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Ethanol-reinforced behaviour in the rat: effects of uncompetitive NMDA receptor antagonist, memantine

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Abstract

Ethanol has been reported to alter NMDA receptor-mediated biochemical and electrophysiological responses in vitro. The aim of the present study was to evaluate the effects of an uncompetitive NMDA receptor antagonist memantine, in animal models of alcoholism. Male Wistar rats were trained to drink 8% ethanol in a free-choice, limited access procedure. A separate group of animals was trained to lever press for 8% ethanol in an operant procedure where ethanol was introduced in the presence of sucrose. The selectivity of memantine's actions was assessed by studying its effects on food or water consumption in separate control experiments. Memantine (4.5–24 mg/kg) significantly, but not dose dependently, affected ethanol drinking in the limited access procedure. However, only 6 mg/kg memantine selectively decreased ethanol drinking. Memantine did not alter ethanol intake in rats trained to lever press for ethanol in the operant procedure. Only 9 mg/kg memantine reduced operant responding in the extinction procedure in the rats trained to lever press for ethanol. The same dose of memantine significantly reduced the operant behaviour of rats trained to respond for water. These results indicate that: (i) single doses of memantine only moderately and not dose dependently reduce alcohol drinking in the limited access procedure; (ii) memantine produces non-selective effects on operant behaviour in rats trained to lever press for ethanol in an oral self-administration procedure.

Keywords: Ethanol drinking; Ethanol self-administration; Extinction; NMDA receptor; Memantine; (Rat)

1. Introduction

Ethanol (alcohol) has been reported to alter a diverse group of neurotransmitters in the mammalian brain (Ollat et al., 1988). A large body of experimental evidence, for example, indicates that clinically relevant concentrations of alcohol (< 50 mM) may interact with glutamatergic neurotransmission, decreasing NMDA receptor-associated channel conductance (for review, see Grant, 1994; Lovinger, 1997). Accordingly, data from biochemical and behavioural studies reveal that NMDA receptors may contribute to ethanol intoxication, tolerance and withdrawal syndrome (Danysz et al., 1992; Sanna et al., 1993). Furthermore, results from drug discrimination experiments strongly suggest that the interoceptive (discriminative) cue of ethanol may be, at least partially, mediated by changes in the function of the NMDA receptor complex (Grant et al., 1991; Bienkowski et al., 1996). However, little is known about the role of NMDA receptors in ethanol reinforcement.

During the last decade, the NMDA receptor complex has been the subject of considerable interest due to its suggested role in memory formation, neurodegeneration, and adaptive processes associated with chronic exposure to drugs of abuse (for review, see Danysz et al., 1995, 1997; Inturrisi, 1997). Interestingly, NMDA receptor antagonists have been reported to attenuate the development of tolerance to opioids (Trujillo and Akil, 1991; Popik and Skolnik, 1996) and ethanol (Khanna et al., 1993). In addition, NMDA receptor antagonists inhibit establishment of psy-

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chostimulant sensitisation (Karler et al., 1991; Wolf and Khansa, 1991). However, from a clinical perspective it would be of particular importance to block the expression of already established, drug-induced adaptive changes in an addict's brain. Some authors have reported that NMDA receptor antagonists do not alter the expression of psychostimulant-induced locomotor sensitisation, conditioned locomotion and conditioned place preference (Karler et al., 1991; Cervo and Samanin, 1995, 1996). These findings fit with the common view that NMDA receptor antagonists may attenuate acquisition of new information but not storage or recall of associations that are well established (Danysz et al., 1995). In contrast, drugs that block the NMDA receptor complex have been shown to decrease the expression of both amphetamine- and morphine-induced conditioned place preference (Bespalov, 1996; Popik and Danysz, 1997). It has been also reported that conditioned activation of intracranial self-stimulation by visual stimuli is antagonised by NMDA receptor antagonists (Bespalov and Zvartau, 1997).

Drug craving is usually defined as the incentive motivation to use a drug which was previously consumed by the addict (Markou et al., 1993; Tiffany, 1997). The extinction procedure is a commonly used animal model of craving (Chiamulera et al., 1995; Ranaldi and Roberts, 1996). This procedure provides several measures of the incentive–motivational effects of drugs and drug-related stimuli by assessing the persistence of operant behaviour, e.g., lever pressing, in the absence of drug reinforcement. Thus, extinction sessions are identical to self-administration sessions except that drug is not available after completion of the operant response requirement. The total number of responses during a session is typically used to assess the intensity of experimental craving (Markou et al., 1993; Chiamulera et al., 1995). Operant behaviour in the extinction procedure is thought to be initiated (cued) and maintained by drug-related conditioned stimuli (Ranaldi and Roberts, 1996; for review, see Bouton and Swartzentruber, 1991). However, extinction of lever pressing does not mean that these original associations have been eliminated. Instead of this, extinction seems to be a process of new learning (Bouton and Swartzentruber, 1991). Accordingly, both the expression of already established associations and the acquisition of novel information may occur during extinction of either Pavlovian or operant conditioning. Bearing in mind the role of NMDA receptors in mechanisms of learning and memory, one could hypothesise that NMDA receptor antagonists might affect extinction behaviour. In line with this hypothesis, it has been recently reported that NMDA receptor antagonists attenuate extinction of Pavlovian conditioned fear (Falls et al., 1992; Baker and Azorlosa, 1996) and conditioned hypoalgesic responses (Cox and Westbrook, 1994).

Memantine has been used for many years in Europe in the treatment of dementia (Görtelmeyer and Erbler, 1992; Pantev et al., 1993). It is a selective, low-affinity uncompetitive NMDA receptor antagonist which decreases conductance of the NMDA receptor-associated channel at low micromolar concentrations (0.5–3 μM). (Kornhuber et al., 1989; Parsons et al., 1996; for review, see Danysz et al., 1997). These concentrations are similar to therapeutic serum levels of memantine found in humans (0.4–1 μM) (Kornhuber and Quack, 1995; Danysz et al., 1997). In a drug discrimination procedure, memantine has been shown to substitute for the uncompetitive NMDA receptor antagonists, phencyclidine and dizocilpine (Sanger et al., 1992; Grant et al., 1996). Recently, Popik and Danysz (1997) have shown that memantine attenuates both acquisition and expression of morphine-induced conditioned place preference. Moreover, in the drug discrimination procedure memantine neither substituted for nor antagonised the cueing effects of morphine (Popik and Danysz, 1997). In contrast, memantine has been shown to substitute for the cueing effects of ethanol (Bienkowski et al., 1998). This latter finding would suggest that memantine could be a substitution therapy for alcohol dependence. However, the relationship between the interoceptive cue of ethanol and its rewarding and aversive effects is far from clear (for recent discussion, see Stefanski et al., 1996). In addition, the relationship between the discriminative stimulus effects of drugs and drug craving has not yet been fully explained (Wise et al., 1990; Benkelfat et al., 1991; Krystal et al., 1994).

In the present study, the effects of memantine in preclinical models of alcoholism were assessed. First, the effects of memantine on ethanol drinking in alcohol-preferring Wistar rats were studied in a limited access procedure. Second, the effects of memantine on lever pressing for ethanol were tested in rats trained in an operant procedure (oral self-administration). In the same rats, the effects of memantine on operant behaviour in the extinction procedure were evaluated. The doses used in the present study were selected on the basis of previous experiments. Thus, a single i.p. administration of 5 mg/kg memantine in the rat leads to serum levels similar (or slightly higher) to therapeutic concentrations of memantine found in human studies (Danysz et al., 1997). Importantly, memantine has been reported to produce several NMDA receptor-related effects at doses lower than 5 mg/kg (Dimpfel et al., 1987; Schmidt et al., 1991; Wenk et al., 1995). Our drug discrimination experiments have indicated that doses of memantine equal to or higher than 6 mg/kg (Bienkowski et al., 1998) or 9 mg/kg (Koros et al., unpublished) may strongly suppress or even completely eliminate operant behaviour in rats trained to discriminate ethanol from saline. To further address the problem of the selectivity of memantine, two separate control experiments were conducted (Hubner and Koob, 1990; Tomkins et al., 1994). In Control Experiment 1, the effects of memantine on food consumption were assessed. In Control Experiment 2, the effects of the drug on water intake were studied in rats trained to lever press for water in an operant procedure.

2. Materials and methods

2.1. Subjects

Male Wistar rats (360–400 g at the beginning of each experiment) were housed individually in wire cages ($20 \times 25 \times 28$ cm, $W \times L \times H$). The animals were supplied by a licensed breeder (HZL, Warsaw, Poland) at least 14 days before the start of each experimental procedure. The rats were kept under standard laboratory conditions at 22 ± 1°C, 60% humidity and a 12-h light–dark cycle (light on at 6:00 a.m.). Food (Bacutil, Poland) and tap water were available ad libitum except as noted below. Treatment of the rats in the present study was in a full accordance with the ethical standards laid down in the Declaration of Helsinki and was approved by our institutional ethics committee.

2.2. Food intake—Control Experiment 1

Access to standard laboratory chow was limited to 4 h/day. Rats ($n = 7$) were allowed to adapt to limited food availability for at least 7 days. After this period, test sessions with memantine were initiated. Memantine (4.5, 6, 12, 24 mg/kg) or its vehicle was administered i.p. 30 min before the test session. (The order of testing various doses of memantine was randomised across subjects in every experiment described in the present paper.) Preweighed food pellets (Bacutil) were placed in small wire-mesh containers located near the top of the home cage. The pellets were weighed and replaced 1, 2 and 4 h after the start of food presentation. Food intake (g) was assessed by recording the differences between the initial and final weights of the pellets. Spillage was monitored and included in the final calculations.

In order to be tested in each subsequent test session, the rat had to maintain a stable food intake ($\pm 20\%$) for at least 3 consecutive drug-free sessions. Except for a small reduction in the beginning of the food deprivation period, the mean weight of the rats remained constant during the course of the experiment.

2.3. Ethanol drinking in alcohol-preferring rats maintained on a limited access procedure

To obtain alcohol-preferring rats the procedure described by Kostowski et al. (1993) was used. Briefly, during the first week the subjects ($n = 30$) received i.g. 20% (v/v) ethanol twice daily (5 g/kg per day). Tap water and food were available ad libitum. During the next 2 weeks the rats had free access to tap water for 1 h/day and for the remaining 23 h the only source of fluid was 5% ethanol (the second week) or 8% ethanol (the third week). During the fourth week, the subjects were presented with a free choice between two graduated drinking tubes—one containing tap water and the other one containing 8% ethanol. Measurement of fluid intake (ml) and refilling of the tubes were conducted each day (at 9:00–10:00 a.m.), when the position of the tubes was also changed to prevent a bias in consumption. The animals consuming ≥ 5 g/kg per day of absolute alcohol ($n = 8$) were considered alcohol-preferring rats and were used in the limited access procedure. In the limited access procedure the rats were trained to drink 8% ethanol in 4-h training sessions (8:00–12:00 a.m.) (Dyr and Kostowski, 1995, 1997). Ethanol intake was measured (to nearest 0.1 ml) 1, 2 and 4 h after insertion of the tube. Food and tap water (in the second drinking tube) were always available in the home cages. The rats were allowed to stabilise their ethanol intake for at least 10 days. Criteria of stable intake were defined as $\pm 20\%$ of the previous day's total intake of ethanol for 3 consecutive sessions and ≥ 2 ml of 8% ethanol consumed (Heyser et al., 1997). Memantine (4.5, 6, 12, 24 mg/kg) or its vehicle was injected i.p., in a randomised order, 30 min before the start of the test session. In order to be tested in each subsequent test session the rat had to meet the criteria of stable drinking for at least 3 consecutive drug-free sessions.

2.4. Apparatus

Operant responding for water or ethanol (oral self-administration) was tested in standard operant conditioning chambers (Coulbourn Instruments, Allentown, PA, USA). The chambers (for details, see Bienkowski et al., 1997) consisted of modular test cages enclosed within sound-attenuating cubicles with fans for ventilation and background white noise. A white house light was centered near the top of the front of the cage. The start of the training or test sessions was signalled by turning the house light on. The cage was also equipped with 2 response levers, separated by a liquid delivery system (the liquid dipper, E14-05, Coulbourn). Only one lever ('active' lever) activated the liquid dipper. Presses on the other lever ('inactive' lever) were recorded but not reinforced. The liquid delivery system presented water or ethanol in a 0.1-ml portion for 5 s. The availability of reinforcer was signalled by a brief click and a small white light (4 W) located inside the liquid dipper hole. Programming of training/test sessions as well as data recording made use of the L2T2 Software package (Coulbourn) running on an IBM-PC compatible.

2.5. Operant responding for water—Control Experiment 2

To facilitate lever pressing for water, the rats ($n = 7$) were deprived of water throughout the course of the experiment. Their access to tap water in the home cages was limited to 2 h/day and started ~ 30 min after the end of an operant session. Food was available ad libitum. The subjects were trained to lever press on a fixed ratio 1 (FR1) schedule of water reinforcement (0.1 ml/response)

in the 30-min daily sessions. All rats learned to self-administer water within the first 4 days of the experiment. After additional 5–7 sessions water intake stabilised and test sessions with memantine were initiated. Memantine (2.25, 4.5, 9, 18 mg/kg) or its vehicle was administered, in a randomised order, 30 min before the start of the test session. In order to be tested in each subsequent test session the subject had to show stable (±20%) responding for at least 3 consecutive drug-free sessions.

The effects of the same range of memantine doses were also studied in the extinction procedure. The extinction sessions were identical to the training/test sessions except that no water was delivered after the rats responded on the 'active' lever. The liquid delivery system was off and the stimuli associated with dipper presentation were absent. Memantine (2.25, 4.5, 9 mg/kg) or its vehicle was injected as described above.

2.6. Operant responding for ethanol

The rats ($n = 8$) were trained to respond for 8% ethanol according to the Samson's sucrose-fading procedure with some minor modifications (Samson, 1986; Files et al., 1997). The animals were deprived of water for 22 h/day during the first 4 days of training and shaped to lever press for 10% sucrose solution on a FR1 schedule of reinforcement. As soon as lever pressing was established, water started to be freely available in the home cages. Food was always available ad libitum. All training sessions were 30 min long and one session was given each day. Starting on day 5, the animals received 2% ethanol–10% sucrose. Then over the next 8–10 sessions ethanol concentrations were gradually increased (from 2% to 8%) and sucrose concentrations were decreased (from 10% to 0%). The rats were allowed to stabilise their ethanol consumption for 15–20 days after which test sessions with memantine started. The criterion for stable responding was defined as ±20% of the previous session's total number of responses for 3 consecutive sessions (Rassnick et al., 1992). Memantine (2.25, 4.5, 9 mg/kg) or its vehicle was injected as described above.

2.7. Extinction procedure

To examine whether presentation of the reinforcement-paired stimuli (the noise and light associated with operation of the dipper system) altered the rat's resistance to extinction and/or effects of memantine, the animals were tested in two extinction conditions. In the first type of extinction session, the liquid delivery system was off, i.e., responding on the 'active' lever had no consequences. Memantine (2.25, 4.5, 9 mg/kg) or its vehicle was injected, in a randomised order, 30 min before the start of the session. In order to be tested in each subsequent extinction session the subject had to maintain stable responding for ethanol for at least 3 consecutive days. In the second type of extinction session, the liquid dipper system was active. Responding on the 'active' lever resulted in the delivery of the ethanol-associated stimuli, i.e., in the activation of the liquid dipper, brief click, illumination of the stimulus light and delivery of water. Memantine (2.25, 4.5, 9 mg/kg) or its vehicle was injected as described above. In addition, the 4.5 mg/kg dose of memantine was injected 30 min before 3 consecutive extinction sessions with the liquid delivery system on. Only the subjects ($n = 7$) with the most stable pattern of the ethanol self-administration were used in the extinction procedure.

2.8. Drugs

Ethanol solutions were prepared daily from a 95% stock solution and tap water. Memantine hydrochloride (Merz, Frankfurt am Main, Germany) was dissolved in sterile physiological saline and administered in a volume of 1 ml/kg. The doses of memantine refer to the salt form. All solutions were prepared immediately prior to use.

2.9. Statistics

The results of the limited access procedures (see Sections 2.2 and 2.3) are reported as mean cumulative food (g/rat per 1, 2 or 4 h) or 8% ethanol (ml/rat per 1, 2 or 4 h) consumption as these were fully comparable with data for absolute food or ethanol intake (g/kg), which are not shown. A two-way (Drug × Time) analysis of variance (ANOVA) with repeated measures was used to compare the data from the above experiments. The results of the operant procedures (see Sections 2.5, 2.6 and 2.7) are shown as mean number of responses on the 'active' lever during the entire 30-min session. These data were analysed by a one- or two-way ANOVA with repeated measures. The data from the control sessions with saline were treated as an additional dose level (i.e., 0.0 mg/kg) and included in the ANOVA. Newman–Keuls test was used for individual post hoc comparisons. A significance level of $P < 0.05$ was used for all statistical analyses.

3. Results

3.1. Food intake—Control Experiment 1

Memantine affected food intake, as revealed by a significant Drug effect; $F(4,30) = 24.11$, $P < 0.001$. The ANOVA indicated also a significant effect of Time; $F(2,60) = 123.63$, $P < 0.001$, and a significant Drug × Time interaction; $F(8,60) = 4.23$, $P < 0.05$. The post hoc analysis showed that only the highest dose of memantine (24 mg/kg) significantly suppressed food intake (Table 1, left).

Table 1
The effect of memantine on cumulative food consumption (g) and ethanol drinking (ml) in separate groups of rats maintained on a limited access to ethanol ($n = 8$) or food ($n = 7$) schedule, respectively

Dose of memantine (mg/kg)	Food intake			Ethanol drinking		
	1 h	2 h	4 h	1 h	2 h	4 h
Saline	10.6 (0.8)	14.4 (0.6)	21.2 (1.1)	3.3 (0.6)	3.8 (0.5)	4.8 (0.7)
4.5	9.8 (0.8)	14.6 (1.1)	20.0 (1.0)	2.7 (0.6)	3.4 (0.9)	4.0 (1.0)
6	8.7 (1.3)	15.1 (1.2)	18.2 (1.3)	2.0 (0.5)[a]	2.8 (0.6)	3.0 (0.7)[a]
12	9.1 (1.4)	16.3 (1.8)	22.0 (2.3)	3.4 (0.9)	4.5 (1.3)	5.3 (1.5)
24	0.2 (0.2)[b]	0.5 (0.3)[b]	5.8 (1.8)[b]	0.1 (0.1)[b]	0.3 (0.2)[b]	1.5 (0.6)[b]

The results are shown as the mean (with S.E.M.) ethanol or food consumption 1, 2 and 4 h after the start of the test session.
[a] $P < 0.05$.
[b] $P < 0.01$.

3.2. Ethanol drinking in alcohol-preferring rats maintained on a limited access procedure

The mean ($\pm$S.E.M.) baseline intake of 8% ethanol was 5.3 ± 0.51 ml/rat per 4 h (or 0.65 ± 0.06 g/kg per 4 h). Typically, more than 70% of the ethanol solution was consumed within the first hour of the session. The water intake during the 4-h sessions did not exceed 0.5 ml/rat (data not shown).

Memantine significantly, but not dose dependently, altered the ethanol drinking behaviour ($F(4,35) = 13.60$, $P < 0.01$). The ANOVA revealed also a significant effect of Time; $F(2,70) = 23.01$, $P < 0.001$. A Drug $\times$ Time interaction was not significant ($F < 1$). Two doses of memantine (6 and 24 mg/kg) significantly decreased the ethanol intake. However, the effect of 6 mg/kg memantine was less consistent than that of 24 mg/kg memantine, which caused a strong suppression of drinking (Table 1, right). This latter finding was in close agreement with the results of Control Experiment 1, where almost complete elimination of food intake was found after 24 mg/kg memantine (see above). In both experiments 24 mg/kg memantine induced a marked locomotor stimulation and stereotypy.

3.3. Operant responding for water—Control Experiment 2

The ANOVA showed a significant effect of Drug; $F(4,24) = 16.52$, $P < 0.001$. Post hoc analysis indicated that the two higher doses of memantine (9 and 18 mg/kg) caused significant decreases in operant responding for water (Table 2). Similarly, operant behaviour in the extinction procedure was significantly affected by memantine ($F(3,13) = 4.68$, $P < 0.05$). The highest dose of the drug tested in the extinction procedure (9 mg/kg) significantly suppressed operant responding (Table 2).

3.4. Operant responding for ethanol

The mean ($\pm$S.E.M.) baseline number of ethanol deliveries was 41 ± 3 dipper deliveries/30 min, with a mean ($\pm$S.E.M.) ethanol consumption of 0.51 ± 0.06 g/kg per 30 min. Typically, more than 90% of the ethanol solution was consumed within the first 10 min of the session.

Table 3 presents the results of the initial extinction sessions performed under the two different conditions. The ANOVA indicated a significant effect of Day for both the first (the liquid delivery system off; $F(2,17) = 3.60$, $P < 0.05$) and the second (the liquid delivery system on; $F(2,17) = 10.87$, $P < 0.01$) type of extinction session. Post hoc analysis showed that the number of lever presses in the extinction sessions was significantly lower than in the respective training sessions. These results demonstrated that ethanol served as a reinforcer in the present experiment. A direct comparison of operant responding in the two different types of extinction sessions did not reveal any significant difference ($P = 0.25$; t-test).

Memantine did not alter operant responding for ethanol ($F(3,17) = 1.91$, $P = 0.17$). As shown in Table 4 the highest dose of the drug (9 mg/kg) tended to reduce, though not significantly, the ethanol-reinforced behaviour.

Table 2
The effect of memantine on the operant behaviour of rats ($n = 7$) responding for water reinforcement during the test sessions (water reinforcement present) and the extinction sessions (water reinforcement absent)

Dose of memantine (mg/kg)	Procedure
Test sessions	
Saline	134 (21)
2.25	136 (18)
4.5	99 (29)
9	75 (19)[a]
18	10 (6)[b]
Extinction sessions	
Saline	20.0 (1.6)
2.25	26.6 (5.0)
4.5	25.6 (5.5)
9	6.25 (3.6)[a]

The results are expressed as the mean (with S.E.M.) number of responses/30 min.
[a] $P < 0.05$.
[b] $P < 0.01$.

Table 3
The responses in extinction sessions performed under two different conditions of rats ($n = 7$) trained to respond for ethanol

Baseline	Extinction	Re-exposure
Liquid delivery system inactive		
35.4 (4.1)	11.2 (1.5)[a]	32.2 (3.9)
Liquid delivery system active		
35.0 (4.7)	15.3 (3.8)[b]	40.1 (10.1)

The results are shown as the mean (with S.E.M.) number of responses/30 min.
For comparison, results of the ethanol-reinforced sessions performed on the day before ('Baseline') and on the day after ('Re-exposure') the respective extinction session are also presented.
[a] $P < 0.05$, [b] $P < 0.01$ vs. the respective 'Baseline' and 'Re-exposure' session.

Memantine significantly altered operant responding during the first type of extinction session (i.e., without response-contingent presentation of the ethanol-associated stimuli; $F(3,19) = 3.34$, $P < 0.05$). However, as shown in Table 4 only 9 mg/kg memantine significantly suppressed lever pressing. As shown above, this dose of memantine decreased also operant behaviour in the rats trained to respond for water.

Memantine did not significantly change operant behaviour during the second type of extinction session (i.e., with response-contingent presentation of the ethanol-associated stimuli; $F(3,18) = 2.03$, $P < 0.14$). However, 9 mg/kg memantine clearly tended to reduce the number of lever presses.

The administration of 4.5 mg/kg memantine before 3 consecutive extinction sessions (Table 5) did not alter operant behaviour, as revealed by a non-significant Drug effect ($F(1,12) = 2.28$, $P = 0.19$) and a non-significant Drug × Session interaction ($F(2,24) = 1.19$, $P = 0.32$).

The mean number of 'inactive' lever presses in the above experiments (see Section 3.3 and this section) was negligible (< 1.6 presses/30 min; data not shown).

Table 4
The effect of memantine on operant responding for ethanol and on responding during extinction sessions ($n = 6$–7 rats)

Dose of memantine (mg/kg)	Procedure
Responding for ethanol	
Saline	44.2 (6.0)
2.25	43.0 (6.9)
4.5	50.0 (10.9)
9	25.5 (7.1)
Extinction	
Liquid delivery system inactive	
Saline	11.2 (1.8)
2.25	7.8 (2.0)
4.5	7.7 (2.3)
9	2.0 (0.5)[a]
Liquid delivery system active	
Saline	17.1 (4.1)
2.25	17.2 (3.7)
4.5	18.5 (7.4)
9	3.0 (2.0)

The results are shown as the mean (with S.E.M.) number of responses/30 min.
[a] $P < 0.05$.

Table 5
The effect of 4.5 mg/kg memantine on operant responding during the 3 consecutive extinction sessions in rats ($n = 6$) trained to respond for ethanol

Treatment	Extinction session		
	#1	#2	#3
Saline	15.7 (2.3)	12.9 (4.1)	6.6 (3.1)
Memantine	27.7 (6.4)	20.1 (5.9)	7.6 (1.5)

The results are shown as the mean (with S.E.M.) number of responses/30 min.

4. Discussion

The ethanol intake and the pattern of drinking in the present study were comparable with those of previous studies (Tomkins et al., 1994; Davidson and Amit, 1996; Dyr and Kostowski, 1997). Similarly, the operant behaviour motivated by ethanol did not substantially differ from that previously described (Rassnick et al., 1992; Files et al., 1997; Heyser et al., 1997). However, the extinction of the ethanol-reinforced behaviour seemed to be more rapid than in the study of Chiamulera et al. (1995). In the present study, the rats significantly reduced their operant responding during the first extinction session while in the previous study 8–10 sessions were required for a substantial decrease in lever pressing (Chiamulera et al., 1995). Importantly, Chiamulera et al. used the FR4 schedule of reinforcement, which could increase the rat's resistance to extinction. Moreover, a different strain of rats (Long–Evans) was used in the previous study. Significant strain differences in the operant responding for ethanol have been found by many authors (Samson, 1986; Ritz et al., 1994; Files et al., 1997).

Pairing of the stimulus complex with each ethanol delivery did not increase responding in the extinction procedure. Accordingly, the stimulus complex used in the present study did not function as a conditioned reinforcement. This result is in line with recent findings of other authors (Slawecki et al., 1997). However, in another study (Bienkowski et al., unpublished) we demonstrated that the same stimulus complex may enhance responding in the extinction procedure but only in rats with a long history (> 4 months) of ethanol self-administration.

The results of the Control Experiment 1 revealed that the highest dose of memantine (24 mg/kg) suppressed food intake in the food-deprived rats. This finding could be at least partially explained by the marked behavioural activation induced by 24 mg/kg memantine. Thus, the

effects of 24 mg/kg memantine on ethanol drinking found in the present study were non-selective and will not be further discussed.

Although food intake is commonly used as a control experiment in ethanol self-administration studies (e.g., Dyr and Kostowski, 1997), one can argue that water consumption under a limited access procedure would be a better control for ethanol drinking. The results of the present study would suggest, for example, that the pattern of water consumption (intense drinking in the beginning of the operant session) is more similar to that of ethanol drinking than to that of food intake.

The lower doses of the drug (4.5–12 mg/kg) were devoid of any effects on food consumption. The lower doses of memantine produced rather weak, and not dose-dependent, effects on ethanol consumption in the alcohol-preferring rats (the limited access procedure). Recently, we have found that a competitive NMDA receptor antagonist, D-(*E*)-2-amino-4-methyl-5-phosphono-3-pentanoate (CGP 40116), does not selectively alter ethanol intake when studied in the above procedure. Interestingly, even doses of CGP 40116 which have been shown to substitute for ethanol in the drug discrimination test (Bienkowski et al., 1996) do not affect ethanol intake in the limited access procedure (Bienkowski et al., unpublished).

In line with the above, memantine did not alter operant responding for ethanol (oral self-administration) in the present study even at the dose of 9 mg/kg, which suppressed other kinds of operant behaviour (Control Experiment 2). Recently, similar results have been published by Shelton and Balster (1997). In this latter study, rats were trained to lever press for ethanol or saccharin solution under a FR4 multiple schedule of liquid availability. Neither a competitive NMDA receptor antagonist, D-3-(2-carboxypiperazine-4-yl)-1-propenyl-phosphonic acid (CP-Pene), nor the uncompetitive NMDA receptor antagonist, phencyclidine, selectively altered ethanol-reinforced behaviour. In contrast, Rassnick et al. (1992) reported that a competitive NMDA receptor antagonist, 2-amino-5-phosphonopentanoic acid (AP-5), injected into the nucleus accumbens selectively attenuated operant responding for ethanol in a free-choice operant task (10% ethanol vs. water). However, the rate of water self-administration was considerably lower than the rate of ethanol self-administration. Moreover, ethanol preference was not affected in this latter study (Rassnick et al., 1992).

Only a relatively high dose of memantine (9 mg/kg) decreased operant behaviour in the extinction procedure in rats trained to respond for ethanol. However, this effect should be considered non-selective because 9 mg/kg memantine suppressed also operant behaviour in the test/extinction sessions in Control Experiment 2. In line with this latter finding, the 9 mg/kg (and higher) dose of memantine has been found to suppress operant responding for food in rats trained in the drug discrimination procedure (Bienkowski et al., 1998; Koros et al., unpublished). Therefore, the present results do not exclude the possibility that memantine might possess anti-craving properties in rats trained to self-administer ethanol but indicate that the doses needed to suppress lever pressing for ethanol in the extinction procedure may decrease other kinds of operant behaviour.

The alcohol deprivation effect, i.e., the increase in ethanol consumption after a period of forced abstinence, has been recently suggested to be a simple model of alcohol craving (Spanagel et al., 1996). Hölter et al. (1996) reported that memantine delivered by osmotic minipumps significantly influenced the alcohol deprivation effect in rats allowed to drink ethanol in a long-term, free-choice model of alcohol addiction. Importantly, memantine did not reduce alcohol drinking when compared with that of the baseline pre-abstinence period. Instead of this, the drug selectively eliminated the increase in ethanol consumption induced by a 2-week period of abstinence. Interestingly, using a similar procedure Spanagel et al. (1996) demonstrated that a new, clinically active, anti-craving compound, acamprosate, fully eliminated the alcohol deprivation effect in the rat. The direct comparison between the results of Hölter et al. and the results from the present study is difficult due to obvious procedural differences. Hölter et al. used rats which, according to previously described criteria (Spanagel et al., 1996), presented signs of 'psychological' dependence on alcohol. Besides, in the previous study memantine was continuously delivered both before and after re-exposure to ethanol. Thus, memantine-induced adaptive changes in the brain as well as direct pharmacodynamic interactions between ethanol and memantine (Beleslin et al., 1997) might be responsible for the elimination of the alcohol deprivation effect. Further studies using different models of alcohol craving are needed to fully assess the possible anti-craving actions of memantine in rats with a long-term history of alcohol drinking.

5. Conclusion

The present results indicate that: (i) memantine decreased ethanol consumption in the limited access procedure but this effect was not clearly dose dependent and, at least for the highest dose of the drug, was probably related to non-specific behavioural actions; (ii) the doses of memantine which suppressed responding in the extinction procedure produced general inhibition of operant behaviour. Accordingly, at the doses used in the present study memantine did not produce selective anti-craving effects in rats trained to lever press for ethanol. Taken together with some previous findings (Bienkowski et al., unpublished; Shelton and Balster, 1997), our results argue against an important role of the NMDA receptor complex in ethanol reinforcement, at least in rats not dependent on ethanol.

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