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Accumbal dopamine overflow after ethanol: Localization of the antagonizing effect of mecamylamine

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Abstract

It has been suggested that ethanol exerts its mesolimbic dopamine activating effects and its reinforcing effects via interaction with central nicotinic acetylcholine receptors, thus providing a basis for the often observed covariation between ethanol and nicotine consumption. We have previously demonstrated that the central nicotinic acetylcholine receptor antagonist mecamylamine totally counteracts the ethanol-induced elevation of extracellular dopamine in the nucleus accumbens, as measured by in vivo microdialysis. A contribution of peripheral nicotinic receptor blockade could, however, not be excluded. In the present study, mecamylamine (1.0 mg/kg, i.p.) again totally counteracted the ethanol-induced dopamine overflow, as measured by in vivo microdialysis, while the quarternary nicotinic receptor antagonist hexamethonium (10 mg/kg, i.p.) did not. Furthermore, the increase in accumbal dopamine overflow after systemic ethanol (2.5 g/kg, i.p.) was counteracted by local perfusion of mecamylamine (50 μM) in the ipsilateral ventral tegmental area, but not by mecamylamine perfusion in the nucleus accumbens. Ethanol-induced accumbal dopamine overflow was also counteracted by perfusion of hexamethonium (250 μM) in the ventral tegmental area. These results provide further evidence that ethanol-induced activation of the mesolimbic dopamine system is mediated via stimulation of central nicotinic acetylcholine receptors, and that the receptor population within the ventral tegmental area may be the most important in this regard. It is suggested that antagonists of central nicotinic acetylcholine receptors may be useful in the treatment of alcoholism. © 1997 Elsevier Science B.V.

Keywords: Dopamine; Ethanol; Hexamethonium; Microdialysis, in vivo; Mecamylamine; Nicotinic acetylcholine receptor

1. Introduction

It is well established that ethanol and several other drugs of abuse activate the mesocorticolimbic dopamine system (Engel and Carlsson, 1977; Lichtensteiger et al., 1982; Grenhoff et al., 1986; Imperato and Di Chiara, 1986; Imperato et al., 1986; Mereu et al., 1987; Clarke et al., 1988; Engel et al., 1988; Grenhoff and Svensson, 1988; Mifsud et al., 1989; Blomqvist et al., 1992, 1993). Furthermore, this system is regarded as an important neuroanatomical substrate for drug dependence (Wise and Rompre, 1989). 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 the enhanced rate of catecholamine synthesis 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). In a recent study (Blomqvist et al., 1996), mecamylamine but not hexamethonium reduced voluntary ethanol intake in rats marked with a preference for alcohol but not in rats with no preference for alcohol, while subchronic nicotine treatment increased ethanol consumption in rats with a modest preference for alcohol. Thus central nicotinic receptors may play a role in the molecular events mediating the reinforcing properties not only of nicotine (cf. Clarke et al., 1988) but also of ethanol.

Nicotinic receptors have been demonstrated presynaptically on the nerve terminals in the nucleus accumbens (Clarke and Pert, 1985), and these receptors may regulate the release of dopamine (Wonnacott et al., 1990). Nicotinic

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acetylcholine receptors are, however, located also on the dopamine cell bodies in the ventral tegmental area (Schwartz et al., 1984; Clarke and Pert, 1985). Systemic nicotine has been shown to increase the firing rate and burst activity of midbrain dopamine neurons (Lichtensteiger et al., 1982; Grenhoff et al., 1986; Mereu et al., 1987) and to increase dopamine overflow preferentially in the nucleus accumbens, as measured by in vivo microdialysis (Imperato et al., 1986). Local perfusion of nicotine through a microdialysis probe elevates extracellular dopamine in the nucleus accumbens both when perfused in the nucleus accumbens (Mifsud et al., 1989) and in the ventral tegmental area (Nisell et al., 1994). However, local nicotine results in a more profound locomotor stimulation in rats when administered in the ventral tegmental area than in the nucleus accumbens (Reavill and Stolerman, 1990; Leikola-Pehlo and Jackson, 1992). Furthermore, accumbal dopamine overflow after systemic nicotine was antagonized by local perfusion of mecamylamine in the ipsilateral ventral tegmental area but not in the nucleus accumbens (Nisell et al., 1994). These findings suggest that mesolimbic dopamine activation after systemic nicotine may depend mainly on nicotinic receptors situated in the ventral tegmental area.

The present in vivo microdialysis study was undertaken to localize the nicotinic receptors involved in the previously described antagonizing effect of systemic mecamylamine on ethanol-induced dopamine overflow in the nucleus accumbens (Blomqvist et al., 1993). The first objective was to differentiate between the involvement of peripheral and central nicotinic receptors in the above effect. Thus, the possible influence of hexamethonium, which blocks peripheral nicotinic receptors, on ethanol-induced dopamine overflow in the nucleus accumbens was compared to that of mecamylamine, which blocks both peripheral and central nicotinic receptors. The second objective was to determine the relative importance of different nicotinic receptor populations located within the mesocorticolimbic dopamine system. For this purpose, the effect of mecamylamine perfused via reverse microdialysis in the nucleus accumbens or in the ventral tegmental area on ethanol-induced accumbal dopamine overflow was compared. The third objective was to challenge the specificity of the mecamylamine antagonizing effect by investigating whether also locally perfused hexamethonium influences ethanol-induced accumbal dopamine overflow.

2. Materials and methods

2.1. Animals

Male Wistar rats, supplied by Beekay (Stockholm), weighing 230–350 g were housed 5 per cage (55 × 35 × 20) at a constant cage temperature (25°C) and humidity (65%). The animals were kept under regular light–dark conditions (light on at 5.00 a.m. and off at 7.00 p.m.) and had free access to 'rat and mouse standard feed' (Beekay Feeds) and tap water. In all experiments drug-naive animals were used. Animals were allowed to adapt for at least 7 days to the animal maintenance facilities of the department prior to the start of the experiments.

2.2. Microdialysis technique

Microdialysis was performed with a modified version of the I-shaped probe described by Santiago and Westerink (1990) (for details, see Waters et al., 1993). Both the inlet and the outlet of the probe were made of PE20 plastic tubing. During manufacture and implantation of the probe a glass tube was used as a holder. The dialysis membrane was prepared from a copolymer of polyacrylonitrile and sodium methallyl sulfonate (Hospal-Gambro, Bologna) with an o.d./i.d. of 310/220 μm. The length of the exposed tip of the dialysis membrane was 2 mm, and the remaining area was covered with silicon glue (CAF 3; Rhodorsil Silicones, Saint-Fons Cedex). Typical in vitro recovery of the probes was around 10%. Data presented are not corrected for recovery. Before implantation, the dialysis probes were perfused (2 μl/min) with 35 μl ethanol (70%) and thereafter rinsed with 100 μl Ringer solution. The inlet and the outlet of the probes were finally sealed by heating.

The rats were anaesthetized with a mixture of ketamine, 165 mg/kg (Parke-Davies, S.A., Barcelona), and xylazin, 15 mg/kg (Bayer, Leverkusen), and were mounted in a Kopf stereotaxic instrument (David Kopf). During surgery, the rat was kept on a heating pad to prevent hypothermia. The skull was exposed, and holes were drilled for placement of the dialysis probe/probes and two anchoring screws. The dura was removed with a sharp needle. Probe coordinates relative to the bregma and according to Paxinos and Watson (1986) were for nucleus accumbens A/P +1.85, L/M -1.3, V/D -7.8 and for ventral tegmental area A/P -5.2, L/M -0.7, V/D -8.4. The probes were fixed to the skull and to the two anchoring screws with Phosphatine dental cement (Svedia dental industri AB, Sweden). Dialysis experiments were performed 48 h later. Rats were allowed to habituate to the experimental spherical cage for at least 12 h prior to the experiment.

On the experimental day the sealed inlet and outlet of the probes were cut open and the inlet cannula of the probe was connected to a perfusion pump (CMA/100, Carnegie Medicin, Sweden) via a swivel (CMA/120, Carnegie Medicin, Sweden), which allowed the animal to move freely. The outlet was connected to a collecting test tube. The probe was perfused at a rate of 2 μl/min with a Ringer solution containing in mM: NaCl 140, $CaCl_2$ 1.2, KCl 3.0 and $MgCl_2$ 1.0. Dialysate (40 μl) was collected every 20 min. After the experiments the locations of the dialysis probes were controlled by microscopic examination of coronal sections of the brains prepared with a Leitz

freezing microtome. Only results derived from rats with correctly positioned dialysis probes were included in the statistical analysis.

2.3. Biochemical assay

For detection of dopamine the main portion of the 40 μl sample was introduced to a cation-exchange system via a 10-port injector. The high-performance liquid chromatography with electrochemical detection (HPLC-ED) system consisted of a HPLC pump (CMA 250, Carnegie Medicin, Sweden), a stainless steel column (0.21 × 15 cm) packed with Nucleosil, SA, 5 mm (Phenomenex, Chesire) and an amperometric detector (Waters 460, Millipore Waters, Milford, MA) operated at 0.60 V versus Ag/AgCl and with the time constant set at 1.0 s plus active filter. The silica-based cation exchanger includes a hydrophobic spacer in the stationary bonded phase and thus exhibits both ion-exchange and reverse-phase retention properties. Two different mobile phases, both giving roughly the same retention time for dopamine, but with completely different retention mechanisms, were used. Mobile phase A: Citric acid 300 mM, NaOH 700 mM, Na_2-EDTA 0.054 mM. Mobile phase B: Citric acid 75 mM, NaOH 175 mM, Methanol 30% v/v, Na_2-EDTA 0.054 mM. A mixture of these two mobile phases was used to optimize chromatographic selectivity. The most common mixture was A/B 20/80 with a pH of 5.6. The flow rate was 0.27 ml/min. To identify the peaks an external standard was used containing 3.26 fmol/μl of dopamine. When at least three stable (±10%) basal values of dopamine were obtained the first drug was injected.

The currents produced were monitored on a Macintosh 480 by using Dynamax II MacIntegrator 1.4 software and Rainin Control Interface Module (Rainin Instrument Company, MA).

2.4. Drugs

Ethanol (AB Svensk Sprit) was dissolved in saline isocamphane hydrochloride purchased from Sigma), a nicotine receptor antagonist able to penetrate the blood–brain barrier, was dissolved in 0.9 (15% w/v) and administered intraperitoneally (i.p.). Mecamylamine HCl (2-[methylamino] % NaCl and administered i.p. in a volume 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 i.p. in a volume of 2 ml/kg. Control animals were given the corresponding vehicles.

2.5. Statistics

Data were statistically evaluated by using a two-factor analysis of variance (ANOVA) with repeated measures. 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. Effects of systemic mecamylamine and hexamethonium on ethanol-induced dopamine overflow in the nucleus accumbens

Ethanol 2.5 g/kg i.p. produced a significant elevation of extracellular dopamine in the nucleus accumbens, compared to that of saline-injected controls, that was maximal (approximately 30% above baseline) in the first dialysate sample 20 min after injection (Figs. 1 and 2). Mecamylamine 1.0 mg/kg i.p. did not affect dopamine levels per se, but totally counteracted ethanol-induced dopamine overflow when administered 20 min prior to ethanol 2.5 g/kg i.p. (Fig. 1).

Hexamethonium 10 mg/kg did not alter dopamine levels per se, and did not alter ethanol-induced dopamine overflow when administered 20 min prior to ethanol 2.5 g/kg i.p. (Fig. 2).

3.2. Effects of local mecamylamine perfusion in the nucleus accumbens and the ventral tegmental area on ethanol-induced dopamine overflow in the nucleus accumbens

Ethanol 2.5 g/kg i.p. statistically significantly elevated extracellular dopamine levels in the nucleus accumbens

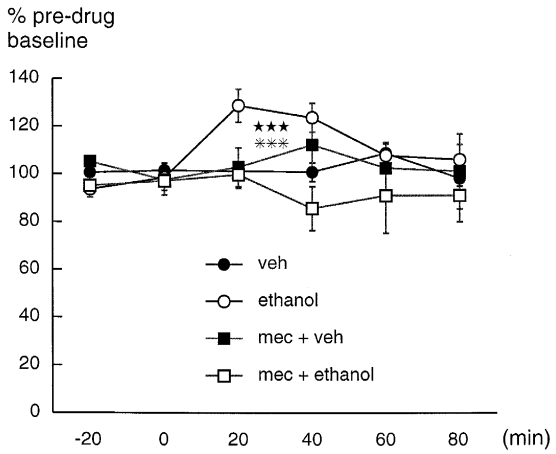

Fig. 1. Effect of mecamylamine (mec) 1.0 mg/kg (i.p.) on the increase in dopamine levels in the rat nucleus accumbens after ethanol 2.5 g/kg (i.p.). All values are expressed as means ± S.E.M., $n = 4–9$, all groups. Statistics on values at $t = 20–40$ min: Two-factor analysis of variance (ANOVA) with repeated measures. ★★★ Vehicle (veh) vs. ethanol, $P < 0.001$; *** mec + ethanol vs. ethanol, $P < 0.001$; mec + veh vs. veh, non-significant. P-values are corrected for multiple comparisons using Holm's procedure.

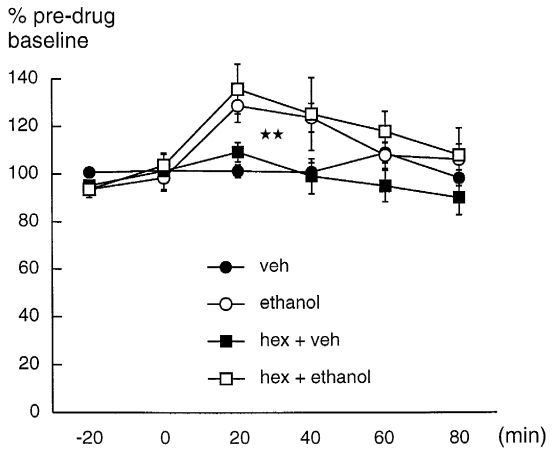

Fig. 2. Lack of effect of hexamethonium (hex) 10.0 mg/kg (i.p.) on the increase in dopamine levels in the rat nucleus accumbens after ethanol 2.5 g/kg (i.p.). All values are expressed as means ± S.E.M., $n = 4–9$, all groups. Statistics on values at $t = 20–40$ min: Two-factor analysis of variance (ANOVA) with repeated measures. ★★ Vehicle (veh) vs. ethanol, $P < 0.01$; hex + ethanol vs. ethanol, non-significant; hex + veh vs. veh, non-significant. *P*-values are corrected for multiple comparisons using Holm's procedure.

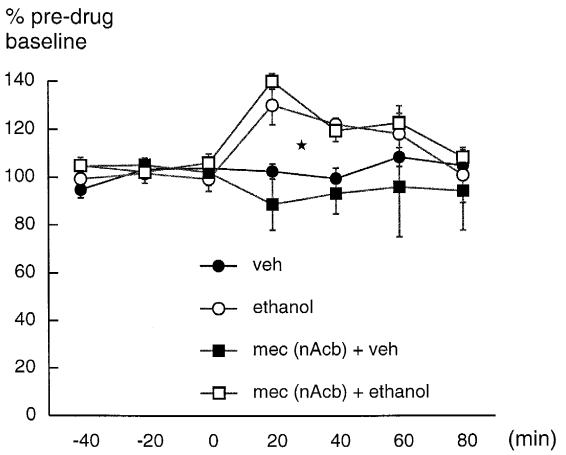

Fig. 4. Effect of mecamylamine (mec) 50 μM perfused in the nucleus accumbens (nAcb) on the increase in dopamine levels in the rat nucleus accumbens after ethanol 2.5 g/kg (i.p.). All values are expressed as means ± S.E.M., $n = 5–8$, all groups. Statistics on values at $t = 20–40$ min: Two-factor analysis of variance (ANOVA) with repeated measures. ★ Vehicle (veh) vs. ethanol, $P < 0.05$; mec (nAcb) + ethanol vs. ethanol, non-significant; mec (nAcb) + veh vs. veh, non-significant. *P*-values are corrected for multiple comparisons using Holm's procedure.

compared to those of controls (Figs. 3 and 4). Mecamylamine 50 μM perfused through the microdialysis probe in the ventral tegmental area had no effect per se, but completely antagonized ethanol-induced (2.5 g/kg i.p.) dopamine overflow in the nucleus accumbens (Fig. 3). Ethanol was injected 40 min (at $t = 0$) after the start of mecamylamine perfusion (at $t = -40$ min). The delay time for the mobile phase to reach the tip of the microdialysis probe was approximately 12 min, indicating that mecamylamine had reached the brain 28 min before ethanol was injected.

Mecamylamine 50 μM perfused through the microdialysis probe in the nucleus accumbens had no effect on accumbal dopamine overflow compared to that of controls, although a slight but not statistically significant tendency to a decrease was seen 60 min after the start of perfusion (at $t = 20$ min) (Fig. 4). Mecamylamine perfused in the nucleus accumbens did, however, not reduce the ethanol-induced elevation of accumbal dopamine levels (Fig. 4). Ethanol was injected 40 min (at $t = 0$) after the start of mecamylamine perfusion (at $t = -40$ min). The delay time for the mobile phase to reach the tip of the microdial-

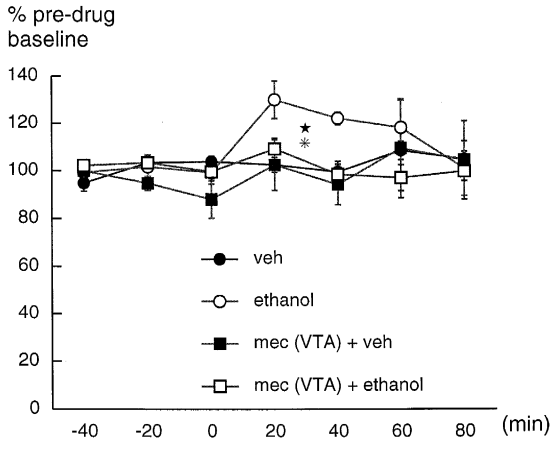

Fig. 3. Effect of mecamylamine (mec) 50 μM perfused in the ventral tegmental area (VTA) on the increase in dopamine levels in the rat nucleus accumbens after ethanol 2.5 g/kg (i.p.). All values are expressed as means ± S.E.M., $n = 6–9$, all groups. Statistics on values at $t = 20–40$ min: Two-factor analysis of variance (ANOVA) with repeated measures. ★ Vehicle (veh) vs. ethanol, $P < 0.05$; * mec (VTA) + ethanol vs. ethanol, $P < 0.05$; mec (VTA) + veh vs. veh, non-significant. *P*-values are corrected for multiple comparisons using Holm's procedure.

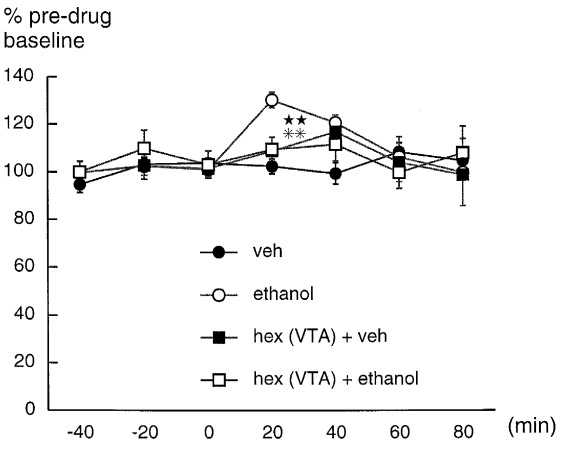

Fig. 5. Effect of hexamethonium (hex) 250 μM perfused in the ventral tegmental area (VTA) on the increase in dopamine levels in the rat nucleus accumbens after ethanol 2.5 g/kg (i.p.). All values are expressed as means ± S.E.M., $n = 4–6$, all groups. Statistics on values at $t = 20–40$ min: Two-factor analysis of variance (ANOVA) with repeated measures. ★★ Vehicle (veh) vs. ethanol, $P < 0.01$; ** hex (VTA) + ethanol vs. ethanol, $P < 0.05$; hex (VTA) + veh vs. veh, non-significant. *P*-values are corrected for multiple comparisons using Holm's procedure.

ysis probe was also in this case approximately 12 min, indicating that mecamylamine had reached the brain 28 min before ethanol was injected.

3.3. Effect of local hexamethonium perfusion in the ventral tegmental area on ethanol-induced dopamine overflow in the nucleus accumbens

Hexamethonium 250 μM perfused through the microdialysis probe in the ventral tegmental area did not statistically significantly alter accumbal dopamine levels per se, although a tendency to an increase was observed at $t =$ 20–40 min (Fig. 5). However, this treatment antagonized the ethanol-induced accumbal dopamine overflow, when ethanol was injected 40 min (at $t = 0$) after the start of hexamethonium perfusion (at $t = -40$ min), i.e. approximately 28 min after hexamethonium had reached the ventral tegmental area.

4. Discussion

In the present study, an i.p. injection of ethanol elevated accumbal extracellular dopamine levels, a finding in accordance with previous reports (Imperato and Di Chiara, 1986; Yoshimoto et al., 1991; Blomqvist et al., 1993). Ethanol increases accumbal dopamine levels also after local perfusion into the nucleus accumbens (Yoshimoto et al., 1991), after oral self-administration (Weiss et al., 1992, 1993) or when injected into anaesthetized rats (Engel et al., 1988). Thus, the ethanol-induced dopamine activation is most likely a specific pharmacological effect and not an unspecific stress effect. The route of activation of the ethanol-induced accumbal dopamine overflow is, however, not clarified.

A low dose (1 mg/kg) of systemically injected mecamylamine, which passes through the blood–brain barrier, completely antagonized the ethanol-induced dopamine overflow. Thus, previous findings (Blomqvist et al., 1993) suggesting that, in the rat, the ethanol-induced elevation of dopamine overflow in the nucleus accumbens depends on activation of central nicotinic acetylcholine receptors were confirmed. Furthermore, hexamethonium (10 mg/kg), a quarternary compound that does not readily pass the blood–brain barrier, neither affected dopamine levels per se nor altered the ethanol-induced increase in dopamine levels. This finding argues against the involvement of peripheral nicotinic acetylcholine receptors in the antagonizing effect of mecamylamine, and further confirms the above hypothesis. In the present dose-ratio (10:1) the antagonistic potency for peripheral nicotinic acetylcholine receptors appears to be about 5 times greater for hexamethonium than for mecamylamine, as estimated by gross observation of ptosis (Blomqvist et al., 1996). Mecamylamine (2 mg/kg) or hexamethonium (10 mg/kg) did not alter the levels of ethanol in serum, collected 90 min after ethanol (2.5 g/kg i.p.) administration, indicating that neither compound affected ethanol metabolism (unpublished observations).

Recently we reported that mecamylamine but not hexamethonium reduced voluntary ethanol intake in high- but not low-preferring rats, classified according to their ethanol preference during a pre-experimental screening period (Blomqvist et al., 1996). Although dopaminergic neurochemistry was not studied in these animals, it is tempting to suggest that the reduction of ethanol intake was related to neurochemical alterations similar to those observed here, i.e. a reduction in the mesolimbic dopamine activating properties of ethanol due to central nicotinic receptor blockade.

In order to differentiate between the relative importance of the ventral tegmental area and the nucleus accumbens as a possible central site of action for the above-described antagonizing effect of systemically administered mecamylamine on ethanol-induced accumbal dopamine overflow, ethanol was systemically administered while mecamylamine was perfused locally in one of the two areas. When mecamylamine was perfused in the ventral tegmental area, the increase in extracellular dopamine after ethanol was totally counteracted. When perfused in the nucleus accumbens, mecamylamine per se tended to decrease dopamine levels. However, when ethanol was co-administered, accumbal dopamine levels were of the same magnitude as after ethanol alone.

Mecamylamine was perfused for 40 min (i.e. approximately 28 min in the brain due to tubing delay) before ethanol was injected in order to achieve an adequate concentration in the area intended. When mecamylamine was perfused in the nucleus accumbens, dopamine was collected through the same probe, indicating that dopamine was collected from the extracellular space most probably reached by mecamylamine. This was not the case in animals receiving mecamylamine through the probe in the ventral tegmental area. In spite of this, mecamylamine perfused in the latter but not the former area was effective in antagonizing accumbal dopamine release after ethanol. Although the diffusion of mecamylamine to other areas close to the ventral tegmental area can not be excluded, it is concluded that nicotinic receptors in the ventral tegmental area are the more important targets for systemically administered ethanol, as previously suggested also for nicotine (Nisell et al., 1994), in producing accumbal dopamine release and, tentatively, reinforcement.

Interestingly, recent studies (McBride et al., 1991; Gatto et al., 1994) have shown that rats may self-administer ethanol directly in the ventral tegmental area, suggesting that ethanol directly activates this region, and that this effect is sufficient to activate the neuronal network mediating ethanol reinforcement, unless locally administered ethanol diffuses to other regions of importance in sufficient concentrations. There is also evidence that systemically administered ethanol increases neuronal firing in the

ventral tegmental area both in vitro (Brodie et al., 1990) and in vivo (Gessa et al., 1985).

Although mecamylamine has been considered a selective antagonist at central as well as peripheral nicotinic acetylcholine receptors, recent reports have indicated that this compound may interfere also with NMDA receptors. Thus, mecamylamine may inhibit both NMDA stimulated currents as measured by patch clamp (O'dell and Christensen, 1988) and NMDA-induced noradrenaline release in the rat hippocampus (Snell and Johnson, 1989). These effects are suggested to be due to non-competitive blockade of the NMDA receptor ion channel via an action of mecamylamine at the PCP (phencyclidine) site (Snell and Johnson, 1989). However, non-competitive NMDA receptor antagonists like PCP and MK-801 (dizocilpine maleate) have been shown to increase rather than decrease dopamine turnover and release (Imperato et al., 1990; Rao et al., 1990; Svensson et al., 1991). Interestingly, local perfusion of high doses (1000 μM) of mecamylamine in the nucleus accumbens produces a profound increase in accumbal dopamine overflow (Nisell et al., 1994; own unpublished observations), probably due to unspecific effects of the compound. In addition, there are findings indicating that ethanol by itself may act as a functional antagonist at the NMDA receptor complex (Lovinger et al., 1989, 1990; Carlsson and Engberg, 1992). These circumstances make it unlikely that the observed effects of mecamylamine are mainly due to its NMDA receptor blocking properties. In addition, the specificity of mecamylamine in the present study is strengthened by the finding that hexamethonium perfused in the ventral tegmental area was also able to block ethanol-induced accumbal dopamine overflow.

The question arises whether anaesthesia with ketamine two days prior to the experiments could have affected the results. Ketamine is a potent non-competitive NMDA receptor antagonist that in sedative doses transiently increases accumbal dopamine overflow (own unpublished observations). Chronic ketamine administration has been reported to increase the responsiveness to dopamine agonists, indicating a hypersensitivity of the dopamine receptors, but no alterations of dopamine levels and turnover have been demonstrated (Lannes et al., 1991). Although it can not be excluded, it appears unlikely that the anaesthesia used in the present study significantly affected the results obtained in awake animals 48 h later.

In conclusion, the mecamylamine-induced blockade of the dopamine-activating effects of ethanol is most likely due to antagonism of central nicotinic acetylcholine receptors located in the ventral tegmental area. Consequently, the ethanol-induced activation of the mesocorticolimbic dopamine system may be brought about either by a direct agonistic effect at the somatodendritical nicotinic acetylcholine receptors on the dopamine cell bodies in this area, or by facilitation of the response of these nicotinic receptors to endogenous acetylcholine. Another possibility would be that ethanol releases acetylcholine, which in turn activates the nicotinic acetylcholine receptors. However, most studies have suggested that ethanol decreases rather than increases acetylcholine turnover and release (cf. Pohorecky and Brick, 1988). Interestingly, electrophysiological studies have demonstrated that ethanol may or may not alter nicotine responsiveness depending on the rat brain area investigated, indicating that there are ethanol-sensitive and ethanol-insensitive nicotinic acetylcholine receptors (Criswell et al., 1993). Furthermore, recent studies using Xenopus oocytes with different nicotinic receptor subunit compositions indicate that in oocytes with $\alpha 2\beta 2$, $\alpha 3\beta 2$ and $\alpha 4\beta 2$ receptor subtypes, ethanol tended to increase nicotine responsiveness (De Fiebre et al., 1995), while in $\alpha 3\beta 4$ receptor subtypes ethanol 100 mM and 300 mM clearly potentiated nicotine-induced inward currents (Covernton et al., 1995). In $\alpha 7$ homomers, ethanol inhibited agonist-induced currents (Yu et al., 1995; De Fiebre et al., 1995). Although in vivo conditions may be significantly different, these preliminary findings provide exciting indications for direct interactions between ethanol and central nicotinic acetylcholine receptors, and most of the nicotinic receptor subtypes listed above appear to be present in the ventral tegmental area (Deneris et al., 1989; Duvoisin et al., 1989; Wada et al., 1989, 1990).

Whatever the exact mechanism, the present results indicate a close interaction between the ethanol-induced mesolimbic dopamine activation and ventral tegmental area nicotinic receptors that may have implications for the understanding of ethanol reinforcement. Furthermore, it is suggested that pharmacological compounds showing antagonistic properties at central nicotinic acetylcholine receptors could reduce alcohol consumption in humans.

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