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Discrimination of Ethanol in Rats: Effects of Nicotine, Diazepam, CGP 40116, and 1-(m-Chlorophenyl)-biguanide

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BIENKOWSKI, P. AND W. KOSTOWSKI. *Discrimination of ethanol in rats: Effects of nicotine, diazepam, CGP 40116, and 1-(m-chlorophenyl)-biguanide*. PHARMACOL BIOCHEM BEHAV **60**(1) 61–69, 1998.—The drug discrimination paradigm was used to evaluate the role of certain ligand-gated ion channels in the discriminative stimulus properties of ethanol. Rats were trained to discriminate ethanol (1.0 g/kg) from saline vehicle under the FR10 schedule of sweetened milk reinforcement. The discrimination of lower ethanol doses was enhanced by either the $GABA_A$ receptor positive modulator, diazepam (0.5 mg/kg), or nicotinic acetylcholine receptor agonist, nicotine (0.3 mg/kg). Neither diazepam nor nicotine produced any effect on the rate of responding. Both the NMDA receptor competitive antagonist, CGP 40116 (0.5 mg/kg) and the $5\text{-}HT_3$ receptor agonist, 1-(m-chlorophenyl)-biguanide (5.0 mg/kg) enhanced the cueing properties of lower ethanol doses, but these effects were associated with a significant reduction in the response rate. The ethanol-like stimulus effects produced by diazepam or CGP 40116 were not influenced by 0.3 mg/kg nicotine. In contrast, CGP 40116 moderately enhanced the ethanol-like stimulus effects of diazepam. The present results show that: 1) pretreatment with nicotine, diazepam, CGP 40116 or 1-(m-chlorophenyl)-biguanide enhance the ethanol discrimination; 2) neither the $GABA_A$ nor the NMDA receptor complex alone is critically involved in the nicotine-induced enhancement of the ethanol discrimination; 3) NMDA receptor competitive antagonist and GABAergic benzodiazepine derivative may produce moderate additive effects in rats trained to discriminate ethanol. © 1998 Elsevier Science Inc.

Ethanol Nicotine Ligand-gated ion channels Drug combinations Drug discrimination Rat

THE drug discrimination procedure has proven to be a useful behavioral test for studying the actions of drugs upon the central nervous system (1,11). With this task, the interoceptive effects of training drugs are used as discriminative stimuli to indicate which of two (or more) responses may lead to reinforcement (11,16). Once a drug effect has been established as a discriminative stimulus (an interoceptive cue) it can be tested for generalization to other drugs or antagonized by still other compounds (11,19,23). Moreover, it has been reported that compounds that neither substitute for nor antagonize the stimulus effects of the training drug may alter its cueing properties (i.e., shift the discrimination dose–response curve) when given in combination with lower doses of the training substance (16,30,50). The test procedures mentioned above are especially useful for identifying receptor mechanisms involved in the formation of drug-induced interoceptive stimuli (1,11,25,51).

Many articles published during the last decade have indicated that ethyl alcohol (ethanol) may interact in a specific manner with several subtypes of central ionotropic receptors (ligand-gated ion channels) (12,22,35,37,38,41). For example, both electrophysiological and biochemical studies have shown that alcohol enhances GABA-mediated inhibition of neuronal activity and increases chloride transport through the $GABA_A$/benzodiazepine/Cl^- receptor complex-associated channel (12,22,55). The above findings were confirmed by several behavioral experiments showing that changes in the $GABA_A$ receptor conductance may be responsible for the ethanol-induced intoxication and stimulus effects (1,22,46, 49,52).

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Ethanol enhances the ion transport through the channels associated with either the serotonergic 5-HT_3 receptor (22,37) or the nicotinic acetylcholine receptor (nAChR) [(6,12,15); but see also (17,61)]. The functional significance of these interactions is less clear because ethanol seems to accelerate the rate of desensitization of both nAChRs and 5-HT_3 receptors (10,22,41,43). Notably, in line with the former results both 5-HT_3 receptor antagonists and a noncompetitive nAChR antagonist, mecamylamine reverse certain biochemical (6,9) and behavioral effects of ethanol (7,8,33,35). On the other hand, neither a nAChR agonist, nicotine, nor mecamylamine generalized from the ethanol interoceptive cue. In addition, mecamylamine did not antagonize the discriminative stimulus effects of ethanol (5,18,27).

The first experiments with 5-HT_3 receptor antagonists gave some support for the involvement of 5-HT_3 receptors in the mediation of the ethanol cue in pigeons (23) and rats (27). Some, but not all, 5-HT_3 receptor antagonists attenuated the discriminative stimulus properties of alcohol. At least in the case of the latter study with rats rather pharmacokinetic factors than a direct interaction at the receptor level were responsible for the result described above (27). Other investigations did not reveal any role of 5-HT_3 receptors in the ethanol cueing effects. Thus, relatively selective 5-HT_3 receptor agonist, 1-(m-chlorophenyl)-biguanide (mCPBG) (40) did not substitute for ethanol (53), and none of the widely used 5-HT_3 receptor antagonists (i.e., tropisetron, bemesetron or ondansetron) attenuated the ethanol cue (34,53).

Ethanol has been found to antagonize the *N*-methyl-D-aspartate (NMDA) receptor-mediated biochemical and electrophysiological responses, leading to the hypothesis that at least certain central effects of ethanol may result from interaction with the NMDA receptor complex (22,38,46). In line with this hypothesis are the data showing that NMDA receptors may contribute to ethanol-induced discriminative stimulus effects, intoxication, and withdrawal symptoms (2,13,24,38,49). In rats, both competitive and uncompetitve NMDA receptor antagonists have been shown to produce either full or partial substitution for ethanol (2,24,45,49). Thus, at least two ionotropic receptors, i.e., the $GABA_A$ and the NMDA receptor, seem to be directly involved in the formation of the discriminative stimulus effects of ethanol (1,2,24,25).

Importantly, the extent to which drugs acting at the $GABA_A$ and the NMDA receptor substitute for ethanol depends strongly upon the ethanol training dose (24,25). Notably, studies with drug mixtures have shown that components of the mixed cue change with relative prominence with changes in training dose ratios (19,59). Taken together, the results cited above have led some authors to the hypothesis that the discriminative cue of alcohol can be viewed as a mixed (or compound) stimulus (24–26,59). Thus, the cueing effects of a given ethanol dose may be composed of both "major" and "minor" component(s) with the latter element(s) overshadowed by the former (19,24–26,50,51,59). If it is true, this hypothesis has important implications for the interpretation of the data coming from antagonism tests. For example, elimination of "minor" component(s) from the mixed ethanol stimulus may be insufficient for the successful attenuation of the ethanol discrimination (1,3,25,59). This may lead to a "false negative" result when one attempts to identify "minor" components of the mixed drug stimulus (3,59). Moreover, one could hypothesize that the ethanol cue might be mimicked by the cueing effects of a specific drug combination. Such combination might consist of drugs representing both "major" and/or "minor" components of the ethanol stimulus.

In previous reports (2,4) we have shown that the 1.0 g/kg ethanol stimulus generalized to diazepam and the competitive NMDA receptor antagonist (48), CGP 40116. In contrast, neither the 5-HT_3 receptor agonist, mCPBG, nor nicotine substituted for 1.0 g/kg alcohol (5,53). Given the mixed nature of the ethanol stimulus and evidences for the involvement of both 5-HT_3 receptors and nAChRs in the central effects of ethanol, one could try to explain the latter negative results by an overshadowing phenomenon (11,19,59). Thus, both the 5-HT_3 receptor- and the nAChR-related component of the alcohol stimulus may be overshadowed by the "stronger" of the $GABA_A$ receptor- and the NMDA receptor-related components.

The purpose of the present study was twofold. First, we wanted to assess the ability of diazepam, CGP 40116, nicotine, and mCPBG to shift the dose–response curve of the ethanol discrimination (Experiment 1). In agreement with previous reports (18,50), nicotine has been found to enhance the ethanol discrimination in the present study. The second aim of the present investigation was to identify possible mechanisms of the nicotine–ethanol interaction (Experiment 2). For this purpose, we examined the ability of nicotine to alter the ethanol-like stimulus effects produced by diazepam and CGP 40116. In addition, the ability of CGP 40116 to alter the ethanol-like stimulus effects of diazepam was studied. Thus, in Experiment 2 we have also tested the hypothesis (see above) that certain drug mixtures might mimic the cueing properties of alcohol.

The doses of the test compounds were selected on the basis of our previous experiments [(2,4,53); Bienkowski et al., unpublished]. These doses produced a submaximal level of ethanol-like responding without affecting the rate of responding.

METHOD

Subjects

Sixteen male Wistar rats (300–330 g at the beginning of the study) were individually housed in plastic cages, in a temperature-controlled (22–23°C) vivarium with 12 L:12 D cycle (lights on at 0700 h). Rats were maintained at ~80% of weight of the free-feeding control group by restricting daily food (Bacutil, Poland) to 15–20 g. Tap water was available ad lib. Experimental and test sessions were conducted between 1400 and 1800 h. All animal care and experimental procedures were approved by our institutional ethical committee.

Apparatus

Standard two-lever operant conditioning chambers (Coulbourn Instruments, Inc., Allentown, PA) consisted of modular test cages (E10-10TC) enclosed within sound-attenuated cubicles with fans for ventilation and background white noise. A white house light was centered near the top of the front panel of the cage, which was also equipped with two response levers, separated by a liquid dipper (Coulbourn model E14-05, module size ½), all positioned 4.0 cm above the grid floor. The liquid dipper presented sweetened milk in a 0.01-ml portion for 5 s during each operation. Experimental sessions and data recording were accomplished using the L91-04 interface and the D91-12 L2T2 software package (Coulbourn) running on a IBM-PC compatible.

Drug Discrimination Procedure

The procedure, similar to the fixed-ratio 10 (FR10) drug discrimination paradigm described by Colpaert (11), was essentially the same as the procedure described in previous re-

ports from this laboratory (2,53). The animals were initially trained to press both levers under a fixed-ratio 1 (FR1) schedule of sweetened milk delivery. Drug discrimination training began only after all of the animals responded reliably on both levers under the FR 10 condition. The rats were trained to press one lever following IP ethanol injection (1.0 g/kg, 10% v/v, 13.0 ml/kg) and to press the other lever following saline vehicle (0.9% NaCl) injections under an FR10 schedule of sweetened milk reinforcement. Injections occurred 15 min prior to the start of 15-min sessions. The lever corresponding with ethanol and saline treatment remained fixed for the duration of the study for a given animal and was counterbalanced across the group of rats. The sessions were conducted Monday through Friday (or Saturday) under the alternating drug sequence: drug-vehicle-vehicle-drug-drug and vehicle-drug-drug-vehicle-vehicle. To avoid the possibility that the correct lever for rats previously tested in the chambers could serve as an olfactory cue, the sequence of treatments on the training days was alternated for successive groups (i.e., half of the animals received vehicle and half received ethanol). Also, the levers were carefully cleaned with 50% ethanol solution after each session. The responses emitted on the incorrect lever were recorded but did not result in sweetened milk delivery. The animals continued to be trained under these conditions until they exhibited the acquisition criteria, which were defined as both correct first-lever selection ($\geqslant$80%) and greater that 90% correct-lever responding during the entire session, for 9 out of 10 consecutive sessions. In addition, the animals were also required to maintain response rates greater than 0.45 responses/s throughout the entire 10-session period (11,53).

After the animals reached the criteria experimental test sessions were initiated. Typically, the test sessions were conducted twice per week with training sessions intervening during the remaining days. To be tested in each subsequent test session the rat must have reached the acquisition criteria for at least 3 days. During the test session the lever on which 10 responses occurred first continued to be reinforced for the remainder of the 15-min session. Response on the other lever were recorded but not reinforced. In preliminary ethanol dose–response sessions, which were performed in the beginning of the study, rats were tested after the administration of various doses of ethanol (0.0–1.0 g/kg, IP; 10% v/v) 15 min before start of the test session. Other experimental protocols are described below.

Experiment 1

In Experiment 1 the ability of different psychoactive compounds to shift the ethanol dose–response curve was determined. Rats were given the generalization tests with saline vehicle or different doses of ethanol (0.25, 0.5, or 1.0 g/kg) after pretreatment with either diazepam (0.5 mg/kg, IP; 15 min before ethanol), CGP 40116 (0.5 mg/kg, IP; 45 min), nicotine (0.3 mg/kg, SC; 5 min), or mCPBG (5.0 mg/kg, IP; 5 min). The order in which the various doses of ethanol and each test compound were administered was counterbalanced for all rats.

Experiment 2

In Experiment 2 the ability of different drug mixtures to generalize from the ethanol cue was investigated. After the completion of Experiment 1, rats were tested with different doses of diazepam (0.0, 0.5, or 1.0 mg/kg, IP) or CGP 40116 (0.0, 0.5, or 1.25 mg/kg, IP) given in combination with 0.3 mg/kg nicotine. Diazepam and CGP 40116 were injected 30 and 60 min, respectively, before start of the session. Nicotine was administered 20 min before start of the session, i.e., 10 and 40 min, respectively, after diazepam and CGP 40116 injection. In addition, the ability of diazepam given in combination with CGP 40116 to substitute for ethanol was studied. CGP 40116 (0.5 mg/kg) was given 30 min before diazepam administration, i.e., 60 min before start of the test session.

Drugs

Ethanol (95%) was obtained from the hospital pharmacy and diluted to the final concentration with 0.9% NaCl. In the dose–response tests ethanol was administered in appropriate volumes to obtain a desired dose. Diazepam (Polfa, Warsaw, Poland) was suspended in 1% Tween and administered in a volume of 2.0 ml/kg. CGP 40116 (D-(E)-2-amino-4-methyl-5-phosphono-3-pentanoate), nicotine di-d-tartrate (RBI, Natick, MA) and 1-(m-chlorophenyl)-biguanide hydrochloride (mCPBG; RBI) were dissolved in 0.9% NaCl and administered in a volume of 2.0 ml/kg. The nicotine solution was adjusted to pH = 7.0 with diluted NaOH. All drug solutions were prepared immediately prior to use. Only the dose of mCPBG referred to the salt form.

Data Analysis

The method of data presentation and analysis was adapted from Druhan et al. (16). The percentage of ethanol-appropriate responding was calculated for each session, using only the responses that occurred before the first reinforcement, by dividing the responses made on the ethanol-appropriate lever by the total number of responses on both levers, and multiplying the result by 100. The percentage data were transformed (arc-sin transformation) prior to the analysis (24,26). A two-way analysis of variance (ANOVA) for repeated measures was used to assess whether the percentage of ethanol-appropriate responding across several doses (between group factor) of ethanol in Experiment 1, diazepam or CGP 40116 in Experiment 2, differed in the presence vs. absence of a test drug pretreatment (repeated measure factor). In the cases where the test drug was administered alone, the data were treated as an additional ethanol, diazepam, or CGP 40116 dose level (i.e., 0.0 mg/kg) and included in the two-way ANOVA. The operational definition of complete stimulus substitution was 80% (or more) of responding on the ethanol appropriate lever (2,24). The response rates were calculated as the total number of responses (on both levers) during the session divided by the session time in seconds (900 s). The two-way ANOVAs were performed on the response rate data. When the significant main effect of the test drug pretreatment or the test drug pretreatment $\times$ ethanol dose interaction was found, post hoc analyses were done using the Newman–Keuls test. Differences revealed with both the ANOVAs and the Newman–Keuls tests were treated as significant when the probability level (p) was less than 0.05. The discrimination data, but not the response rate data, were excluded from the analysis if the rat failed to complete at least one FR10 during the 15-min test session, i.e., failed to obtain at least one reinforcement. The ED_50 (and 95% C.L.) was calculated for the preliminary dose–response tests (with ethanol alone) according to the method of the Litchfield and Wilcoxon (56).

RESULTS

All animals acquired the ethanol–saline discrimination (range: 29–62 training sessions). The ED_{50} (0.57 g/kg; C.L.:

0.33–0.71) for ethanol was similar to the values (0.45–0.56 g/kg) reported in the previous articles from this laboratory (2–5,53).

Experiment 1

Diazepam. The two-way ANOVA indicated a significant effect of ethanol dose, $F(3, 26) = 38.59$, $p < 0.001$; diazepam pretreatment, $F(1, 26) = 13.01$, $p < 0.01$, and significant ethanol dose × diazepam pretreatment interaction, $F(3, 26) = 5.49$, $p < 0.01$. Diazepam increased the 0.5 g/kg ethanol discrimination (from 19 to 89%, $p < 0.01$; the Newman–Keuls test) above the 80% criterion of complete stimulus substitution (Fig. 1A, upper panel). The ANOVA did not show any significant changes in the mean response rate (Fig. 1A, lower panel).

CGP 40116. The ANOVA showed a significant effect of ethanol dose, $F(3, 32) = 18.24$, $p < 0.001$, and CGP 40116 pretreatment, $F(1, 32) = 10.38$, $p < 0.01$. The interaction was not significant, $F(3, 32) = 1.54$, $p = 0.22$. CGP 40116 significantly enhanced the discrimination of 0.5 g/kg ethanol (from 23 to 72%, $p < 0.01$) (Fig. 1B, upper panel). The ANOVA revealed a significant effect of CGP 40116 pretreatment on the mean response rate, $F(1, 32) = 7.61$, $p < 0.01$. The effect of ethanol dose, $F(3, 32) = 2.57$, $p = 0.07$, and the interaction, $F(3, 32) = 2.67$, $p = 0.06$, was not significant (Fig. 1B, lower panel). CGP 40116 given in combination with 1.0 g/kg ethanol significantly suppressed the rate of responding.

Nicotine. The ANOVA found a significant effect of ethanol dose, $F(3, 32) = 18.81$, $p < 0.001$, and nicotine pretreatment, $F(1, 32) = 8.47$, $p < 0.01$. The interaction was not significant, $F(3, 32) = 1.47$, $p = 0.24$. Nicotine pretreatment increased the cueing properties of 0.5 g/kg ethanol (from 32 to 80.5%, $p < 0.01$) above the 80% criterion of complete stimulus substitution (Fig. 2A, upper panel). The ANOVA did not reveal any effect of nicotine on the rate of responding, $F(1, 32) = 0.007$, $p = 0.93$ (Fig. 2A, lower panel). Similarly, there was no significant ethanol dose × nicotine pretreatment interaction, $F(3, 32) = 1.74$, $p = 0.17$. However, the effect of ethanol dose was marginally significant, $F(3, 32) = 3.01$, $p = 0.045$.

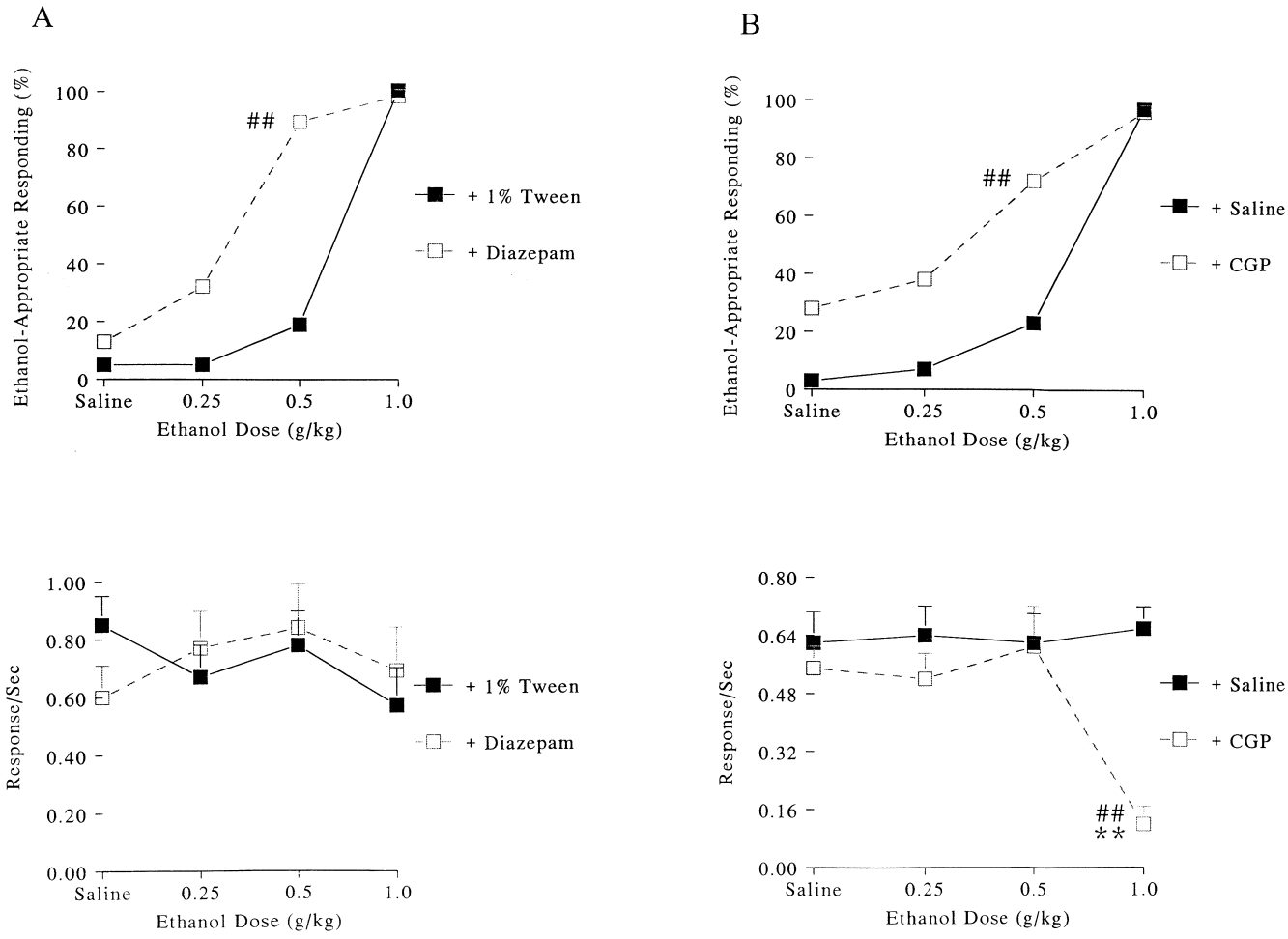

FIG. 1. Effect of diazepam (A) and CGP 40116 (B) on the discriminative stimulus effects of ethanol. The data (upper panel) are presented as the mean percentage of ethanol appropriate responding as a function of increasing dosages of ethanol after pretreatment with diazepam (0.5 mg/kg) or CGP 40116 (0.5 mg/kg). Mean (±SEM) response rates are presented below. *$p < 0.05$, **$p < 0.01$ vs. the respective control group treated only with vehicles, #$p < 0.05$, ##$p < 0.01$ vs. the respective group treated with ethanol and the test drug vehicle; n = 8–11 rats. CGP = CGP 40116.

mCPBG. The ANOVA indicated a significant effect of ethanol dose, $F(3, 28) = 33.42$, $p < 0.001$, and mCPBG pretreatment, $F(1, 28) = 5.00$, $p < 0.05$. The interaction was also significant, $F(3, 28) = 3.07$, $p < 0.05$. mCPBG significantly enhanced the cueing effects of 0.5 g/kg ethanol (from 11 to 59%, $p < 0.05$) (Fig. 2B, upper panel). The ANOVA showed significant effect of mCPBG pretreatment on the mean rate of responding, $F(1, 28) = 10.23$, $p < 0.01$. The effect of ethanol dose and the interaction were not significant (Fs < 0.8) (Fig. 2B, lower panel).

Experiment 2

Nicotine–diazepam combination. The ANOVA showed only a significant effect of diazepam dose, $F(2, 25) = 7.08$, $p < 0.01$. Neither the effect of nicotine pretreatment, $F(1,25) = 1.04$, $p = 0.31$, nor the interaction, $F(2, 25) = 0.49$, $p = 0.61$, was significant (Fig. 3A, upper panel). The ANOVA on the response rate data revealed significant effect of diazepam dose, $F(2, 26) = 4.99$, $p < 0.05$, and nicotine pretreatment, $F(1, 26) = 9.71$, $p < 0.01$. The interaction was not significant, $F(2, 26) = 1.29$, $p = 0.29$. Nicotine given in combination with diazepam suppressed the response rate when compared with the saline-treated control group (Fig. 3A, lower panel). The combination of nicotine with 1.0 mg/kg diazepam made one out of nine rats unable to complete even one FR10.

Nicotine–CGP 40116 combination. The ANOVA revealed a significant effect of CGP 40116 dose, $F(2, 26) = 9.17$, $p < 0.01$. Neither the effect of nicotine pretreatment nor the interaction was significant (both Fs < 0.7) (Fig. 3B, upper panel). The ANOVA for the response rate results showed a significant effect of CGP 40116 dose, $F(2, 16) = 5.12$, $p < 0.05$, and nicotine pretreatment, $F(1, 26) = 10.49$, $p < 0.01$. The interaction was not significant, $F(2, 26) = 1.94$, $p = 0.16$. Nicotine given in combination with 1.25 mg/kg CGP 40116 significantly decreased the rate of responding (Fig. 3B, lower panel) and made two out of nine rats unable to complete even one FR10.

CGP 40116–diazepam combination. The ANOVA showed a significant effect of diazepam dose, $F(2, 25) = 6.04$, $p < 0.01$, and CGP 40116 pretreatment, $F(2, 25) = 8.05$, $p < 0.01$. The

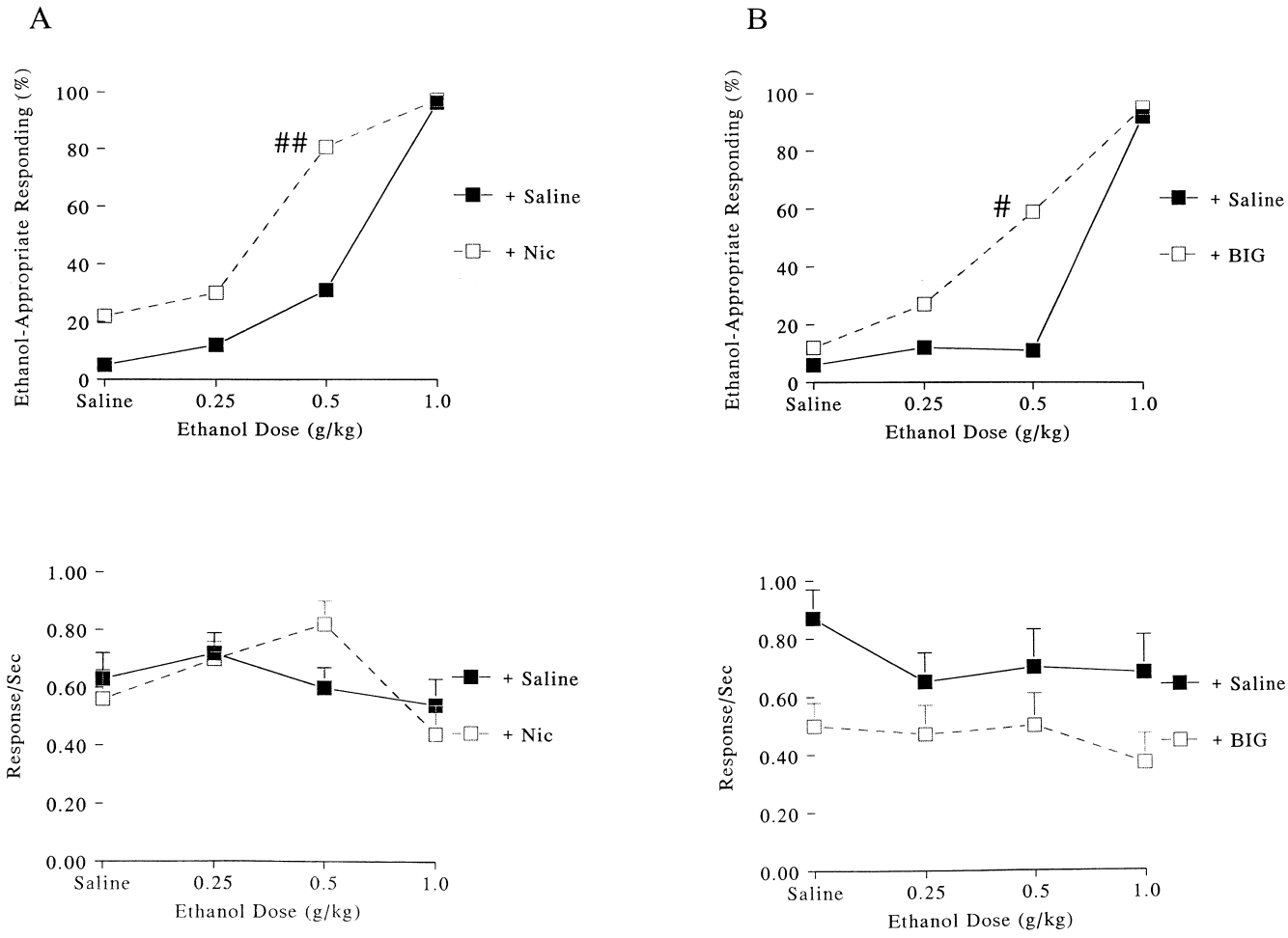

FIG. 2. Effect of nicotine (A) and mCPBG (B) on the discriminative stimulus effects of ethanol. The data (upper panel) are presented as the mean percentage of ethanol appropriate responding as a function of increasing dosages of ethanol after pretreatment with nicotine (0.3 mg/kg) or mCPBG (5.0 mg/kg). Mean (±SEM) response rates are presented below. *$p < 0.05$, **$p < 0.01$ vs. the respective control group treated only with saline, #$p < 0.05$, ##$p < 0.01$ vs. the respective group treated with ethanol and saline; n = 8–11 rats. NIC = nicotine; BIG = mCPBG = 1-(m-chlorophenyl)-biguanide.

interaction was not significant, $F(2, 25) = 0.21$, $p = 0.8$. CGP 40116 increased the ethanol-like responding after 0.5 mg/kg diazepam above the 80% criterion of complete stimulus substitution. However, none of the groups treated with the drug combination differed significantly from their respective control group treated with saline–diazepam combinations. Moreover, effects of CGP 40116–diazepam mixtures were smaller than expected from the combined effects of the two constituent drugs (Fig. 4) The response rates were not affected by the mixtures (Fs < 0.9).

DISCUSSION

The results of Experiment 1 revealed (Fig. 1) that both diazepam and CGP 40116 enhanced the ethanol discrimination (i.e., shifted the ethanol dose–response curve to the left). These findings are not particularly surprising because both compounds produce behavioral effects similar in many aspects, including the discriminative stimulus effects, to ethanol (2,13,22,45,48,49,60). Our results are in line with those of Järbe and McMillan (30), who reported a marked enhancement of the ethanol discrimination by diazepam in pigeons. On the other hand, Schechter and Lovano (47) did not observe additive effects after coadministration of ethanol and another full benzodiazepine agonist, chlordiazepoxide, in rats trained to discriminate 0.6 g/kg ethanol from its vehicle. Lower ethanol training dose and different benzodiazepine receptor agonist used in the latter study may explain the discrepancy between the results mentioned above.

The 5-HT_3 receptor agonist, mCPBG, moderately increased the cueing properties of ethanol (Fig. 2). In contrast to diazepam and nicotine, it reduced the overall response rate as well. Notably, in our previous investigation (52) mCPBG did not produce any remarkable ethanol-like cueing effects. The release of many neurotransmitters (e.g., GABA, serotonin, or dopamine) has been reported to be under control of 5-HT_3 receptors (9,31,40). Thus, many receptor systems may be indirectly involved in the mCPBG-induced enhancement of the ethanol discrimination. Anyway, our finding suggests that 5-HT_3 receptors are involved, though not primarily, in the formation of the ethanol stimulus. Certainly, further pharmacological studies are needed to confirm this hypothesis.

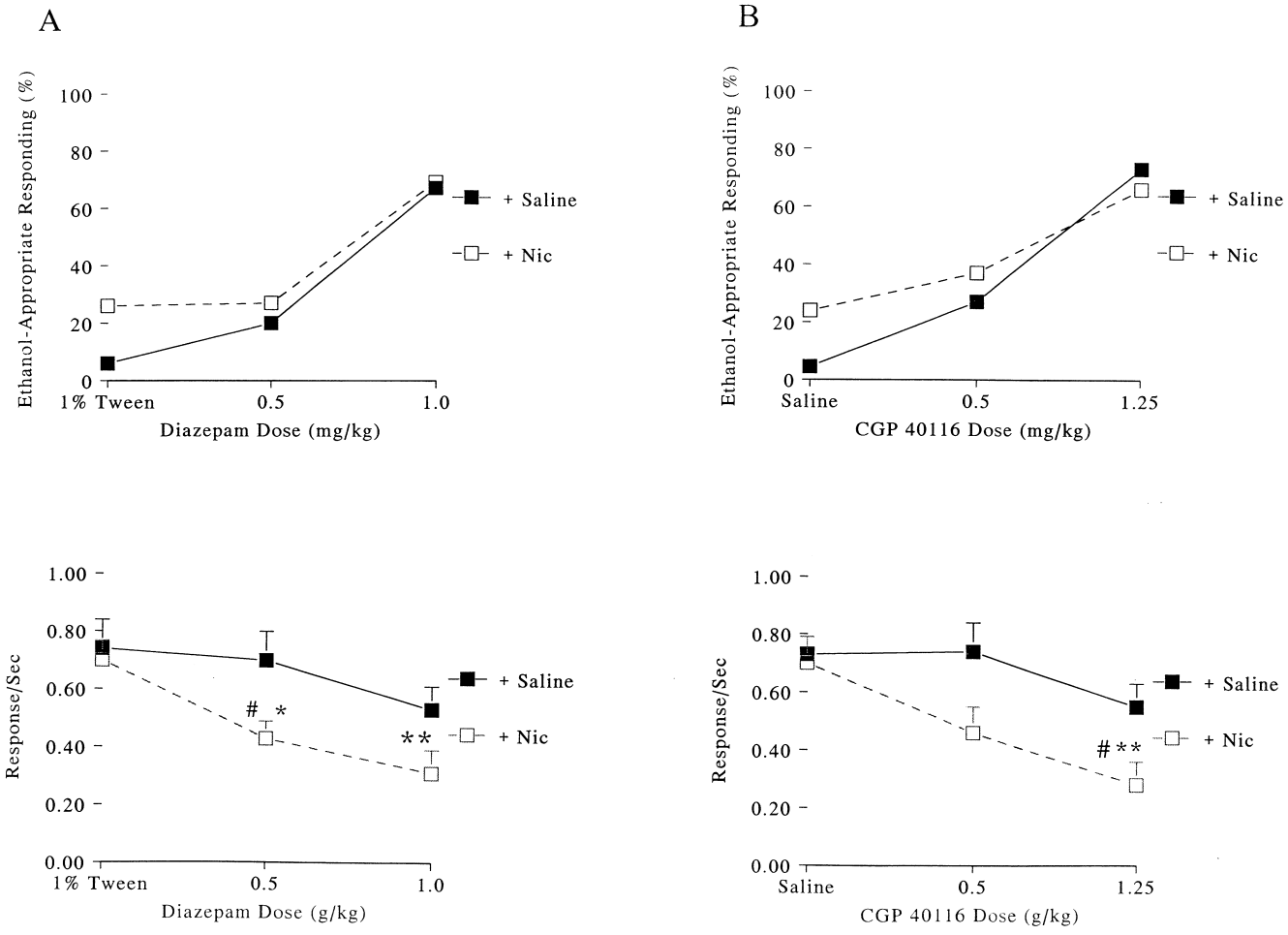

FIG. 3. Effect of nicotine (0.3 mg/kg) on the ethanol-like stimulus effects of diazepam (A) and CGP 40116 (B). The data (upper panel) are presented as the mean percentage of ethanol appropriate responding as a function of increasing dosages of diazepam or CGP 40116 after pretreatment with nicotine or saline. Mean (±SEM) response rates are presented below. *$p < 0.05$, **$p < 0.01$ vs. the respective control group treated only with vehicles, #$p < 0.05$, ##$p < 0.01$ vs. the respective group treated with diazepam or CGP 40116 and saline; n = 9–11 rats. NIC = nicotine.

Recently, the selectivity of mCPBG has been questioned because a large part of its behavioral effects was not affected by selective 5-HT_3 receptor antagonists (28). Besides, the pharmacokinetic interaction between ethanol and any substance tested in Experiment 1 cannot be ruled out.

Furthermore, we have also shown that nicotine (0.3 mg/kg) increased the cueing effects of ethanol in the present study. This finding is especially important in the light of many clinical reports that nicotine is the most commonly coabused substance in alcohol-abusing humans (10,29,39). Nicotine (0.2–0.4 mg/kg) has been previously reported to enhance the ethanol discrimination in rats (50). More recently, Gatto et al. (18) have reported that nicotine (0.56 mg/kg) shifted the dose–response curve of the ethanol discrimination to the left. Importantly, the blood ethanol level was not affected by nicotine in the latter study. In rats trained to discriminate nicotine, however, ethanol, up to the dose of 0.75 g/kg, neither substituted for nicotine nor affected the nicotine discrimination dose–response curve (18). Moreover, nicotine has been found to substitute for ethanol in the genetically selected for high alcohol-preference P line of rats but not in the low alcohol-preferring NP line of rats (21). Based on the results mentioned above, one could hypothesize that the ethanol action at the nAChR is involved, although not primarily (see the introductory paragraphs), in the formation of the ethanol cueing effects. This hypothesis is supported by several recent studies that have shown that ethanol alters the function of nAChRs (10,12,41,61). For example, ethanol accelerates the desensitization of both central and peripheral nAChRs (14,41,44). Moreover, ethanol has been reported to decrease excitatory responses to nicotine in the rat locus coeruleus neurons (17) and to inhibit the function of the $\alpha2\beta2$ and the $\alpha7$ nAChR subtype in Xenopus oocytes (15,61). In line with the above, high doses of ethanol have been reported to attenuate the stimulus effects of nicotine (32). In mice, a nonanticonvulsant dose of ethanol added to nicotine accelerated the behavioral desensitization to subsequent nicotine-induced seizures (14). On the other hand, ethanol enhanced the excitatory effects of nicotine in the majority of cells in the rat substantia nigra reticulata and ventral pallidum (12) and potentiated acetylcholine responses in the $\alpha2\beta4$ nAChR subtype (15). In line with these latter reports, mecamylamine blocked the ethanol-induced dopamine release and hyperlocomotion (6,8). Thus, it still remains an open question whether ethanol enhances or inhibits the function of central nAChRs in vivo.

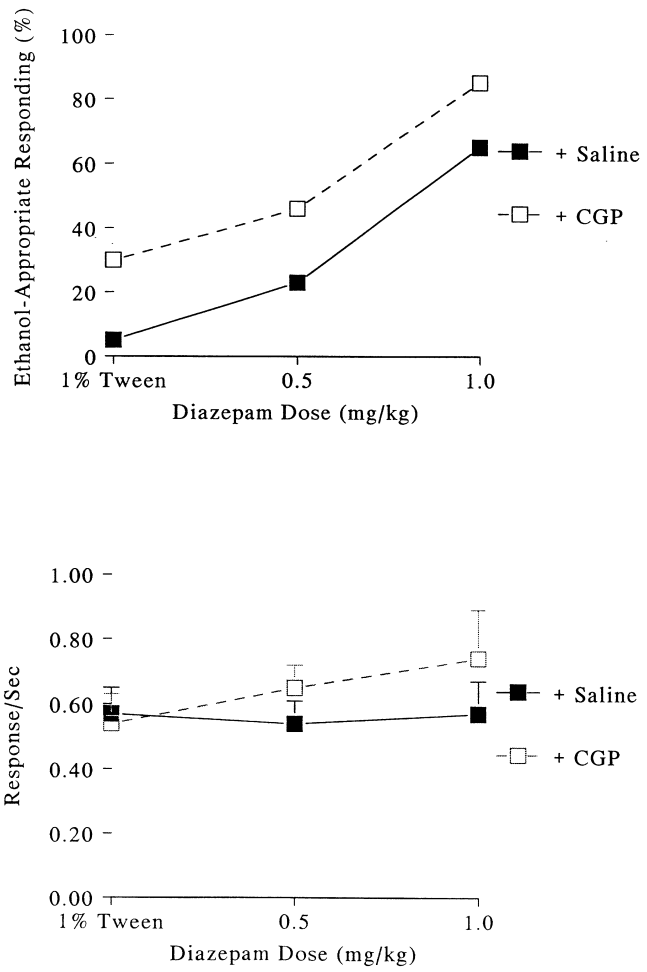

FIG. 4. Effect of CGP 40116 (0.5 mg/kg) on the ethanol-like stimulus effects of diazepam. The data (upper panel) are presented as the mean percentage of ethanol appropriate responding as a function of increasing dosages of diazepam after pretreatment with CGP 40116 or saline. Mean (±SEM) response rates are presented below. n = 9–11 rats. CGP = CGP 40116.

Apart from direct interactions at the receptor level, nicotine might improve the ethanol discrimination by several other mechanisms. As suggested by some authors, nicotine could increase the cueing effects of ethanol by the nonspecific enhancement of performance in the drug discrimination experiments (18). However, this explanation seem rather unlikely because in the present study nicotine was not able to increase the ethanol-like cueing effects of diazepam or CGP 40116 (Experiment 2).

Although usually classified as a psychostimulant, nicotine has been suggested to possess anxiolytic and/or antistress properties in rats and humans (20,42,54,57). One could speculate that nicotine increases the ethanol cueing effects by a nonspecific enhancement of its tranquilizing properties (of course, assuming that this part of ethanol subjective profile has anything to do with its discriminative stimulus properties). The results from Experiment 2 argue against this possibility because nicotine did not influence the ethanol-like cueing effects of another potent anxiolytic compound, diazepam.

Nicotine has been shown to alter the activity of many neurotransmitter systems within the CNS via its agonist action on nAChRs on presynaptic nerve terminals. For example, nicotine may enhance GABA and glutamate release in different regions of the brain (36,44,54,58). These latter properties of nicotine could tend to increase or decrease the ethanol cueing effects, respectively. Our results showing that nicotine do not alter the ability of diazepam or CGP 40116 to substitute for ethanol argue against any specific interaction between nAChRs and $GABA_A$ or NMDA receptors in the formation of the ethanol interoceptive cue.

As shown in Experiment 2, CGP 40116 enhanced the ethanol-like stimulus effects of diazepam. However, this effect was rather modest and less evident than could have been expected from a simple summation of the ethanol-lever responding produced by CGP 40116 and diazepam when given alone. This would support the notion that the ethanol stimulus is formed from distinct components, which when given together, do not produce a completely new entity but can be, to a certain extent, processed separately (24–26,59). Our results should be, however, treated with caution because only one

class of $GABA_A$ receptor positive modulators and NMDA receptor blockers has been studied. As pointed out before, drugs from different classes of $GABA_A$ and NMDA receptor ligands might generalize from the ethanol cue (1–4,25).

In conclusion, the results of the present investigation suggest that: (a) both nAChRs and 5-HT_3 receptors may contribute to the formation of the ethanol cue; (n) neither the $GABA_A$ nor the NMDA receptor complex alone is critically involved in the nicotine-induced enhancement of the ethanol discrimination; (c) there is an additive interaction between the NMDA receptor competitive antagonist, CGP 40116, and the GABAergic benzodiazepine, diazepam, in rats trained to discriminate ethanol.

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