INVolvEmENt OF NicOTINIC ACETYLchOLINE RECEPTORS IN THE REGULATION OF alCOHOL DRINKING IN WISTAR RATS

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Abstract — The aim of the present study was to determine if nicotinic acetylcholine receptors (nAChRs) might be involved in the regulation of alcohol intake by Wistar rats. A non-selective nAChR agonist, nicotine, and a non-competitive nAChR antagonist, mecamylamine, were tested in alcohol-prefering Wistar rats maintained on a limited access (4 h/24 h) to ethanol (10%, v/v). In addition, the effects of nicotine and mecamylamine on intake of standard laboratory chow were studied in a separate control experiment. Nicotine (0.1 — 0.6 mg/kg, s.c.) decreased ethanol consumption, but had no effect on food intake. In contrast, mecamylamine (1 — 3 mg/kg, s.c.) did not alter ethanol drinking even at the dose (3 mg/kg) which significantly decreased food intake. These results suggest that activation of nAChRs may selectively reduce ethanol consumption in outbred Wistar rats.

INTRODUCTION

Cholinergic transmission in the brain has been suggested to be involved in regulation of alcohol (ethanol) drinking in rodents. Thus, the muscarinic acetylcholine receptor antagonists scopolamine and atropine have been reported to reduce ethanol consumption both in genetically selected alcohol-preferring (P) rats and outbred Sprague—Dawley rats (Rezvani et al., 1990; Katner et al., 1997). Furthermore, choline acetyltransferase inhibitors have been reported (Ho et al., 1975) to reduce ethanol intake in mice.

Interestingly, a nicotinic acetylcholine receptor (nAChR) agonist, nicotine, has also been shown to reduce ethanol consumption while a non-competitive nAChR antagonist, mecamylamine, did not alter ethanol consumption in P rats (Katner et al., 1997). Both nicotine and mecamylamine were administered intracerebroventriculally in this latter study.

Like other drugs of abuse, ethanol and nicotine have been reported to enhance dopamine (DA) transmission in the nucleus accumbens septi (NAS) (Imperato et al., 1986; Lapin et al., 1989; Yoshimoto et al., 1991). DA release in the NAS is thought to be involved critically in the processing of reinforcing stimuli (Koob, 1996). Notably, Blomqvist et al. (1993) have demonstrated that mecamylamine attenuates ethanol-induced DA release in the NAS, suggesting that nAChRs might be involved in ethanol reinforcement.

In order to assess further the role of nAChRs in ethanol reinforcement, we tested the effects of nicotine and mecamylamine on ethanol consumption in alcohol-preferring Wistar rats maintained on a limited access procedure. In addition, the effects of nicotine and mecamylamine on food intake were studied in a separate control experiment. Doses of both cholinergic agents were selected on the basis of previous behavioural studies (Blomqvist et al., 1993; Stolerman et al., 1995; Bienkowski et al., 1997).

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 180—200 or 300—350 g at the beginning of the ethanol drinking and food intake experiments, respectively, were housed individually in wire-mesh cages (20 × 25 × 35 cm). The animals were kept in a
room with constant environmental conditions: 21–22°C, 60% relative humidity and 12 h:12 h light:dark cycle (lights on at 08:00). Food (Bacutil, Poland) and tap water were available ad libitum, except where noted. All procedures described in the present study were in accordance with the relevant Polish and European regulations for care and use of experimental animals.

Selection of alcohol-prefering rats and limited access procedure

To obtain alcohol-prefering rats, a procedure described in detail by Dyr and Kostowski (1995, 1997) was used. Briefly, during the first week of the experiment, the rats (n = 30) received 20% (v/v) alcohol solution intragastrically, twice daily at 09:00–10:00 and 16:00–17:00 (the daily dose was 5 g/kg body wt). During the next 2 weeks, the animals had free access to tap water for 1 h per day, whereas during the remaining 23 h the only source of fluid was 5% (v/v) ethanol (second week) or 10% (v/v) ethanol (third week). During the fourth week, the rats were presented with a free choice (2 bottles) between water and 10% (v/v) ethanol. The volumes of ethanol and water drunk by each animal were measured every day at 09:00–10:00. The animals (n = 8) consuming >5 g/kg/24 h of absolute alcohol were considered alcohol-prefering and were accordingly used in the limited access procedure.

In the limited access procedure (Dyr and Kostowski, 1997), 10% (v/v) ethanol was available for 4 h per day (09:00–13:00; Monday–Sunday). Food and tap water were always available ad libitum. The subjects were allowed to stabilize their ethanol consumption for 10–14 days. After this period, test sessions with nicotine (0.1 or 0.6 mg/kg) and mecamylamine (1 or 3 mg/kg) were initiated. The doses were injected s.c. immediately prior to the start of the test session. Ethanol consumption (ml/kg) was measured 60, 120, and 240 min after the start of the test session. The animals with nicotine were done first. Then, after at least 7 drug-free days, the sessions with mecamylamine were started. In order to be tested in each subsequent test session, the rat had to present stable ethanol drinking behaviour for at least 2 consecutive drug-free sessions. The doses of both compounds, including saline, were tested in a randomized order in the six to seven rats with the most stable pattern of ethanol consumption.

Food intake

The effects of nicotine (0.1 or 0.6 mg/kg) and mecamylamine (1 or 3 mg/kg) on food intake were studied in a separate group of rats (n = 8). Access to standard laboratory chow was limited to 4 h per day (Zarrindast and Oveis, 1997). The rats were allowed to stabilize their food intake for 5–7 days, after which the test sessions with nicotine started. Then, after at least 7 drug-free days, the test sessions with mecamylamine were initiated. The doses of both compounds were injected s.c. in a randomized order in the six rats with the most stable pattern of food intake. The amount of food consumed (g/rat) was measured at 60, 120, and 240 min after the start of the test session. Except for a small reduction at the beginning of the food deprivation period, the mean body weight of the subjects remained relatively constant during the course of the experiment.

Statistics

All results were analysed by a two-way ANOVA (drug × time) with repeated measures. The Newman–Keuls test was used for individual post-hoc comparisons. A significance level of P < 0.05 was used for all statistical analyses.

Drugs

All ethanol solutions were prepared from stock 95% ethanol solution obtained from a hospital pharmacy. Nicotine dihydrogen-D-tartrate (RBI, Natick, MA, USA) and mecamylamine hydrochloride (RBI) were dissolved in sterile physiological saline and administered in a volume of 1 ml/kg. The pH of nicotine solutions was adjusted to ~7.0 with dilute NaOH. The doses of nicotine and mecamylamine refer to the free base and salt form respectively.

RESULTS

The alcohol-prefering rats consumed 6.23 ± 0.7 g/kg/24 h of absolute alcohol during the final week of the selection procedure. In the limited access procedure, the mean (±SEM) baseline intake of 10% ethanol was 1.09 ± 0.1 g/kg/4 h of absolute alcohol. The water intake during the 4-h sessions was low; it did not exceed 0.2 ml/rat/4 h (data not shown).
Nicotine significantly decreased ethanol consumption ['Drug effect': F(2,17) = 3.87, P < 0.05; Fig. 1A]. In contrast, nicotine did not exert any effect on the food intake [F(2,15) = 2.01, P = 0.17; Table 1]. Mecamylamine failed to influence the ethanol intake [F(2,15) = 1.16, P = 0.35; Fig. 1B], but dose-dependently decreased the amount of food consumed [F(2,15) = 4.40, P < 0.05; Table 1].

DISCUSSION

Nicotine strongly suppressed ethanol consumption in alcohol-prefering Wistar rats maintained on the limited access to 10% ethanol. Our finding is in agreement with the recent report of Katner et al. (1997) showing that centrally administered nicotine selectively decreases ethanol consumption in the P line of rats. In line with this latter study, we have also shown that the non-competitive nAChR antagonist, mecamylamine, did not reduce ethanol intake, even at doses which decreased food consumption. Although Blomqvist et al. (1996) found that mecamylamine decreased ethanol drinking in Wistar rats, the fact that the drug did not alter ethanol preference argues against any selective effect of mecamylamine on ethanol intake in this latter study. Taken together, the above results suggest that tonically active nAChRs do not play any critical role in alcohol drinking behaviour.

The mechanism of the nicotine effect on ethanol consumption remains unexplained. Importantly, nicotine did not alter food intake in the present study. Moreover, our recent experiments have shown that the same doses of nicotine did not affect spontaneous locomotor activity in the Wistar rat (Bienkowski et al., 1997). Thus, non-specific behavioural inhibition seems not to be responsible for the nicotine-induced suppression of ethanol consumption. Although acute injections of nicotine (0.2–0.6 mg/kg) have been shown to reduce food intake (Zarrindast and Oveis, 1997), in other studies higher doses of nicotine did not influence food consumption (Clarke and Kumar, 1984; Wellman et al., 1986).

As mentioned above (see the Introduction), both nicotine and ethanol increase DA release in the brain.
NAS. This effect has been postulated to be involved in the rewarding properties of many drugs of abuse (Imperato et al., 1986; Koob, 1996). Therefore, it could be speculated that, in the present study, a possible nicotine-induced DA release in the NAS could have reduced the rat’s need to stimulate this neurotransmitter system with ethanol. However, nicotine is a relatively weak reinforcer in rodents. Acute injections of nicotine actually produce conditioned place avoidance rather than conditioned place preference (Jorenby et al., 1990; Shoab et al., 1994; Stolerman et al., 1995). Therefore, it could be hypothesized that the aversive effects of nicotine might be responsible for the nicotine-induced decrease in ethanol intake.

The effects of nicotine on the ethanol interoceptive (discriminative) cue should also be taken into account. Nicotine has been reported to enhance ethanol discrimination when given in combination with low doses of ethanol (Signs and Schechter, 1986; Bienkowski and Kostowski, 1998). Accordingly, in the present study, nicotine might alter ethanol consumption by increasing its discriminative stimulus effects. However, this possibility also seems unlikely, as the higher dose of nicotine almost completely suppressed ethanol drinking behaviour, and nicotine also failed to substitute for ethanol when given alone (Bienkowski et al., 1998).

In contrast to acute effects, chronic nicotine treatment has been shown to increase ethanol consumption in rats (Potthoff et al., 1983). More recently, Blomqvist et al. (1996) reported that chronic nicotine injections increase ethanol intake and preference in Wistar rats. Clinical studies indicate that concomitant cigarette consumption may lead to increased ethanol intake (Bien and Burge, 1990). Thus, it seems that there would be no benefit from nicotine treatment in alcoholics.

Adaptive changes in nAChR numbers and sensitivity may be responsible for the different effects of acute and chronic nicotine treatment on ethanol consumption. Notably, in contrast to its acute effects, chronic nicotine administration causes nAChR desensitization (Collins and Marks, 1996).

In conclusion, the results of the present study indicate that stimulation of nAChRs may selectively alter ethanol drinking behaviour in the rat.

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