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mTOR activation is required for the anti-alcohol effect of ketamine, but not memantine, in alcohol-preferring rats

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Abstract

Glutamate NMDA receptors mediate many molecular and behavioral effects of alcohol, and they play a key role in the development of excessive drinking. Uncompetitive NMDA receptor antagonists may, therefore, have therapeutic potential for alcoholism.

The first aim was to compare the effects of the NMDA antagonists memantine and ketamine on ethanol and saccharin drinking in alcohol-preferring rats. The second aim was to determine whether the effects of the two NMDA receptor antagonists were mediated by the mammalian target of rapamycin (mTOR).

TSRI Sardinian alcohol-preferring rats were allowed to self-administer either 10% *w/v* ethanol or 0.08% *w/v* saccharin, and water. Operant responding and motor activity were assessed following administration of either memantine (0–10 mg/kg) or ketamine (0–20 mg/kg). Finally, ethanol self-administration was assessed in rats administered with either memantine or ketamine but pretreated with the mTOR inhibitor rapamycin (2.5 mg/kg).

The uncompetitive NMDA receptor antagonists memantine and ketamine dose-dependently reduced ethanol drinking in alcohol-preferring rats; while memantine had a preferential effect on alcohol over saccharin, ketamine reduced responding for both solutions. Neither antagonist induced malaise, as shown by the lack of effect on water intake and motor activity. The mTOR inhibitor rapamycin blocked the effects of ketamine, but not those of memantine.

Memantine and ketamine both reduce alcohol drinking in alcohol-preferring rats, but only memantine is selective for alcohol. The effects of ketamine, but not memantine, are mediated by mTOR. The results support the therapeutic potential of uncompetitive NMDA receptor antagonists, especially memantine, in alcohol addiction.

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Disclosure/Conflict of Interest

The authors declare no conflict of interest.

Keywords

Animal model; Ethanol; NMDA; Drinking; Rapamycin; Glutamate; Addiction; Rat; Reinforcement; Reward

1. Introduction

Although 140 million people worldwide have been estimated to suffer from alcohol dependence [1], few medications are currently available to treat this disease. In recent years, major advances have been made in our understanding of the neurobiological basis of alcoholism, opening novel avenues in the development of new pharmacotherapeutics.

It has been suggested that many of the behavioral effects of ethanol are mediated by the blockade of the N-methyl-d-aspartate (NMDA) type of excitatory glutamate receptor, which is among the highest affinity targets for ethanol in the brain [2, 3]. Furthermore, ethanol blocks NMDA receptor function in a dose-related manner, by binding to a hydrophobic pocket that is distinct from other modulatory binding sites [4, 5]. In response to the chronic blockade of NMDA receptors associated with sustained ethanol administration, ligand binding, as well as mRNA and protein levels of the NMDA receptors increase in several brain areas. These changes are thought to, in turn, sustain heavy drinking and promote relapse [6].

A promising pharmacological target for the treatment of alcohol dependence is, therefore, the NMDA receptor; thus, antagonists to this receptor may have therapeutic potential by suppressing withdrawal, hindering the development of tolerance and targeting glutamatergic alterations that might contribute to cognitive dysfunction [7].

Several NMDA receptor antagonists have been tested in humans as potential drugs for the treatment of alcoholism. The anti-craving drug acamprosate has been shown to modulate the activity of NMDA receptors, which suggests that its therapeutic effects may be due, at least in part, to its influence on this channel [8, 9]. Administration of the NMDA antagonist ketamine to recovering alcoholics has been shown to reduce negative symptoms and dysphoria [10]. More recently the NMDA receptor antagonist memantine, clinically used and well-tolerated for the treatment of dementia [11], has been shown to reduce cue-induced alcohol craving in recovering alcohol-dependent patients, without producing negative effects on cognitive performance [12]; these findings suggest that well-tolerated NMDA receptor antagonists, such as memantine, could potentially be useful to treat alcohol addiction [6, 10, 12–15].

The potential pharmacological use of NMDA receptor antagonists for the treatment of alcoholism has also been demonstrated in several preclinical studies. Indeed, memantine has been shown to reduce alcohol consumption in a number of animal models, including volitional intake and relapse models [16–18], and to possess ethanol-like discriminative stimulus properties [19–21]. Similarly, other uncompetitive NMDA channel blockers, including dizocilpine ((+) MK-801) and ketamine, can reduce ethanol-seeking behavior and substitute for alcohol in drug discrimination tasks [10, 21, 22].

Both memantine and ketamine are classified as “uncompetitive” NMDA antagonists, i.e. they require that the receptor pore be open, in order to bind to the internal sites, which is different from those of the agonists, and they can remain trapped inside the channel following its closure [23]. Open channel blockers, therefore, only enter a channel opened by an agonist and block excessively activated NMDA receptors, while sparing normal glutamatergic neurotransmission.

However, whether memantine and ketamine reduce alcohol consumption and craving with the same mechanism of action is currently unknown. Recently, the rapid antidepressant effects of ketamine have been shown to be mediated by the mammalian target of rapamycin (mTOR) [24]; however the relationship between memantine's effects and mTOR is presently unclear. To the best of our knowledge, only a single study investigated the relationship between memantine's effect and mTOR, demonstrating that memantine treatment decreases mTOR activity [25].

Moreover, to the best of our knowledge, no studies have compared head to head the effects of the uncompetitive NMDA receptor antagonists memantine and ketamine on alcohol-related experimental paradigms. In addition, whether the anti-alcohol effects of these compounds are mediated by mTOR is unknown. For this purpose, the first aim of this study was to evaluate the effects of memantine and ketamine on ethanol and saccharin self-administration under a fixed ratio 1 (FR1) schedule in TSRI Sardinian alcohol-preferring (Scr:sP) rats trained for operant self-administration. The second aim of this study was then to determine whether the effects of the two NMDA receptor antagonists were mediated by mTOR.

2. Materials and Methods

2.1 Subjects

Subjects of this study were adult male rats derived from the TSRI Sardinian alcohol-preferring rats (Scr:sP, 29–30th generation, http://rgd.mcw.edu/rgdweb/report/strain/main.html?id=2302666), then maintained for 7–8 generations at Boston University without further selective breeding. Scr:sP rats were generated from intra-line breeding at The Scripps Research Institute from sP rats generously provided after 32 generations of selective breeding from Prof. G.L. Gessa (University of Cagliari, Italy). Rats, 55–60 days old at study onset, were housed in groups of two-three per cage in a humidity- and temperature (22 °C)-controlled vivarium on a 12-h light–dark cycle (lights off at 10:00 am) with water and regular rodent chow available *ad libitum* at all times. Experiments were conducted during the rats' dark cycle. Rats were n=8–10/group for the memantine and ketamine ethanol dose-response studies, n=9/group for the memantine and ketamine saccharin dose-response studies, n=7/group for the reversal studies, n=7/group for the motor studies (all separate sets of rats). All experimental procedures adhered to the guidelines of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of Boston University.

2.2 Drugs

Ethanol solution (10% *w/v*) was prepared using 95% ethyl alcohol and tap water. Saccharin solution (0.08% *w/v*) was prepared using saccharin sodium salt hydrate (Sigma Aldrich) and tap water. Memantine hydrochloride (1,3-dimethyl-5-aminoadamantane hydrochloride) was purchased from Acros Organics (Geel, Belgium); ketamine hydrochloride ((±)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride) was purchased from Sigma-Aldrich (St. Louis, MO); rapamycin was purchased from AK Scientific (Union City, CA). Memantine and ketamine were freshly dissolved in isotonic saline, while rapamycin was dissolved in a mixture of ethanol/cremophor/saline (1/1/18 ratio). Drugs were administered intraperitoneally (i.p.) in a volume of 1 ml/kg of body weight, using a within-subject, Latin square design. Memantine (0–10 mg/kg) and ketamine (0–20 mg/kg) were administered 30 min before the self-administration session; a dose of rapamycin without intrinsic action on ethanol self-administration (2.5 mg/kg) was administered 30 min before memantine or ketamine (60 min prior to the beginning of the self-administration sessions). The dose of rapamycin we used (2.5 mg/kg, i.p.) was based on a previous study showing that rapamycin

itself can reduce alcohol drinking at doses of 5 mg/kg and higher [26], as well as other in vivo reports [27, 28]. The doses of memantine and ketamine were chosen based on previous reports with abused drugs [16, 26, 29–32]. Test days were spaced by at least 2 intervening treatment-free days, until intake had returned to baseline levels.

2.3 Apparatus for operant oral ethanol self-administration

The test chambers used for operant oral self-administration (Med Associates, Inc., St. Albans, VT) were located in sound-attenuating, ventilated environmental cubicles. Syringe pumps (Med Associates, St. Albans, VT) dispensed ethanol or water into two stainless steel drinking cups mounted 2 cm above the grid floor in the middle of one side panel. Two retractable levers were located 3.2 cm to either side of the drinking cups [33]. Fluid delivery and recording of operant responses were controlled by microcomputers.

2.4 Self-administration procedure

Scr:sP rats were trained to self-administer 10% *w/v* ethanol, as previously reported, without any fading procedure [34, 35], under a continuous fixed ratio 1 schedule of reinforcement, wherein each response resulted in the delivery of 0.1 ml of fluid. Briefly, Scr:sP rats were first allowed continuous (24 hr/day) two-bottle choice access to ethanol (10% *w/v*) and water in their home cages for 1 week. Rats were then allowed one or two overnight (16 hr) operant sessions to ethanol with chow available *ad libitum*. Following these initial overnight sessions, all subsequent ethanol self-administration daily sessions were 30 min in duration. Lever presses had no scheduled consequences for 2.01 sec after the activation of the pumps, in order to avoid double responses as previously reported [36].

For saccharin self-administration, separate sets of rats were used; Scr:sP rats were trained to self-administer a saccharin solution (0.08% *w/v*) during 30-min FR1 sessions [34, 35]. This concentration maintained response rates similar to those elicited by ethanol.

During all sessions, rats were also allowed to press for water (0.1 ml) on the opposite lever (FR1). Testing began when performance stabilized (<15% variation across three consecutive sessions).

2.5 Motor activity

Motor activity of individually-housed, ethanol experienced Scr:sP rats was measured in Plexiglas chambers (27×48×20 cm) using an Opto-M3 activity system (Columbus Instruments, Columbus, OH) as in [37]. Activity was recorded by a computer using the Multi Device Interface software. Rats were given 3 days of acclimation to daily saline injections and allowed to habituate to the motor apparatus for 30 min before the drug treatments. Rats were then treated with either memantine (0, 5, 10 mg/kg, *i.p.*) or ketamine (0, 10, 20 mg/kg, *i.p.*) (within-subject Latin square design); motor activity was recorded for 60 min. To control for potential effects of the two antagonists on motor activity, the first 30 min post-injection were excluded from the analysis, because it represented the pretreatment time used for the self-administration tests.

2.6 Statistical analysis

Intake data were expressed as Mean ± SEM, normalized for body weight (ethanol, g/kg; saccharin and water, ml/kg). The effects of memantine and ketamine on ethanol, saccharin or water self-administration were analyzed by repeated measures one-way ANOVAs, with Dose as a within-subject factor. Data resulting from the motor experiment were analyzed using repeated measures two-way ANOVAs, with Dose and Time as within-subject factors. Data resulting from the antagonist reversal experiment were analyzed using repeated measures two-way ANOVAs, with Memantine/Ketamine and Rapamycin as within-subject

factors. For *post hoc* interpretation of effects having more than two levels, Student Newman–Keuls pairwise comparisons were used. Student's *t*-tests were used for within-subject factors having only two levels. The software packages used were Systat 12.0 and InStat 3.0 (GraphPad, San Diego, CA, USA); SigmaPlot 10.0 (Systat Software Inc., Chicago, IL, USA) was used as graphic software.

3. Results

3.1 Experiment 1: Effects of memantine on ethanol self-administration in alcohol-preferring rats

As shown in Figure 1, panel A, treatment with the NMDA antagonist memantine reduced ethanol intake (Treatment: $F(3,21)= 3.82$, $p<0.001$). Pairwise post hoc comparisons revealed that the doses of 5 and 10 mg/kg both significantly reduced ethanol intake (34.0 and 87.9% reduction, respectively, compared to vehicle-treated rats). In contrast, memantine did not alter responding for water (Treatment: $F(3, 21)= 2.06$, *n.s.*), shown in Figure 1, panel B.

3.2 Experiment 2: Effects of ketamine on ethanol self-administration in alcohol-preferring rats

As shown in Figure 1, panel C, treatment with the NMDA antagonist ketamine reduced ethanol intake (Treatment: $F(2,18)= 4.12$, $p<0.05$) in Scr:sP rats. Pairwise post hoc comparisons revealed that only the dose of 20 mg/kg significantly reduced ethanol intake (33.3% reduction compared to vehicle-treated rats). In contrast, ketamine did not alter responding for water (Treatment: $F(2, 18)= 1.94$, *n.s.*), as shown in Figure 1, panel D.

3.3 Experiment 3: Effects of memantine on saccharin self-administration in alcohol-preferring rats

As desired, the 0.08% *w/v* saccharin solution elicited levels of responding in 30 min under vehicle conditions that were comparable to response levels for 10% *w/v* ethanol (Mean ± SEM, 45.7 ± 6.9 and 43.8 ± 5.7, saccharin and ethanol, respectively). As shown in Figure 2, panel A, treatment with memantine reduced saccharin responding (Treatment: $F(2,18)= 15.13$, $p<0.001$). Pairwise post hoc comparisons revealed that only the dose of 10 mg/kg significantly reduced saccharin intake, in contrast with the doses of 5 and 10 mg/kg which were both effective in reducing ethanol intake. In addition, the degree of reduction in ethanol responding elicited by memantine 10 mg/kg was slightly greater than the reduction in saccharin responding (degree of reduction 88 vs. 70%, ethanol and saccharin, respectively), suggesting both a greater potency and a greater efficacy of memantine towards alcohol as compared to the non-drug reinforcer saccharin.

3.4 Experiment 4: Effects of ketamine on saccharin self-administration in alcohol-preferring rats

As shown in Figure 2, panel B, treatment with ketamine reduced saccharin responding (Treatment: $F(2,16)= 6.35$, $p<0.01$). Pairwise post hoc comparisons revealed that only the dose of 20 mg/kg significantly reduced saccharin intake, the same dose effective in reducing ethanol intake; therefore, ketamine was equally potent in reducing intake of ethanol and saccharin. Notably, the effective dose of ketamine (20 mg/kg) was slightly more efficacious in reducing saccharin responding than ethanol (degree of reduction 33.3 vs. 57.4%, ethanol and saccharin, respectively).

3.5 Experiment 5: Effects of memantine and ketamine on motor activity in alcohol-preferring rats

As shown in Fig. 3, neither memantine nor ketamine affected motor activity during the 30-min session (Memantine: Dose, $F(2,10)= 2.36$, *n.s.*; Dose × Time, $F(10,50)= 0.46$, *n.s.* Ketamine: Dose, $F(2,10)= 1.82$, *n.s.*; Dose × Time, $F(10,50)= 1.74$, *n.s.*), confirming the specificity of the self-administration results.

3.6 Experiment 6: Effects of the mTOR inhibitor rapamycin on the reduction of ethanol self-administration induced by memantine and ketamine in alcohol-preferring rats

As shown in Fig. 4, panel A, pretreatment with the mTOR inhibitor rapamycin - administered at a dose without intrinsic action on ethanol self-administration (2.5 mg/kg)- had no effect on memantine-induced (5 mg/kg) reduction in ethanol self-administration (Rapamycin × Memantine: $F(1,6)= 0.18$, *n.s.*).

In contrast to the lack of effect on memantine, pretreatment with a sub-threshold dose of the mTOR inhibitor rapamycin completely prevented the ketamine-induced (20 mg/kg) reduction in ethanol intake as shown in Fig. 4, panel B (Rapamycin × Ketamine: $F(1,6)= 10.37$, $p<0.05$).

4. Discussion

The main findings of the present study were as follows: *i)* The uncompetitive NMDA receptor antagonist memantine dose-dependently reduces responding for ethanol in TSRI Sardinian alcohol-preferring rats without affecting water intake or motor activity; *ii)* memantine also reduces responding for the non-drug reinforcer saccharin but at higher doses than those required to reduce ethanol; *iii)* the uncompetitive NMDA receptor antagonist ketamine reduces responding for ethanol without affecting water intake or motor activity; *iv)* ketamine reduces responding for the non-drug reinforcer saccharin at the same doses which reduce ethanol; *v)* the mTOR inhibitor rapamycin prevents the anti-alcohol effects of ketamine, but not those of memantine.

We observed that the uncompetitive NMDA receptor antagonist memantine potently blocked ethanol self-administration in Scr:sP alcohol-preferring rats. The minimum efficacious dose, 5 mg/kg, selectively reduced ethanol, but not saccharin self-administration, suggesting selectivity of action; conversely, the highest dose of memantine, 10 mg/kg, reduced both ethanol and saccharin self-administration. The 5 mg/kg dose of memantine, found here to be selective for ethanol, has been reported in rats to result in serum levels similar to therapeutic concentrations in humans [38]; in addition a 4.5 mg/kg dose has been previously shown to produce a 50% level of the ethanol-like stimulus effects without affecting the rate of operant behavior [39]. The uncompetitive NMDA receptor antagonist ketamine, a dissociative anesthetic, was also able to reduce ethanol self-administration, and, to the best of our knowledge, this is the first demonstration of such an effect. However, differently from memantine, the same dose of ketamine (20 mg/kg) which was effective in reducing ethanol responding, decreased responding for the non-drug reinforcer saccharin, suggesting a more general effect on motivated behaviors. Neither memantine nor ketamine however induced sickness or malaise in Scr:sP ethanol-experienced rats, as shown by the lack of effect motor activity. A trend towards a reduction of responding for water could be observed after ketamine (but not memantine) administration, which again would suggest a lack of specificity.

Our observation that memantine is able to reduce ethanol self-administration in Scr:sP rats supports the anti-alcohol effects of this drug demonstrated in humans and in other animal models of alcoholism, while substantiating the overall hypothesis that the NMDA receptor is

a promising pharmacological target for alcohol dependence. Indeed, NMDA receptor blockade has been shown to reduce ethanol self-administration and reward-related responses to ethanol, as well as to attenuate withdrawal from chronic ethanol exposure [16, 40–43]. Memantine, a clinically well-tolerated, low-affinity uncompetitive NMDA receptor antagonist, has been shown to reduce ethanol drinking, to attenuate alcohol deprivation effect, and to reverse cognitive impairment associated with chronic alcohol consumption and withdrawal [16, 40, 44, 45]. Ethanol responding in outbred rats is also reduced by competitive NMDA receptor antagonists, e.g. AP-5 administered intra accumbens [46] and LY 274614 administered systemically [47]. The uncompetitive NMDA receptor antagonist dizocilpine, as well as the partial agonists of the glycine site (+)-HA-966 and ACPC, also reduce the consumption of ethanol in rats [47, 48]; however, it is important to acknowledge that it has been reported that the same doses of antagonists that reduce ethanol consumption also impair the motor activity [47].

Although it is now well-established that NMDA receptors are involved in the reinforcing effects of ethanol, the mechanism by which blockade of NMDA receptors leads to a decrease in ethanol intake is still debated. It has been suggested that NMDA receptor antagonism may decrease ethanol intake by acting as a reinforcing stimulus, therefore "substituting" for ethanol, but the existing literature is controversial. This hypothesis is supported by the findings that both ethanol and NMDA receptor antagonists increase dopamine release in the nucleus accumbens and stimulate the activity of dopaminergic neurons in the ventral tegmental area [49–51]; additionally, both dizocilpine and AP-5 reinstate cocaine-seeking behavior following an extinction period [52, 53]. However, it should be noted that not all NMDA receptor antagonists increase dopamine levels in the nucleus accumbens [54–56], and that memantine reduces cue-induced alcohol craving without stimulating the craving [12]. Furthermore, although some antagonists have been shown to block ethanol-induced conditioned place preference (CPP), most of them – including memantine- do not induce CPP on their own [30, 31, 57, 58], ruling out a possible reinforcing efficacy of these drugs. These observations lead to the alternative interpretation that ethanol reinforcement requires the *activation* of NMDA receptors, and, therefore, that NMDA receptor antagonists would block ethanol drinking by preventing the reinforcing properties of ethanol [59].

In this context, it should be noted that, although most agents that block NMDA receptors have been shown to substitute for ethanol in drug discrimination studies and to produce ethanol-like effects [10, 20–22, 60], which would suggest a common mechanism of action, it is becoming progressively clearer that not all NMDA receptor antagonists are alike. Besides the general classification into competitive, non-competitive and uncompetitive [23, 61], differences have emerged even within the uncompetitive class of antagonists both in the mechanism of action and in the exerted behavioral effects. Our findings show that the effects of memantine are stronger, as well as more selective for alcohol than for the non-drug reinforcer saccharin, compared to those of ketamine. The difference between the effects of ketamine and memantine on ethanol and saccharin self-administration is an interesting point of discussion. The two antagonists share many pharmacological properties: they both bind to the internal portion of the pore and can remain trapped inside the channel following its closure; in addition, at higher agonist concentrations the two drugs inhibit with faster kinetics, and they both also show similar specificity for the NMDA receptor subtypes [23, 62]. However, ketamine has a long dwell time (i.e. slow off-rate) which causes the blockade of normal functions of the NMDA receptor, therefore producing important side effects; conversely, memantine shows lower receptor affinity, and, therefore, only a partial "trapping" within the NMDA channel, as well as a significantly faster off-rate compared to ketamine [63–65]; these important properties have been proposed to be responsible for the improved clinical tolerability and better therapeutic profile of memantine [66, 67] over the

dissociative anesthetic ketamine. Moreover, a fascinating hypothesis regarding the better profile of memantine relates to its ability, when administered at low doses, to preferentially block the chronic, pathological activation of extrasynaptic NMDA receptors induced by elevated levels of glutamate [67, 68], which may occur as a result of excessive alcohol drinking [69–73], while sparing the normal synaptic activation of NMDA receptors. This peculiar mechanism of action of memantine may therefore be responsible not only for its increased tolerability but also for its higher selectivity towards ethanol observed in the present study, making it a more suitable candidate as a therapeutic agent.

The present series of studies was performed in genetically-selected TSRI Sardinian alcohol-preferring rats, derived from the breeding of the 32nd generation of Sardinian alcohol-preferring rats without further selection. Alcohol-preferring lines voluntarily drink ethanol in high quantities and preference, possessing predictive validity for identifying pharmacotherapies for alcohol dependence [74–76] and exhibiting a heritable component similar to human ethanol dependence [77–79]. Individuals with a family history of ethanol dependence show reduced sensitivity to the dysphoric effects of both ethanol and ketamine, when compared with subjects without such a history; this is of critical importance, since reduced dysphoric responses to ethanol appear to be an important predictor of subsequent ethanol drinking problems [7, 80–82]. Reduced perceptual alterations and dysphoria in response to ketamine were also found in recovering ethanol-dependent patients [10, 14, 83]. Similar to the human findings, preclinical studies support the hypothesis that pre-existing alterations of the NMDA receptor function can influence the propensity to drink ethanol [7]. Animals bred for reduced sensitivity to ethanol effects also show reduced motor stimulation following ketamine exposure [84]; additionally, mice bred for resistance to the sedative effects of ethanol show reduced NMDA antagonist sensitivity [85]. Similarly, inbred rodent strains selected for the their vulnerability to ethanol withdrawal seizures show reduced sensitivity to some acute effects of MK-801, increased MK-801 binding to NMDA receptors, and increased sensitivity to the motivational effects of ethanol [86–88]. Alcohol-preferring Indiana P rats also have reduced sensitivity to the effects of MK-801 on particular spectra of the EEG [89]. Therefore it has been hypothesized that altered NMDA receptor function that reduces the response to antagonists may increase the risk for heavy drinking. This hypothesis is consistent with our present findings, showing the efficacy of NMDA receptor antagonists in Scr:sP alcohol-preferring rats and further supports the proposed use of such antagonists in the treatment of excessive drinking. Our findings are also consistent with a previous study showing the efficacy of NMDA antagonist treatment on home-cage ethanol drinking in Myers alcohol-preferring rats [40].

Finally, in the present paper we found that the mTOR inhibitor rapamycin blocked the anti-alcohol effects of ketamine, but not those of memantine, which indicates that the two antagonists may indeed act via different signal transduction systems. mTOR is a serine/threonine protein kinase, member of the Phosphatidylinositol 3-Kinase-related Kinase family. It regulates cell growth, proliferation, motility, survival, protein synthesis, and transcription; its function is influenced by the activities of surface receptors and ion channels including NMDA and AMPA receptors, TrkB, dopaminergic and metabotropic glutamate receptors. mTOR, therefore, represents a node of convergence downstream of these receptors and several signaling pathways including phosphoinositide dependent kinase-1 (PDK1), phosphatidylinositol 3-kinase (PI3K) and Akt [90–93]. Recently, the activation of mTOR signaling in the PFC has been shown to mediate the rapid antidepressant actions of ketamine, along with the associated elevation of synapse-associated proteins and spine number; indeed, the administration of the mTOR inhibitor rapamycin was able to prevent the antidepressant-like effects of ketamine in mice [24]. Analogously, here we show that rapamycin was able to prevent the reduction in ethanol self-administration induced by ketamine, suggesting that mTOR mediates not only the antidepressant-like but also the anti-

alcohol effects of ketamine. Interestingly, in contrast, rapamycin did not affect the anti-alcohol effects of memantine in this study, suggesting that memantine's effects are mediated by a signaling pathway other than the activation of mTOR. The finding of a differential involvement of mTOR in ketamine vs. memantine's action is on one hand surprising, because these two uncompetitive antagonists have been considered to act with the same mechanism of action (except for the different off-rate). However, our findings seem to fit with a previous study showing that memantine does not, indeed, activate mTOR; rather in this study mTOR was reduced by memantine [25]; in addition, since memantine is clinically used to treat dementia in Alzheimer's disease patients, a mechanism of action involving a stimulation of mTOR would not be consistent with the observation that Alzheimer's disease subjects show a hyperactive mTOR, likely activated by the high levels of soluble beta amyloid [94, 95]. Further studies will be needed to determine the signal transduction pathway responsible for the reduction in ethanol intake observed after administration of memantine.

5. Conclusions

In summary, the present series of studies, performed in alcohol-preferring rats, further support the potential use of uncompetitive NMDA receptor antagonists, especially memantine, for the treatment of alcoholism. In addition, we demonstrated that ketamine also reduces ethanol intake in preferring rats and its effects, unlike those of memantine, are mediated by the activation of mTOR.

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Highlights

- Memantine reduces responding for ethanol but not water intake or motor activity
- Memantine reduces responding for the non-drug reinforcer saccharin at higher doses
- Ketamine also reduces ethanol responding but not water intake or motor activity
- Ketamine reduces responding for the non-drug reinforcer saccharin at the same doses
- mTOR inhibitor rapamycin prevents anti-alcohol effects of ketamine, but not memantine

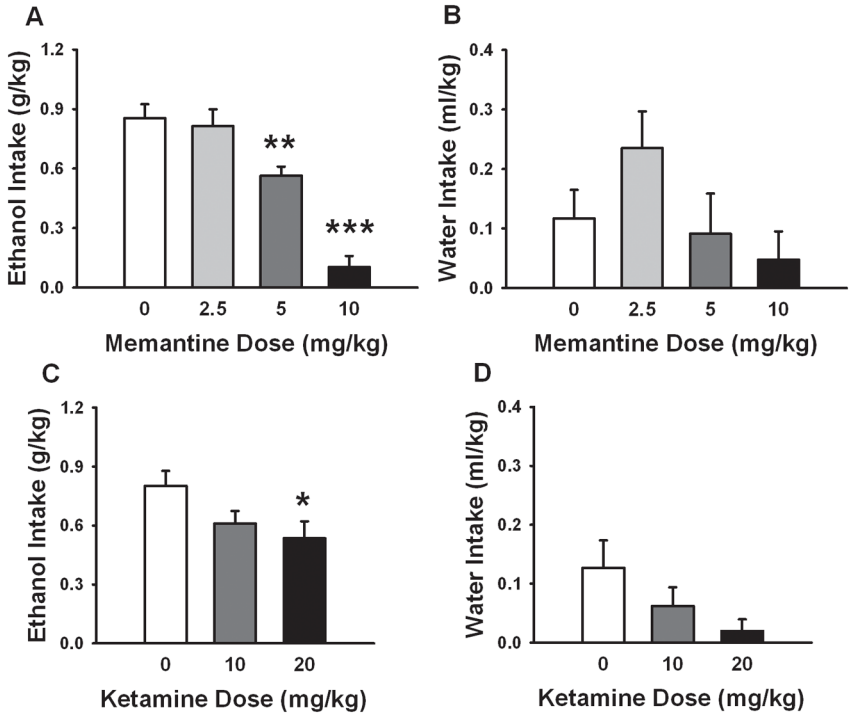

Fig. 1. Effects of memantine and ketamine on ethanol self-administration in alcohol-preferring rats

Effects of pretreament with the uncompetitive NMDA antagonists memantine (A and B, n= 8) and ketamine (C and D, n= 10) on responding for 10% *w/v* ethanol (A and C) and water (B and D) in TSRI Sardinian alcohol-preferring (Scr:sP) rats in an FR1 schedule of reinforcement. Data show M+SEM. * p<0.05, ** p<0.01, *** p<0.001 vs. vehicle-treated group (Student Newman Keuls test).

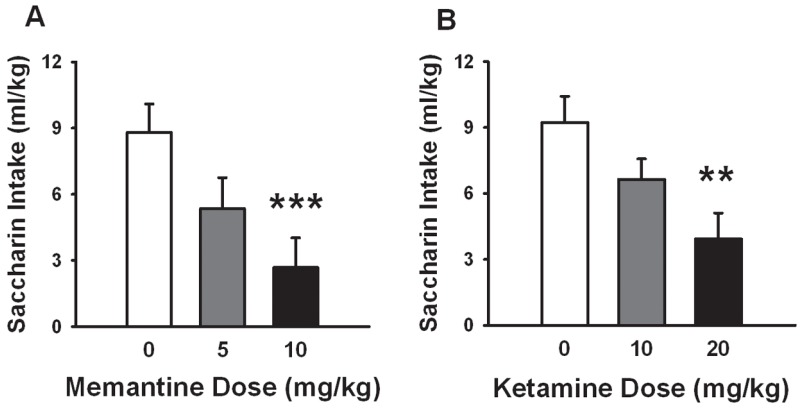

Fig. 2. Effects of memantine and ketamine on saccharin self-administration in alcohol-preferring rats

Effects of pretreament with the uncompetitive NMDA antagonists memantine (A, n= 9) and ketamine (B, n= 9) on responding for a 0.08% w/v saccharin solution in TSRI Sardinian alcohol-preferring (Scr:sP) rats in an FR1 schedule of reinforcement. Data show M+SEM. ** p<0.01, *** p<0.001 vs. vehicle-treated group (Student Newman Keuls test).

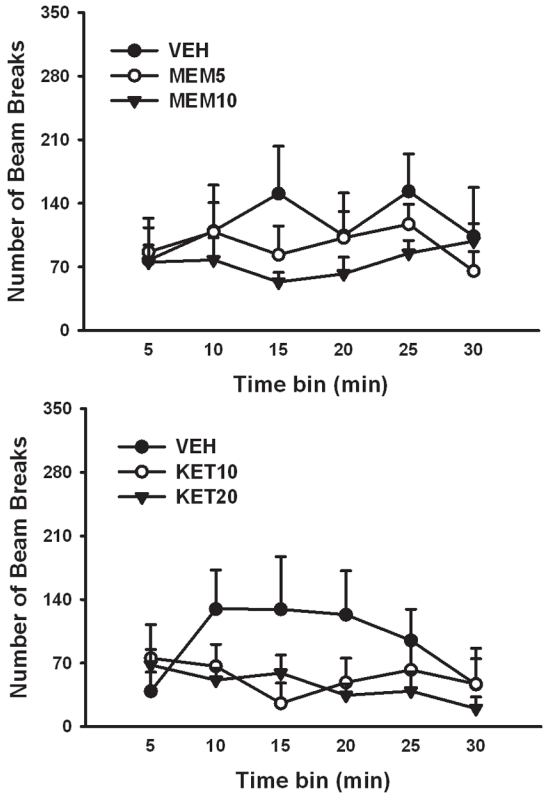

Fig. 3. Effects of memantine and ketamine on motor activity in alcohol-preferring rats

Effects of pretreament with the uncompetitive NMDA antagonists memantine (A, n= 6) and ketamine (C, n= 6) on motor activity in TSRI Sardinian alcohol-preferring (Scr:sP) rats in an FR1 schedule of reinforcement. Data show M+SEM.

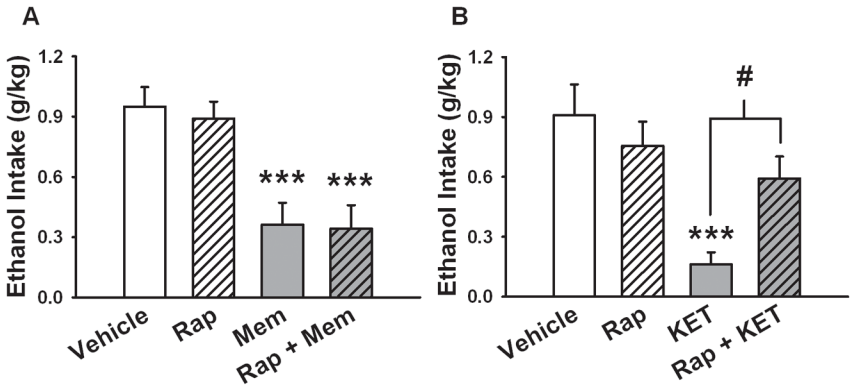

Fig. 4. Effects of the mTOR inhibitor rapamycin on the reduction of ethanol self-administration induced by memantine and ketamine in alcohol-preferring rats

Effects of pretreament with the mTOR inhibitor rapamycin (2.5 mg/kg, i.p.) on the effects of the uncompetitive NMDA antagonist memantine (A, $n = 7$) and ketamine (B, $n = 7$) on responding for a 10% *w/v* ethanol solution in TSRI Sardinian alcohol-preferring (Scr:sP) rats in an FR1 schedule of reinforcement. Data show M+SEM. *** $p < 0.001$ vs. vehicle-treated group, # $p < 0.05$ vs. ketamine-treated group (Student Newman Keuls test).