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Effect of NMDA antagonists, an NMDA agonist, and serotonin depletion on acute tolerance to ethanol

J.M. Khanna[a,b], G.S. Morato[c,*], H. Kalant[a,b]

[a]*Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 1A8*
[b]*Centre for Addiction and Mental Health, Toronto, Ontario, Canada M5S 1A8*
[c]*Departamento de Farmacologia, Universidade Federal de Santa Catarina, Rua Ferreira Lima 82, Florianopolis, SC 88015-420, Brazil*

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Abstract

The effect of *N*-methyl-D-aspartate (NMDA) antagonists [dizocilpine, (+)MK-801, and ketamine], an NMDA agonist (D-cycloserine) and of brain serotonin (5-HT) depletion with *p*-chlorophenylalanine (*p*-CPA) on acute tolerance to ethanol was examined, using the tolerance model proposed by Radlow [Psychopharmacology 114 (1994) 1–8] and Martin and Moss [Alcohol Clin Exp Res 17 (1993) 211–216]. This model is based on the concept of a linear increase of acute tolerance with time; the rate of acute tolerance development is the slope of the output function that relates blood alcohol concentrations (BACs) and intoxication. Pretreatment with NMDA antagonists inhibited the development of acute tolerance to ethanol, whereas pretreatment with D-cycloserine enhanced it. Depletion of 5-HT by *p*-CPA also blocked acute tolerance to ethanol. These results on acute tolerance are similar to those previously found on rapid and chronic tolerance to ethanol.

Keywords: Ethanol tolerance; Acute; NMDA; Antagonist; Agonist; 5-HT; Rat brain

1. Introduction

Tolerance to ethanol has been an important topic in research on alcoholism for many years, partly because it has been seen as a model for many other types of neuroadaptive processes (Kalant, 1988), partly because of its possible importance in relation to alcohol consumption (Waller et al., 1983) and alcohol dependence (American Psychiatric Association, 1994), and partly because of its potential value as a predictor of susceptibility to alcoholism (Schuckit, 1986).

However, the subject is complicated by the fact that tolerance can occur within several different time frames that are commonly designated by different adjectives: *acute* tolerance is that which develops within the course of a single exposure to alcohol (Kalant et al., 1971); *rapid* tolerance is seen in a second exposure that follows the first by 8–36 h (Bitran and Kalant, 1991); and *chronic* tolerance is that which is seen to develop progressively during repeated exposures over the course of many days or weeks (Kalant et al., 1971; Littleton, 1980). Acute tolerance probably represents an inherent adaptive response, whereas rapid and chronic tolerance are generally perceived as consequences of excessive exposure. It is therefore important to clarify as much as possible the relationship among these various forms of tolerance, and for this purpose the effects of various behavioral and pharmacological manipulations on the different forms of tolerance have been compared.

In earlier studies from our laboratory, we found that the *N*-methyl-D-aspartate (NMDA) antagonists dizocilpine [(+)MK-801] and ketamine blocked the development of rapid and chronic tolerance to ethanol in motor-impairment (tilt-plane) and hypothermia tests (Khanna et al., 1991b, 1992a,c, 1993b, 1994). These studies have been confirmed by others with the moving belt, rotarod, sleep-time and hypothermia tests (Karcz-Kubicha and Liljequist, 1995; Szabo et al., 1994; Wu et al., 1993). In contrast to these observations, ketamine failed to block acute tolerance to ethanol as determined by the blood levels of ethanol at recovery from motor impairment (tilt-plane test) after dif-

* Corresponding author. Tel.: +55-48-331-9764 or 331-9491; fax: +55-48-2224164.
E-mail address: gsmorato@farmaco.ufsc.br (G.S. Morato).

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ferent ethanol doses (Khanna et al., 1992c). These results suggested that the NMDA type of glutamate receptor is involved in chronic and rapid tolerance but not in acute tolerance to ethanol. On the other hand, the effect of brain serotonin (5-HT) depletion by selective raphe lesions on acute tolerance development, as measured by the decrease in impairment scores over time, was consistent with the effect of these lesions on chronic tolerance. We found that median raphe lesions delayed the development of both acute and chronic tolerance, and that dorsal raphe lesions had no effect on either (Campanelli et al., 1988; Khanna et al., 1987).

One major difference among the studies described above concerns the method used for measuring acute tolerance. Our 1992 study (Khanna et al., 1992c) with the NMDA antagonists used log dose–response curves for blood ethanol levels at the time of recovery as a measure of acute tolerance, whereas our 1988 study (Campanelli et al., 1988) measured the decrease in response to alcohol over time with approximately steady-state blood alcohol concentration (BAC).

Researchers have used a number of methods to study acute tolerance; the strengths and weaknesses of these methods have been discussed by Radlow (1994) and Martin and Moss (1993). These authors have suggested the use of an output function (BAC–intoxication) and have argued that this approach provides a better measure of acute tolerance than previously available methods.

In view of the different results of 5-HT depletion and NMDA antagonists on acute versus chronic tolerance, we felt it was important to reexamine the effects of both of these manipulations on acute tolerance, using the same method of acute tolerance measurement. The effect of (+)MK-801 and ketamine on the development of acute tolerance to the motor-impairment effect of ethanol in the tilt-plane test was therefore studied using the Radlow procedure (1994), in order to examine further the relationship between acute and chronic tolerance. In other studies, we examined the effect of D-cycloserine, an agonist at the glycine site of the NMDA receptor, and of 5-HT depletion with *p*-chlorophenylalanine (*p*-CPA) on acute tolerance to ethanol.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats obtained from Charles River Canada (Montreal, Quebec) had initial body weights of 225–250 g. They were individually housed in a colony room maintained at 21 ± 1 °C, with lights on from 7 a.m. to 7 p.m. Water was available at all times. Purina Rat Chow was given ad lib for 1 week. Thereafter, the daily ration was restricted and individually adjusted to maintain comparable body weights in the various groups. All procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Test procedure

The tilt-plane test was used as a measure of motor impairment (Arvola et al., 1958; as modified by Khanna et al., 1991a). The apparatus consists of a plane hinged at one end, about which it can be inclined at a fixed angular velocity through a range of 55° above the horizontal. The animal was placed on the slightly roughened surface of the plane, facing the free end, which was then raised until the animal began to slide from the starting position. The test measure used was the angle at which this sliding occurred. The sliding angle was measured before and at various times after the injection of ethanol (E). The degree of postdrug ataxia was expressed as the percentage change in the sliding angle, compared to the predrug value for the same animal. Maximum impairment occurred about 30–60 min after injection of E. Blood samples (50 μl) for E measurement were taken from the tip of the rat's tail. BAC was analyzed by the enzymatic method described previously (Hawkins et al., 1966).

2.3. Statistical methods

Results in these experiments were analyzed by multiple regression analysis and one-way ANOVA as required, using the GLM-ANOVA program in the NCSS statistical package for PCs. Post hoc comparisons were carried out by the Newman–Keuls range test.

2.4. Acute tolerance measurement

Acute tolerance was measured by means of an output function that relates BACs and impairment (Martin and Moss, 1993; Radlow, 1994). The rate of increase of this function over time is the measure of acute tolerance. The basic concept is that in the absence of acute tolerance, the BAC and the effect should follow parallel curves over time; the increasing discrepancy between BAC and effect is therefore the measure of tolerance (Radlow, 1994). First, the BAC values at various times are converted to percentages of the BAC maximum, which is expressed as 100%. Similarly, the impairment values at the different test times are also converted to percentages of the impairment maximum (also expressed as 100%). Next, the percent impairment value (IMP) is subtracted from the percent BAC value at each time of assessment. The output values, BAC (% max) − IMP (% max), are then plotted against time. The slope of this output function was computed separately for each rat.

2.5. Experiment 1: acute ethanol tolerance — dose response study

Thirty-six rats were randomly divided into two batches. The first batch of 20 rats was brought up to the laboratory in the morning. After weighing, they were divided into four groups receiving an intraperitoneal E dose of 2.5, 2.7, 3.0,

or 3.3 g/kg, respectively. Before and at 30, 45, 60, 75, 90, 105 and 120 min after E injection the degree of motor impairment was assessed (tilt-plane test) in all animals. A blood sample for E analysis was taken from the tip of the rat's tail immediately after each measurement of motor impairment. An identical experimental procedure was carried out for the second batch of 16 rats the next day. The individual values of BAC and IMP were used for acute tolerance determinations. A second experiment was carried out with lower doses of E (l.9, 2.3 or 2.8 g/kg), but otherwise following the same procedure as above.

2.6. Experiment 2: effect of NMDA antagonist on acute ethanol tolerance

Twenty-seven rats were randomly divided into three equal groups. The first group was pretreated with saline, the second group with (+)MK-801 (0.25 mg/kg ip) and the third group with ketamine (4 mg/kg ip). Half an hour later, a dose of E (3 g/kg ip) was given to all the animals. Before the injection of E and at 30, 45, 60, 80, 100 and 120 min after it, the degree of motor impairment was assessed in all animals. A blood sample for E measurement was taken from the tip of the rat's tail immediately after each measurement of motor impairment. The rest of the experimental procedure was as described in Experiment 1.

2.7. Experiment 3: effect of NMDA agonist (D-cycloserine) on acute ethanol tolerance

Twenty rats were randomly divided into two equal groups. After weighing, initial performance on the tilt plane was measured for all rats. Then one group was injected with D-cycloserine (CS; 30 mg/kg ip) and the other group received saline (S). Thirty minutes later all rats were injected with E (3.0 g/kg ip). Every 15 min, from 30 up to 135 min after E, the degree of motor impairment was assessed in all animals. A blood sample was taken from the tip of the rat's tail immediately after each measurement of motor-impairment response. The individual values of BAC and IMP were used for acute tolerance determination in all groups.

2.8. Experiment 4: effect of 5-HT depletion with p-CPA on acute ethanol tolerance

Fifteen rats were divided into two groups ($n = 7$ or 8 per group). One group was gavaged with *p*-CPA (100 mg/kg) dissolved in water daily for 5 days to deplete 5-HT, and the other received water alone. Numerous studies from this laboratory have shown that pretreatment with *p*-CPA for 5 days results in >95% depletion of brain 5-HT (Frankel et al., 1977, 1978). On Day 6, all rats were brought up to the laboratory. They were weighed, initial performance was measured, and then all were injected with E (3.0 g/kg ip). The rest of the procedure and times of measurement were identical to those described in the experiments above.

3. Results

At each time of assessment, the BAC and the impairment score (IMP) were converted to a percentage of their respect-

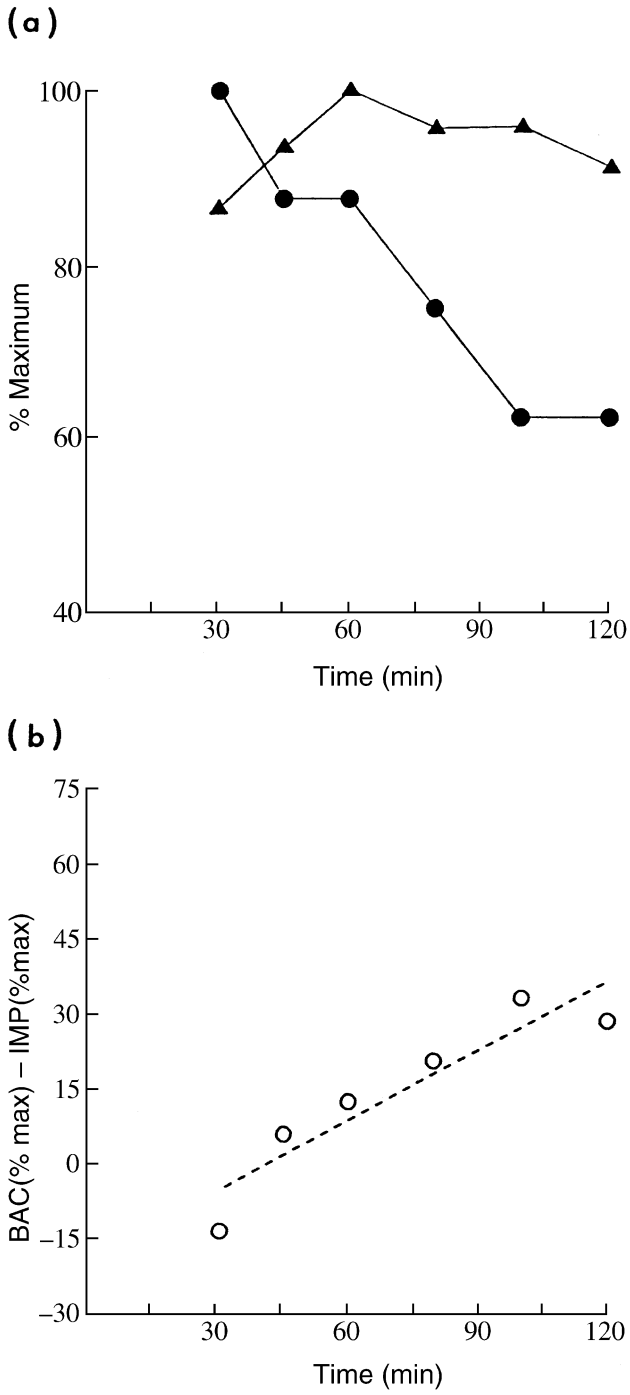

Fig. 1. (a) An example of data presentation from one subject at the 3.0 g/kg E dose in Experiment 2. At each time of assessment, data are presented for BACs (▲——▲% max) and impairment (IMP) ratings (●——● % max). (b) Computation of acute tolerance using the output function measure relating BACs and impairment from the same subject as shown in (a). The values from the output function (% max BAC – % max IMP) increase over time. The slope of this increase is the measure of acute tolerance.

ive maximum value for each rat, as shown in Fig. 1a. The output function is calculated as: % max BAC − % max IMP (Fig. 1b).

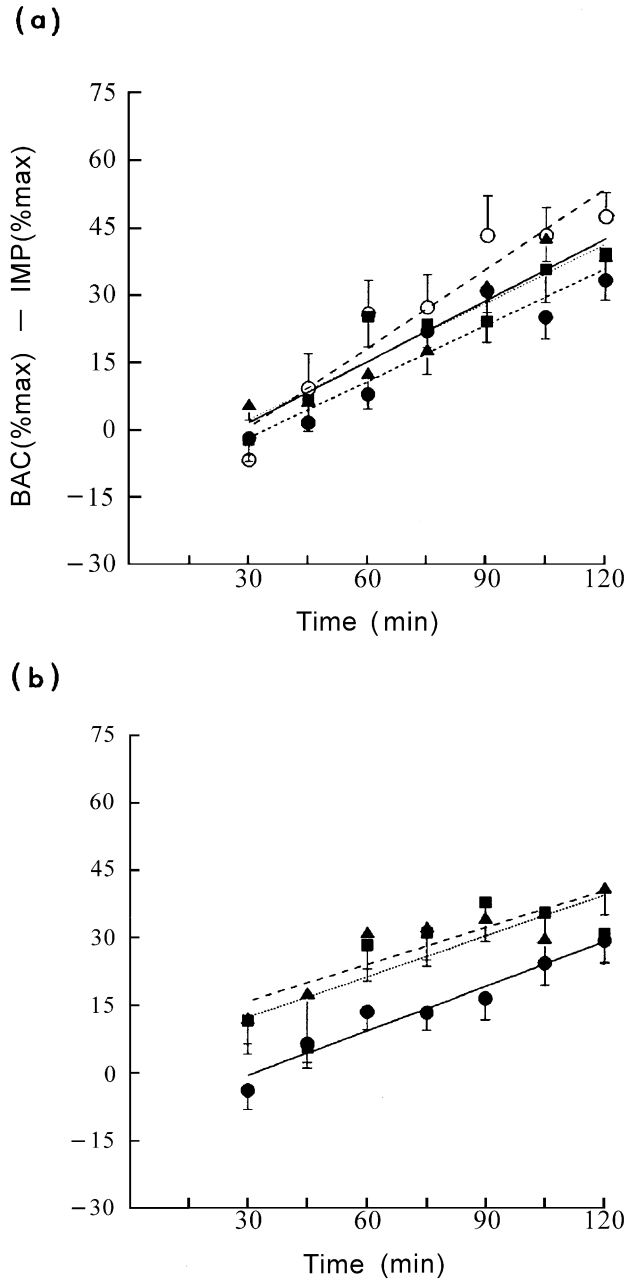

Fig. 2. (a) Effect of different ethanol doses on acute tolerance development. Four different dose groups received either 2.5 (○----○), 2.7 (■·····■), 3.0 (▲——▲) or 3.3 (●-·-·-●) (b) Effect of lower ethanol doses on acute tolerance development. Three different dose groups received either 1.9 (▲....▲), 2.3 (■----■) or 2.8 (●——●) g/kg E at 0 min. E-induced motor impairment was assessed at 30, 45, 60, 75, 90, 105 and 120 min. BAC measurement was taken immediately after each assessment. Acute tolerance development was expressed as in Fig. 1. Results shown are mean ± S.E.M. (n = 9–10 animals per group).

Table 1
Slope values and correlation coefficients for different dose groups

EtOH dose (g/kg)	Slope value (%/min)	Correlation coefficient
2.5	0.688 ± 0.087	.828 ± .024
2.7	0.652 ± 0.056	.849 ± .026
3.0	0.613 ± 0.040	.854 ± .020
3.3	0.558 ± 0.052	.888 ± .022

n = 9 animals at each dose level.

3.1. Experiment 1: acute ethanol tolerance — dose–response study

The output function values for each of the doses employed (Fig. 2a) increased over time (between 30 and 120 min), suggesting acute tolerance development. A multiple regression analysis of slopes (Table 1), with dose as the independent variable, showed a slightly negative correlation between slopes and doses (− .2658). However, the analysis of variance from regression analysis showed that the effect of dose was not significant, $F(1,34) = 2.58$, $P > .117$. These results suggested that the rate of acute tolerance development is independent of dose, over the E dose range used in this experiment. This was also confirmed with one-way ANOVA for slopes against different doses. The main effect of doses was not significant, $F(3,32) = 0.82$, $P > .495$. Similar results were found in the replication with lower E doses, $F(2,26) = 1.16$, $P > .3294$, again indicating that development of acute tolerance to E is independent of the E dose employed (Fig. 2b).

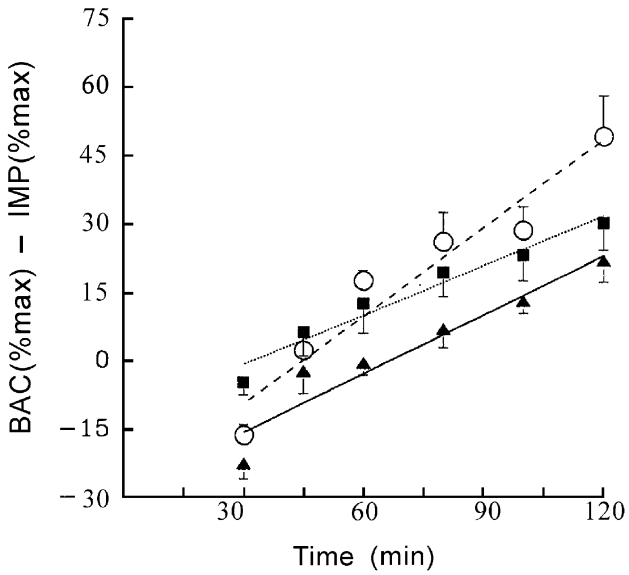

Fig. 3. Effect of NMDA antagonists on the development of acute tolerance to ethanol. One group received (+)MK-801 (▲——▲ 0.25 mg/kg ip), one group received ketamine (■·····■ 4 mg/kg ip) and a control group received saline (○---○), 30 min prior to ethanol (3 g/kg ip). E-induced motor impairment was assessed at 30, 45, 60, 80, 100 and 120 min. BAC was measured immediately after each assessment of performance. Acute tolerance development to E was expressed as in Fig. 1. Results shown are mean ± S.E.M. (n = 9 animals per group).

3.2. Experiment 2: effect of NMDA antagonists on acute ethanol tolerance

The results of these studies are shown in Fig. 3. The individual slopes were calculated from the output function values for each animal receiving saline, ketamine or (+)MK-801 pretreatments (Table 2). The output function values for each pretreatment group increased over time (i.e., slopes were positive), suggesting that acute tolerance developed in all three groups. However, a one-way ANOVA of the individual slope values revealed that the main effect of group was significant, $F(2,24) = 5.07$, $P < .0145$. A post hoc Newman–Keuls range test confirmed that the control group had significantly ($P < .05$) greater slopes than either the (+)MK-801 or the ketamine group, and that there was no difference in slopes for (+)MK-801 versus ketamine groups. There was also no difference between the means of individually calculated correlation coefficient values in the three groups. These results showed that administration of NMDA antagonists reduced the rate of development of acute tolerance to ethanol.

3.3. Experiment 3: effect of NMDA agonist (D-cycloserine) on acute ethanol tolerance

The output function values for each pretreatment group (S and CS) increased over time, suggesting acute tolerance development in both groups (Fig. 4a). The mean slope value (Table 3) for the S control group was significantly lower ($P < .01$) than for the CS group, suggesting that CS pretreatment enhanced acute tolerance to ethanol. This was confirmed by a one-way ANOVA of the individual slope values, which showed a significant main effect of pretreatment, $F(1,18) = 8.56$, $P < .009$.

3.4. Experiment 4: effect of 5-HT depletion with p-CPA on acute ethanol

The output function values for both pretreatment groups increased over time, indicating acute tolerance development in both water and *p*-CPA groups (Fig. 4b). However, the mean slope value for the water group was significantly higher ($P < .02$) than that for the *p*-CPA group (Table 3). This suggests that *p*-CPA pretreatment reduced acute tolerance. A one-way ANOVA of slopes in

Table 2
Slope values and correlation coefficients for different pretreatment groups

Pretreatment	Slope value (% min)	Correlation coefficient
Control	0.74 ± 0.10	.899 ± .014
Ketamine	0.44 ± 0.04 *	.856 ± .026
(+)MK-801	0.43 ± 0.07 *	.875 ± .023

$n = 9$ animals for each group.
* $P < .05$ (Newman–Keuls test) as compared to control.

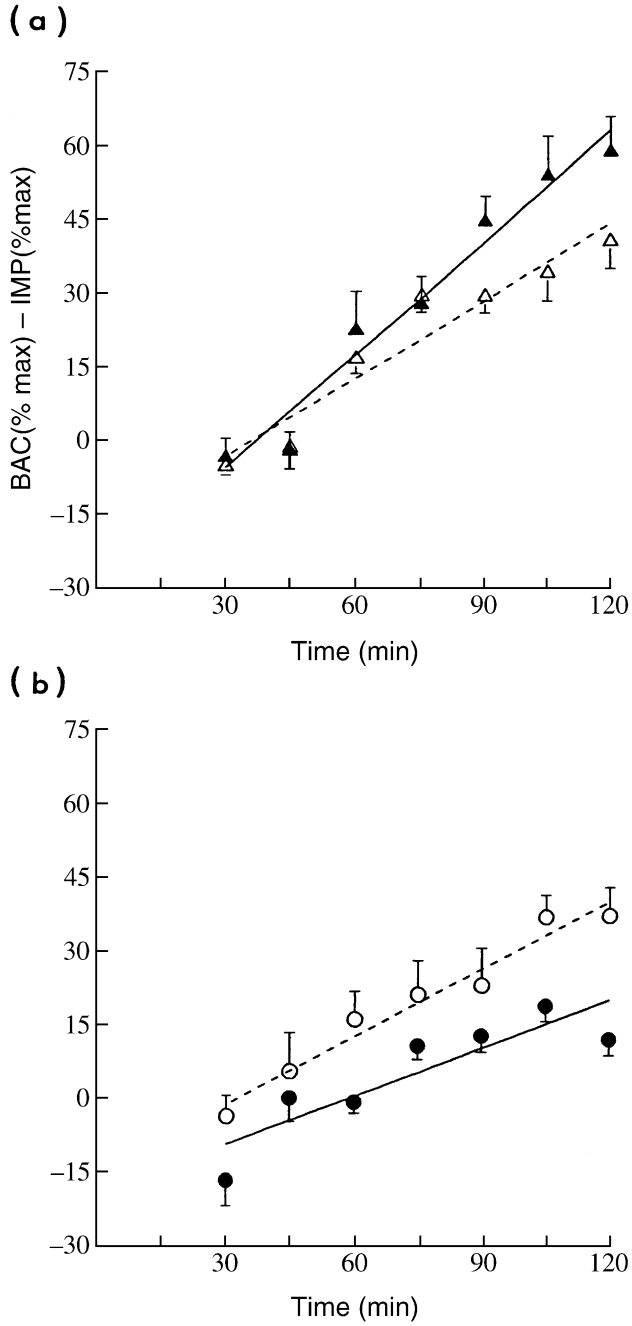

Fig. 4. (a) Effect of NMDA agonist (D-cycloserine) on the development of acute tolerance to ethanol. Two groups received either cycloserine (▲——▲ 30 mg/kg ip) or saline (△----△) 30 min prior to E (3 g/kg ip). E-induced motor impairment was assessed at 30, 45, 60, 75, 90, 105 and 120 min. BAC was measured immediately after each assessment. Acute tolerance development to ethanol was expressed as in Fig. 1. Results shown are mean ± S.E.M. ($n = 10$ animals per group). (b) Effect of 5-HT depletion (*p*-CPA) on the development of acute tolerance to E. Live groups were pretreated with *p*-CPA (●——● 100 mg/kg daily) or water (○---○) by gavage for 5 days. On the test day, E-induced motor impairment was assessed at 30, 45, 60, 75, 90, 105 and 120 min. BAC measurement was taken immediately after each assessment. Development of acute tolerance to E was expressed as in Fig. 1. Results shown are mean ± S.E.M. ($n = 7–8$ animals per group).

Table 3
Slope values and correlation coefficients for Experiments 3 and 4

	Group	Slope value (% min)	Correlation coefficient
Experiment 3[a]	Control	0.58 ± 0.06	.880 ± .022
	Cycloserine	0.85 ± 0.07*	.933 ± .010
Experiment 4[b]	Control	0.63 ± 0.06	.888 ± .016
	p-CPA	0.39 ± 0.06*	.804 ± .054

[a] $n = 10$ animals in each group.
[b] $n = 7$ to 8 animals in each group.
* $P < .05$ (Newman–Keuls test) as compared to control.

the two pretreatment groups confirmed that there was a significant main effect of pretreatment, $F(1,13) = 7.76$, $P < .0155$.

4. Discussion

The time course of change of the output function that relates BAC and impairment is consistent with the development of acute tolerance to the motor impairment effects of ethanol. As changes in blood levels of ethanol were measured simultaneously with changes in motor impairment, pharmacokinetic factors could be ruled out as a possible explanation of acute tolerance. The linear increase in the difference between percent of maximum BAC and percent of maximum IMP is consistent with earlier findings by others, and our findings of the same slope in all E dosage groups supports Radlow's (1994) hypothesis that development of acute tolerance is time dependent but dose independent. This suggests that acute tolerance is the result of an adaptive process that is triggered by the first effects of E and proceeds at an endogenously predetermined rate. It remains to be seen whether the maximum degree of acute tolerance that develops on a given occasion [i.e., the maximum attained value of % max BAC − % max IMP] is affected by dose or maximum BAC.

The difference between the effects of NMDA antagonists on acute tolerance in this study and in our previous study (Khanna et al., 1992c) may be related to the method used for measuring acute tolerance. In the previous study, the criterion for acute tolerance was the blood ethanol level at the time of recovery. This had been found earlier to be greater, the larger the ethanol dose given and the longer the time elapsed between ethanol administration and recovery (Maynert and Klingman, 1960). This procedure for assessing acute tolerance suffers from the drawback that the endpoint is a single measurement of recovery of function and blood alcohol level at that time. Since the time of recovery is progressively later, the higher the dose, there are necessarily two simultaneous variables influenced by dose, which cannot be resolved by a single measurement. The present method involves repeated measures that provide a slope of change in tolerance over time. This is a much better way of measuring acute tolerance than determination of a single point at recovery.

Similarly, comparison of the regression lines of impairment versus blood or brain alcohol concentration at different times after different doses of ethanol (LeBlanc et al., 1975), or in the presence and absence of NMDA antagonist, would be preferable to the Mellanby (1919) procedure or the two-dose technique (Waller et al., 1983). In the Mellanby method, comparison of acute tolerance is based on single-point determinations (just like the single-point recovery method), which may not be as reliable as the slope measurement done here, or the regression line comparison used by LeBlanc et al. (1975). The two-dose technique requires two injections of ethanol or drug and the index of acute tolerance is the difference in blood levels at recovery of function after each of the two doses. The problem with this method is that it does not truly reflect the total acute tolerance developed but only the *additional* acute tolerance observed after the second dose and disregards any acute tolerance development following the first dose. It is also subject to the drawback of individual differences in the time taken to reach recovery.

Although peripheral blood was used for BAC measurement in the present study, several studies have shown no differences between brain and plasma levels measured at the same time after intraperitoneal administration of ethanol (Gostomzyk et al., 1969; LeBlanc et al., 1975; Tabakoff et al., 1986), especially at times later than 10 min post-injection (Sunahara et al., 1978).

Since there was significant difference between NMDA antagonist and control groups with respect to output function, it appears that (+)MK-801 and ketamine did have an inhibitory effect on acute tolerance development (Fig. 3). This decrease in the slope of acute tolerance development by NMDA antagonists is similar to the results obtained previously with these agents on rapid and chronic tolerance (Khanna et al., 1991b, 1992a,c, 1993b, 1994). The earlier finding of a lack of effect of NMDA antagonists on acute tolerance (Khanna et al., 1992c) was in fact rather puzzling. The inhibitory effect of NMDA antagonists on rapid and chronic tolerance had been interpreted as an extension of its inhibitory effect on learning and memory (Khanna et al., 1994), because learning plays an important role in the development of rapid and chronic tolerance. Since learning was also found to play a significant role in acute tolerance (Lê and Kalant, 1992), it had been expected that NMDA antagonists would also hinder the development of acute tolerance. The present findings are consistent with that expectation.

Some caution is necessary in the interpretation of these findings. (+)MK-801 is a potent and highly selective non-competitive NMDA receptor antagonist that binds to a site in the receptor-activated ion channel (Woodruff et al., 1987; Javitt and Zukin, 1989). Ketamine appears to share the same mechanism of action, but is less potent and less selective (Wong et al., 1988). In view of the wide distribution of NMDA receptors throughout the brain, it is not surprising that at the doses used in these experiments, MK-801 and

ketamine have effects of their own on motor and cognitive functions. Wozniak et al. (1990) reported that a dose of 0.2 mg/kg of (+)MK-801 caused marked impairment of a variety of sensorimotor performances in rats, and Rafi-Tari zet al. (1996) found that a dose of 0.15 mg/kg increased the error score on a circular maze test, independently of the motor effects. Some of these effects resemble those of ethanol, and it is conceivable that an apparent reduction in acute tolerance to ethanol might really be due to summation of the effects of the NMDA antagonist with those of ethanol. However, this seems unlikely, because the NMDA antagonists produced a change in the *slope* of the output function, rather than a parallel displacement.

The effect of 5-HT depletion in Experiment 4 is consistent with the results of our previous studies in which electrolytic lesions of the median raphe nuclei had the same effect on acute tolerance as on chronic tolerance (Campanelli et al., 1988; Khanna et al., 1987). Similarly, enhancement of NMDA activity by D-cycloserine enhanced acute tolerance to ethanol (Fig. 4a), just as it increased the development of rapid tolerance to ethanol (Khanna et al., 1993a, 1995). Although we have not examined the effect of D-cycloserine on chronic tolerance, numerous studies from this laboratory have shown that the results of many different manipulations on rapid tolerance and cross-tolerance parallel the results obtained in chronic tolerance and cross-tolerance studies (Khanna et al., 1991a, 1992b, 1994). Similarly, depletion of 5-HT by *p*-CPA was found to inhibit both rapid tolerance (Khanna et al., in preparation) and acute tolerance (present study) in the same way as shown previously for chronic tolerance (Frankel et al., 1977, 1978).

It was suggested many years ago (Kalant et al., 1971; Littleton, 1980) that chronic tolerance might be simply an acceleration of acute tolerance development, as a result of repeated exposure to ethanol. Indeed, such an acceleration of acute tolerance has been described in the course of development of chronic tolerance to ethanol (Kalant et al., 1978; Wu et al., 2001). However, the close resemblance between the effects of various interventions on acute, rapid and chronic tolerance does not in itself permit any conclusions as to whether the cellular mechanisms underlying the three forms of tolerance are identical or not.

In conclusion, the present results show that the NMDA antagonists and 5-HT depletion decrease the development of acute tolerance, whereas an NMDA agonist enhances acute tolerance, just as they have been previously shown to do with respect to rapid and chronic tolerance. This would suggest a close similarity among acute, rapid and chronic tolerance, although the exact nature of the relationship among them remains to be clarified.

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