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Ethanol elevates accumbal dopamine levels via indirect activation of ventral tegmental nicotinic acetylcholine receptors

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Abstract

It was previously demonstrated that the central nicotinic acetylcholine receptor antagonist mecamylamine perfused in the ventral tegmental area (VTA) counteracts the elevation of extracellular dopamine levels in the nucleus accumbens after systemic ethanol, as measured by in vivo microdialysis. In the present study we investigated the effect of different concentrations of ethanol perfused locally in the VTA or in the nucleus accumbens on extracellular accumbal dopamine levels. Ethanol (10–1000 mM) perfused in the VTA did not influence dopamine output in the nucleus accumbens. However, ethanol (300 mM) perfused in the nucleus accumbens increased accumbal dopamine levels to approximately the same extent (30%) as observed after systemic ethanol, whereas ethanol (1000 mM) decreased the dopamine output by approximately 50%. Next, the hypothesis that endogenous acetylcholine is required for the increased accumbal dopamine levels after ethanol was challenged. It was shown that in animals pre-treated with vesamicol, a potent inhibitor of vesicular acetylcholine storage, ethanol (300 mM) in the nucleus accumbens failed to elevate extracellular accumbal dopamine levels. Similarly, in animals perfused with mecamylamine in the VTA, but not in the nucleus accumbens, ethanol in the nucleus accumbens (300 mM) failed to increase accumbal dopamine levels. However, whereas dihydro-β-erythroidine (antagonist for the nicotinic receptor subtype α4β2) perfused in the VTA prevented the increase in accumbal dopamine after systemic nicotine, the antagonist was unable to prevent the dopamine elevating effects of ethanol. Finally, to investigate whether mecamylamine exerts its antagonizing effect of ethanol induced accumbal dopamine levels through an interaction with the NMDA receptor MK-801, the effects of the prototypic NMDA receptor antagonist were examined and compared to those of mecamylamine. After perfusion in the VTA, MK-801 enhanced accumbal dopamine levels by itself but did not antagonize the enhancing effect of ethanol. The present set of experiments indicate that the mesolimbic dopamine activating effects of ethanol may be due to an indirect rather than direct activation of ventral tegmental nicotinic acetylcholine receptors of a subtype composition different from the α4β2. Furthermore, it is argued that the primary site of action of ethanol in its accumbal dopamine elevating effect may be located to the nucleus accumbens or nearby regions.

Keywords: Ethanol; Nicotine; Nicotinic receptor; Dopamine, mesolimbic; Microdialysis; (Rat)

1. Introduction

Drugs of abuse, including ethanol, have repeatedly been shown to activate the mesocorticolimbic dopamine system (Engel and Carlsson, 1977; Grenhoff et al., 1986; Imperato and Di Chiara, 1986; Clarke et al., 1988; Engel et al., 1988; Mifsud et al., 1989; Blomqvist et al., 1993, 1997). This dopamine system, projecting from the ventral tegmental area to the limbic areas, i.a. the nucleus accumbens, the amygdala, the septum and the frontal cortex, is regarded as an important neuroanatomical substrate for drug dependence (Wise and Rompre, 1989) but has also been implicated in mediating natural rewards and hedonia. In order to prevent the dopamine activating effects of drugs of abuse without concomitantly altering the motivation for natural rewards or producing anhedonia, it is seemingly important to unravel the mechanisms of actions by which these drugs activate the system. While the mechanisms of action of psychostimulants, opiates and nicotine in this respect are fairly well established those of ethanol remain unidentified.

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 catecholamine syn-

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thesis rate observed in the limbic forebrain after ethanol were completely antagonized by mecamylamine, a blood–brain barrier penetrating nicotine acetylcholine receptor antagonist (Blomqvist et al., 1993). Furthermore, when perfused in the ventral tegmental area mecamylamine and the quaternary nicotine acetylcholine receptor antagonist hexamethonium completely antagonized the accumbal dopamine overflow after systemic ethanol (Blomqvist et al., 1997), whereas when perfused in the nucleus accumbens mecamylamine did not influence the effects of ethanol. Furthermore, we have demonstrated that acute mecamylamine but not hexamethonium reduces voluntary ethanol intake in high- but not low-preferring rats (Blomqvist et al., 1996), and recently it was shown that this phenomenon most likely involves antagonism of ventral tegmental nicotinic receptors (Ericson et al., 1998). Thus, ventral tegmental nicotinic acetylcholine receptors may play an important role in mediating the mesolimbic activating and reinforcing properties not only of nicotine (cf. Clarke et al., 1988; Corrigal et al., 1992), but also of ethanol, and provide a neurochemical basis for the often observed co-abuse of ethanol and nicotine in man (Dreher and Fraser, 1967; Crowley et al., 1974; for review, see Bien and Burge, 1990).

The present study was undertaken in order to investigate whether ethanol interacts directly or indirectly with ventral tegmental nicotinic acetylcholine receptors and whether the most common nicotine acetylcholine receptor subtype in the brain, the $\alpha4\beta2$, is involved in the ethanol-induced dopamine activation described above. Furthermore, since mecamylamine interacts not only with nicotinic receptors but also with glutamatergic NMDA receptors in the brain (McDonough and Shih, 1995), the possibility that the mecamylamine effects related above derive from an interaction with NMDA receptors rather than with nicotine acetylcholine receptors was also examined. To this end, in vivo microdialysis to measure extracellular accumbal dopamine levels, and reversed microdialysis, to administer ethanol or various pharmacological tools locally, were employed with or without systemic injection of ethanol or other substances.

2. Materials and methods

2.1. Animals

Male Wistar rats, supplied by Beekay (Stockholm, Sweden) and weighing 250–350 g, were housed five per cage ($55 \times 35 \times 20$) at a constant cage temperature (25 °C) and humidity (65%). The animals were kept under regular light–dark conditions (lights on at 7:00 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, Italy) with an o.d./i.d. of 310/220 m. 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, France). 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 120 μl Ringer solution and thereafter the inlets and outlets were sealed by heating.

The rats were anaesthetized with a 1:2 v/v mixture of ketamine, 67 mg/kg (Parke-Davies, Barcelona, Spain) and xylazin, 13 mg/kg (Bayer Leverkusen), and mounted in a Kopf stereotaxic instrument (David Kopf Instruments). 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 (1982) was 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, Sweden). Dialysis experiments were performed approximately 48 h later.

On the experimental day, the sealed inlet and outlet of the probe were cut open and the inlet cannula of the probe was connected to a perfusion pump (CMA/100, Carnegie Medicin, Sweden or U-864 Syringe Pump, AgnTho's Sweden) via a swivel allowing 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.

2.3. Biochemical assay

To determine the concentration of dopamine in the dialysate a high-pressure liquid chromatography system with electrochemical detection was used. To identify the dopamine peak an external standard was used containing 2.64 fmol/μl of dopamine. The external standard was used in order to verify the dopamine levels of the samples, the concentration used was similar to the ones obtained when recording baseline level samples. When at least three stable

basal values of dopamine were obtained (± 5%) the first drug was introduced.

2.4. Experimental design

In the first part of the study, rats were perfused with 10, 100, 300 or 1000 mM ethanol in either nucleus accumbens or in the ventral tegmental area in order to determine mechanism of action of ethanol. No rat was perfused with more than one concentration of ethanol. In the second part of the study, in order to examine the influence of endogenous acetylcholine on the accumbal effects of ethanol, vesamicol was administered (4 mg/kg, i.p.) and after 60 min, 300 mM ethanol was perfused in the nucleus accumbens. In the third part of the study, mecamylamine (100 μM) was perfused either in the nucleus accumbens or in the ventral tegmental area 40 min prior to accumbal ethanol perfusion (300 mM) in order to investigate whether the antagonist was able to block the effects of locally perfused ethanol. In the fourth part of the study, we examined the subtype composition of the nicotine acetylcholine receptor involved in the accumbal ethanol response. Dihydro-β-erythroidine (1 mM), or Ringer solution, was perfused in the ventral tegmental area 40 min prior to an ethanol (2.5 g/kg, i.p.), nicotine (1 mg/kg, s.c.) or saline injection. In the last part of the study, MK-801 (100 μM) was perfused in the ventral tegmental area 40 min before ethanol (2.5 g/kg) or saline was administered i.p. in order to investigate the role of NMDA receptors in the present aspect of ethanol-induced accumbal dopamine release. The perfused concentration of mecamylamine was chosen based on previous studies from the group, whereas the concentrations administered of both dihydro-β-erythroidine and vesamicol were chosen based on dose-finding experiments demonstrating them to be the highest possible producing minimal effects per se.

2.5. Drugs

Ethanol (AB Svensk Sprit) was dissolved in Ringer solution and perfused in the nucleus accumbens or in the ventral tegmental area or was dissolved in 0.9% NaCl and administered i.p. 2-[Methylamino]isocamphane hydrochloride (Mecamylamine HCl; purchased from Sigma), a nicotine receptor antagonist, was dissolved in Ringer solution and perfused in the ventral tegmental area. (−)-Vesamicol hydrochloride (Vesamicol; RBI), a potent inhibitor of vesicular acetylcholine storage, was dissolved in 0.9% NaCl and administered i.p. (+)-MK-801 hydrogen maleate (MK-801; RBI), a highly potent and selective non-competitive NMDA receptor antagonist, was dissolved in Ringer solution and perfused in the ventral tegmental area. Dihydro-β-erythroidine hydrobromide (Sigma), a competitive nicotinic receptor antagonist selective for the α4β2 subtype composition, was dissolved in Ringer solution and perfused in the ventral tegmental area. Control animals were perfused with Ringer solution both in the nucleus accumbens and in the ventral tegmental area.

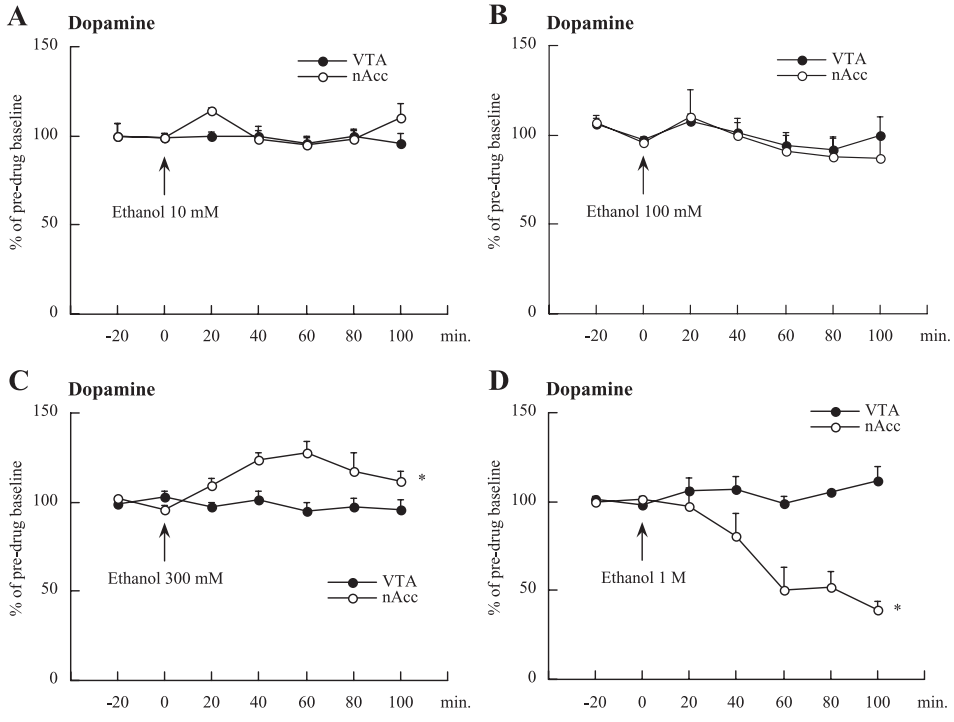

Fig. 1. Effects of ethanol (A) 10 mM, (B) 100 mM, (C) 300 mM and (D) 1 M perfused in the nucleus accumbens or in the ventral tegmental area on extracellular accumbal dopamine levels, as measured by in vivo microdialysis in awake, freely moving animals. Shown are the means ± S.E.M.; $n = 8–11$. Statistics: repeated-measures ANOVA, $*P = \leq 0.05$. Drug administration was initiated as indicated by the arrow.

2.6. Statistics

Data were statistically evaluated by using repeated measures analysis of variance (ANOVA) followed by Fisher's protected least-significant difference test (PLSD). A probability value (P) of less than 0.05 was considered statistically significant. All values are expressed as means ± S.E.M.

3. Results

Ethanol (10, 100, and 300 mM or 1 M in the perfusate) administered by reversed microdialysis into the ventral tegmental area produced no significant change in extracellular accumbal dopamine levels as expressed as % of control. The lower concentrations (10 and 100 mM) of ethanol perfused in the nucleus accumbens also failed to alter the dopamine levels in this region. However, when ethanol was perfused in the nucleus accumbens at a concentration of 300 mM, accumbal dopamine levels increased by approximately 30% compared to ethanol perfused in the ventral tegmental area ($P=0.008$). Perfusion of 1 M ethanol in the nucleus accumbens instead significantly decreased the extracellular dopamine levels in the same area ($P=0.013$) (Fig. 1A–D).

Vesamicol (4 mg/kg i.p.), an acetylcholine vesicular storage inhibitor, slightly, but not significantly, decreased the accumbal dopamine level, as compared to pre-drug baseline levels. Pretreatment with vesamicol (4 mg/kg, i.p., administered 60 min before ethanol), completely antagonized the enhancement of accumbal dopamine levels after perfusion of ethanol (300 mM) in the nucleus accumbens (Fig. 2).

Simultaneous perfusion of the nicotinic antagonist mecamylamine (100 μM) in the ventral tegmental area completely antagonized the accumbal dopamine-elevating effect of ethanol (300 mM) administered into the nucleus accumbens ($P=0.010$; Fig. 3). Mecamylamine (100 μM) perfused in the nucleus accumbens did, however, not alter the dopamine response to ethanol (300 mM) perfused in the same area. The selective α4β2 nicotine acetylcholine receptor antagonist dihydro-β-erythroidine (1 mM) perfused in the ventral tegmental area tended to slowly increase the dopamine overflow in the nucleus accumbens (Fig. 4). Dihydro-β-erythroidine (1 mM) was, however, unable to block the ethanol-induced (2.5 g/kg, i.p.) increase in accum-

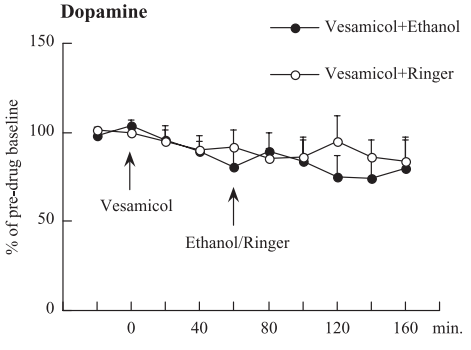

Fig. 2. Effect of vesamicol (4 mg/kg, i.p.) and ethanol (300 mM, perfused in the nucleus accumbens) or Ringer solution on extracellular accumbal dopamine levels, as measured by in vivo microdialysis in awake, freely moving animals. All values are expressed as the means ± S.E.M., $n=10-11$. Statistics: repeated-measures ANOVA. There were no significant differences between the groups. Drug administration was initiated as indicated by the arrows.

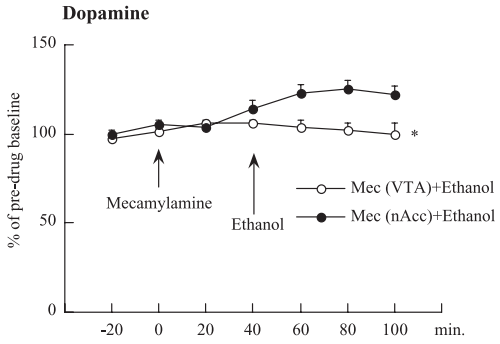

Fig. 3. Effect of mecamylamine (100 μM) perfused in the ventral tegmental area (VTA) or in the nucleus accumbens (nAcc) on extracellular accumbal dopamine levels after ethanol (300 mM) perfused in the nucleus accumbens, as measured by in vivo microdialysis in awake freely moving Wistar rats. Shown are the means ± S.E.M.; $n=8-11$. Statistics: repeated-measures ANOVA, $*P=\leq 0.05$. Drug administration was initiated as indicated by the arrows.

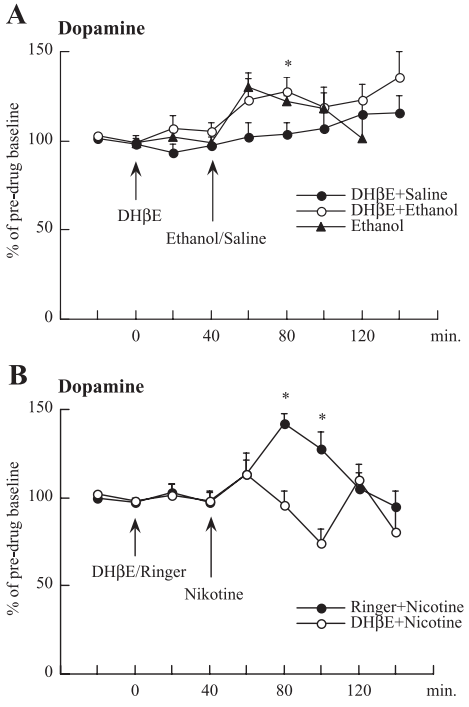

Fig. 4. Effect of dihydro-β-erythroidine (DHβE, 1 mM) perfused in the ventral tegmental area (VTA) on extracellular accumbal dopamine levels after ethanol (2.5 g/kg, i.p.) or after nicotine (1 mg/kg, s.c.), as measured by in vivo microdialysis in awake freely moving Wistar rats. Shown are the means ± S.E.M.; $n=4-8$. Statistics: ANOVA followed by Fishers PLSD, $*P=\leq 0.05$. Drug administration was initiated as indicated by the arrows.

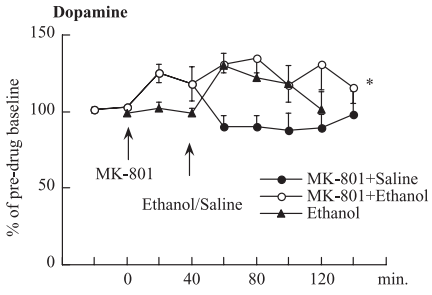

Fig. 5. Effect of MK-801 (100 μM) perfused in the ventral tegmental area (VTA) on accumbal dopamine after ethanol (2.5 g/kg, i.p.) or saline (0.9% NaCl), as measured by in vivo microdialysis in awake freely moving Wistar rats. After 40 min. of MK-801 treatment the animals were split into two groups, in which one received ethanol and the other nicotine. Shown are the means ± S.E.M.; n = 7–11. Statistics: repeated-measures ANOVA, *$P = \leq 0.05$. Drug administration was initiated as indicated by the arrows.

bal dopamine levels ($P = 0.030$ at 40 min after ethanol administration), although the same concentration was able to block the nicotine-induced (1 mg/kg, s.c.) increase in accumbal dopamine levels.

Local perfusion of the NMDA receptor antagonist MK-801 (100 μM) in the ventral tegmental area increased accumbal dopamine levels initially ($P = 0.048$ at 20 min after MK-801 administration). Administration of ethanol (2.5 g/kg, i.p.) to animals with MK-801 simultaneously perfused in the ventral tegmental area significantly increased dopamine levels, as compared to animals treated with the NMDA receptor antagonist per se ($P = 0018$) (Fig. 5).

4. Discussion

Previous studies from this group have demonstrated that local perfusion of mecamylamine in the ventral tegmental area prevents systemic ethanol-induced dopamine release in the nucleus accumbens (Blomqvist et al., 1997) and abolishes ethanol intake as well as the associated accumbal dopamine release in ethanol high-preferring Wistar rats (Ericson et al., 1998). These results indicate that ethanol produces its mesolimbic dopamine activating and reinforcing effects via activation of ventral tegmental nicotinic acetylcholine receptors.

In the present study, two microdialysis probes were implanted in each rat, one in the ventral tegmental area and one in the ipsilateral nucleus accumbens, and, in order to examine whether ethanol interacts directly or indirectly with ventral tegmental nicotinic acetylcholine receptors, the effect on accumbal dopamine levels was investigated after local perfusion of ethanol in either the cell body (ventral tegmental area) or terminal region (nucleus accumbens) of the mesolimbic dopamine system. None of the ethanol concentrations (10, 100 and 300 mM, 1 M) perfused in the ventral tegmental area influenced the dopamine output in the nucleus accumbens. This finding argues against the possibility that ethanol interacts directly with nicotinic receptors in the ventral tegmental area and also does not provide support for the hypothesis that ethanol interferes with mesolimbic dopamine function after ventral tegmental application in vivo. This finding contrasts to the findings of Brodie et al. (1990) and Brodie and Appel (1998), who demonstrated that ethanol excites dopaminergic ventral tegmental neurons and increases the firing rate in brain slices and in isolated neurons. The present in vivo studies may, however, not be directly comparable to the in vitro studies, since major afferent influences have been abolished in the latter studies. The in vitro phenomena may thus not be observable in vivo due to the concomitant influence of ethanol on, e.g. afferent neuronal activity. Another possible reason for the discrepant results is that the placement of our microdialysis probe may not have allowed for activation of the same population of dopaminergic neurons that were studied in the in vitro studies. Such a circumstance could tentatively also explain the contradiction between the present results and the fact that rats may self-administer ethanol directly into the ventral tegmental area (Gatto et al., 1994). However, in that study no connection to an actual activation of the mesolimbic dopamine system was demonstrated. Thus, the self-administering effect observed could be due to some other, dopamine-independent effect. Another possible reason for why ethanol did not produce a dopamine-activating effect in the present experiments is that the concentration steps applied may have been too large. This is an obvious risk when studying a drug like ethanol that may be expected to produce several possibly opposing effects on neuronal activity. However, although preliminary reports are available, this is, to our knowledge, the first paper that has investigated the effect on accumbal dopamine levels after ventral tegmental application of ethanol in vivo, and, taken together with our previous results, the data obtained indicate that even though nicotine acetylcholine receptors in this area are involved in mediating the dopamine activating and reinforcing properties of ethanol there appears to be no *direct* interaction between ethanol and nicotine acetylcholine receptors of dopaminergic neurons.

Local perfusion of ethanol in the nucleus accumbens demonstrated, in line with Yim et al. (1998), that low concentrations of ethanol (10 and 100 mM) had no effect on accumbal dopamine levels, whereas 300 mM ethanol increased the accumbal dopamine levels by approximately 30%. This increase is similar in magnitude to that observed after systemic administration of ethanol (2.5 g/kg, i.p.; Blomqvist et al., 1996) or after ethanol intake in ethanol high-preferring rats (approximately 1 g/kg, p.o.; Ericson et al., 1998). When ethanol was perfused in the nucleus accumbens at a concentration of 1 M, a profound decrease (−50%) of the extracellular dopamine levels was observed instead in the same area. This decrease could perhaps be due to a recruitment of other opposing actions of ethanol, or to, e.g. desensitization of the stimulatory effect of ethanol. Ethanol is known to produce biphasic effects on

several behaviors and neurotransmitter activity (Hunt and Majchrowicz, 1983). However, a toxic effect of ethanol on cell membranes could also be tentatively involved in the dopamine decreasing effect, although this possibility may appear less likely since the same concentration produced no alteration of accumbal dopamine levels when perfused in the cell-body region. The present findings are in line with those of several investigators who have reported elevated accumbal dopamine levels after local injection or perfusion of ethanol into the nucleus accumbens (Yoshimoto et al., 1991; Wozniak et al., 1990) or in the striatum (Wozniak et al., 1990).

It is difficult to judge what concentrations of ethanol that prevail in the area surrounding the probe and how these concentrations compare to, e.g. the concentrations after systemic ethanol injection in the rat. Preliminary data from our laboratory indicate that the ethanol concentration of the perfusate is lowered by approximately 15–20% after in vitro or in vivo perfusion. Thus, perfusion of ethanol 300 mM would result in concentrations of 45–60 mM directly outside the probe. However, it can be expected that the immediate region surrounding the probe is disturbed by, e.g. reactive gliosis, and hence is probably not the most viable and biologically active region. How large a volume of the nucleus accumbens that needs to be reached in order for ethanol to produce a dopamine activating effect big enough to be reflected in an increased dopamine overflow in the dialysis probe remains unclear. A systemic injection of 2.5 g/kg ethanol results in an estimated brain concentration of approximately 30 mM (preliminary results). It thus appears likely that when taking the necessary diffusion and concomitant dilution of ethanol into account ethanol (300 mM) in the perfusion medium probably produces tissue levels in a range similar to that observed after injection of the above dose of ethanol.

As already mentioned, we have previously reported that accumbal dopamine overflow after systemic ethanol is antagonized by mecamylamine, both after systemic administration (Blomqvist et al., 1993) and after local application into the ventral tegmental area, but not after local application in the nucleus accumbens (Blomqvist et al., 1996). We have also reported that nicotinic receptors located in the cell-body region of the mesocorticolimbic dopamine system appear to be of importance for the reinforcing properties of ethanol (Ericson et al., 1998). The present observation that ethanol failed to increase extracellular accumbal dopamine levels after perfusion into the ventral tegmental area taken together with these earlier findings therefore suggest that ethanol does not interact directly with ventral tegmental nicotinic receptors. Thus, the interaction may instead be indirect, possibly involving acetylcholine release in the ventral tegmental area.

Furthermore, since local administration of ethanol in the nucleus accumbens elevated accumbal dopamine levels to approximately the same extent as systemic ethanol, the nucleus accumbens or nearby regions could be the primary locus of action also for systemic ethanol in its dopamine activating effect. In that case the ethanol-induced elevation of dopamine after accumbal application should, similarly to that after systemic administration, be antagonized by local pre-treatment with mecamylamine in the ventral tegmental area, but not in the nucleus accumbens. As shown in the present study, this is indeed the case. Taken together, these findings and our previous results thus suggest that increased ethanol-induced dopamine overflow is primarily mediated via a mechanism located to or nearby the nucleus accumbens and involves, possibly via a neuronal loop, enhanced endogenous (acetylcholine) activation of nicotinic acetylcholine receptors located in or nearby the ventral tegmental area.

To explore the importance of endogenous acetylcholine for the enhancement of accumbal dopamine after local administration of ethanol, we administered vesamicol (4 mg/kg, i.p.), a potent inhibitor of vesicular acetylcholine storage, 60 min before perfusing ethanol (300 mM) in the nucleus accumbens. This dose of vesamicol has previously been reported to reduce extracellular acetylcholine levels by more than 50%, approximately 1 h after systemic administration (Marien et al., 1991). The ethanol-induced increase in accumbal dopamine levels was completely antagonized by vesamicol, indicating that endogenous acetylcholine is important for this response. Interestingly, the combination of systemic vesamicol and ethanol in the nucleus accumbens instead tended to decrease the accumbal dopamine levels. A similar decrease (Blomqvist et al., 1993) or trend for a decrease (Blomqvist et al., 1996) has previously been observed after the combined treatment with ethanol and mecamylamine, although mecamylamine by itself did not alter dopamine levels. Taken together, this could, again, indicate that ethanol produces two opposing effects on dopamine levels in the nucleus accumbens, one that is stimulatory via a neuronal loop involving acetylcholine-mediated activation for nicotinic receptors in the ventral tegmental area, and one that is inhibitory, perhaps via activation of accumbal GABAA/benzodiazepine receptors (Zetterström and Fillenz, 1990). After systemic or accumbal administration of ethanol the stimulatory component may normally prevail, but upon blockade of the stimulatory mechanism the inhibitory component is disclosed, as observed in the present and previous experiments.

The effects of ethanol on acetylcholine neurochemistry and release have previously been investigated. For instance, high concentrations of ethanol have been demonstrated to elevate striatal high-affinity choline uptake, an index of acetylcholine release (Hunt and Majchrowicz, 1983) and later, voluntary ethanol self-administration was demonstrated to increase dopaminergic and cholinergic neurotransmission (Nestby et al., 1999). Acetylcholine levels in the hippocampus may also increase during behavioral signs of ethanol withdrawal, something that persists for approximately 3 days (Imperato et al., 1998).

To our knowledge, there are, however, no studies available studying the effect of systemic ethanol on extracellular acetylcholine levels in the ventral tegmental area. The ventral tegmental cholinergic afferent projections derive from the pedunculopontine tegmental nucleus and the laterodorsal tegmental nucleus (Heimer et al., 1991) and hypothetically ethanol could interact directly or indirectly with the cholinergic cell bodies and/or terminals resulting in an increase in ventral tegmental acetylcholine. Whether there are projections from the nucleus accumbens to the pedunculopontine tegmental nucleus or to the laterodorsal tegmental nucleus are not, to our knowledge, known, whereas GABAergic neurons projecting from the nucleus accumbens shell to the ventral tegmental area have been established (Walaas and Fonnum, 1980; Heimer et al., 1991). The most simple chain of events could be that ethanol inhibits GABAergic influence on acetylcholine terminals located in the ventral tegmental area, leading to an activation of nicotinic receptors located on ventral tegmental dopamine cell bodies. Needless to say, a neuronal circuit involving also the cell-body regions of the cholinergic neurons cannot, however, be excluded.

There are three subtype compositions of the nicotine acetylcholine receptor in the brain that appear to be especially abundant: the α4β2, α3β4 and α7 subtypes. Among them, the α4β2 subtype composition may be the most common. The dopaminergic neurons in the ventral tegmental area appear to express many α and β subtypes although the α7 subtype may be expressed only in 50% of the neurons (Azam et al., 1999). Since the α4β2 subtype composition appears to be one of the most common in the brain and since the β2 receptor subtype has previously been demonstrated to be of importance for the nicotine-induced increase of accumbal dopamine levels (Lena and Changeux, 1999), it was judged to be of interest to explore whether α4β2 nicotinic receptors in the ventral tegmental area are involved in the dopaminergic response to ethanol. To this end the selective α4β2 nicotine acetylcholine receptor antagonist dihydro-β-erythroidine was perfused in the ventral tegmental area and dopamine levels in the nucleus accumbens were monitored after systemic ethanol. Dihydro-β-erythroidine did not counteract the ethanol-induced dopamine release but, at later timepoints, instead tended to increase accumbal dopamine levels by itself and, to possibly potentiate the ethanol effect. Since dihydro-β-erythroidine was administered via the microdialysis probe it could be questioned whether the drug in fact passed over the dialysis membrane. However, since dihydro-β-erythroidine clearly prevented the increase in accumbal dopamine levels observed after systemic nicotine, the drug apparently reached the neurons in sufficient amounts to exert a pharmacologically significant effect. Moreover, this latter finding indicates that nicotine produces its mesolimbic dopamine activating effect via the activation of ventral tegmental nicotinic receptor of the α4β2 subtype, whereas ethanol apparently does not, and that endogenous acetylcholine activation of these neurons, at least of the neurons that are involved in the ethanol effect, is not carried out via the α4β2 subtype.

A recent patch-clamp study by Aistrup et al. (1999) demonstrated that ethanol significantly increases high-affinity neuronal nicotine acetylcholine receptor currents insensitive to α-bungarotoxin in cultured rat cortical neurons, e.g. currents through the α4β2 subtype. In another in vitro study, ethanol produced a highly variable effect on the α4β2 subtype inducing both potentiation and inhibition of acetylcholine-induced currents (Covernton and Connolly, 1997), whereas Cardoso et al. (1999), expressing human nicotine acetylcholine receptor subtypes, demonstrated a consistent ethanol-induced potentiation of the acetylcholine-induced currents through the α4β2 subtype. Interestingly, these studies and others have found that other nicotinic responses may be weakly inhibited by ethanol, e.g. that of the α7 subtype, which suggests that ethanol has different effects on different nicotine acetylcholine receptor subtypes. In the present study blockade of the α4β2 subtype with dihydro-β-erythroidine did not antagonize the ethanol-induced increase in extracellular dopamine levels after systemic administration, which might appear contradictory to the available in vitro results. However, as pointed out above, the ethanol interaction in this respect is most likely indirect, involving release of endogenous acetylcholine, and hence a tentative direct effect of ethanol at α4β2 appears irrelevant in this context. It should, however, be noted that in most in vitro studies regarding the interaction between ethanol and nicotine acetylcholine receptor subtypes ethanol generally fails to activate the receptor by itself but merely functions as a co-agonist, requiring acetylcholine for its activating (potentiating) effect. Therefore, it is possible that in vivo, after systemic ethanol, when ethanol is available both in the nucleus accumbens and in the ventral tegmental area, the mecamylamine-sensitive dopamine activation may derive both from an ethanol-induced liberation of acetylcholine in the ventral tegmental area and an ethanol-induced potentiation of the acetylcholine effect at the relevant nicotine acetylcholine receptor subtype in the ventral tegmental area, which then most likely is not of the α4β2 subtype.

It has been suggested that mecamylamine also interacts with NMDA receptors and thus, the effects obtained after local perfusion with mecamylamine in the ventral tegmental area could tentatively derive from such an interaction rather than from an interaction with nicotinic receptors. However, since locally perfused hexamethonium has been shown to produce a similar effect as mecamylamine (Blomqvist et al., 1997), and, to our knowledge, this compound does not interact with NMDA receptors, this possibility appeared unlikely. In the present study, this possibility was, however, tested more directly. Thus, perfusion of the non-competitive NMDA receptor antagonist MK-801 (100 μM) was found to increase accumbal dop-

amine levels by itself, a finding in line with increased accumbal dopamine levels after systemic MK-801 (Mathé et al., 1999), and the dopamine levels remained increased compared to baseline levels, when MK-801 and ethanol were co-administered. Both the MK-801-induced elevation of dopamine by itself and the failure to counteract the ethanol effect contrast to the effects observed after ventral tegmental mecamylamine and thus argue against the notion that the mecamylamine effect would derive from NMDA receptor antagonism.

5. Conclusion

These findings indicate that the ethanol-induced activation of mesolimbic dopamine neurons may be mediated via an indirect rather than direct stimulation of ventral tegmental nicotine acetylcholine receptors and suggest that the primary locus of action of ethanol in this respect may be in the nucleus accumbens or nearby regions. The findings also suggest that the increase in accumbal dopamine levels after ethanol involves endogenous acetylcholine release in the ventral tegmental area. The nicotine acetylcholine receptor subtype involved is not clear; however, the $\alpha4\beta2$ subtype does not appear to be involved. In contrast, ventral tegmental nicotine acetylcholine receptors of the $\alpha4\beta2$ subtype appear to mediate the mesolimbic dopamine activating effects of systemic nicotine. Ongoing research in the present laboratories is aimed at trying to further identify this primary locus and mechanism(s) of action of ethanol in the nucleus accumbens as well as determining what nicotine acetylcholine receptor subtype(s) that is involved in the ventral tegmental area. The results of these studies may provide targets for developing drugs aimed at specifically interfering with the dopamine activating and reinforcing properties of ethanol.

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