Bachelor's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Chemistry

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Supervisor: Elisabeth Egholm Jacobsen
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Abstract

In this paper stiripentol's mechanisms against epileptic seizures will be discussed. Stiripentol is a drug mostly used for treating Dravet syndrome, a rare childhood epilepsy with a high mortality rate. Stiripentol's mechanisms include enzyme inhibition, $GABA_A$ modulation and inhibition of other antiepileptic drugs. The results of animal and human studies will be considered. The cause for its high potency against prolonged seizures (status epilepticus) is examined. The pharmacokinetics and metabolism of (*R*)-and (*S*)-stiripentol are also included. Based on the studies, stiripentol should be marketed as a racemate since the enantiomers inhibit each other's metabolism. Stiripentol is clinically most potent when taken with valproate and clobazam. It should also keep being recommended for status epilepticus, especially in young children. Cannabidiol is a potential adjuvant treatment for Dravet syndrome.

Sammendrag

I denne oppgaven skal stiripentols mekanismer mot epilepsi diskuteres. Stiripentol er et legemiddel mest brukt mot Dravet syndrom, en sjelden barneepilepsi med høy dødsrate. Mekanismene mot epilepsi består av enzyminhibering, $GABA_A$ modulering og inhibering av andre antiepileptiske legemidlers metabolisme . Resultater av dyre- og menneskestudier vil drøftes. Årsaken til stiripentols effekt mot langvarige anfall (status epilepticus) er diskutert. Farmakokinetikken og metabolismen til (R)-og (S)- stiripentol er inkludert i diskusjonen. Basert på kliniske studier, bør stiripentol gis rasemisk siden enantiomerene hemmer hverandres metabolisme. Stiripentol er mest potent i behandling med valproat og klobazam. Stiripentol bør fortsette å bli anbefalt i bruk mot langvarige anfall. Cannabidiol er en mulig adjuvant behandling mot Dravet syndrom.

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Abbreviations

AED- Antiepileptic Drug

DS- Dravet Syndrome

STP- Stiripentol

GABA- Gamma-aminobutyric acid

CNS- Central Nervous System

LDH- Lactate Dehydrogenase

IPSC- Induced Pluripotent Stem Cells

MDMA- Methylenedioxymethamphetamine

PIMT- Protein-L-isoaspartyl methyltransferase

1. Introduction

According to the World Health Organization 50 million people have epilepsy, and 80% of them live in developing countries.[1] Much of the epilepsy is untreated globally due to economic instability, right care, and lack of diagnoses.[2] However, the use of the right anti-seizure medications could stop seizures in 70% of epilepsy patients.[1] People in developed country are more vulnerable to seizures. The deliverance of antiepileptic drugs to facilities in developing countries needs to be sustainable and affordable.[2] According to neurologist Gretchen Birbeck, delivering older AED's like phenobarbitone is the most effective way to do this.[2] Focus on renewable public funds could increase sustainability. In addition, nutritional deficiencies can change the brain's electrical activity and incite seizures.[3] Vitamin B6 deficiencies in infants have also caused or worsened seizures.[3] Since malnutrition is more common in developing countries, its population is more susceptible to epileptic seizures.

Dravet Syndrome is a childhood epilepsy typically diagnosed before age two. It has a mortality rate of 15-20%.[4-5] In 2022 The United States approved stiripentol for DS patients over the age of six months co-adjunctive with clobazam.[5] It was approved in Canada in 2012. Stiripentol is marketed as a racemate, even though its *R*-enantiomer is the most potent. 60 capsules of 250 g Diacomit® (stiripentol) cost $1,891.[6] A Canadian analysis found that stiripentol's current price needs to be reduced 61.4% for it to be cost-effective.[7]

Stiripentol was first synthesized in 1978.[8] Its Anticonvulsive properties were first observed in monkeys in 1985.[8] In 1988 studies that showed stiripentol's antiseizure effect on humans were published.[8] Stiripentol could also potentially be used in treating Alzheimer's, urine oxalate excretion and glioblastoma.

Children have a high expression of the α-3 receptor.[9] This receptor is replaced by the α-1 receptor before age one. Stiripentol is more effective in child patients, especially infants, than adults. Fisher *et al.* observed that stiripentol was the most potent against the α-3 receptor. Stiripentol's high efficacy on children could be due to its high potency on the receptor that they highly express. This leads to the research question: "How does stiripentol work in the treatment of epileptic seizures?"

2. Theory

2.1 Chiral medications

Half of the drugs in the pharmaceutical industry are chiral compounds.[10] 90% of the drugs are racemates. Racemates contain an equal amount of two enantiomers.[11] The mirror images are called enantiomers and are formed when a carbon atom is connected to four different groups. Enantiomers can be expressed as d and l, or (+) and (-).[10] A chiral switch is the transformation of a racemate to its most bioactive enantiomer. Enantioselectivity is characterized by a chiral compound with one dominant enantiomer. Stereoselective synthesis is the preparation of one enantiomer (often the preferred one). This technique is useful for removing a toxic enantiomer in an otherwise potent drug.

Many pharmaceutical drugs are marketed as racemates.[10] This can be due to the difficulty and high cost of chiral separation. For example, (+)-ketamine is more potent and less toxic than (-)-ketamine, but ketamine still marketed as a racemate. Racemates often have one eutomer, which is a chiral compound with one bioactive enantiomer. Many antiepileptic drugs, antibiotics and psychostimulants are eutomers. Moreover, some drugs are more potent as racemates due to the enantiomers complementing each other's pharmacokinetic effects.

Enantiomers often differ in metabolism and pharmacokinetics. The immune system can distinguish and be selective between the enantiomers of a drug. Drug metabolites need a connective support molecule (often proteins) to form an immunogen.[12] Then the immune system can form an antibody. The difference of enantiomers can be explained by their reactions with enzymes and receptors in the body. *R*-and *S*-enantiomers rotate in plane-polarized light in different directions. This is why they do not bind to the same receptor. Only the enantiomer that binds to the receptor will be active. The inactive enantiomer can bind to other receptors and possibly have toxic effects.

Chiral separation can be divided into classical and modern technologies.[13] Classical chiral separation often involves an acid-base reaction with a racemate and an enantiomer. This yields two different diastereomeric salts that can be separated by crystallization. A subsequent degradation with an acid or base can result in a pure enantiomer. Enzymatic or kinetic resolution can also separate chiral compounds. This chemically destroys one of the two

enantiomers. Microorganisms are often used to "consume" the undesirable enantiomer enzymatically. High-Performance Liquid Chromatography (HPLC) is one of the most common techniques for separating chiral compounds.

Using enantiomerically pure active pharmaceutical ingredients (API) is preferred. In the 1990's the optically pure drugs were recommended by the Food and Drug Administration (FDA).[13] To maintain a pure active pharmaceutical ingredients low-cost chiral separation and resolution is necessary.

The E factor is an important factor of green chemistry. The concept was proposed in 1992 by professor of Biocatalysis Roger Sheldon. The *E* factor is the total mass of waste (kg) divided by the mass of product (kg).[14] A low *E* factor should be the goal of all companies. However, An E factor of zero is ideal. Oil production has an *E* factor of 0.1, while the *E* factor for pharmaceutical processes varies from 25-100.[14] The "12 Principles of Green Chemistry" were published by Paul Anastas in 1999. Some examples of his principles are waste prevention, chemical safety, and catalysis.

2.2 Stiripentol

Stiripentol is the industrial name for 4,4-dimethyl-1-[3,4(methylenedioxyphenol)-phenyl]-1-penten-3-ol] (El-behairy *et al*).[4] It is an aromatic allylic secondary alcohol.[4] Stiripentol is an antiepileptic drug that is used for the treatment of Dravet syndrome.[15] In 2007 stiripentol was approved by the EU for co-administration with clobazam and valproate.[8] Stiripentol has two enantiomers, classified as (*R*)- and (*S*)- Stiripentol. (*R*)-stiripentol is 2.4 times more potent against epilepsy than (*S*)-stiripentol.[4] In the pharmaceutical industry it is marketed as a racemate. Stiripentol is considered an orphan drug, a drug supported financially by the government for a small patient population.[16]

In 1985 the first antiseizure effects of stiripentol were observed in monkeys.[8] Stiripentol's effect on humans was first published in 1988.[8] Stiripentol is most potent against the rare childhood epilepsy Dravet syndrome.[15] It is potent against status epilepticus, a possibly fatal prolonged seizure.[17] Stiripentol has a similar structure to the psychostimulant MDMA.[18]

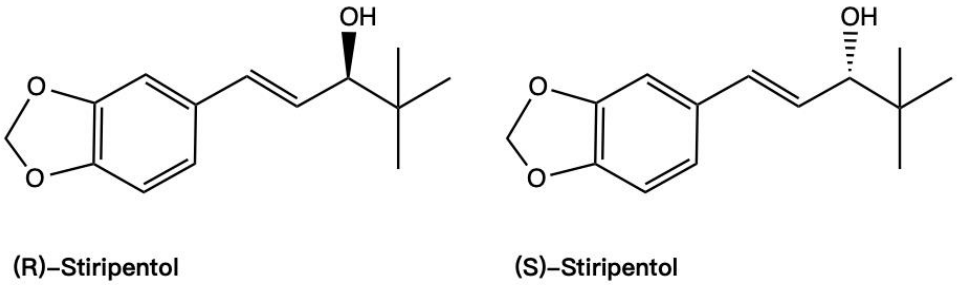

Figure 2.1: The chemical structure of (R)-and (S)-Stiripentol. Made in Chemdraw.

2.3 Synthesis of Stiripentol

Stiripentol was first synthesized in 1978 and has since been synthesized several ways.[8] Jacobsen *et al*. prepared (*R*)-stiripentol from piperonal and pinacolone in 2011.[15] After a reduction with $NaBH_4$, stiripentol was resolved with vinyl butanoate in hexane. The reaction was catalyzed by Lipase A from *Candida Antarctica*.

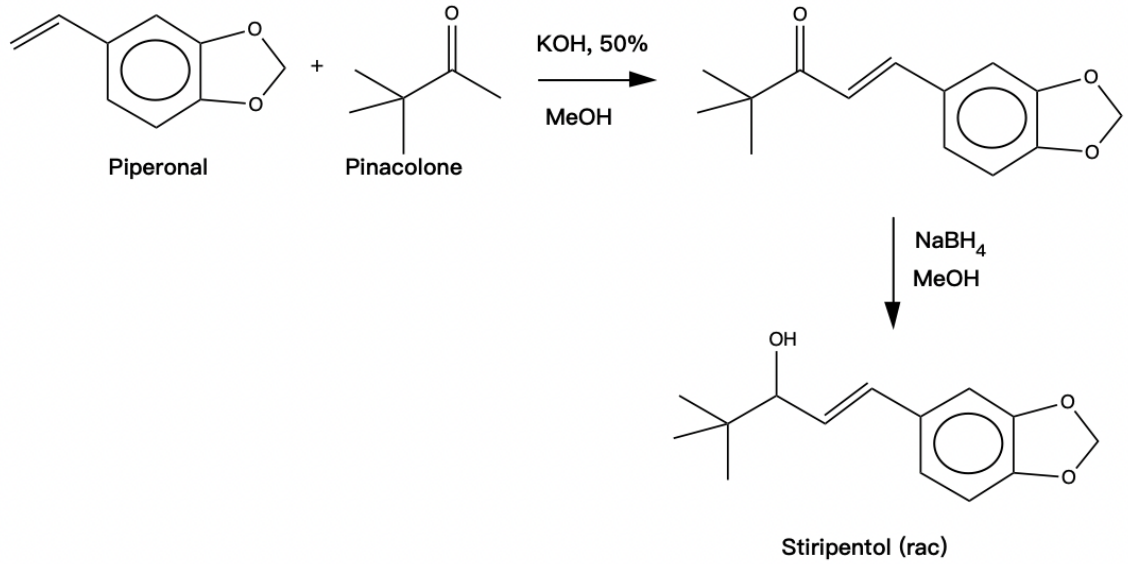

Figure 2.2: Jacobsen *et al.*'s synthesis of racemic stiripentol from piperonal and pinacolone.[15]

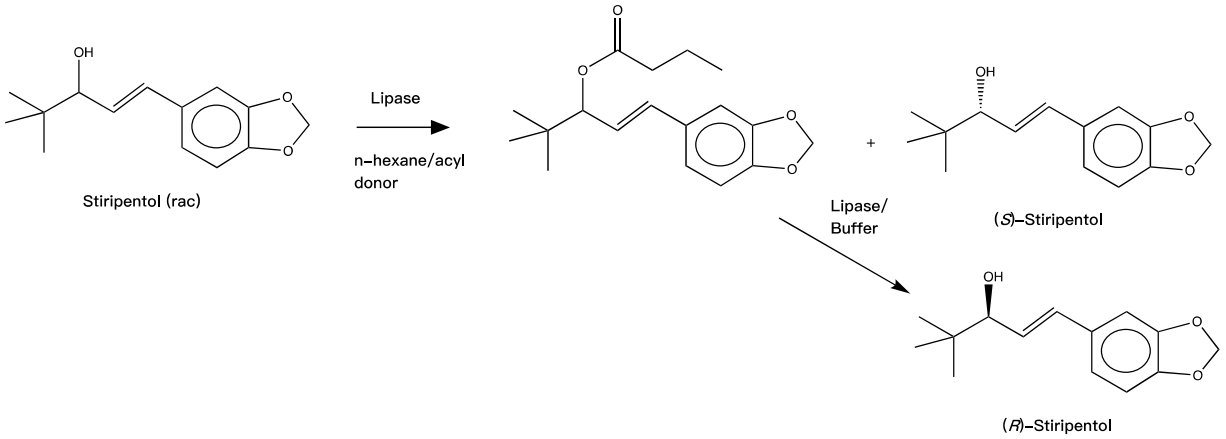

Figure 2.3: Resolution of enantiomeric stiripentol catalyzed by lipase A from *Candida Antarctica.*[15]

The reaction had an enantiomeric excess of 94%. (*R*)-stiripentol reacted quicker than the S-enantiomer. (*S*)-Stiripentol yielded 86% enantiomeric excess. Biocatalysts have become more popular in the synthesis of enantiopure compounds in recent years.[15] Lipases are a good choice of biocatalysts because they don't require cofactors and have stereoselective properties.[4]

In 2019 Almutairi *et al.* also synthesized stiripentol from piperonal and pinacolone.[19] However, they reduced it with sodium sulfate (Na_2SO_4) instead of Sodium borohydride ($NaBH_4$). This reaction yielded 79% (0.8 g)

El-Behairy *et al.* Synthesized (*R*)-stiripentol by cross metathesis of 5-vinylbenzo(d) (1,3)-dioxale and (*R*)-4,4-dimethylpent-1-en-3-ol.[4] They also performed a kinetic resolution of the latter catalyzed by lipase A. This reaction resulted in enantiopure stiripentol (>99%).
They used cross metathesis to synthesize (*R*)-stiripentol with Grubbs catalyst in dry CH_2Cl_2. The reaction yielded 15% (*R*)-stiripentol (5 mg). El-Behairy *et al.* thought the low yield was caused by homo-coupling. Homo-coupling occurs when two identical species combine to one product.[20]

They also studied 11 different lipases for enantioselective transesterification of racemic 4,4-dimethylpent-1-ol.[4] Three lipases gave acceptable enantiomeric ratios. Lipase A from *Candida Antarctica* and lipase from *Candida Rugosa* caused the R-enantiomer to react fastest. The most favorable enantiomeric ratio was by lipase from *Candida Antarctica* in toluene.

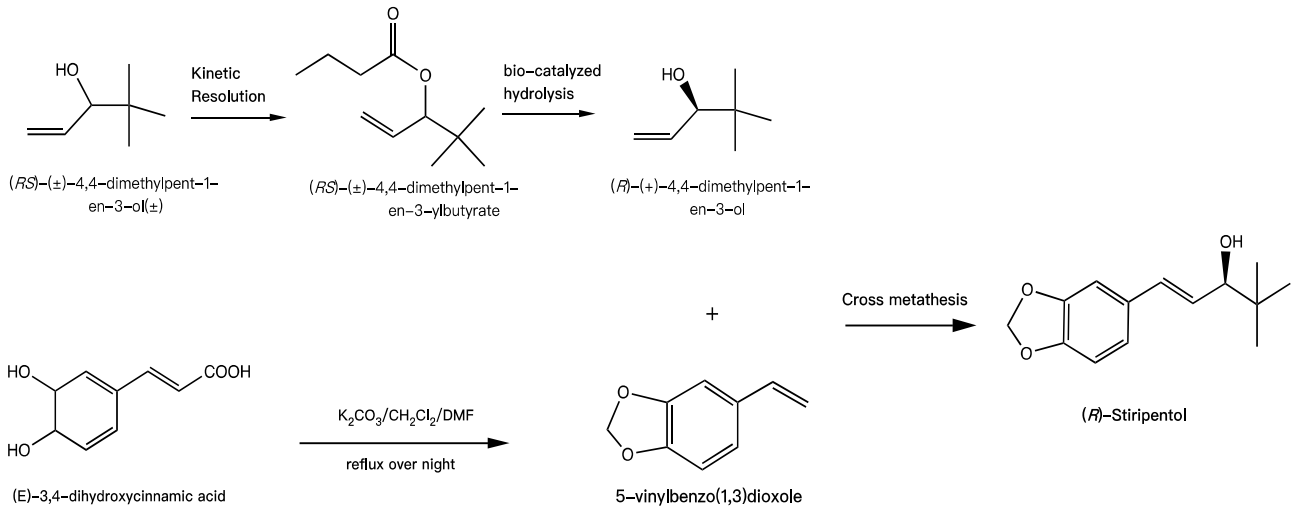

Figure 2.4: The synthesis of (*R*)-stiripentol performed by El-Behairy. *et al*.[4]

2.4 GABA receptors

GABA (γ -aminobutyric acid) is an amino acid that mediates neurotransmission in mammals.[21] The amino acid glutamate is its precursor.[18] GABA receptors are ligand-gated ion channels in the synapses of the central nervous system. GABA acts through the $GABA_A$ and $GABA_B$ receptors.[21] Irregularities in the $GABA_A$ receptor are linked to epilepsy, insomnia, and anxiety disorders. There are three types of GABA receptors: $GABA_A$, $GABA_B$ and $GABA_C$.[22] The GABA receptor is structurally heterogeneous and has 19 subunits.[21] The main $GABA_A$ receptor isoform in adults is the α1β2 γ2 receptor.[9]

GABA receptors bind to GABA by opening a transmembrane that is permeated by chloride ions.[21] A high chloride flux through the GABA receptors can lead to neuronal excitation, action potential and a possible seizure.

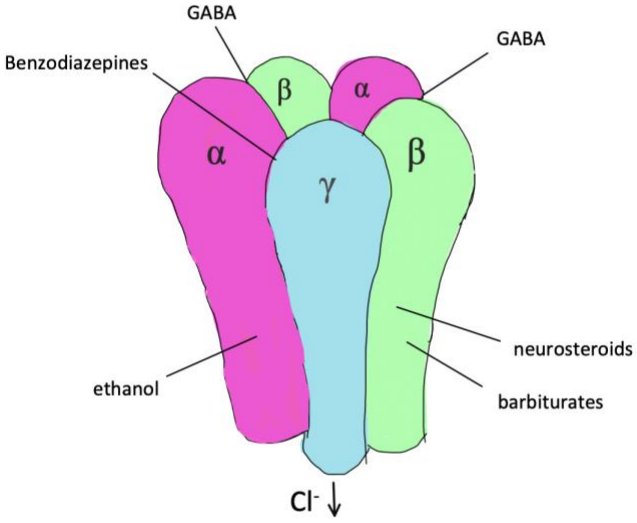

Figure 2.5: An example of a subunit structure of a GABA receptor. Benzodiazepines bind between the γ and α subunits. GABA binds between the α and β subunits. Barbiturates (sedatives) and neurosteroids act on the β subunit.[23] Ethanol (alcohol) mainly acts on the α subunit. A high flux of chloride ions into the transmembrane of the GABA receptor can incite seizures. The figure is inspired by Chen. *et al.*[24]

The γ-2 subunits in the $GABA_A$ receptor have two possible mutations that can impair its function: K289M and R43Q.[25] These mutations have been proven to be connected to childhood epilepsy. However, the α-1 subunit is the most likely cause of Dravet syndrome.[25]

Stiripentol is the most potent on the α-3 receptor, a receptor that contains the amino acid methionine.[9] Stiripentol was not dependent on a γ subunit, but strongly modulated the δ -subunits. Expression of certain subunits change during development.[9] The α-3 subunit is highly expressed in children and its expression is limited in adults. Changes in the α-3 expression is seen in epileptogenesis (development of an epileptic brain after head trauma).[9]

Moreover, Stiripentol increases synthesis of the $GABA_A$ receptor.[9] This can increase the receptors flow into extrasynaptic areas and prevent seizures. For example, increased membrane flow of the $GABA_A$ receptor has been linked to status epilepticus. In children the activation of the $GABA_A$ receptor can lead to excitation and initiate seizures. Seizures often occur when inhibitory transmitters don't function correctly.[24]

2.5 Epilepsy and Dravet syndrome

Epilepsy is a brain disorder that causes recurring unprovoked seizures.[26] Unprovoked seizures don't have a known cause, like alcohol abuse or a brain injury. The two main types of epilepsy are focal (partial) and generalized epilepsy.[27] Focal epilepsy begins with increased electricity in one brain hemisphere. Focal seizures can spread throughout the brain and become tonic-clonic. Tonic-clonic seizures are generalized seizures that lead to a loss of consciousness. Furthermore, generalized seizures cause an electricity surge in both brain hemispheres. Symptoms include prolonged staring, muscle contraction and limp stiffening. Myoclonic seizures are generalized seizures that cause muscle jerks. These seizures typically begin in early childhood. Dravet Syndrome is an example of myoclonic epilepsy.[27]

Imbalance between inhibitory and exhibitory conductances can cause seizures in a healthy brain.[28] Blocking inhibition and activating excitation can also induce seizures. In chronic epilepsy the timing of the seizures is caused by the shift in inhibitory and exhibitory conductances. In 2015 Professor of Child Neurology Kevin Staley wrote about epilepsy mutations in the acclaimed science journal *Nature*.[28] He suggested that changes in ionic homeostasis is the root cause of epilepsy.

Different concentrations of ions have been connected to ictogenesis.[28] Ictogenesis is the development of an epileptic brain. Maintenance of ionic homeostasis relies on specific proteins. Mutations coding for these proteins are connected to epilepsy. Gene mutations that encode the AMPA receptor membrane family are the LGI1 and ADAM22 proteins. In addition, chemically induced seizures lead to an increase in a potassium efflux. This hinders the sodium pump Na^+-K^+-ATPase's ability to maintain a cation gradient. Consequently, membrane potential becomes impaired. Damaged membrane potential causes depolarization of neurons and thus action potential, which can initiate a seizure. On the contrary, hyperpolarization of neurons inhibits the neuron from firing an impulse, which terminates action potential.[29]

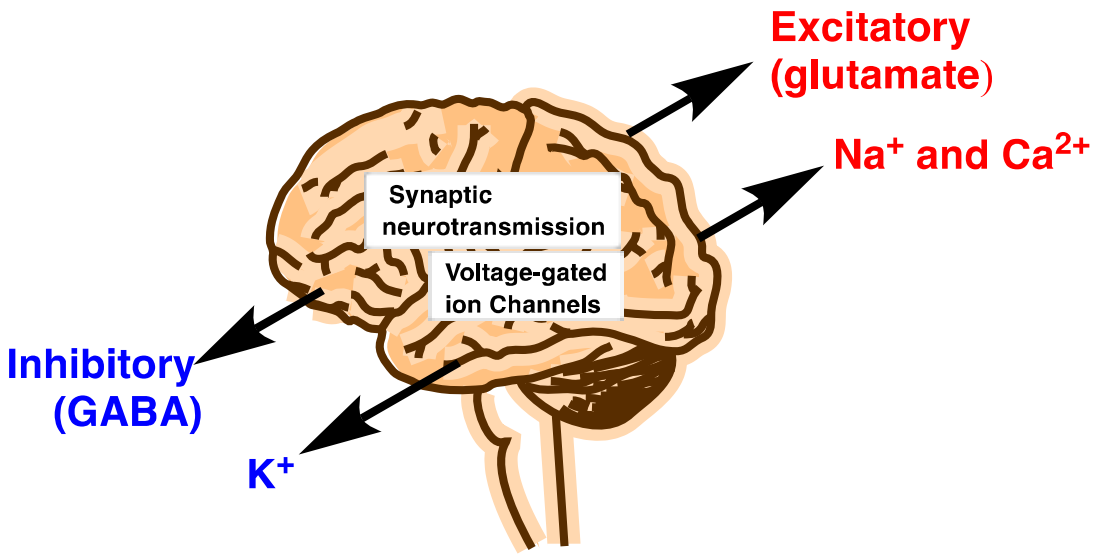

Figure 2.6: An illustration of the imbalance of excitation and inhibition in the mammalian brain. Imbalances between the voltage-gated potassium (K^+), sodium (Na^+) and calcium (Ca^{2+}) channels can cause action potential. Glutamate impairment can have excitatory effects on the central nervous system.[30] GABA is an inhibitory neurotransmitter in the CNS.[30] The figure is inspired by Stafstrom. *et al.*[31]

Some anti-convulsive drugs can increase seizures in epileptic patients.[32] In addition, medications targeting the CNS can induce epileptogenesis in patients that previously have never had seizures.[32]

Dravet syndrome was discovered by the French psychiatrist Charlotte Dravet in 1978.[33] She observed children experiencing myoclonic seizures, cognitive delay, and movement disorders. Dravet syndrome affects approximately one in 15700 children.[34] The syndrome usually starts with febrile seizures. After age ten seizures often stabilize, but behavior and cognitive issues can exacerbate. Between 15-20% of patients die before adulthood, often caused by prolonged seizures (status epilepticus).[34]

The goal for treating Dravet syndrome is to reduce frequency and duration of seizures. Valproate and clobazam are the antiepileptic drugs that are often used in Dravet syndrome treatment. However, in 2018 stiripentol was approved as adjunctive medication in patients two years or older. In 2023 more than 50% of Dravet syndrome patients are treated with stiripentol.[33] The recommended dose of stiripentol was 5 mg/kg/day for children.[8] Adults are administered a lower dose. Buck *et al.* discourages Dravet syndrome patients from using drugs that block sodium channels.[33] These include carbamazepines and phenytoin.[25]

3. Discussion

Stiripentol is a positive allosteric modulator of the $GABA_A$ receptor.[9] Fisher *et al*. investigated stiripentol's effect on the GABA receptor subunits. The α-3 subunit had the highest potency, and it's the subunit that is highly expressed in embryo brains. This could explain stiripentol's high efficacy in children. Development of the brain limits expression of the α-3 subunit, but epilepsy patients have higher α-3 production. The GABA receptors that were tested were positively modulated by stiripentol. Stiripentol amplified the receptors by increasing their sensitivity to GABA. Evidence that Stiripentol influenced GABA receptor activity was discovered in 2006.[35] Stiripentol increased the velocity and duration of the receptor's postsynaptic actions. Direct modulation by stiripentol was proven when stiripentol increased the length of the opening of GABA-activated channels.

Dravet syndrome (formerly called SMEI- Severe Myoclonic Epilepsy in Infancy) is often treated with stiripentol.[17] In 2000, 41 patients with Dravet syndrome were administered stiripentol for 2 months.[36] Stiripentol was co-administered with valproate and clobazam. 71% of the patients reduced seizures. There was a big difference in results between the stiripentol and the placebo. Stiripenol's high inhibition of seizures in DS patients its popularity.

Mutations in the gene SCN1A are connected to Dravet syndrome.[37] In 2001 a relationship between Dravet syndrome and the SCN1A gene was discovered. The gene SCN1A codes for the sodium channel α-1 subunit.[37] The α-1 subunit replaces the α-3 subunit before age 1. 80-90% of Dravet syndrome patients have a mutation in the SCN1A gene. The SCN2A and SCN3A genes code for the α-2 and α-3 sodium channel subunits, respectively. Mutations in these genes are connected to more intense cases of Dravet syndrome. Cho *et al*. studied stiripentol's clinical effect on patients with the SCN1A mutations. Stiripentol had a more potent effect on Dravet syndrome patients with this mutation. Stiripentol reduced seizure frequency by 72% in patients with a mutation, compared to 51% with no mutation. Cho also found a strong correlation with high-frequency seizures and gene mutation. 72% of people with the SCN1A gene experienced frequent seizures. The SCN1A gene was only found in 80-90% of Dravet syndrome patients, meaning it can't the only predictor for the disease.

The enzyme lactate dehydrogenase (LDH) is inhibited by stiripentol.[38] Lactate dehydrogenase converts pyruvate to lactate in the lactate astrocyte shuttle. In 2015 Sada *et al*. discovered that stiripentol is a lactate dehydrogenase inhibitor. Stiripentol was the only antiepileptic drug out of 20 drugs tested that inhibited the enzyme. They tried to mimic the metabolism of the ketogenic diet; a low-carbohydrate diet often used by epilepsy patients.[38] Inhibiting the lactate dehydrogenase pathway reduced epileptic seizures in rodents. Neurons were hyperpolarized following the inhibition. High concentrations of lactate are connected to some types of epilepsy.[39] By inhibiting the enzyme that catalyzes the conversion of pyruvate to lactate, it decreases the lactate concentration in the brain. Sada *et al*. also reported that isosafrole inhibits lactate dehydrogenase. Isosafrole is a structural analog of stiripentol that doesn't have a tert-butyl or hydroxyl group. Seizures were more suppressed by isosafrole than stiripentol. In the future synthesizing new stiripentol analogs can yield a compound with maximum anticonvulsive effect and undiscovered anti-seizure mechanisms. In conclusion, inhibiting the enzyme lactate dehydrogenase is likely one of stiripentol's anticonvulsive mechanisms.

Stiripentol inhibits the metabolism of other antiepileptic drugs.[40] Levy *et al*. reported that phenytoin metabolism is inhibited by stiripentol. The plasma clearance of phenytoin decreased 78% at a 2400 mg/day stiripentol dose. Kerr *et al*. found that stiripentol inhibits the plasma clearance of clobazam 50% and facilitates its reduction to its metabolite carbamazepine-10,11-epoxide.[41] Stiripentol also inhibits the metabolism of valproate, phenobarbital, and primidone.[42] Stiripentol increased cannabidiol (cannabis oil) concentration in the plasma of epileptic children. Kerr *et al*. recommended a reduction in clobazam when co-administered with stiripentol.[41] The inhibiting effect of stiripentol on clobazam's metabolism increases after 7-10 days. They suggested that stiripentol's methylenedioxyphenyl ring causes its inhibitory effects and can inhibit cytochrome P-450 (CYP450).[43] CYP450 is a group of enzymes that metabolize pharmaceuticals.[44] The cytochrome P450-3A4 is member of the CYP450 family. Its inhibition is linearly correlated to the plasma concentration of stiripentol in epilepsy patients.[45]
The inhibition of the metabolism of other antiepileptic drugs suggests that stiripentol should be co-administered.

The metabolism of stiripentol is accelerated by other antiepileptic drugs.[40] Levy *et al*. discovered that co-medication with clobazam or phenytoin increases the plasma clearance of stiripentol threefold.[40] In conclusion, stiripentol should be co-administered with AED's that don't increase its plasma clearance.

(*R*)-stiripentol is 2.4 times more potent than the *S*-enantiomer.[46] Arends *et al*. analyzed brain and blood samples to see if the racemate's tolerance is caused by a change in the enantiomeric composition of regularly administered stiripentol. Acute (short-lasting) administration of stiripentol is more anticonvulsive than subacute (regular) administration.[47]Subacute administration of the drug led to a 5-fold increase in the *S*/*R* enantiomeric ratio.[46] The increase of the less potent S-enantiomer would likely decrease the anticonvulsive effect by 67%, according to Arends *et al*. This suggests that acute administration of stiripentol would highly increase its anti-seizure potency. Perez *et al*. reported that subacute administration of racemic stiripentol lead to a 40% decrease of its anticonvulsive effect.[48] The peak *S*/*R* ratio was 3 after one dose of stiripentol. They speculated that higher doses of stiripentol would increase the *S*/*R* ratio and thus decrease its anticonvulsive properties. To conclude, acute administration of (R)-stiripentol would maximize its anticonvulsive properties.

(*S*)-stiripentol has a longer half-life than (*R*)-stiripentol.[46] Arends *et al*. observed that the S-enantiomer has a half-life of 6.5 hours, compared to 2.8 hours for (*R*)-stiripentol. This could explain the change in the enantiomeric ratio in subacute administration. In addition, racemic stiripentol has lower plasma clearance than each of the enantiomers.[46] This could be explained by the two enantiomers mutually inhibiting each other's metabolism. Since racemic stiripentol has a lower plasma clearance, it should be favorable for pharmaceutical use.

Stiripentol reduces seizure frequency in most clinical trials. Stirpentol's anticonvulsive potency was first studied in 1989 by Rascol *et al*.[49] Seven patients with frequent seizures were treated with co-administered stiripentol. Many of the patients had a seizure frequency reduction. Bebin *et al*. conducted a clinical trial with seven epilepsy patients in 1994.[42] Stiripentol was co-administered and over 60% of the patients reported a decrease in seizure frequency. No stiripentol tolerance developed over time. Kerr *et al*. reported that stiripentol was less potent than the antiepileptic drugs phenobarbital, phenytoin, and carbamazepine.[41] However, in this trial only 12/47 (25%) of patients that were administered stiripentol had a reduction in seizure frequency. In 1999 Perez *et al*. conducted a clinical trial where 212 epilepsy patients received stiripentol.[48] 49% of the patients responded to stiripentol. Patients with focal (partial) epilepsy had the best response (57%). All the responding patients developed anorexia. Myers *et al*. speculated that some cases of pancreatitis were connected to the co-administration of valproate and stiripentol. Stiripentol was the most potent against focal epilepsy when administered with

carbamazepine (CBZ). In clinical trials stiripentol often reduces approximately 50-60% of seizure frequency.

Stiripentol is effective in the treatment of status epilepticus. Status epilepticus is characterized by prolonged seizures.[17] Myers *et al.* co-administered stiripentol to 41 Dravet syndrome patients in a 12-year study. Stiripentol was co-administered with the antiepileptic drugs valproate and clobazam. 41% of the patients reduced frequency of status epilepticus. Grosenbaugh *et al.* studied the anticonvulsive effect of stiripentol and diazepam in status epilepticus in rodents.[50] 100% of the seizures terminated after stiripentol administration. The potentiation was especially high in juvenile rodents. The $GABA_A$ receptor subunits also change during status epilepticus. However, the receptors with α-4 subunits remain on the membrane surface. Grosenbaugh *et al.* thought subunit selectivity caused stiripentol's efficacy in status epilepticus. In addition, they speculated that stiripentol's maintained effect on a prolonged seizure is also due to its potentiation of IPSC's (Induced Pluripotent Stem Cells). Stiripentol's IPCS potency was higher in juvenile animals like its antiseizure potency. Myers *et al.* concluded with advocating for stiripentol use in the treatment of status epilepticus in infants and children.

The structure of stiripentol is similar to methamphetamines' structure.[18] The methamphetamine MDMA (methylenedioxymethamphetamine) and stiripentol both have a methylenedioxy group. MDMA has an amide and two methyl groups, while stiripentol has a secondary alcohol connected to the tert-butyl group. In 2023 Zimmerman *et al.* used magnetic resonance spectroscopy to investigate the levels of GABA and glutamate in the brains of frequent MDMA users.[18] Chronic MDMA use resulted in elevated levels of glutamate in the left striatum. The striatum is the part of the brain that controls movement, function, and reward.[51] In addition, higher frequency of MDMA use was also linked to lower GABA levels. A decrease of GABA is linked to epileptic seizures, a known MDMA side effect. However, it's worth to note that the GABA level did not differentiate from the control group. Stiripentol increases cerebral GABA levels, observed by Grosenbaugh *et al.*[50] In conclusion, stiripentol and MDMA have different effect on brain GABA levels despite their similar chemical structure.

The synthesis of new stiripentol analogs can improve its anticonvulsive properties. Aboul-Enein *et al.* synthesized new stiripentol analogues in 2012.[52] The addition of a semicarbazone molecule to a stiripentol moiety yielded the analogue with the highest potency (Figure 3.1). On a MES (Maximal Electroshock Seizure) screen this analogue displayed 100% protection, while

stiripentol only displayed 66% seizure protection. This analogue also showed more antiepileptic activity than stiripentol on scPTZ screens. Furthermore, the addition of a (4-bromophenyl) methanone moiety displayed the highest potency on the scPTZ screens of all the analogs.
The removal of the amide group on a stiripentol analogue also increased its potency. The substitution of the amide group on with an aryl group reduced its anticonvulsive properties. In conclusion, the addition of semicarbazone and (4-bromophenyl) methanone moieties had the highest increase in anticonvulsive activity.

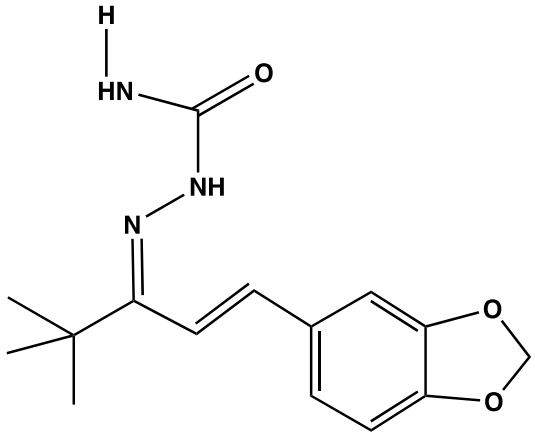

Figure 3.1: The structure of the stiripentol analogue 2-[(1E)-1-(1,3-benzodioxol-5-yl)-4,4-dimethylpent-1-en-3-ylidene] hydrazinecarboxamide. Synthesized by Aboul-Enein *et al*. in 2012.[52] The figure is made in Chemdraw.

Hyperammonemia is a possible side effect of stiripentol. In 2020 Ali *et al*. co-administered stiripentol to 28 adult Dravet syndrome patients.[53] 77% of the patients developed elevated ammonia levels and got hyperammonemic encephalopathy. Encephalopathy refers to a negative change in brain function.[54] The fatty acid oxidation cofactor carnitine treated the hyperammonemia and decreased side effects. Stiripentol was co-administered with valproate, which has hyperammonemia as a common side effect. A mild version of hyperammonemia has the side effects lethargy, confusion, and headaches. These are also side effects of stiripentol. Stiripentol's side effects could possibly be masqueraded as valproate's side effects, but more research is necessary for more knowledge and better treatment.

Stiripentol increased the presence of protein-L-isoaspartyl methyltransferase (PIMT), which has positive neurological effects.[55] PIMT is an enzyme that repairs different residues in pathways leading to cell death. This suggest that stiripentol could possibly be effective in repairing damaged cells. However, there's limited research on stiripentol's connection to

cancer. Glioblastoma is a type of cancerous brain tumor that is linked to the LDH enzyme, which stiripentol inhibits. Some cancers convert glucose to lactate in the Warburg effect. In three different studies stiripentol decreased lactate production in mice with glioblastoma. Since stiripentol is connected to GABA and glucose levels in the brain it could possibly be useful in the treatment of Alzheimer's disease.[8]

4. Conclusion

Stiripentol is a positive allosteric modulator of the $GABA_A$ receptor. It is mainly used in the treatment of Dravet syndrome. Mutations in the gene SCN1A are connected to Dravet syndrome. Stiripentol is an inhibitor of the enzyme lactate dehydrogenase. Stiripentol inhibits the metabolism of other AED's. In addition, its own metabolism is accelerated by other AED's. (*R*)-stiripentol is more potent than (*S*)-stiripentol. However, (*S*)-stiripentol has a longer half-life. Stiripentol reduces seizure frequency in most clinical trials. It is especially effective in treating status epilepticus. New stiripentol analogs can improve its anticonvulsive properties. Furthermore, hyperammonemia is a possible side effect of stiripentol and causes lethargy and headaches. Stiripentol increased the presence of the enzyme protein-L-isoaspartyl methyltransferase, suggesting that stiripentol can increase reparation of damaged cells.

The clinical studies suggest that stiripentol is most effective when given to Dravet syndrome patients with valproate and clobazam. Stiripentol should also keep being recommended for treating status epilepticus, especially in young children. However, cannabidiol is a potential stiripentol adjuvant. Stiripentol's potency against other epilepsy diseases has limited research. Discovering more stiripentol mechanisms is important for understanding its effect on patients and reducing side effects. Future studies could be focused on stiripentol's connection to other neurological diseases and cancer.

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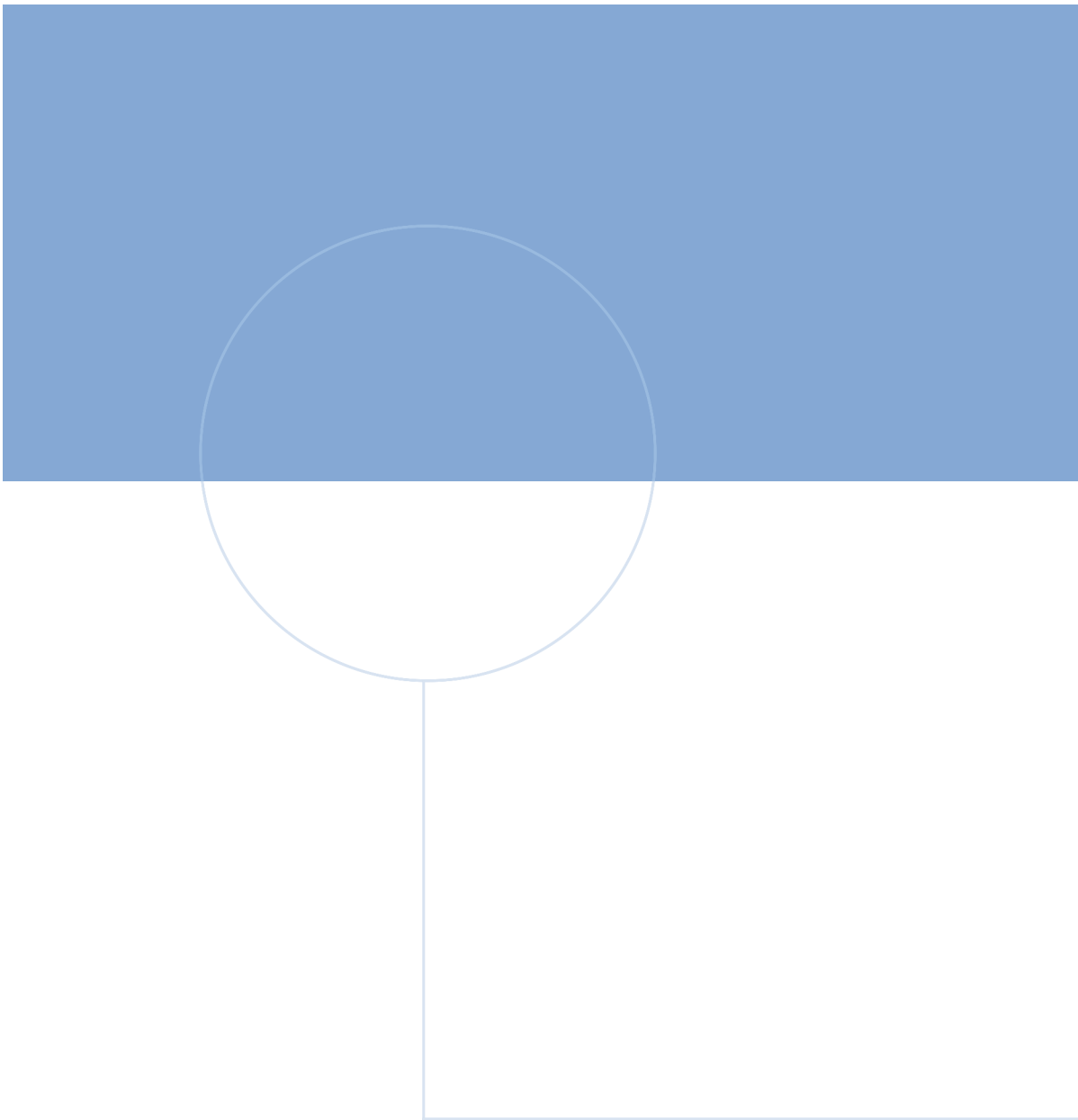

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