Charles University

Faculty of Science

Study programme: Special Chemical and Biological Programmes

Branch of study: Molecular Biology and Biochemistry of Organisms

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Neuroprotective effects of ketamine and memantine on NMDA receptors from molecular to systemic level

Neuroprotektivní účinky působení ketaminu a memantinu na NMDA receptory od molekulární po systemickou úroveň

Bachelor´s thesis

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Prague, 2021

Acknowledgement

I would like to thank my supervisor Mgr. Martin Horák Ph.D. and the whole team of Department of Neurochemistry at the Institute of Experimental Medicine CAS for feedback and valuable advice. Special thanks go to my family for all the support.

Declaration

I declare that I have prepared the final thesis independently and that I have listed all used information sources and literature. Neither this work nor a substantial part of it has been submitted for another or the same academic degree.

Prague, 3.5.2021 Matěj Hotovec

Abstract

The goal of this thesis is to present the evidence for the role of NMDA receptor excitotoxicity in pathology of Alzheimer's disease, epilepsy, major depressive disorder and ischemic stroke, evaluate the neuroprotective effects of NMDA receptor antagonists ketamine and memantine based on excitotoxicity research, and review the current body of evidence on their potential use as a treatment for these conditions.

Keywords: excitotoxicity, ketamine, memantine, NMDAR, neurodegeneration.

Abstrakt

Cílem této práce je prezentovat důkazy pro roli excototoxicity NMDA receptoru v patologii Alzheimerovy choroby, epilepsie, depresivní poruchy a ischemické mrtvice, zhodnotit neuroprotektivní účinky antagonistů NMDA receptorů ketaminu a memantinu na základě výzkumu excitotoxicity a posoudit současný soubor důkazů pro jejich potenciální využití v léčbě těchto nemocí.

Klíčová slova: excitotoxicita, ketamin, memantin, NMDAR, neurodegenerace.

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1. Introduction

Various neurological and mental disorders have been plaguing humanity throughout history. Although most of these conditions are still somewhat shrouded in mystery today, we have gained some insight into the mechanisms behind these disorders. One such mechanism is NMDA receptor excitotoxicity, which plays a role in the pathophysiological processes leading to neurodegenerative diseases through increasing neuronal apoptosis and necrosis via increased Ca^{2+} influx. The following text aims to elucidate the basic mechanisms of NMDA receptor excitotoxicity and its role in Alzheimer's disease, epilepsy, major depressive disorder and ischemic stroke and lay an argument for the therapeutic use of NMDA receptor antagonists. Ketamine and memantine are among the most thoroughly studied NMDA receptor inhibiting drugs to date and both are already widely used in medical practice. They both have been more or less extensively studied as potential treatment options for Alzheimer's disease, epilepsy, major depressive disorder and ischemic stroke.

2. Characteristics of NMDA receptors

2.1.General

NMDA receptors are a group of ionotropic glutamate receptors, occurring on the neuronal membranes. They are involved in several physiological and pathophysiological processes e.g. long-term potentiation, associated with memory formation and neural plasticity (Artola and Singer 1987) and excitotoxicity (Rothman and Olney 1987).

2.2.Subunits

The individual NMDA receptors are assembled into heterotetramers consisting of two obligatory GluN1 subunits and combination of two GluN2 or GluN3 subunits. The GluN1 subunit occurs in a total of eight variants resulting from alternative splicing from a single gene. GluN2 occurs in four variants (A-D) and GluN3 in two variants (A, B). Each GluN2 and GluN3 subunit variant is coded by a separate gene. (Traynelis et al. 2010) NMDARs are formed into GluN1/GluN2 or GluN1/GluN3 diheteromers (Sanz-Clemente et al. 2013; Traynelis et al. 2010) but can also occur as triheteromeric GluN1/GluN2A/GluN2B (Rauner and Köhr 2011) or GluN1/GluN2/GluN3A receptors (Sasaki et al. 2002). Different subunit combinations are present in different parts of the nervous system and vary during development having a unique function (Traynelis et al. 2010). The subunit composition of each NMDA receptor dictates its Ca^{2+} permeability, agonist/antagonist affinity, desensitization and deactivation periods. GluN2A containing NMDA receptors have the highest open probability, but the lowest glutamate affinity compared to the other subunit types. GluN2C, GluN2D and GluN3 all lower the Ca^{2+} permeability as well as sensitivity to Mg^{2+} and open channel blockers compared to GluN2A and GluN2B (Paoletti et al. 2013).

2.3.Functional domains

All types of GluN subunits show high structural homology and consist of the same four functional domains connected by linkers that carry the conformational changes throughout the receptor. The amino-terminal domain (ATD) and the ligand-binding domain (LBD) are extracellular. They both have a clamshell-like shape which is formed by two subdomains - S1 and S2 in LBD and R1 and R2 in ATD. The ATD has a role in the inter-subunit connection, interaction with extracellular proteins, and also contains binding sites for Zn^{2+} and various drugs which in turn regulate the function of the receptor. The ligand binding domain as the name suggests contains binding sites for agonists. The transmembrane domain (TMD) is anchored in the membrane and forms the channel of

the NMDA receptor. It consists of three transmembrane helices (M1, M3, M4) and a return loop (M2). The pore of the channel is composed of the M3 helices, carrying the amino acid motif responsible for ion selectivity and the M2 loop, which contains the binding site for Mg^{2+} and open channel blocker inhibitors. The carboxy terminal domain (CTD) is located intracellularly and interacts with several associated proteins (Traynelis et al. 2010).

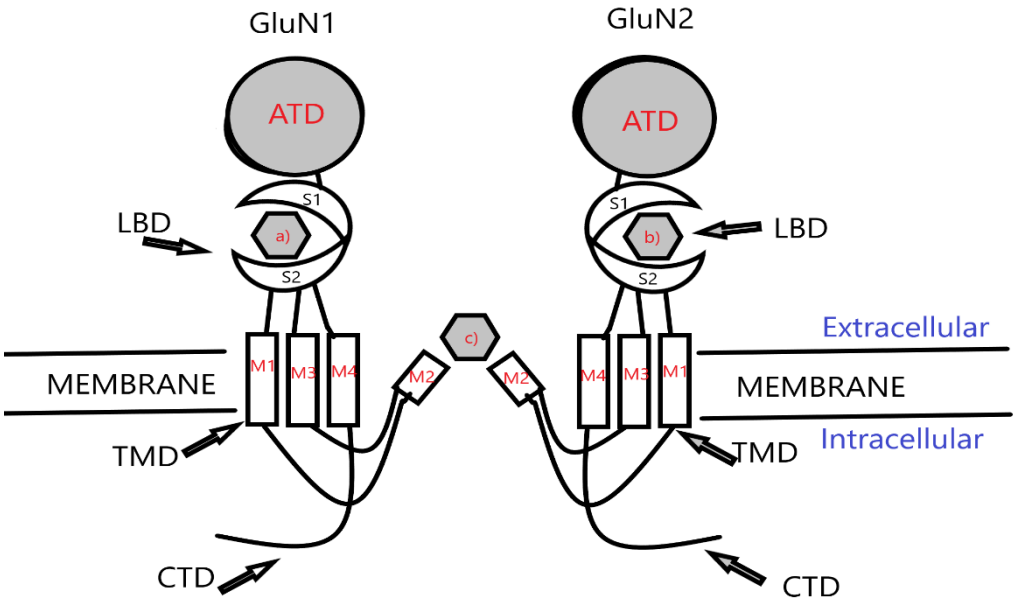

Figure 1: Simplified cross section of NMDA receptor showing GluN1 and GluN2 subunits. a) binding site for L-glycine/D-serine b) binding site for L-glutamate/D-aspartate c) binding site for Mg^{2+} and open channel blockers. (adopted from Traynelis et al. 2010; edited)

2.4.Activation and functional properties

The mechanism of activation was deduced after investigating these mechanisms in related channels from the glutamate receptor family (AMPA, kainate) due to the strong homology of key structures, but since then, many studies examined the properties of NMDA receptor itself (Ladislav 2018). To open the channel, binding of L-glycine or D-serine to the LBD of each GluN1 subunit and L-glutamate, D-aspartate or NMDA to the LBD of the GluN2 subunit (or another L-glycine in case of the GluN3 subunit containing variant) is required (Patneau, Mayer 1990; Chatterton et al. 2002). After ligand binding, the S2 domain is bend towards the S1, closing the clamshell structure and causing conformational changes carried by linkers to the TMD and subsequent channel opening

and the flow of ions such as Na^+, K^+ and Ca^{2+} through the pore. NMDA receptor function is also regulated by Mg^{2+} voltage-dependent block. Many glutamate-activated synapses work with glutamate receptors in tandem, meaning that the membrane is first depolarized by the activation of a non-NMDA receptor, which releases Mg^{2+} from its binding site within the NMDA receptor channel, allowing the flow of cations (Ladislav 2018).

2.5.Basic antagonist pharmacology

There are three types of NMDA receptor inhibitors, competitive inhibitors that bind to the ligand binding domain and don't allow the agonist to bind, allosteric inhibitors that change the steric conformation of the NMDA receptor and uncompetitive inhibitors (open channel blockers) that bind directly into the channel of NMDA receptor. Ketamine and memantine are both mainly uncompetitive antagonists, but can also modify the NMDA receptor allosterically (Traynelis et al. 2010).

3. Role of NMDA receptors in excitotoxicity

As stated earlier, NMDA receptors play a role in phenomenon known as NMDA excitotoxicity. The term first used by J.W. Olney in the early 1970s (Olney 1971), however, the toxic effects of high doses of glutamate were already observed by T. Hayashi in 1954 (Hayashi 1954). At that time no connection between excitotoxicity and NMDA receptors was made since they weren´t yet discovered. The mechanism of excitotoxicity itself was described later, after the discovery, that NMDA receptors are permeable for Ca^{2+} (MacDermott et al. 1986). Ca^{2+} is an important second messenger in several cascades, and cell death is one of them (Schanne et al. 1979). In cases where NMDA receptor channels are more active than is physiologically normal, the intracellular concentration of Ca^{2+} ions increases, which in turn triggers processes leading to cell death. This can be a result of pathogenic mutations or pathological processes in the body making the NMDA receptors more easily activated, staying open for long periods, or being expressed on the cell surface in larger numbers than in healthy individuals (XiangWei, Jiang, and Yuan 2018). Another reason for NMDA receptor hyperactivity can be simply too high concentrations of its agonists, arising from another pathological state, such as in the case of stroke and brain injury or administration of certain drugs (Castillo, Dávalos, and Noya 1997). It is then logical to predict that pharmacological inhibition of such hyperactivated receptors can alleviate the pathological manifestations of some neurological disorders caused by increased neuronal death due to NMDA receptor excitotoxicity. Studies have shown improved neuronal survival of excitotoxic insult when treated with NMDA receptor antagonists (Choi, Koh, and Peters 1988). Note, that not all NMDA receptors are equal in their excitotoxic effects. NMDA receptor localization and subunit composition determining the pathways they trigger upon activation. NMDA receptors containing the GluN2B subunit tend to be localized more on the extrasynaptic membrane and seem to be the dominant culprit when it comes to NMDA receptor-related excitotoxicity, but there is still a great deal of contradictory data for this hypothesis to be fully affirmed (Parsons and Raymond 2014).

4. Role of excitotoxicity in selected neurological and mental diseases

4.1.Alzheimer's disease

The cause of Alzheimer's disease has long been a mystery to science, but in the last few decades many plausible mechanisms have been proposed and glutamate excitotoxicity is among them. A dysregulation in glutamate transporters and receptors and subsequent impaired extracellular glutamate clearing have been found in Alzheimer's patients (Jacob et al. 2007). Beta-amyloid plaques, another studied factor in AD was also connected to increased excitotoxicity (Koh, Yang, and Cotman 1990). The connection between glutamate excitotoxicity and Alzheimer's disease was thoroughly reviewed by Hynd et.al. in Neurochemistry 2004 (Hynd, Scott, and Dodd 2004).

4.2.Epilepsy

It has been shown that impaired NMDA receptor signaling plays a substantial role in the epileptic episodes. In epilepsy models, there is evidence for increased concentrations of extracellular glutamate (Soukupova et al. 2015). Moreover, the normal expression of glutamate transporters seems to be disrupted in epilepsy (Hubbard et al. 2016). Many patients that suffer from frequent epileptic seizures have been diagnosed with a form of NMDAR mutation which can increase neuron susceptibility to excitotoxic events by changing the NMDA receptor sensitivity to agonists and channel open probability (Sibarov et al. 2017). These NMDA receptor signaling impairments are perfect conditions for the development of excitotoxic neuronal damage which often leads to other neurological and cognitive impairments in epilepsy patients.

4.3.Major depressive disorder

For a long time, monoamine disruption was the main theory behind the mechanism of depression development, but recently more evidence for the role of glutamate has come to light. A meta-analysis of brain imaging studies found a significant volumetric reduction of either certain brain regions or even total cerebral volume in patients diagnosed with depression (Koolschijn et al. 2009), which in conjunction with dysregulations of glutamate reuptake systems (Niciu et al. 2014) point to excitotoxicity possibly playing a role in treatment-resistant depression. Similar claims of this connection have already been made in review papers discussing the glutamate hypothesis of depression (Sanacora, Treccani, and Popoli 2012).

4.4.Ischemic stroke

According to the World Health Organization statistics, ischemic stroke is among leading causes of death in developed countries. Its survivors are often struck with debilitating long-term effects caused by massive neuronal death in the oxygen-deprived brain. Although it cannot be held accountable for all the damage, NMDA receptor excitotoxicity seems to have a non-negligible role in stroke pathophysiology. Elevated levels of excitatory amino acids have been measured in animal and *in vitro* models as well as in actual stroke patients (Benveniste et al. 1984; Baker et al. 1991; Castillo, Dávalos, and Noya 1997; Kimura et al. 1998). In the case of progressing stroke, the excitatory amino acid concentrations stayed elevated up to 24 hours after onset (Dávalos et al. 1997). One case report measured a 300-fold increase in extracellular levels of excitatory amino acids persisting up to 6 days after a massive stroke (Bullock et al. 1995). These findings show solid evidence for the role of NMDA receptor excitotoxicity in the pathology of ischemic stroke, further backed by *in vitro* hypoxia studies that linked neuronal damage to NMDA receptors (Goldberg et al. 1987).

5. Memantine

5.1.General characteristics

Memantine is a medication of the adamantane class. It is widely used to treat Alzheimer's and Parkinson's disease. It was first synthesized by Eli Lily and Company in 1968 as potential diabetes medication. After its mind-altering effects were discovered, research has focused on using it for treating Alzheimer´s disease, but it wasn´t until 1980 that its interaction with NMDA receptors came to light.

5.2.NMDA receptor inhibition

There are two direct ways and one indirect way how memantine inhibits the NMDA receptors' activity. Memantine binds directly into two distinct binding sites. First is located in directly into the ion channel pore and block NMDA receptors in a use-dependent block manner, same way as ketamine does (Schmitt, Ryan, and Cooper 2007). Memantine can also bind to a site located in the ligand-binding domain and cause allosteric inhibition (Glasgow, Wilcox, and Johnson 2018). There is also evidence that memantine when applied to rodent brain slices increases the production of kynurenic acid, an endogenous NMDAR antagonist (Kloc et al. 2008). This indirect effect of memantine can be the third route of NMDA receptor inhibition. Memantine has lesser affinity for NMDA receptors compared to other antagonists, so it is more suitable for certain treatments since it allows for some level of physiological NMDAR activity to persist (Rammes, Danysz, and Parsons 2008).

5.3.In vitro studies

A large body of evidence for memantine's neuroprotectivity *in vitro* has been accumulated throughout the 1990s and early 2000s. Most of the studies share a similar methodic of applying memantine and NMDA receptor agonists to neuronal cultures, with the only differences being the types of neuronal culture used, different memantine and agonist concentrations, and times of memantine application. Despite the minor methodical differences, all the studies presented similar results. All cultures treated with memantine had significantly more surviving cells after the insult than those without treatment (Erdö and Schäfer 1991; Volbracht et al. 2006). A different approach was chosen in another study, where rat cortical neurons were plated on a microelectrode array and their firing patterns were recorded. The study found that memantine application preserved the synchronized firing of neuronal networks after glutamate insult (Kutzing, Luo, and Firestein 2012). Apart from studies focused on direct glutamate insult,

memantines potential to protect neurons exposed to beta-amyloid peptide was also examined. Although memantine was effective at decreasing neuronal damage, it did not affect beta-amyloid clearance or decreasing extracellular glutamate hyper-release (Song et al. 2008).

5.4.Animal studies

5.4.1. Artificial excitotoxicity experiments

After successfully proving memantine's anti-excitotoxic ability *in vitro*, research shifted focus to animal models. Although different methods of inducing glutamate-induced excitotoxicity were deployed, all animal groups injected with memantine showed a significant reduction in neuronal loss compared to those without treatment. In one study, they repeatedly injected rats with different compound cocktails intraperitoneally or intravitreally for three weeks. Injections consisted of either pure low dose glutamate, low dose glutamate with memantine and control groups got saline or low dose memantine. Rats given memantine together with glutamate retained more healthy systemic and retinal neural tissue, compared to the control group injected with glutamate only. The group treated with only memantine without inducing excitotoxic insult by glutamate exhibited no difference compared to the group injected with only saline (Vorwerk et al. 1996). A very different approach was chosen in another study, where supraphysiological glutamate concentrations were induced by methylmercury injections. Here again, the group injected with memantine prior to methylmercury administration had a better outcome in terms of less neuronal damage and fewer morphological malformations of the cortex. However, memantine had no effect on reducing the excess glutamate and it seems it might have negatively affected the mercury clearing since the treated group had slightly higher mercury levels in the brain when compared to the untreated group (W. Liu et al. 2013). These studies however focused only on the histological side of treatment and didn't take memantine's cognitive effects into account.

5.4.2. Alzheimer's disease

Since the discovery of the role of beta-amyloid in Alzheimer's disease, many treatment options were studied in this context and memantine is no exception. Two main approaches were used in the studies. In the first approach, they directly injected beta-amyloid into the brains of animals. In the second, they used transgenic animals expressing human presenilin-1 (PS1) and amyloid precursor protein (APP) which caused high production of beta-amyloid plaques. In the case of direct beta-amyloid injections, the

results were determined by histological examination of the brain slices. The groups treated with memantine had significantly less neuronal damage in their hippocampi compared to the animals who received no treatment (Miguel-Hidalgo et al. 2002; Colom et al. 2013). Although these results are very promising for memantine efficacy, simply injecting animals with beta amyloids does not fully simulate the whole complex of processes that undergo in Alzheimer patients. Also, the results only show decreased neuronal loss, but that does not mean there is no other damage to the functional structure of the neural tissue as a whole. The transgenic mice studies, on the other hand, implemented a variety of cognitive-behavioral tests which better reflect the overall outcome of memantine treatment. After a single oral dose of 5 mg/kg of memantine the transgenic mice exhibited substantial improvement in memory and cognitive performance, compared to other groups receiving either donepezil or no treatment (Nagakura et al. 2013). In another study both the transgenic and wild type mice received 30 mg/kg memantine daily for four months. The transgenic group receiving memantine performed significantly better in the water maze test than their untreated transgenic counterparts. In this case, memantine had no apparent effect on the locomotor ability, exploration or aggression in neither transgenic nor wild type group (Minkeviciene, Banerjee, and Tanila 2004). One of the most thorough studies used mice expressing also human tau protein in addition to PS1 and APP and examined their performance in water maze test, object recognition test and passive inhibitory avoidance test. After consuming 30 mg/kg memantine daily for three months the mice improved significantly their cognitive performance. Additionally, an immunohistochemical assay showed that memantine treatment successfully lowered the total beta-amyloid accumulation in the brains of the transgenic mice. Again, no apparent effect was noticed in healthy mice treated with memantine (Martinez-Coria et al. 2010). These results form a solid case for memantine's efficacy in the treatment of Alzheimer's disease.

5.4.3. Ischemic stroke

The administration of memantine was repeatedly proven able to reduce the impact of reversible ischemia in animal models, but the differences in experiment design and subsequent differences in results make it hard to compose a definitive resolution on its effectiveness in rodents. While all studies found a reduction of lesion size and neuronal damage, the dose-response varied substantially. One team saw the neuroprotective effect at dosages as low as 0.2 mg/kg, but 10 mg/kg was ineffective for others. Also, while one

behavioral study had successfully improved cognitive performance with doses of 20 mg/kg, another study examining the histology of treated mice brains reported that this dose actually causes more neuronal damage (Block and Schwarz 1996). In another study, mice consuming 30 mg/kg memantine daily for 28 days after the induction of ischemia started improving their motor skills after the first 7 days of treatment (López-Valdés Héctor E. et al. 2014). One study found that daily subcutaneous injections of 20 mg/kg of memantine starting 3 days after ischemia induction and continued for 28 days improved mice's performance in motor skill and spatial memory tests. An improvement was also noted in brain histology, notably improved plasticity and injury site remodeling and decreased atrophy. Biochemical assays found increased concentrations of brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, and vascular endothelial growth factor and reduced expression of GluN2B while GluN2A expression was increased. None of these effects were seen with 4 mg/kg daily doses (Wang et al. 2017).

5.4.4. Depression

With the advent of the glutamate hypothesis of depression memantine naturally came up as a potential treatment candidate. Both acute and chronic memantine administration in doses 5-20 mg/kg effectively reduced depression-like behavior in rats subjected to forced swimming. Additionally, acute administration of 20 mg/kg memantine increased brain-derived neurotrophic factor production in these rats (Réus et al. 2010). Another study subjecting the rats to chronic unpredictable stress while dosing them with 20 mg/kg memantine daily reported a reduction in depression-like behavior and also spatial memory impairment in treated animals, speculating it may be caused by dysregulated GluN2B expression (Quan et al. 2011). Memantine also reduced depression symptoms, improved stress tolerance and reduced neural apoptosis in mice after olfactory bulbectomy (Takahashi et al. 2018).

5.4.5. Epilepsy

Memantine has proven effective at reducing neuronal damage caused by pilocarpine-induced status epilepticus when administered both before and after the seizure induction in rats. The effect was shown to be dose and time-dependent with higher doses given close to the seizure occurrence were the most effective (Zenki et al. 2018). Another study used lithium chloride and pilocarpine to induce seizures and deployed behavioral tests to quantify the cognitive impairment following the seizure. The rats showed signs of impaired spatial memory and orientation which were ameliorated in the group treated

with memantine (Kalemenev et al. 2016). On the other hand, memantine was shown to induce seizures in amygdala kindled rats, but this method is surrounded by a bit of controversy because although being a good model for studying the collateral effects of seizures in healthy animals, kindling is not thought to play a role in most epilepsy patients (Löscher and Hönack 1990; Bertram 2007).

5.5.Clinical studies

5.5.1. Stroke

Two clinical studies on the use of memantine in recovering stroke patients have been completed so far. One of them was open-label, randomized, and placebo-controlled. They enrolled 53 patients admitted to the hospital with mild to moderate stroke within 24 hours and divided them into two groups with roughly the same age, sex and comorbidity factor distribution. Both groups were treated according to the standard practice while one group received 20 mg memantine three times a day and the other placebo for a total of five days. The patients were scored according to the National Institute of Health Stroke Scale at the time of the admission and then every day of the duration of the study. In the end patients treated with memantine on top of standard treatment had improved their average NIHSS score by 2.96 compared to just 1.24 in the placebo group (Kafi et al. 2014). Another study looked at using memantine for the treatment of chronic poststroke aphasia. 28 patients have enrolled in the double-blind randomized phase of the study, where they received either 5 – 10 mg of memantine a day or a placebo for a total of 24 weeks. All patients were undergoing constraint-induced aphasia therapy during this time. Both groups had improved their communicative abilities, but the one receiving memantine had a significantly better outcome. After that a 24-week open-label phase continued and all patients received memantine. Here the former placebo group experienced greater improvement, but the effect was not as pronounced in the group who received memantine prior. This is probably due to established tolerance to the compound. This study does not directly show memantine's anti-excitotoxic qualities, since there is no evidence for increased glutamate signaling several years after the stroke occurred, more likely, this stems from memantine's neuro-regenerative properties via brain-derived neurotrophic factor and other neuroregenerative factors increase (Berthier et al. 2009).

5.5.2. Alzheimer's disease

A meta-analysis from 2015 looking at 9 clinical memantine monotherapy studies with total of 2433 patients found a consistent improvement in all measured scales of cognition and quality of life compared to placebo without any serious side effects (Matsunaga, Kishi, and Iwata 2015). Another meta-analysis from 2017 examined 30 clinical studies with total of 7567 patients consisting of monotherapy, combination with cholinesterase inhibitors and comparison studies. Here again memantine was assessed as successful at improving cognition and quality of life in all patients without causing any notable side effects (Kishi et al. 2017).

5.5.3. Epilepsy

A study done on epilepsy patients with memory dysfunction showed significant improvement in both the double-blind and subsequent open-label phase, however, the researchers speculated whether memantine was the main factor or the patients got better at solving the memory test due to acquired practice. Apart from memory, memantine also improved the general cognitive performance and overall quality of life (Leeman-Markowski et al. 2018). Another study looked at 50 epilepsy patients with cognitive impairment. All patients were given 5 mg memantine a day for two months and their cognitive performance was frequently evaluated. Although memantine had no effect on seizure frequency or severity it did improve patients' cognitive performance significantly, but these results should be taken lightly since the study didn't include any placebo control (Solomatin et al. 2016). Memantine was also well tolerated when given to patients already treated with antiepileptic drugs and also managed to improve their cognition in phase III double-blinded placebo-controlled clinical trial (Marimuthu et al. 2016). When compared with an acetylcholinesterase inhibitor donepezil, memantine provided greater cognitive and memory improvements for epilepsy patients at the same daily dose of 10 mg (Oustad et al. 2020).

5.5.4. Depression

Two studies found that memantine in doses 5 – 20 mg a day for 8 weeks or 10 – 20 mg for 12 weeks did not produce different outcome from placebo in patients diagnosed with major depressive disorder (Lenze et al. 2012; Zarate et al. 2006). But an open-label study using a progressive increase of dosage up to 40 mg a day found a significant reduction in depressive symptoms without major side effects (Ferguson and Shingleton 2007). One study examining the effects of memantine combined with escitalopram in patients with

geriatric depression found a greater increase in gray matter volume compared to escitalopram with placebo, but neither group had any changes in mood following the treatment (Krause-Sorio et al. 2020).

6. Ketamine

6.1.General characteristics

Ketamine is a dissociative anesthetic from the group of arylcyclohexylamines. Dissociative anesthesia refers to a state in which a person under the influence of a given anesthetic is still partially conscious and shows signs of alertness such as eye movement, but no reaction to pain. It was first synthesized by Calvin Lee Stevens in 1962 in search of possible alternatives to phencyclidine. During the first years of testing it was known as Cl-581. Two enantiomers are available (R)-ketamine and (S)-ketamine, from which the latter exhibits a much greater affinity for NMDA receptors (Lodge, Anis, and Burton 1982).

6.2.NMDA receptor inhibition

Arylcyclohexanimes such as ketamine have a common binding site directly in the NMDA receptor channel, so their binding can only occur after the channel is opened. As in the case of Mg^{2+} inhibition, the change in membrane current pulls the positively charged ketamine molecule into the channel pore. The more NMDA receptor channels are activated on a given neuron the more ketamine can bind into them. This mechanism is referred to as the use-dependent block. The subsequent activation and opening of the NMDA receptor channel are also needed to release the arylcyclohexamines from the binding site, so the molecule may be "trapped" within the closed channel and dissociate only after the next activation which opens the ion channel. Apart from binding directly into the channel, ketamine can also bind to an allosteric site located on the intermembrane portion of the receptor and cause a drop in the open probability of NMDA receptors (Orser, Pennefather, and MacDonald 1997).

6.3.Metabolism

Ketamine is known to be turned into several metabolites after administration, some of which show varying levels of affinity for NMDA receptors themselves. This has to be taken into consideration whenever a study moves from *in vitro* to *in vivo* stage (Zanos et al. 2018).

6.4.In vitro studies

To date, ketamine's neuroprotective qualities have been fairly well studied *in vitro*. Several studies confirmed ketamine's ability to protect cultured neurons from glutamate-induced excitotoxicity. Out of these, there are two that laid the foundation for a lot of future ketamine-mediated neuroprotection research. One of them found that the introduction of ketamine to cultured cortical neurons exposed to supraphysiological

levels of extracellular glutamate had a net positive effect on their survival (Choi et al. 1990). In the other study, ketamine application to cultured hippocampal neurons made them more likely to survive the glutamate insult and axotomy. This study also compared the potency of the two ketamine isomers and found that (S)-ketamine is more neuroprotective than its counterpart (Himmelseher, Pfenninger, and Georgieff 1996). Interestingly, it seems that neuroprotective effect of ketamine is not only associated with protecting neurons from excess glutamate exposure by decreasing the opening of NMDA receptor. (S)-ketamine has been shown to upregulate the expression of neuroregenerative factors *in vitro*, however further examination is needed to prove this effect *in vivo* (Himmelseher et al. 2000) Ketamine was also successful in protecting neurons exposed to cyanide and hypoxic environment, which served as a basis for making it one of the candidates for treatment of ischemic stroke (Weiss, Goldberg, and Choi 1986). As good as this all sounds, there are some drawbacks when it comes to ketamine's overall effect on neurons. A major one would be ketamine's own inherent neurotoxicity especially in high doses or with frequent administration. When applied to cultured rat neurons, ketamine not only induced generation of reactive oxygen species, but also upregulated NMDA receptor expression in cells which is can be more detrimental for a brain with pathologically high levels of extracellular NMDA receptor agonists or otherwise increased Ca^{2+} inflow through these channels (F. Liu et al. 2013). However, it is possible to reduce this effect when ketamine is stacked together with other drugs such as thiopental sodium (Shibuta, Varathan, and Mashimo 2006).

6.5.Animal studies

6.5.1. Artificial excitotoxicity experiments

So far only one animal study examining ketamine in the context of neuroprotection against NMDA receptor agonist insult has been made. In this study rats were injected with either NMDA to cause the excitotoxic event in the brain. Researchers found that a dose of 180 mg/kg given up to two hours after, but not before NMDA injection was able to lessen the neuronal death in rats' hippocampi. They also noted that dividing the total dose into several smaller ones worked better than if given all at once (Lees 1995). Ketamine was actually more studied in the context of its neurotoxicity rather than neuroprotective activity in animals. For this reason, scientists often look at studying ketamine as a neuroprotectant with skepticism (Rudin et al. 2005).

6.5.2. Depression

Single subanesthetic doses of ketamine have been effective at acutely reducing depression behavior in mice subjected to chronic unpredictable stress, but the effects were short-lived and continuous treatment saw no benefit (Jiang et al. 2017). No anti-depressive effect was exhibited in rats treated acutely nor continuously with anesthesia-inducing doses (Popik et al. 2008). However, another study using both mice and rats found a significant acute improvement in depressive behavior after (S)-ketamine administration and even long-lasting effect after (R)-ketamine administration (Fukumoto et al. 2017). Another study suggested, that the anti-depressive effects might be age-dependent since no effect was seen in juvenile mice (Nosyreva et al. 2014). A comparative study found that administration of GluN2B selective antagonist Ro-25-6981 or early growth response protein 1 (Erg-1) siRNA produced the same anti-depressive effects as ketamine in mice subjected to chronic unpredictable stress and therefore theorized that ketamine produces these effects through antagonizing GluN2B-containing NMDA receptors and subsequent regulation of Egr-1 expression (Zhang et al. 2018). Another study subjecting mice to chronic mild stress found increased expression of GluN1 in stressed mice, which was ameliorated by ketamine and their depression behavior was also reduced (Tang et al. 2015).

6.5.3. Ischemic stroke

Ketamine was quite thoroughly studied in animal models of ischemic stroke with mixed results. One of the first experiments was carried out in 1987 on Mongolian gerbils whose brains are especially vulnerable to carotid blockade. The researchers found that injecting the gerbils with 100, 150, and 200 mg/kg of ketamine before inducing ischemia reduced the number of deaths compared to the animals anesthetized by ether before the procedure. Histopathological examination of the brains was carried out after sacrificing the recovered animals. The groups treated with ketamine had retained significantly more healthy neurons than the untreated in a dose-dependent manner (Marcoux, Goodrich, and Dominick 1988). Another study saw no benefit from injecting rats with 10 mg/kg before and after inducing ischemia but bringing the total dose up to 200 mg/kg divided into several doses over 8 hours preserved more neurons in rats' hippocampi (Church, Zeman, and Lodge 1988). On the other hand, in a study administering 24 mg/kg before inducing forebrain ischemia and then 120 mg/kg a day intramuscularly to rats for seven days, there was no difference in brain necrosis compared to the untreated group (Jensen and Auer

1988). One study used sodium fluoride injections instead of blood flow blockage to simulate ischemic conditions. They found that simultaneous injection of ketamine with sodium fluoride had no effect on mice survivability, but pretreating them with 150 mg/kg ketamine prolonged the survival time, however the mice still died after two hours (Vasilev et al. 1998).

6.5.4. Alzheimer's disease

There are currently no animal nor clinical studies examining the use of ketamine in the treatment of Alzheimer's disease. There are two main reasons why it is not considered a viable route to be worth examining. One being ketamine's neurotoxic effects when taken chronically or in high doses and the other would be ketamine's plethora of undesirable physical and mental side effects. Simply put, unless the patient enjoys being high on ketamine for the rest of their life it would likely have no positive effect on their quality of life but rather the opposite.

6.5.5. Epilepsy

In one study, seizures were induced in rats by intracranial application of bicuculline. A 10 mg/kg dose of ketamine was injected intraperitoneally 15 minutes before and then every 30 minutes the bicuculline application for a total of four hours. Although ketamine did not stop the seizures from occurring nor reduced the epileptiform EEG activity, it was effective at reducing the subsequent neuronal damage. 80% of animals treated with ketamine had no signs of necrosis in the brain, while all of the untreated ones did (Clifford, Zorumski, and Olney 1989). Another study induced seizures by injecting rats with lithium chloride mixed with pilocarpine intraperitoneally. Here ketamine decreased the intensity and duration of status epilepticus and post epileptic anxiety. Apart from this, ketamine also reduced neuronal death by 85 - 100 % (Loss, Córdova, and de Oliveira 2012).

6.6. Clinical studies

6.6.1. Stroke

Given the mixed results from rodent studies and the many known side effects of ketamine in dosages needed to induce neuroprotection, it never managed to move into a clinical phase of testing for stroke treatment and it is quite unlikely to ever be tested.

6.6.2. Epilepsy

A review examining 41 case studies of 248 refractory epilepsy patients reported ketamine to be highly effective at stopping the seizures in these patients, but no controlled clinical trials for the use of ketamine in epilepsy exist to date (Rosati, De Masi, and

Guerrini 2018). Although it was not studied in the context of neuroprotection in epilepsy patients, the fact that ketamine can ameliorate the frequency and severity of seizures in some patients resistant to treatment, might mean it can also indirectly reduce the neuronal damage produced by the seizures.

6.6.3. Depression

A meta-analysis published in 2020 looked at 20 randomized placebo-controlled studies examining the anti-depressive effects of single-dose or repeated ketamine administration. They reported single dose of ketamine being effective at improving mood up to 7 days after administration and up to 3 weeks after repeated dosing (Kryst et al. 2020). Two studies deploying brain imaging techniques reported that ketamine administration can reverse structural changes and volumetric loss in the brains of depressed patients (Dai et al. 2020; Zhou et al. 2020).

7. Conclusions and discussion

The current knowledge suggests that dysregulation of glutaminergic system and NMDA receptor dysfunction play a significant role in Alzheimer's disease, epilepsy and ischemic stroke. For this reason, the study of NMDAR antagonists as treatment for these diseases is highly desirable.

Memantine proved itself effective at improving the outcomes and quality of life in Alzheimer's disease, epilepsy and stroke patients and at ameliorating the pathological processes in these diseases. However, it still falls short of being able to treat these diseases on its own, rather it makes a good supplement drug for already used therapies. Ketamine, on the other hand, was most effective only *in vitro* conditions, and apart from helping with controlling seizures in few cases of epilepsy, it got no further attention to be studied as a treatment for Alzheimer's disease or ischemic stroke, which, given its undesirable effects, is quite understandable.

The glutaminergic hypothesis of depression is gaining attention in the scientific community partly thanks to the success of ketamine in recent clinical trials, but since memantine failed to replicate these results it is more likely that ketamine's anti-depressant effect comes from affecting a different pathway. However, ketamine's ability to reverse structural changes in the depressed brain may come from preventing excitotoxicity and is responsible for the long duration of the anti-depressant effects.

Overall, ketamine and memantine are both very interesting and important substances with a lot of potentials. Rationally developed memantine and ketamine derivatives can suppress side effects and open a new way in the treatment of neurological and mental diseases in the future.

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