ELSEVIER

European Journal of Pharmacology 314 (1996) 257–267

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Voluntary ethanol intake in the rat: effects of nicotinic acetylcholine receptor blockade or subchronic nicotine treatment

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Received 9 May 1996; revised 4 July 1996; accepted 12 July 1996

Abstract

It has been suggested that the mesolimbic dopamine activating and the reinforcing properties of ethanol involve activation of central nicotinic acetylcholine receptors. To test this hypothesis, the effects of two nicotinic receptor antagonists and of subchronic nicotine treatment on voluntary ethanol consumption (ethanol 6% v/v or water) were studied in ethanol low-, medium- or high-preferring Wistar rats. After systemic mecamylamine (2 mg/kg) but not hexamethonium (10 mg/kg) high- but not low-preferring rats decreased their ethanol intake but, however, not their ethanol preference. When subchronically exposed to nicotine (0.35 mg/kg, s.c. daily) medium-preferring rats markedly increased their ethanol intake and preference. This effect lasted for more than 1 week after interrupting nicotine administration. Ethanol intake levels did not correlate with locomotor activity scores after nicotine challenge (0.35 mg/kg, s.c.) or with exploratory locomotor activity. However, exploratory locomotor activity correlated with locomotor activity scores both after nicotine (0.35 mg/kg, s.c.) and ethanol (0.125 g/kg i.p.) challenge. Dopamine release, as indicated by accumulation of 3-methoxytyramine after monoamine oxidase inhibition, was increased in the limbic forebrain (including the nucleus accumbens, the olfactory tubercles, the amygdala and the septum) after acute nicotine (0.35 mg/kg s.c.) or ethanol (2.5 g/kg i.p.) in animals subchronically exposed to nicotine compared to subchronically vehicle-treated controls. The present results further implicate central nicotinic receptors in the molecular events mediating the reinforcing properties of ethanol, and suggest that subchronic nicotine enhances the responsiveness of mesolimbic dopamine neurons both to nicotine and to ethanol. Clinical implications are discussed.

Keywords: Dopamine; Ethanol; Nicotine; Nicotinic acetylcholine receptor; Preference; Sensitization; (Rat)

1. Introduction

A common feature of ethanol, nicotine, cocaine, amphetamine and opiates, is their ability to activate the mesocorticolimbic dopamine system (Engel and Carlsson, 1977; Grenhoff et al., 1986; Imperato and Di Chiara, 1986; Mereu et al., 1987; Clarke et al., 1988; Mifsud et al., 1989; Koob, 1992; Blomqvist et al., 1993), a key structure in the so-called brain reward system. This property has been suggested to be important for the development and maintenance of dependence to these drugs of abuse (Wise and Rompre, 1989). The primary mechanisms of action for this effect of the psychostimulants, the opiates and nicotine are fairly well established, whereas that for ethanol remains to be elucidated.

We have recently suggested that ethanol activates the mesocorticolimbic dopamine system via direct or indirect stimulation of central nicotinic acetylcholine receptors. Thus, both ethanol-induced dopamine release in the rat nucleus accumbens and enhanced catecholamine synthesis rate observed in the limbic forebrain after ethanol were completely antagonized by mecamylamine, a blood brain barrier penetrating nicotinic acetylcholine receptor antagonist (Blomqvist et al., 1993). Moreover, in the mouse, ethanol-induced enhancements of locomotor activity and brain dopamine turnover were partially counteracted by mecamylamine, but not by the quarternary nicotinic acetylcholine receptor antagonist hexamethonium (Blomqvist et al., 1992). Thus central nicotinic receptors may play an important role in the molecular events mediating the reinforcing properties not only of nicotine (cf., Clarke et al., 1988) but also of ethanol, and provide a neurochemical

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PII S0014-2999(96)00583-3

basis for the often observed co-abuse of ethanol and nicotine in man (Dreher and Fraser, 1967; Crowley et al., 1974). To further test this hypothesis, we have studied whether pharmacological manipulations of central nicotinic acetylcholine receptors alter voluntary ethanol consumption in rats classified as low- (< 25% ethanol), medium- (25–65% ethanol) or high- (> 65% ethanol) preferring based on their ethanol preference in a free choice drinking situation (ethanol 6% v/v or water, see Materials and methods 2.2).

First the effect of central and peripheral nicotinic acetylcholine receptor blockade by means of mecamylamine and hexamethonium, respectively, on ethanol intake and preference was investigated. Secondly, the effect of subchronic intermittent nicotine treatment on ethanol consumption was studied. To this end rats were given daily injections of a small dose of nicotine, a treatment previously described to consistently enhance the locomotor activating properties of acute nicotine in animals that have been habituated to the test apparatus before injection (e.g., Ksir et al., 1985, 1987; Benwell and Balfour, 1992; Johnson et al., 1995a), or to switch initial behavioral depression into activation in non-habituated animals (e.g., Clarke and Kumar, 1983a,b; Clarke et al., 1988). This phenomenon can be established after approximately 5 days of nicotine administration and has been reported to persist for 1–2 weeks after drug cessation (Ksir et al., 1985; Suemaru et al., 1993). This treatment also increases [^{3}H]nicotine binding (Ksir et al., 1985, 1987), but it is not clear whether this upregulation of central nicotinic acetylcholine receptors is functionally linked to the behavioral sensitization.

Since previous investigators have suggested a link between exploratory locomotor activity and/or amphetamine-induced hyperlocomotion and self-administration of amphetamine (Piazza et al., 1989) or ethanol (Bisaga and Kostowski, 1993; Fahlke et al., 1995a), it was of interest to study also if exploratory locomotion or locomotor activity after nicotine challenge in the rat correlated with the sensitivity to ethanol in terms of ethanol consumption, and, conversely, if voluntary ethanol intake during 5 weeks would alter exploratory locomotion and/or locomotor activity after nicotine challenge.

Recent data from our laboratories have demonstrated that daily nicotine treatment in mice enhances ethanol-induced locomotor stimulation and the dopamine turnover increasing effect of ethanol (Johnson et al., 1995b). It was therefore also of interest to examine if the subchronic intermittent nicotine treatment procedure used in the present study altered the sensitivity of the mesolimbic dopamine neurons to nicotine and/or ethanol. As an indication of dopamine release accumulation of 3-methoxytyramine after monoamine oxidase inhibition with pargyline was determined in the rat limbic forebrain (cf., Kehr, 1976).

Parts of the present data were previously presented in a more preliminary form (Söderpalm et al., 1993).

2. Materials and methods

2.1. Animals

Male Wistar rats, about 100 days old, were supplied by Möllegaard Breeding Laboratories (Denmark). Upon arrival in the laboratory, the animals were housed in groups of 5 per cage (55 × 35 × 20 cm) at a constant cage temperature (25°C) and humidity (65%) for 2 weeks to adapt to the novel environment. The animals were kept under artificial light-dark conditions (light on at 9:00 p.m. and off at 9:00 a.m.) and had free access to 'rat and mouse standard feed' (Beekay Feeds) and tap water.

2.2. Screening for ethanol preference

Rats had continuous access to a bottle of ethanol solution in addition to the water bottle. The ethanol concentration was gradually increased (2–4–6% v/v) over a 2-week period. The animals were subsequently housed individually in clear plastic cages (Macrolon 3; 40 × 24 × 15 cm). They had continuous access to two bottles (plastic 300 ml bottles with ballvalve spouts; ALAB, Sweden) containing either tap water or 6% ethanol solution. This concentration of ethanol solution was used since previous observations (Fahlke, 1994) indicate that the consumption of ethanol is maximal at about this concentration in the strain of rats used in the present study. Water and ethanol intake was measured for a 3-week period, twice a week, when the bottles also were cleaned and filled with fresh beverages. Body weight was recorded once a week during the whole test period. The amount (g) of ethanol solution consumed in per cent of total fluid intake (g) was used as an index of ethanol preference. Rats were classified as low- (< 25% ethanol), medium- (25–65% ethanol) or high- (> 65% ethanol) preferring based on their ethanol preference.

According to previous tests in our laboratories most rats (90%) do not show side preference that affects their ethanol preference. Some animals (25–30%), appear disturbed by the side switch, but return to their baseline ethanol preference after 1–2 days. The animals were not tested for side preference in order to avoid such disturbances.

2.3. Nicotinic acetylcholine receptor blockade

High- and low-preferring rats were injected with one of the nicotinic receptor antagonists (mecamylamine 2 mg/kg or hexamethonium 10 mg/kg) or saline in a counterbalanced cross-over fashion. Injections were given twice a day (at 8 a.m. and 12 a.m.) for two periods of 3 consecutive days, 4 days between periods. Ethanol consumption was measured between 9 a.m. and 3 p.m. all treatment days, and the 3-day mean values for each animal was calculated. The dose of mecamylamine was chosen based on previous experience (Blomqvist et al., 1993), whereas the hexamethonium dose was selected based on pilot ex-

periments indicating that in the present dose ratio hexamethonium produced a more than 2-fold peripheral anticholinergic action than mecamylamine. This was indicated by ptosis, which was estimated by an observer blind to the given dose and compound; mecamylamine 8 mg/kg produced a tendency for ptosis while mecamylamine 2 and 4 mg/kg did not. Hexamethonium 10 mg/kg produced a slight tendency for ptosis in 2 of 5 animals while hexamethonium 20 mg/kg produced marked ptosis and moderate motor impairment ($n = 5$ in all groups).

2.4. Subchronic intermittent nicotine treatment

After the preference screening procedure was completed (see above), rats involved in subchronic nicotine treatment experiments had no access to the ethanol solution for at least 3 weeks prior to experiments. The medium-preferring rats were divided in two equally preferring groups, one receiving daily s.c. injections of 1.0 mg/kg nicotine ditartrate salt (equals 0.35 mg/kg nicotine base) and one receiving vehicle injections for 10 days. The animals had no access to ethanol during this period. The rats were tested for locomotor activity after nicotine on day 1 and 10 of the nicotine treatment period. Thereafter the animals were returned to the two-bottle free choice paradigm with access to both water and ethanol solutions. Preference was measured for 14 days with concomitant nicotine treatment and for an additional period of 25 days after tapering of the nicotine treatment during 2 days by reducing the nicotine dose to 50% and 25% before cessation. For reasons of clarity once a week ethanol consumption data are presented.

2.5. Locomotor activity

Locomotor activity was measured by photocell recordings. The instrument ('M/P 40 Fc Electronic Motility Meter', Motron Products, Stockholm, Sweden) was equipped with 40 photoconductive sensors (5 rows × 8, centre/centre distance 40 mm) covered by a translucent floor, upon which a plexiglass test cage (21 × 32 × 25 cm) was placed. A black-painted chimney-like cover was put on top of the test cage to prevent reflex light scattering and animal escape. The light source (15 W light bulb) was placed in the ceiling of the sound-proof chambers in which the equipment was housed. The test chambers were equipped with semitransparent mirrors, allowing observation of the animals. The number of counts, representing all light beam interruptions of any of the sensors, were printed by external, timer-controlled counters. Locomotor activity was measured both when animals were habituated to the test cage for 60 min and after drug administration for an additional period of 60 min. Since both exploratory locomotor activity and locomotor activity after nicotine are displayed mainly during the first half of the two test periods, locomotor activity during the first 30 min is presented in the present study, while locomotor activity after ethanol is presented for the entire 60 min period.

2.6. 3-Methoxytyramine accumulation after monoamine oxidase inhibition

3-Methoxytyramine is formed after metabolisation of extracellular dopamine by catechol-*O*-methyltransferase. Pargyline is an inhibitor of monoamine oxidase and thus inhibits the conversion of 3-methoxytyramine to homovanillic acid and the conversion of intracellular dopamine to 3,4-dihydroxyphenylacetic acid (DOPAC). Upon administration of pargyline there is a time-dependent increase in the 3-methoxytyramine content of the brain, which can be used as an in vivo measure of dopamine release (Kehr, 1976).

Pargyline (75 mg/kg i.p.) was coadministered with vehicle, ethanol (2.5 g/kg i.p.) or nicotine (0.35 mg/kg s.c.) to rats that had been pretreated for 10 days with nicotine or vehicle (these animals were not screened for ethanol preference). The animals were decapitated 30 min later and the dopamine-rich limbic forebrain (including the nucleus accumbens, the olfactory tubercles, the amygdala and the septum) was dissected out (Carlsson and Lindqvist, 1973) on a chilled Petri dish and stored at −70°C until analysed by high performance liquid chromatography with electrochemical detection (HPLC-ED).

2.7. Drugs

Ethanol (AB Svensk Sprit) was diluted (2–4–6% v/v) with regular tap water and presented in regular plastic 300 ml bottles or dissolved in distilled H_2O (15% w/v) and administered intraperitoneally (i.p.). Mecamylamine HCl (2-[methylamino]isocamphane hydrochloride purchased from Sigma), a nicotine receptor antagonist able to penetrate the blood–brain barrier, was dissolved in 0.9% NaCl and administered i.p. in volumes of 2 ml/kg. Hexamethonium Cl (hexane-1,6-bis[trimethyl-ammonium chloride] purchased from Sigma), a nicotine receptor antagonist not able to penetrate the blood–brain barrier, was dissolved in 0.9% NaCl and administered subcutaneously (s.c.) in volumes of 2 ml/kg. Nicotine ditartrate salt ([−]-1-methyl-2-[pyridyl]pyrrolidine di-[+]tartrate salt purchased from Sigma) was dissolved in 0.9% NaCl and administered (s.c.) in volumes of 2.0 ml/kg. Pargyline-HCl (*N*-methyl-*N*-propargylbenzylamine hydrochloride purchased from Aldrich) was dissolved in distilled H_2O and administered i.p. in volumes of 2 ml/kg. Control animals were given the corresponding vehicles.

2.8. Statistics

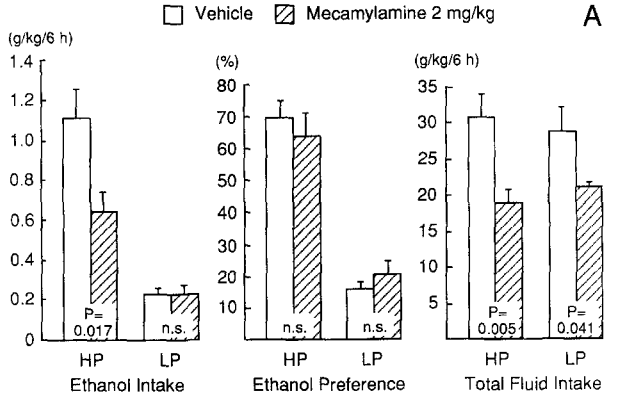
The continuous ethanol consumption data were statistically evaluated by using a two-factor analysis of variance (ANOVA) with repeated measures. Ethanol consumption data from the nicotinic acetylcholine receptor blockade

experiments, locomotor activity data and biochemical data were statistically evaluated by using Paired t-test within groups and Student's t-test between groups. Correlations were evaluated with Paired Correlation Analysis followed by Fisher's r to z (P value). When appropriate, multiple comparisons were corrected for using Holm's procedure, a weighted improvement of the Bonferroni procedure (Holm, 1979). A probability value (P) less than 0.05 was considered statistically significant. All values are expressed as means ± S.E.M.

3. Results

3.1. Ethanol intake and preference

After two injections of mecamylamine (2 mg/kg i.p.) at 8:00 a.m. and 12:00 a.m., high- but not low-preferring



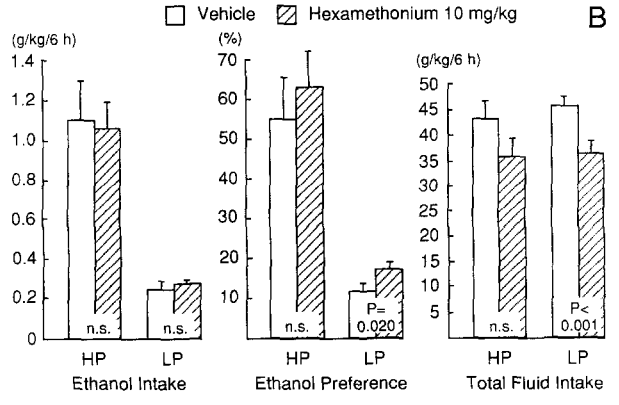
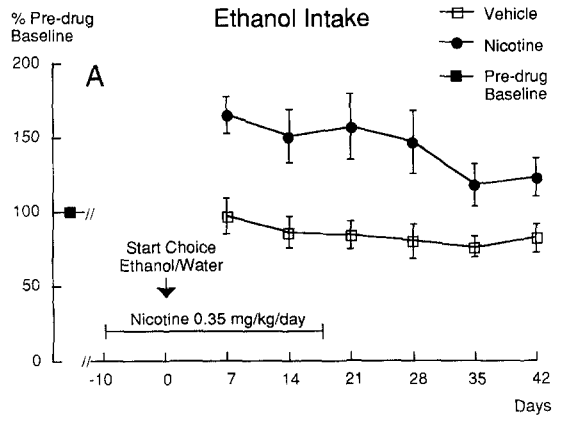
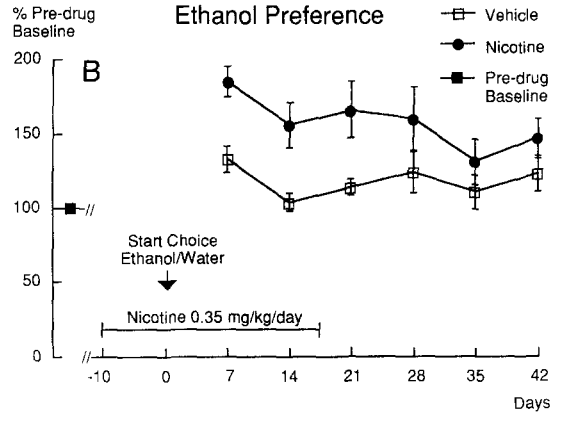


Fig. 1. (A) Effect of mecamylamine 2.0 mg/kg, administered i.p. at 8:00 a.m. and 12:00 a.m. on ethanol intake, ethanol preference and total fluid intake during 6 h (between 9:00 a.m. and 3:00 p.m.) in high-preferring (HP) and low-preferring (LP) rats. Control = saline injection. All values are expressed as means ± S.E.M., $n = 7–8$, all groups. Statistics: Paired t-test. (B) Effect of hexamethonium 10.0 mg/kg, administered s.c. at 8:00 a.m. and 12:00 a.m. on ethanol intake, ethanol preference and total fluid intake during 6 h (between 9:00 a.m. and 3:00 p.m.) in high-preferring (HP) and low-preferring (LP) rats. Control = saline injection. All values are expressed as means ± S.E.M., $n = 8–10$, all groups. Statistics: Paired t-test.





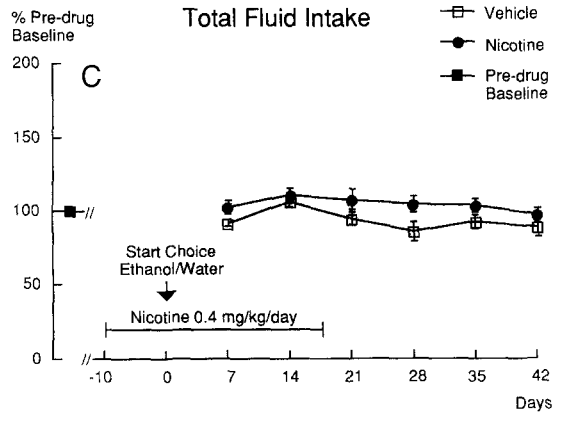


Fig. 2. Effect of daily treatment with nicotine 0.35 mg/kg (s.c.) and subsequent nicotine withdrawal on (A) ethanol intake, (B) ethanol preference and (C) total fluid intake in medium-preferring rats. Data are presented as percentages of pre-drug baseline values. All values are expressed as means ± S.E.M., $n = 7$ both groups. Statistics: two-factor analysis of variance (ANOVA) for repeated measures. (A) $F(1, 60) = 12.14$, $P = 0.0045$ nicotine vs. vehicle, (B) $F(1, 60) = 7.21$, $P = 0.020$ nicotine vs. vehicle, (C) $F(1, 60) = 3.24$, $P = 0.097$ nicotine vs. vehicle.

rats decreased their ethanol intake significantly, when measured between 9:00 a.m. and 3:00 p.m. (Fig. 1A). There was no significant change in ethanol preference in any group. Both groups decreased their total fluid intake signif-

icantly during this period. This change appeared to be more pronounced in the high-preferring group.

After two injections of hexamethonium (10 mg/kg s.c.) at 8:00 a.m. and 12:00 a.m., there was no change in ethanol intake in high- or low-preferring rats, when measured between 9:00 a.m. and 3:00 p.m. (Fig. 1B). There was a significant increase in ethanol preference in low- but not high-preferring rats and a significant decrease in total fluid intake in low-preferring, and a trend to such an effect in high-preferring rats.

Subchronically nicotine-treated medium-preferring rats increased their ethanol intake significantly compared to subchronically vehicle-treated controls. This increase persisted throughout the entire experimental period, i.e., 25 days after nicotine cessation (Fig. 2A). Nicotine-treated medium-preferring rats also increased their ethanol preference significantly (Fig. 2B). This change lasted for at least 1 week after tapering of nicotine. Thereafter the preference level tended towards that of the vehicle-treated group. Nicotine treatment had no effect on the total fluid intake in medium-preferring rats (Fig. 2C).

3.2. Locomotor activity

When challenged with an acute dose of nicotine (0.35 mg/kg s.c.) or neutralized nicotine (0.35 mg/kg s.c., brought to pH 7.2–7.4 with NaOH) after a 60 min habituation period, drug-naive rats displayed a significantly higher locomotor activity than after vehicle challenge (Table 1A–C). There was no difference in locomotor activity score between the two nicotine-treated groups, neither when expressed as the total score during 30 min (Table 1B,C) or when considered over time (not shown). Also as judged from gross observation the present dose of nicotine appeared to arouse the animals, causing an increase in motor behavior including locomotion, rearing and grooming lasting approximately 20 min after injection, whereas stereotypies or tail tremor were not observed. Vehicle controls, on the other hand, displayed an increase in motor behavior that lasted approximately 5 min after the initial stress of being handled and injected, whereafter locomotor activity returned to the low baseline level observed during the last 30–40 min of the habituation period.

Table 1
Effect of acute nicotine on locomotor activity in various groups of rats

Group	Pre-experimental condition	Acute treatment	Locomotor activity (counts/30 min)
A. Drug-naive rats	–	Vehicle	765 ± 168
B. Drug-naive rats	–	Nicotine	1522 ± 172 [a]
C. Drug-naive rats	–	Neutr. nic.	1743 ± 217 [b]
D. MP rats	–	Nicotine	1464 ± 360
E. MP rats	Vehicle 9 days	Nicotine	1730 ± 376
F. MP rats	Nicotine 9 days	Nicotine	3086 ± 465 [c,d]
G. LP rats	Before P screening	Nicotine	1424 ± 126
H. LP rats	After P screening	Nicotine	1756 ± 169 [e]
I. MP rats	Before P screening	Nicotine	1181 ± 69
J. MP rats	After P screening	Nicotine	1756 ± 118 [f]
K. HP rats	Before P screening	Nicotine	1559 ± 297
L. HP rats	After P screening	Nicotine	2167 ± 351 [g]

Nicotine, neutralized nicotine (neutr. nic.) or vehicle was injected s.c. in a dose of 0.35 mg/kg. LP = low-preferring, MP = medium-preferring, HP = high-preferring, P = ethanol preference. All values are expressed as means ± S.E.M. Statistics: Student's *t*-test between groups, Paired Student's *t*-test within groups. [a] $P < 0.05$ B vs. A, [b] $P < 0.01$ C vs. A, [c] $P < 0.01$ D vs. corresponding rats in F, [d] $P < 0.05$ F vs. E, [e] $P < 0.05$ H vs. G, [f] $P < 0.001$ J vs. I, [g] $P < 0.01$ L vs. K. *P* values for comparisons a–d are corrected for multiple comparisons using Holm's procedure.

Medium-preferring rats subchronically treated with nicotine (0.35 mg/kg per day) displayed a significantly higher locomotor score after nicotine challenge (0.35 mg/kg s.c.) on day 10 compared to day 1 (Table 1D,F). Vehicle-treated medium-preferring rats did not significantly differ in locomotor score after acute nicotine (0.35 mg/kg s.c.) on day 10 compared to day 1 (Table 1D,E). According to gross observation both groups appeared stimulated (see above) after nicotine at both challenge occasions, and the locomotor score on day 1, and on day 10 in the chronically vehicle-treated group, was similar to that observed after nicotine challenge in drug (and ethanol)-naive animals.

When challenged with an acute dose of nicotine (0.35 mg/kg s.c.), rats displayed a significantly higher locomotor activity immediately after an ethanol preference screening period (5 weeks) than before (Table 1G–L). In the same animals, exploratory locomotor activity was significantly lower after the ethanol preference screening period than before (data not shown). There was no significant difference in locomotor activity after nicotine between high-, medium- and low-preferring rats at any of the challenge occasions. According to gross observation all groups appeared stimulated (see above) after acute nicotine at both challenge occasions, and, again, the locomotor activity score on the first test occasion (when both drug- and ethanol-naive) was similar to that observed in other drug (and ethanol)-naive animals.

3.3. Correlations

In rats challenged with ethanol (0.125 g/kg i.p.) there was a positive correlation between exploratory locomotor activity and locomotor activity after ethanol (Table 2A). In rats challenged with nicotine (0.35 mg/kg s.c.) before and after an ethanol preference screening period (1st and 2nd test occasion), there was a positive correlation between exploratory locomotor activity and subsequent locomotor activity after nicotine challenge on both test occasions (Table 2B,C). There was no correlation between locomotor activity after nicotine and subsequent ethanol intake (Table 2D), between ethanol intake and locomotor activity after nicotine, after the preference screening period (Table 2E), or between the relative increase in locomotor activity after

Table 2
Correlations between exploratory locomotor activity, drug-induced locomotor activity and ethanol intake

Observation	Correlation	P value
A. Exploratory locomotor activity (30 min) vs. ethanol-induced (0.125 g/kg) locomotor activity (60 min)	0.879	0.0003
B. 1st exploratory locomotor activity (30 min) vs. 1st nicotine-induced locomotor stimulation (30 min)	0.391	< 0.001
C. 2nd exploratory locomotor activity (30 min) vs. 2nd nicotine-induced locomotor stimulation (30 min)	0.549	< 0.001
D. 1st nicotine-induced locomotor stimulation (30 min) vs. subsequent ethanol intake	0.056	0.583
E. 2nd nicotine-induced locomotor stimulation (30 min) vs. previous ethanol intake	0.069	0.502
F. 2nd/1st nicotine-induced locomotor stimulation (30 min) quotient vs. ethanol intake	0.009	0.932
G. 1st exploratory locomotor activity (30 min) vs. subsequent ethanol intake	0.186	0.070
H. 2nd exploratory locomotor activity (30 min) vs. previous ethanol intake	0.088	0.390

n = 96–98 for all groups except correlation A (n = 10). Statistics: Paired Correlation Analysis followed by Fisher's r to z (P value) test. P values for correlations B and C are corrected for multiple comparisons using Holm's procedure.

nicotine (2nd/1st test occasion) and ethanol intake (Table 2F). There was no correlation between exploratory locomotor activity on any of the test occasions and ethanol intake (Table 2G,H).

3.4. 3-Methoxytyramine accumulation after monoamine oxidase inhibition

The limbic 3-methoxytyramine levels after pargyline treatment were not significantly altered in drug-naive rats when acutely challenged with nicotine (0.35 mg/kg s.c.) or ethanol (2.5 g/kg i.p.), compared to vehicle controls (Fig. 3). The limbic 3-methoxytyramine levels after pargyline were, however, significantly higher in subchronically nicotine- compared to vehicle-treated rats, after acute nicotine (0.35 mg/kg s.c.) or ethanol (2.5 g/kg i.p.) challenge (Fig. 3).

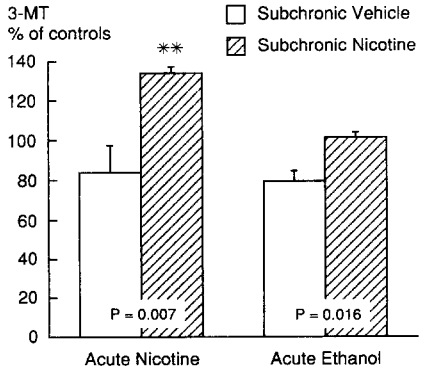


Fig. 3. Effect of daily treatment with nicotine 0.35 mg/kg (s.c.) for ten days on the accumulation of 3-methoxytyramine in the limbic area after an acute challenge with nicotine (0.35 mg/kg s.c.) or ethanol (2.5 g/kg i.p.) in pargyline (75 mg/kg i.p.) pretreated rats. All values are expressed as means ± S.E.M., n = 4–5, all groups. Statistics: Student's t-test. ** P < 0.01 vs. vehicle controls.

4. Discussion

The present study shows that mecamylamine, a blood brain barrier penetrating nicotinic acetylcholine receptor antagonist, decreases ethanol intake in high- but not low-preferring Wistar rats, whereas blockade with the quarternary nicotinic receptor antagonist hexamethonium does not. However, hexamethonium similarly to mecamylamine reduced or tended to reduce total fluid intake in both groups. Since these effects of hexamethonium and mecamylamine were of a similar magnitude in the low-preferring rats they may be suggested to involve the peripheral anticholinergic action these compounds have in common, for example via vasopressin-induced antidiuresis (cf., Cadnapaphornchai et al., 1974). In high-preferring animals, however, the reduction of total fluid intake was much larger after mecamylamine than hexamethonium, apparently due to the contribution of a decrease in ethanol intake. Taken together, these findings indicate that central rather than peripheral nicotinic receptors are involved in the ethanol intake-reducing effect of mecamylamine, whereas peripheral receptors may be involved in the concomitant reduction of water intake, which in turn explains the lack of reduction in ethanol preference in the high-preferring animals.

The mecamylamine dose used was intermediate to those previously demonstrated to antagonize ethanol-induced dopamine release in the nucleus accumbens and catecholamine synthesis rate in the limbic forebrain, respectively (Blomqvist et al., 1993). Although, in that study, rats were drug-naive and did not self-administer ethanol, it is tempting to speculate that the effects on the mesolimbic dopamine system were present also in this study and causally related to the lowered ethanol intake. Indeed, previous work has demonstrated a decrease in voluntary

ethanol intake after both dopamine receptor antagonists and lesioning of dopamine neurons in the ventral striatum by means of 6-hydroxydopamine (Myers and Melchior, 1975; Panocka et al., 1993). There is, however, also evidence for a lack of effect of dopamine receptor antagonists or lesions on ethanol consumption (Brown et al., 1982; Rassnik et al., 1993) as well as for an increase in ethanol consumption after the dopamine receptor antagonist haloperidol (Gauvin et al., 1993). Although strain differences and incomplete dopaminergic lesions may explain some of these contradictory results, it is difficult to conclude to what extent and in what direction mesolimbic dopamine influences voluntary ethanol consumption.

Mecamylamine has been reported to have antagonistic properties at the NMDA receptor (O'Dell and Christensen, 1988), a property that also ethanol is believed to possess (Lovinger et al., 1989). The NMDA system has been suggested to be involved in mediating ethanol tolerance and withdrawal symptoms (Hoffman et al., 1990). Danysz et al. (1992) found, however, no reduction of ethanol intake in high- or low-preferring rats after the NMDA antagonist MK-801 (5-methyl-10,11-dihydro-5*H*-dibenzocyclohepten-5,10-imine maleate), a finding that makes it unlikely that NMDA receptors are involved in the present effects of mecamylamine.

We next investigated whether voluntary ethanol intake could be influenced by subchronic nicotine administration, a treatment previously demonstrated to consistently produce sensitization to nicotine-induced locomotor stimulation in rats that have been habituated to the test-apparatus before injection (e.g., Ksir et al., 1985, 1987; Benwell and Balfour, 1992; Johnson et al., 1995a). After a 60 min habituation period, when the spontaneous locomotor activity was low, acute nicotine (0.35 mg/kg s.c.), as compared to vehicle, produced locomotor stimulation in drug-naive rats lasting for approximately 20 min, whereas no depressant effect of nicotine could be detected either by the locomotor activity score or by gross observation of the animals. An almost identical locomotor stimulation was observed in animals receiving a neutralized nicotine solution, a result argueing against the possibility of the nicotine-induced locomotor activation being due to the pain reaction that might occur in immediate connection with injection of nicotine di-tartrate (pH 3.5). Previous findings in habituated animals of locomotor stimulation after nicotine sulfate (Ksir et al., 1985) or a neutralized nicotine hydrogen tartrate solution (Benwell and Balfour, 1992) lasting for a similar period of time are in line with this reasoning. Drug-naive rats that subsequently were screened for ethanol preference and medium-preferring rats that had been withdrawn from ethanol for 3 weeks displayed similar locomotor activity scores and gross behavior as drug-naive rats after acute nicotine, indicating that nicotine produced stimulation also in these animals, although vehicle controls were not included in these experiments.

Daily subchronic nicotine treatment (10 days) in rats that had been withdrawn from a limited ethanol exposure (medium-preferring) produced an increase in locomotor activity after nicotine challenge that was similar to that previously observed in the present laboratories after an identical treatment regimen in ethanol-naive rats (cf., Johnson et al., 1995a). In contrast, no such enhancement was observed in subchronically vehicle-treated medium-preferring rats. Although a similar increase in locomotor activity after vehicle challenge in rats subchronically treated with nicotine can not be excluded (these control experiments were not performed), it appears unlikely, since previous studies have not observed such an effect (Clarke and Kumar, 1983a,b; Ksir et al., 1987). Hence, in all likelihood behavioral sensitization to the locomotor-activating effect of nicotine had developed, a finding in line with several previous investigations (Ksir et al., 1985, 1987; Benwell and Balfour, 1992; Suemaru et al., 1993; Johnson et al., 1995a).

The effect of nicotine treatment on ethanol consumption was studied in medium-preferring rats, since this would allow alterations of the drinking behavior in both directions in a homogeneous population. Interestingly, medium-preferring rats clearly increased their ethanol intake and preference, but not their total fluid intake, after subchronic nicotine treatment and during continuous daily nicotine treatment. This finding is in line with that of Pothoff et al. (1983), who observed increased voluntary ethanol intake in rats chronically treated with nicotine. Those animals were, however, not investigated with regard to locomotor activity after nicotine and thus no indication of whether the animals were sensitized to nicotine or not before presentation of ethanol was obtained.

In the present study, the enhanced ethanol intake did not, in contrast to that observed by Pothoff et al. (1983), increase further after presentation of the ethanol solution, indicating that the neurochemical events leading to this enhanced consumption were present already after the subchronic nicotine treatment period and probably do not require the concomitant administration of ethanol and nicotine. This is further indicated by the fact that both ethanol intake and preference remained significantly enhanced, compared to that in the vehicle-treated group, for a substantial period of time also after tapering of nicotine. This finding also argues against the interpretation made by earlier workers that the enhanced ethanol consumption observed during nicotine administration would reflect 'self-medication' to counteract tentative tension-enhancing effects of nicotine (Pothoff et al., 1983).

Ethanol preference remained enhanced for 11 days after tapering of nicotine, but then slowly decreased towards control levels. The discrepancy to the results obtained regarding ethanol intake may be due to a slight and non-significant preference increase after discontinuation of subchronic vehicle treatment in the control group, which in turn is explained by a slight decrease in water intake. The time course for the persistence of ethanol preference after

nicotine cessation is otherwise similar to those reported for sensitization to the behavioral stimulatory effects of nicotine (Ksir et al., 1985; Kita et al., 1992) and for nicotine receptor upregulation (Ksir et al., 1985; Collins et al., 1988) at withdrawal after a comparable subchronic nicotine treatment period. Although no binding studies were performed in the present study, a mechanistic connection between the three phenomena may be suggested (see below).

Although it is known that ethanol and nicotine increase extracellular dopamine levels in the nucleus accumbens in rats (e.g., Imperato and Di Chiara, 1986; Benwell and Balfour, 1992), the accumulation of 3-methoxytyramine in the limbic forebrain after pargyline pretreatment instead tended to decrease after acute nicotine or ethanol in the present study. This discrepancy may be explained by the fact that in addition to the nucleus accumbens also the olfactory tubercles, the amygdala and the septum were included in the analysis. In addition, ethanol may interact with the enzyme catechol-*O*-methyltransferase catalyzing the conversion of dopamine to 3-methoxytyramine (Reniläa et al., 1995). Nevertheless, the accumulation of 3-methoxytyramine after pargyline pretreatment was significantly higher in subchronically nicotine-treated animals than in vehicle-treated controls not only after acute nicotine but also after ethanol challenge. These findings are in line with previous in vivo microdialysis and in vitro release studies, showing enhanced dopamine release after nicotine challenge in animals treated subchronically with nicotine (Benwell and Balfour, 1992; Wonnacott et al., 1990). However, this is, to our knowledge, the first study suggesting that subchronic nicotine increases also the mesolimbic dopamine releasing effect of ethanol. These findings are in accord with the results showing that nicotine pretreatment increases the dopamine turnover (as estimated by the DOPAC/dopamine quotient)-enhancing effect of ethanol in mice (Johnson et al., 1995b). The dopamine release-enhancing effect of subchronic nicotine treatment appeared, however, more pronounced after nicotine than after ethanol challenge.

Considering that amphetamine-induced hyperlocomotion correlates with both amphetamine self-administration (Piazza et al., 1989) and ethanol preference (Fahlke et al., 1995a) the question arose as to whether high- and low-preferring rats were differentially sensitive also to locomotor activity after nicotine. As mentioned above the nicotine challenge used here clearly increased the locomotor activity score as compared to that observed after vehicle. However, when rats were challenged with nicotine before and after an ethanol preference screening period, there was no difference in locomotor activity between rats that subsequently ended up high-, medium- or low-preferring on any of the test occasions. It was thus not possible to predict future ethanol consumption in the rat by the locomotor score obtained after a nicotine challenge.

It should be noted that there was a clear increase in locomotor activity after nicotine in all rats on the second test occasion compared to the first. This finding is in agreement with the observation that an 8-week ethanol treatment period (3% v/v in drinking water) resulted in a higher amphetamine-induced locomotor response compared to controls (Lograno et al., 1993). In the present study, there was, however, no correlation between the locomotor activity score after nicotine and the amount of ethanol consumed during the ethanol preference screening period, argueing against that long-term ethanol consumption cross-sensitizes rats to the locomotor stimulant effect of nicotine. Furthermore, the present experiments did not control for the possible influence of unspecific factors, such as, e.g., isolation and stress, that follow upon placement of the animals in single cages. This procedure may result in elevated plasma levels of corticosterone, a hormone that is suggested to play an important role for the induction of behavioral sensitization to ethanol (Roberts et al., 1995; Fahlke et al., 1995b) and nicotine (Johnson et al., 1995a).

Previous investigators have shown that animals with a delayed habituation to a novel environment may be more vulnerable to self-administration of amphetamine (Piazza et al., 1989), or ethanol (Bisaga and Kostowski, 1993). In the present study there was, however, no correlation between ethanol consumption and spontaneous locomotor activity during habituation to the motility boxes when measured before or after the ethanol preference screening period. This is supported by similar findings by Fahlke et al. (1995a). There was, however, a linear correlation between exploratory locomotor activity and locomotor activity after ethanol, but not vehicle (not shown), in rats without previous ethanol experience, although the locomotor score after ethanol was not significantly increased compared to controls. Also in nicotine-challenged animals there was a correlation, although not as clear, between exploratory locomotor activity and subsequent locomotor activity after nicotine. These data are in line with the findings that exploratory locomotor activity correlates with amphetamine-induced hyperlocomotion (Piazza et al., 1989).

Taken together, blockade of central but not peripheral nicotinic receptors reduces ethanol intake in high-preferring rats, an effect that may involve a decrease in ethanol-induced activation of the mesolimbic dopamine system. Chronic nicotine treatment, a procedure known to upregulate central nicotinic receptors and to alter the responsiveness of the mesolimbic dopamine system, increased the locomotor score after nicotine. This treatment increased ethanol preference and intake in medium-preferring rats, an effect that persisted for several days after nicotine withdrawal. Subchronic intermittent nicotine treatment also increased both nicotine- and ethanol-induced dopamine release in the limbic forebrain. Thus, ethanol may activate the mesolimbic dopamine system via direct or indirect interaction with central nicotinic receptors (Blomqvist et

al., 1992, 1993), and activation of this system appears to be important for the regulation of voluntary ethanol consumption. Whether the present effects of subchronic nicotine on ethanol consumption are connected to upregulation of central nicotinic receptors and/or to an altered responsiveness in the mesolimbic dopamine system remains to be elucidated.

The locomotor stimulatory effect of several dependence-producing drugs, including ethanol, nicotine, amphetamine, cocaine, heroine and morphine, has been proposed to be homologous to the rewarding and reinforcing effect, since both involve mesolimbic dopamine activation (Engel and Carlsson, 1977; Wise and Bozarth, 1987). However, although subchronic nicotine appeared to cross-sensitize rats to ethanol-induced mesolimbic dopamine release, there was no linear relationship between the locomotor activity score after nicotine and ethanol consumption in the present study. Interestingly, there also appears to be a lack of correlation between ethanol consumption and ethanol-induced locomotor activity in rats (Criswell et al., 1994; Fahlke et al., 1995a; see however Waller et al., 1986), although ethanol-induced accumbal dopamine release, as measured by in vivo voltammetry, is larger in high- than low-preferring rats (Engel et al., 1992). These findings indicate that the neuronal networks regulating ethanol self-administration and locomotor activity after nicotine or ethanol are overlapping but not identical. Thus, other neurotransmitter systems may regulate the mesolimbic dopamine system or act independently to influence the two behaviors differentially depending, e.g., on the species, the experimental setting and the drug. Indeed, both ethanol (cf., Engel et al., 1992) and nicotine (cf., Benowitz, 1994) display complex pharmacodynamic profiles involving, e.g., the serotonergic, GABAergic and opioid systems that also are involved in the regulation of voluntary intake of ethanol (Myers and Veale, 1968; Wise, 1987; Pulvirenti and Kastin, 1988; Engel et al., 1992).

The present findings in rats give further support for a mechanistic relation between ethanol and nicotine, possibly via interaction with central nicotinic receptors. It is well known that most alcoholics smoke, and alcoholism has been estimated to be 10 times more common among smokers than non-smokers (DiFranza and Guerrera, 1990). Even more intriguing is that there appears to be a strong correlation between onset of tobacco addiction at early age and addiction to alcohol (DiFranza and Guerrera, 1990) as well as other drugs of abuse (Loimer et al., 1991) later in life. This could of course be explained by a predisposition to drug abuse in general among these individuals, but it is tempting to speculate that nicotine abuse, especially during adolescence, might initiate sensitization mechanisms that make the individual more inclined to develop dependence for ethanol and other substances. The present results also give further support to the notion that alcoholics might maintain alcohol abstinence more easily if they also gave up smoking. Finally, the animal model used in the present study has previously proven to be of predictive value for identifying pharmacological compounds, e.g., naltrexone (O'Malley et al., 1992; Volpicelli et al., 1992) and selective serotonin reuptake inhibitors (Naranjo and Sellers, 1989), that may reduce ethanol intake also in man. Thus, since mecamylamine reduced ethanol consumption in the present study in the rat, it is suggested that antagonists at central nicotinic receptors may be a useful complement in the treatment of alcoholics.

Acknowledgements

The expert technical assistance of Mrs Gun Andersson and Mr Kenn Johannessen is gratefully acknowledged. B.S. was a recipient of the Berth von Kantzow Scholarship during parts of these studies. This study was financially supported by grants from the Swedish Medical Research Council (no. 11583 and no. 4247), the Swedish Alcohol Monopoly Foundation for Alcohol Research, Göteborg Medical Society, Swedish Society for Medical Research, Magnus Bergvalls Stiftelse, Lundbecks Fond för Psykofarmakologisk Forskning, O.E. och Edla Johanssons Vetenskapliga Stiftelse, Åke Wibergs Stiftelse, Leons minnesfond, Wilhelm och Martina Lundgrens vetenskapsfond and Åhlén-stiftelsen.

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