find drugs that affect this receptor to hinder the symptoms of these diseases. There are only five drugs for Alzheimer's disease that have been launched on the market for the past 40 years[7]. The blockage of a magnesium ion (Mg^{2+}), inside the NMDA-receptor, hinder the influx of calcium ions (Ca^{2+}) to occur, which regulates many signals ionvolved in e.g. cell survival[1, 8]. However, if the calcium influx is too high, cell death can occur[4]. The activation of the NMDA-receptor has to occur to alleviate the magnesium blockage, which relies on the binding of glutamate and glycine to the receptor[1, 9].

The BBB controls the passage of molecules from the CNS, which is a network of capillaries, and there are strict limits for the penetration through the BBB[10, 11, 12]. One drug of interest is memantine which is a weak NMDA blocker, and is thought to block the receptor reversibly[13, 14]. This is a promising territory if the drugs work on the glutamatergic system for people suffering of the neurodegenerative diseases.

Based on the study of the NMDA-receptor, the glutamatic system is of interest in the treatment of neurodegenerative diseases, such as Alzheimer's disease and depression. The drugs in the treatment have to cross the BBB, which can be difficult, due to the strict limits of penetration. It is shown that drugs with permeability above $4.54 \times 10-6$ are able to cross the BBB to enter the CNS[12]. There is a large field of research on medication for neurodegenerative disorders, and derivatives of quinolines are interesting molecules in this research[15].

Contents

Introduction

People with Alzheimer's disease lose their memory gradually. There is an estimation that 33.9 million people have this disease worldwide (2011), and the cases are expected to triple in the next 40 years[2, 16]. The central nervous system (CNS) is a target in the treatment of Alzheimer's and other neurodegenerative diseases, which involves the crossing of the blood-brain barrier (BBB)[2]. The main neurotransmitters in the CNS are glutamate (**1**) and the γ-aminobutyric acid (GABA, **2**), which are parts of a complex network that is required for the complexity of CNS function[17]. The molecular structures of the neurotransmitters **1** and **2** are represented in Figure 0.1.

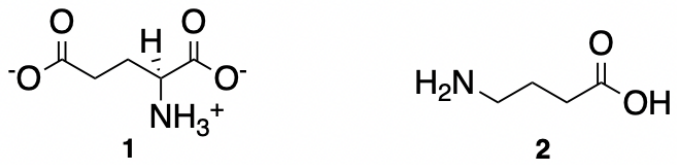


Figure 0.1: The molecular structures of the neurotransmitters glutamate (**1**) and GABA (**2**). The molecular structures are made in Chemdraw.

Glutamate (**1**) is the primary neurotransmitter in the brain, and composes the transmission of signals for approximately 60% of the neurons in the brain[3, 5]. The N-methyl-D-aspartate (NMDA) receptor controls the ligand-voltage ion channel, and it is likely that over-stimulation of this receptor causes the loss of neurons. This over-stimulation happens as a result of increased infusion of **1** , which transports synaptical signals between two neurons in the synaptic cleft [18].

An over-stimulation of **1** in the synaptic cleft results in dysregulation of the calcium ion (Ca^{2+}) homeostasis, mitochondrial dysfunction and generation of reactive oxygen species (ROS). The amount of **1** is usually regulated, otherwise, it will lead to neurodegenerative diseases due to the loss of neurons, such as Alzheimer's and depression[19, 1].

> Depression is one of the most common mental illnesses, and many people are suffering from this disease. The World Health Organization (WHO) considers depression one of the most common causes of disability, and it is assumed that 850 000 people lose their life every year because of depression[1]. The decrease in synaptic connections in the hippocampus, prefrontal cortex and the limbic brain is associated with depression. It is believed that the glutamateric system plays an important role in the process in the brain when interfering with the mental illness. The blockade of the NMDA-receptor on the GABA neurons leads to increased levels of **1**, which further leads to disinhibition of glutamate transmission[1].

The glutamate and glycine ionotropic NMDA-receptor is important in the treatment of different diseases, including depression and Alzheimer's disease[6]. The receptor allows cation flux into the cell, which is important for synaptic activity and learning[4].

The NMDA-receptor has different binding sites, the glutamate- and glycine binding sites being the main focus in this paper. Furthermore, I will be discussing the similarities and differences in the binding sites, and their function. The central question is:

How can the NMDA-receptor be affected to attenuate the symptoms for neurodegenerative diseases?

1 Theory

1.1 The N-Methyl-D-Aspartate Receptor

The glutamate receptor family is divided into two groups; ionotropic- and metabotropic receptors. The ionotropic receptors comprehend upon their selective synthetic agonist; NMDA- and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor, together with the kainate acid (KA) receptor. The AMPA- and KA receptors classify the majority of the fast glutamateric in the brain. The AMPA-receptor is important for the NMDA-receptor, as the AMPA-receptor provides the necessary depolarization to unblock the NMDA-receptors to allow cation flux into the cell. The KA receptor primarily modulates neuronal excitability and synaptic transmission at both presynaptic and postsynaptic sites[20]. The high levels of **1** could affect synaptic and extrasynaptic glutamate receptors on neurons, and therefore, the location of the glutamate receptors is important. The NMDA-receptors trigger different protective molecular pathways in the various neurodegenerative disorders, but it is also believed that the extrasynaptic NMDA-receptor arbitrates neuronal damage[21]. The metabotropic receptors are divided into groups on the basis of effector coupling and ligand sensitivity[3].

The NMDA-receptor is a glutamate- and glycine dependent ionotropic receptor, and is located in the CNS. The receptor has voltage-gated ion channels, and the opening of the pores of the cell allows non-selective cation flux[6]. The voltage gated ion channels are truly resembled by **1**, instead of the acethylcholine/GABA family. It is believed that the total number of binding agonists bound to the receptor infects the conductance level of the channel[22]. The NMDA-receptor is blocked by a magnesium ion (Mg^{2+}) in a voltage sensitive manner inside the NMDA ion channel[3], as illustrated in Figure 1.1. This blocking prohibits the calcium flux[1], which is a second messenger and regulates many signals involved in e.g. cell survival[8]. The sensitivity of the receptor increases because of the calcium, which leads to a long term potential. The decrease of the membrane potential is caused by a high intensity period of excitatory synaptic activity, which leads to removement of the magnesium block[1]. The influx of calcium into cells is important for peoples' learning ability, memory and synaptic plasticity, but if the influx is too high it can cause excitotoxicity which can result in cell death[4]. Figure 1.1 illustrates the NMDA-receptor with the magnesium blockage inside, which needs to be removed in order for the calcium flux to flow through the receptor, in addition to the different binding sites of the receptor [4].

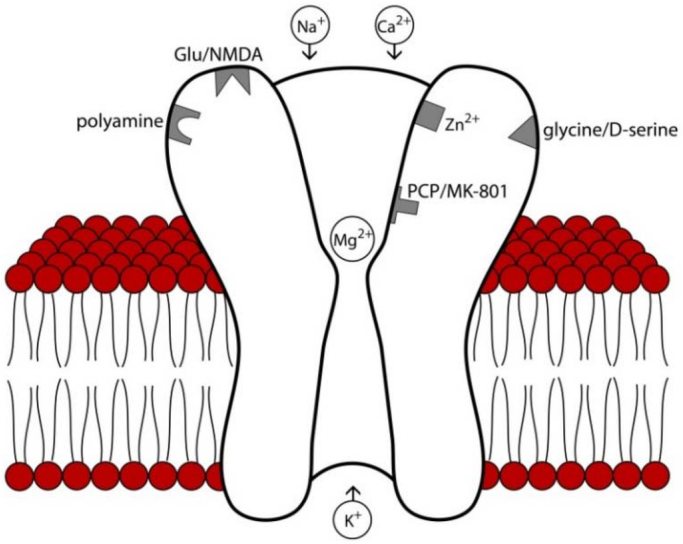


Figure 1.1: An overwiew of structure and binding sites of the NMDA-receptor. Magnesium blocks the pore, and has to be removed to allow cation flux. The illustration is obtained from Pharmaceuticals, Copyright[4].

On the background of the sequence identities of **1**, the NMDA-receptor can be divided into three subunits; NR1, NR2 and NR3, respectively[22]. The NR1 accesses the neurons which are retained in the endoplasmic reticulum until they assemble with the NR2 units[3].

To alleviate the magnesium blockage from the channel pore, the activation of the NMDA-receptor has to occur. The activation relies on two synergistic processes to open the channel, which are the binding of **1** at GluN2 and a co-agonist (either L-glycine or D-serine) at GluN1, so the depolarization of the membrane can occur[1, 9]. The GluN2A subunit corresponds to the glutamate binding, and the GluN1 unit corresponds to the glycine binding[23]. The GluN2B subunit of the NMDA-receptor is phosphorylated at Ser1303, and the status at this phosphorylation site affects the conductance of the NMDA-receptor channel[24]. The depolarization that occurs can lead to presynaptic or axomyelinic release of **1** which can cause toxic effects[21]. Further, **1** is recycled in both neurons and glia by excitatory amino acid (EAA)[3, 5]. The (*S*)-glutamatic acid is the main excitatory neurotransmitter in the CNS, and is important for different functions[22, 25]. The ionotropic receptors, NMDA and AMPA, are coexpressed in the most excitatory terminals, but some of the terminals only express the NMDA-receptor. These receptors need a membrane depolarization to allow action[22]. The receptor proteins that are bound to the membrane has alternating hydrophobic and hydrophilic regions, which can make it difficult for the proteins to stabilize themselves outside the membrane matrix[22]. The binding sites of the different receptors are not catalysts for chemical reactions, in contrast to the active sites in enzymes[22]. The membrane depolarization causes the membrane potential to be more positive than the resting potential, and Ca^{2+} flows into the cell[26], as shown in Figure 1.2.

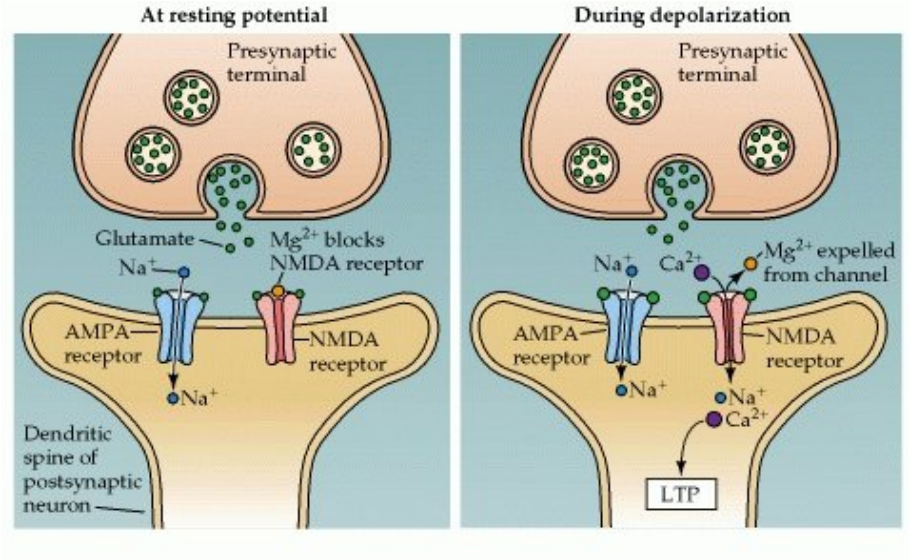


Figure 1.2: An illustration of the depolarization of the NMDA-receptor, where the magnesium block is expelled from the pore and allows the flow of calcium flux, leading to a long term potential. The illustration is obtained from Sinauer,[26]. The original illustration is from Nicoll *et.al* 1988, Copyright [27].

The KA receptor seems to reduce the glutamine release when activated on the presynaptic receptor. Figure 1.3 points out the interactions between the kainate molecule (**3**) and the amino acids. The α-carboxyl group of **3** interacts with the Arg485, along with the guanidinium group through ionic interactions, and with Thr480 through the NH_2-group. The interactions of Thr480 also involve the protonated amino group of KA, and the interactions are involved in domain one. The interactions in domain two contain the positively charged amino group which interacts with Glu705 and the ω-carboxyl group, which again interacts with the backbone NH groups of Ser654 and Thr655, in addition to the hydroxyl group of Thr655[22].

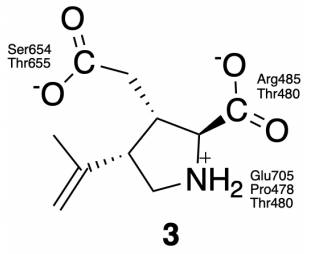


Figure 1.3: The molecular structure for kainate (**3**) with the respective amino acids that interact with the molecule. The molecular structure, with the respective amino acids, is made in Chemdraw.

The NMDA-receptor consists of two main binding sites; the glutamate- and glycine binding site. Figure 1.4 shows the different binding sites, and where the magnesium block is alleviated, so the influx of calcium can occur.

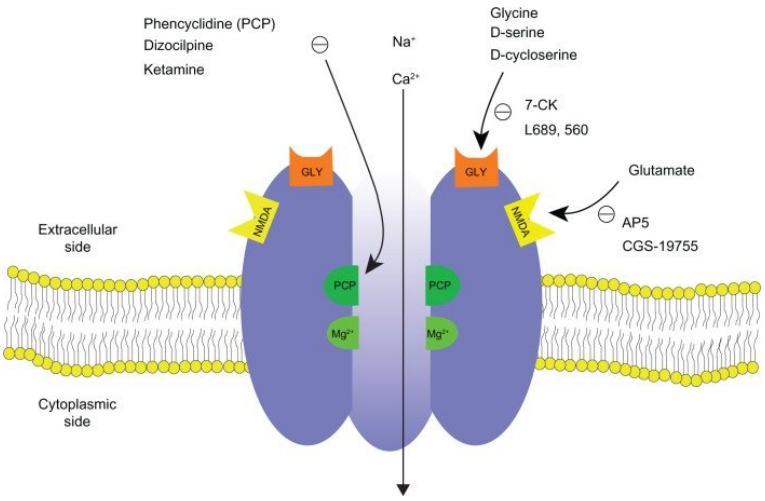
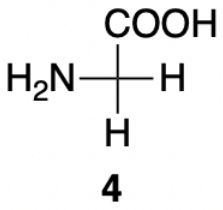


Figure 1.4: An illustration showing the binding sites of the NMDA-receptor. The illustration is obtained from Dovepress, Copyright. [28]

1.2 The Glycine Binding Site

The amino acid, glycine (**4**), is involved in neurochemical transmission, both inhibitory and excitatory, in the CNS[29]. The molecular structure of **4** is shown in Figure 1.5. The glycine site of the NMDA-receptor is at present a favored target for therapeutic treatment[3]. The binding of **4** to the NMDA-receptor occurs via an unguided-diffusion mechanism[23]. The binding of **4** is through the ligand binding domain (LBD), where **4** binds as a result of random fluctuations[23].



4

Figure 1.5: The molecular structure of glycine (**4**). The structure is made in Chemdraw.

Concentrations of **4** in the CNS are regulated by two high affinity transporters; glycine transporter-1 (GlyT1-1a-e) and glycine transporter-2 (GlyT-2a-c). When the local synaptic concentrations of **4** increase, it leads to selective inhibition of GlyT-1 which has an attractive approach that influences the NMDA-receptor[9].

The glycine site affects the channel opening time and desensitization rate in the presence of the agonist (**1**), but does not induce channel opening by itself[3]. The binding of **4** to the LBD, GluN1, is not characterized by binding through interactions outside

the binding pocket[23]. The interactions in the protein ligand binding occurs between the open LDB and the ligand. Figure 1.6 illustrates the binding of **4** to the LDB of the NMDA-receptor. The hydrogen bonding holds (a) **4** and (b) the kynurenic acid, which is an important antagonist for the NMDA-receptor binding with its glycine site[30].

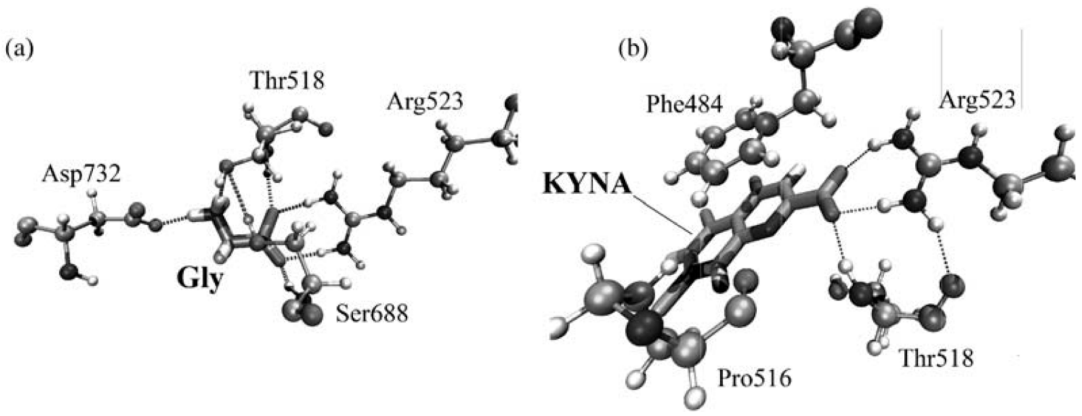


Figure 1.6: The binding of **4** to the NMDA-receptor (a), forming six hydrogen bonds, together with the binding of the kynurenic acid (b), forming three direct hydrogen bonds with the NMDA-receptor. The picture is obtained from Springer, Copyright[30].

The interactions occur between the Arg523 and the α-carboxylate of the ligand first, and then between the Phe484 and the carbons in **4**[23]. Phe484 is rotated 108 degrees around the χ_1 dihedral angle, and recruits the ligand[23]. Furthermore, the ligand rotates so that the amine comes in contact with the Pro516 carbonyl oxygen and the hydroxyl group of the Thr518 sidechain. The ligand is seen like in the crystal structure, when glycine forms these contacts, and the LDB does not immediately close[23]. This either guides the water molecules in bulk solvent or frees glycine ligands displacing the bound glycine from the first lobe[23]. The global free energy is very low, and it is located inside the binding pocket, near to the residues which interact with the ligand in the bound complex[23]. The potential of mean force does not form ligand densities into the global free energy minimum inside the binding pocket, which indicates a lack of binding pathways[23].

The sensitivity of **4** in the NMDA-receptor depends on the subunit of the receptor. The NR2A-subunit shows less affinity to the receptor than the NR2B-subunit. The binding of **4** and D-serine to the NMDA-receptor has an affinity to the receptor of approximately 100 nM. The transport process has to be present to regulate the relative intrasynaptic amino acid level, which corresponds to the extracellular pool to protect the glycine binding site from the overall brain glycine and D-serine levels[3]. The transportation of **4** into the brain is done by several transporters, including type 1 and type 2. The transporter of type 1 is bound to two Na^+-ions, but the type 2 transporter binds to three of the ions, which causes the type 1 to maintain the synaptic concentration of **4** in high nanomolar range[3].

1.3 The Glutamate Binding Site

The neurotransmitter, glutamate, which is formed in neurons from the amino acid glutamine, is released in extracellular space and taken up by neurons[1]. The binding of **1** to the NMDA-receptors occurs via a guided-diffusion mechanism[23]. The positively charged residues on the surface of the glutamate LDB assist the binding. **1** can also be bound in an inverted orientation, but it is not confirmed if the binding has any effect on the receptor in binding this way[23].

The binding of **1** to GluN2A LBD occurs with a series of metastable interactions, and the positive side chains in **1** guide the ligand into its binding pocket [23]. There are interactions between the α-carboxylate of glutamate and a helix arginine (Arg692), and also between a γ-carboxylate and Arg518. While inside the binding pocket, the glutamate ligand contacts the residues on lobe 1 prior, so it can react with lobe 2[23]. Simulations done by Yu and Lui[23], show that the contact of **1** to Arg518 alternates between α- and γ-carboxylate, flipping between the inverted structure and the constructions we see in the crystal structures[23]. The binding of **1** is estimated to bind in three different ways. Two of the bindings may involve metastable interactions between the ligand carboxylates and helix E arginins, Arg692 and Arg695, and Arg518.

The model of Laube *et. al*[31] has predicted that the amino acids, Arg493 and Asp706, occupy the positions corresponding to the Arg485 and Glu705 of GluR2 of the NR2B subunit, respectively. The interactions of bound **1** in the GluR2 subunit are between the latter residues in the α-amino- and carboxylate group. The residues, Arg493 and Asp706, are believed to provide the key interactions with the charged α-amino- and carboxylate groups of the bound amino acids in all iGluR subunits. These residues can function as a key marker for the orientation of the ligand in the binding pocket, based upon the fact that these residues were found in close contact with the α-ionic groups of **1**.

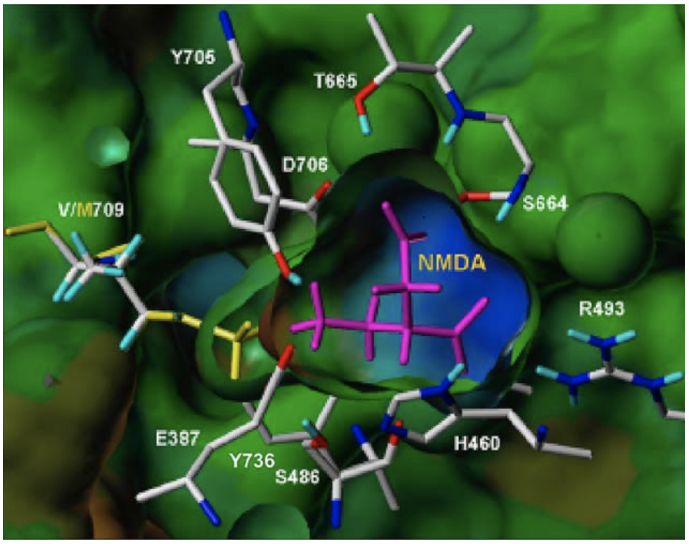


Figure 1.7: The docking of the NMDA (showed in pink) in the glutamate binding cleft of the NR2B subunit. The orientation of NMDA is similar within the binding site as glutamate, and interacts with the same residues of both lobes (I+II). The figure is obtained from Neuro pharmacology, and the article by Laube *et. al*[31].

The glutamate (**1**) binding mechanism can be assumed based on the presented model[31], and an overview of the binding of **1** is shown in Figure 1.7. The interactions of the α-carboxylate group in **1** with four potential hydrogen bonds with Thr488, Arg493 and Ser664, along with the binding of the distal carboxylate group to lobe II via hydrogen bonds to Thr665 and Ser664. Hydrogen bonding between the α-amino group of **1** to Thr488, Ser486 and Asp706 results in a tetrahederal arrangement. The cation π-interactions seem important for binding to the cationic region of the ligands, together with the stabilizing effect of the positively charged residues within the glutamate-binding site, indicated by strong effects of the aromatic side-chain substitutions. The model also indicates the development of an aromatic ring between two aromatic side chains (Tyr705 and Tyr736) around the amino group of **1** in a relative distance of 4-6 Å. The hydrophobic environment, provided by His450, stabilizes the glutamic backbone. This model implies that **1** is bound in a folded conformation.

1.4 Synthesis of Useful Drugs in the Treatment of Neurodegenerative Diseases

One of the most promising heterocycles is the heterocycle-fused quinoline, which is one of the privileged structures on the drug market. Different derivatives of quinolines have reported biological activity, and are synthesized with different structures and positions of the substituents[15]. Derivatives of oxazolo[4,5-c]quinolin-4-one are synthesized and biologically evaluated for the NMDA-, AMPA- and KA receptors. The study of developing new drugs that have the NMDA-receptor as a target[32], has been in focus the last years. Three cyclic derivatives have been in focus, and some of them have shown affinity to the NMDA-receptor, among them 4-oxo-1,2,4-triazolo[1,5-a]quinoxaline-2-carboxylate derivatives (TQX series) and 5-oxo-pyrazolo[1,5-c]quinazoline-2-carboxylate compounds (PQZ series).

Some structural features are pointed out, that are important for the NMDA-receptor antagonists; "(i) *a flat hydrophobic area represented on the fuzed benzo ring*; (ii) *a*

NH hydrogen bond donor that binds to a proton acceptor of the receptor; and (iii) *a γ-negatively charged moiety which could form a hydrogen bond with a cationic hydrogen bond donor-receptor site*" [32]. The ethyl 2-carboxylate function is important for the anchoring to the NMDA binding site, and the acidic carboxyl group can work even better. The affinity of the NMDA-receptor to the TQZ- and PQZ series is even better with groups withdrawing electrons in precise position(s) of the fused benzo ring. The different derivatives of the different substituents (**5**) of oxazolo[4,5-c]quinolin-4-one are shown in Figure 1.8. The 2-marcapto group (SH-group) acts like a proton acceptor group and is therefore able to form hydrogen bonds, which is important for the binding ability. Several tautomers were synthesized in this study, but these are not of relevance for the topic at hand[32].

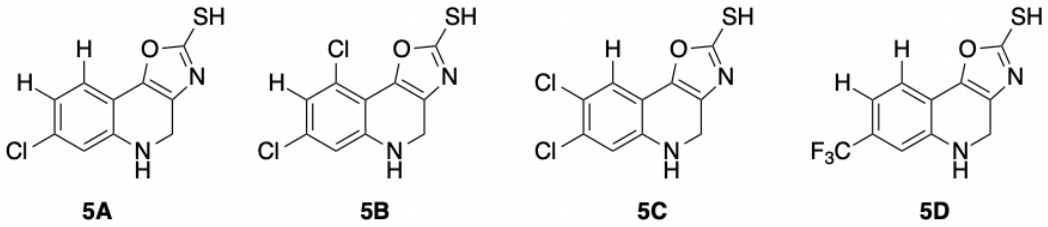


Figure 1.8: The molecular structures of some of the reported derivatives of oxazolo[4,5-c]quinolin-4-one (2004) (**5A-5D**). The molecular structures of the derivatives are made in Chemdraw.

1.5 The Blood-Brain-Barrier

The control of passage from the CNS to the brain is done by the blood-brain barrier (BBB), which is a network of capillaries[10, 11]. The network of the capillaries is a thin monolayer of tightly attached endothelial cells between the blood and the brain[11]. This barrier is very important for regulation of drug delivery to the brain[10]. There are strict limits for penetration through the BBB, and it has been discovered that nearly 100% of the molecules that could be useful for treatment of different CNS illnesses do not reach their target, as a result of their size. Water, oxygen and most lipid-soluble molecules can penetrate the BBB, together with small amounts of the ions sodium (Na^+), potassium (K^+) and chloride (Cl^-) that contribute to the signaling between the neurons[10]. The drugs with permeability above 4.54×10^{-6} cm s^{-1} are able to cross the BBB and enter the CNS[12].

The functionality of the BBB can be disturbed when different diseases occur, such as Alzheimer's, stroke and epilepsy, among others[33]. The NMDA-receptor´s subunits are not exclusively expressed in neurons in the brain, but also present in inter alia astrocytes and in peripheral neurons. The exposure of brain microvascular endothelial cells to glutamate results in formation of reactive oxygen species (ROS) and phosphorylation myosin light chains among others, but many of these effects are blocked by the NMDA-receptor antagonist dizosipline maleate (MK801, **6**), with the molecular structure shown in Figure 1.9. This result proposes the possible involvement of the NMDA-receptor in the BBB, and therefore the BBB becomes an interesting target for treatment of different neurodegenerative diseases[33].

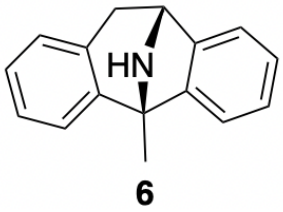


Figure 1.9: The molecular structure of the NMDA-receptor antagonist dizosipline maleate (MK801) (**6**). The molecular structure is made in Chemdraw.

The BBB is a challenge for the drug delivery to the CNS. For Alzheimer's disease, the drug donepezil (**7**), shown in Figure 1.10, penetrates the BBB with the organic cation transporter. The drug is also flow dependent, which means that it rapidly enters the CNS. The disruption of drugs within the brain tissue is limited to 100 µm of the diffusion[2].

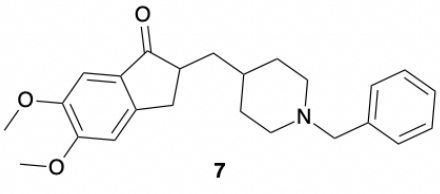


Figure 1.10: The molecular structure of donepezil (**7**). The structure is made in Chemdraw.

2 Discussion

The progressive loss of functional neurons, often referred to as neurodegeneration, is related to changes in structure, in addition to neuromorphological changes[34]. There are not any drugs on the market today that can hinder these diseases, but there are some medicaments that slow down their development[35]. One of the most used drugs against Alzheimer's disease is memantine, however the benefits are minor, as using the drug does not stop or cure the disease[14]. However it has proven slow down the disease efficiently and safely[13].

2.1 The Binding Sites of Glycine and Glutamate

The study done by Yu and Lau[23] simulated the binding of **4** and **1** to GluN1 and GluN2 in the LDB, respectively. The simulations showed that **4** binds in the orientation we see in crystal structures, while **1** can bind in an inverted orientation as well. There is on the other hand no evidence that the NMDA-receptor will be activated when **1** binds in the inverted conformation. One factor of the activation may be the orientation of the agonist inside the binding pocket. The different binding mechanisms of **1** and the co-agonist **4** to the NMDA-receptor is distinct. The potential of mean forces for **1** to GluN2A and GluA2 attributes to the continuous densities which link the periphery of the ligand binding domain to the binding pocket. On the other hand, does the glycine binding potential mean force (PMF) for GluN1 have a disconnected density between the periphery and the pocket.

This manifests two opposing paradigms in protein binding, one in which the peripheral residue-ligand interactions contribute substantially to the binding process, contrary to the other, where the only important factor in the ligand binding is the protein-ligand interactions. "The binding of glutamate to GluN2A occurs along several preferred pathways where positively charged residues mediate protein-ligand interactions at the periphery of the ligand binding domain, and assist the binding via a guided diffusion mechanism"[23]. The binding of **4** to GluN1 takes place via an unguided diffusion mechanism, where the binding primarily is a random diffusive process. The most important protein-ligand interactions are made between the Lobe 1 and the ligand in binding site residues. There is observed conformational changes in the LDB after ligand binding, which include the partial cleft closure for Glu2A and the complete cleft closure for GluN1. Additional conformational rearrangements in the protein and ligand have to occur for GluN2A before the ligand can fit lobe 2. The glutamate ligand must truly rotate so the α-carboxylate interacts with lobe 1, so the full cleft closure can occur[23].

2.2 The Function of Memantine

The NMDA-receptor antagonist, memantine (**8**), is clinically used in the treatment of moderate to severe Alzheimer's disease[14]. The drug is used in an attempt to slow down the excitotoxic neurodegeneration[3], and it seems like it is a promising therapeutic option in the treatment of Alzheimer's disease[13]. **8** is a weak NMDA

channel blocker[13], and is thought to function by blocking open NMDA channels reversibly. Further it has a three-ring cyclic structure, with a protonated amino group (NH_3^+) under psychological conditions[14]. The binding of **8** occurs at the site which is deep within the ion channel, where the NMDA-receptor blocker Mg^{2+} is bound[3, 36]. The molecular structure of **8** is shown in Figure 2.1.

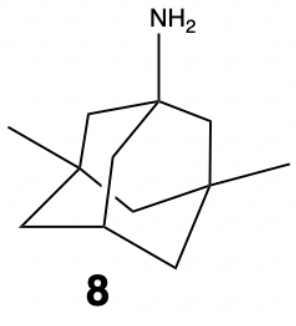


Figure 2.1: The molecular structure of the drug memantine (**8**). The molecular structure is made in Chemdraw.

The favored interactions/binding sites are between the charged amino group and the amino acid, asparagine, in the GluN1 subunit. The two methyl groups will bind to the alanine residues, Ala645 and Ala644, on the third transmembrane GluN1 and GluN2B, respectively[14]. The low-affencienty blocker seems to elevate the synaptic glutamate concentrations, and therefore potentially block the neurotoxic effects in Alzheimer's disease[3]. The problem of the excitotoxity of Alzheimer's disease is proposed as a result from tonic glutamatic over-activation, due to the loss of the normal Mg^{2+} voltage blockade of the NMDA-receptor. Other glutamate-reducing treatments, like metabotropic antagonists, have been used to reduce the damaging effects of Alzheimer's disease. The group I reduces the NMDA-receptor activation, and the group II/III are reported to prevent neurodegeneration in a variety of clinical models[3]. There are some NMDA-receptors in the human erythrocytes, where the activity is controlled by plasma glutamate and glycine[14].

The studies of the glycine-site agonist in the NMDA-receptor in treatment of schizophrenia has shown that the glycine site works as a positive allosteric modulator for the NMDA-receptor complex. Three different agents have been used in the study; **4**, D-serine and D-cycloserine. The first two function as full agonists, and the last one works as a partial agonist. Some studies confirm that this treatment has good effects for people with the lowest pretreatment levels. In some studies, it has been documented that there are negative symptoms correlated significantly with the glycine levels[3].

Both **4** and D-serine have proven to have good effect in the treatment of schizophrenia, but have to be given in doses of gram to penetrate into the CNS. It has been proven that the glycine level can be increased by the use of glycine transport inhibitors (GTIs). The GTIs raise the level of **4** in the synapses by the removement of **4** from the synaptic cleft. Glycyldodecylamide (GDA) is a glycine transport antagonist, which has been shown to inhibit the glycine transport in hippocampal or cortical synaptosomes in

rodents. The drug was shown to work in rodents by inhibition of the amphetamin-induced dopamine release, along with induced phencyclidine (PCP) hyperactivity. In 2004, there was a study that showed that sacrosine (N-methyl glycine) reduced the negative symptoms of schizophrenia with approximately 15%, in addition to reduction in positive symptoms and cognitive symptoms[3].

2.3 The Up-and-Coming Drugs

Derivatives of oxazolo[4,5-c]quinolin-4-one are synthesized and biologically evaluated for the NMDA-, AMPA- and KA receptors. It has been proven that different substitutes show different efficiencies to the receptors. The substitutes were introduced in the second position and on the fused benzo ring, where the 2-mercapto-derivatives showed the best efficiency to the NMDA-receptor. The 7-chloro substituted derivative showed the best efficiency to the NMDA-receptor compared to the AMPA- and KA receptor, with 50- and 500-fold, respectively. The structure activity relationship (SAR) studies mentioned the importance of electron rich moieties in both 2- and 3-position of the oxazolo[4,5-c]quinolin-4-one framework. It is believed that the 3-sp^2-nitrogen atom plays an important role in reinforcing the hydrogen bond which the 4-carbonyl oxygen probably forms with the residue Arg523 of the NMDA-receptor side. It is proved that the presence of 2-substituent (SH) is important for the binding due to the hydrogen bonding interaction. The protonated arginine residue, Arg523, is reported to have an essential contribution to the binding of the antagonist and agonist of the NMDA-receptor. The contribution is mainly to the cationic hydrogen bond donor-receptor site. A docking study of the the TQZ- and PQZ series pointed out a bidentate interaction of Arg523 with both the N-group and the adjacent carbonyl group with derivatives of TQZ and PQZ, this was done to a homology-based model of the NMDA-receptor. The molecular structures of the two series are presented in Figure 2.2. Some chemical calculations show that the arginine residue has better efficiency to the derivatives (**A-D**) than the derivatives that have a carbonyl group in place of the mercapto group. These stronger interactions are a result of the the presence of the 3-sp^3 N-atom, which can reinforce the hydrogen bond that the 4-carbonyl oxygen forms with Arg523[32].

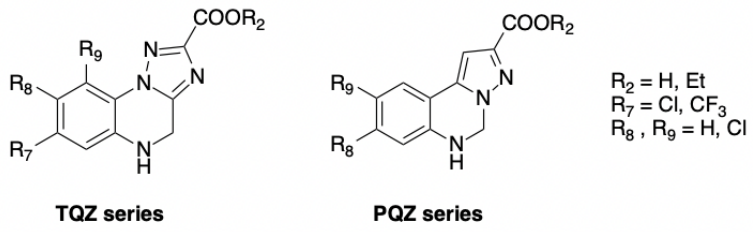


Figure 2.2: TQZ and PQZ series reported earlier. The molecular structures are made in Chemdraw.

3 Conclusion

There are many people worldwide who suffer from neurodegenerative diseases, and it is therefore important to develop drugs specified for blocking of different receptors which have a critical role in the flow of intracellular calcium, such as the NMDA-receptor.

The NMDA-receptor is a glutamate and glycine dependent ionotropic receptor, together with the AMPA- and KA receptors. The blockage of the NMDA-receptor by a magnesium ion hinders the influx of calcium. The calcium influx is important in memory and learning, among other functions, but neuronal death can occur with too high influx of calcium. The activation of the receptor involves the binding of both **1** and **4**/D-serine at GluN2 and GluN1, respectively.

Glutamate (**1**) is the primary neurotransmitter in the CNS, and glutamatic targeted drugs are the main focus when developing drugs. The crossing of the BBB is important for the drug delivery to the brain, but it has limitations for penetration through the BBB. The strict limits make it difficult for the drugs to enter the brain, and the limit for permeability is above 4.54×10^{-6} cm s^{-1}. The BBB is however a promising target for the treatment of neurodegenerative diseases.

The glycine site of the NMDA-receptor is a favored target for therapeutic treatment at present. The binding of **4** to the NMDA-receptor is an unguided diffusion, contrary to the binding of **1** which is a guided-diffusion. The arginine residue, Arg523, is important for the binding on the glycine site, among others, to the derivatives of oxazolo[4,5-c]quinolin-4-one.

The heterocycled-fused quionoline is one of the most privileged structures on the drug market, and is promising for future studies on drugs against neurodegenerative diseases. There are different criteria that are important for the binding to the glycine site of the NMDA-receptor; "(i) *a flat hydrophobic area represented on the fuzed benzo ring*; (ii) *a NH hydrogen bond donor that binds to a proton acceptor of the receptor*; and (iii) *a γ-negatively charged moiety which could form a hydrogen bond with a cationic hydrogen bond donor-receptor site*" [32].

Memantine (**8**), which is a NMDA-receptor antagonist, is clinically used in the treatment of moderate to severe Alzheimer's. The drug is a weak NMDA channel blocker, and is thought to function by blocking reversable open NMDA channels. The binding of **8** occurs at the site deep within the ion channel, where the magnesium ion is binding.

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