ELSEVIER

European Journal of Pharmacology 358 (1998) 189–196

ejp

Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow are blocked by ventral tegmental mecamylamine

Mia Ericson, Ola Blomqvist, Jörgen A. Engel, Bo Söderpalm *

Department of Pharmacology, Institute of Physiology and Pharmacology, Göteborg University, Box 431, SE 405 30 Göteborg, Sweden

Received 14 May 1998; revised 29 July 1998; accepted 14 August 1998

Abstract

The mesocorticolimbic dopamine system is believed to be involved in mediating the positive reinforcing effects of drugs of abuse, including ethanol. The nicotinic acetylcholine receptor antagonist mecamylamine perfused via reversed microdialysis in the ventral tegmental area antagonizes the increase of accumbal extracellular dopamine levels after systemic ethanol, and, after systemic injection, lowers ethanol intake in the rat. In the present study the effect of ventral tegmental mecamylamine on ethanol intake and preference, as well as on extracellular accumbal dopamine levels, was investigated in the same animal. To this end, in vivo microdialysis using a double probe approach (one in the nucleus accumbens and one in the ventral tegmental area) was combined with an ethanol preference model invoking a free choice between a bottle of water and a bottle of ethanol 6% (v/v) solution. Wistar rats drinking more than 60% of their total daily fluid intake from the ethanol solution (ethanol high-preferring animals) were selected during a screening period and used for the experiments. The animals received vehicle or mecamylamine (100 μM) in the ventral tegmental area and were then presented with a choice between water and ethanol in a limited access paradigm to which they previously had been adapted. On the next day the rats that received vehicle day 1 now received mecamylamine, and vice versa. When treated with vehicle, ethanol intake and preference were unaltered, as compared to baseline behavior, and accumbal dopamine levels increased significantly to approximately 130% of the pre-drug baseline level. When receiving mecamylamine, ethanol intake and preference were reduced markedly and dopamine levels were unaltered, as compared to pre-drug baseline levels. The present results further indicate that nicotinic acetylcholine receptors in the ventral tegmental area are involved in the positive reinforcing effects of ethanol. Thus, mecamylamine or other antagonists specifically aimed at ventral tegmental nicotinic acetylcholine receptors could represent a new pharmacological treatment principle against alcohol abuse, the efficacy of which should be explored in high-scale alcohol consumers or alcoholics.

Keywords: Dopamine; Ethanol; Microdialysis; Nicotinic receptor; Positive reinforcement; (Rat)

1. Introduction

Ethanol, like other drugs of abuse, has repeatedly been demonstrated to activate the mesocorticolimbic dopamine system after systemic injections (Engel and Carlsson, 1977; Imperato and Di Chiara, 1986; Mereu et al., 1987; Koob, 1992; Blomqvist et al., 1993, 1997). This dopamine pathway appears to be a crucial component of the so-called brain reward system, and its activation has been suggested to be important for the development of addictive behavior towards various drugs of abuse (Wise and Bozarth, 1987; Koob and Bloom, 1988; Robinson and Berridge, 1993).

Whereas several studies are available demonstrating activation of the mesocorticolimbic dopamine system in association with self-administration of psychostimulants in experimental animals, to our knowledge only one study has been presented addressing this issue in animals self-administering ethanol (Weiss et al., 1993). The first aim of the present study was therefore to examine whether voluntary oral ethanol intake in ethanol high-preferring Wistar rats choosing between ethanol and water consumption in a limited access free-choice paradigm is associated with increased accumbal dopamine levels, concomitantly measured by in vivo microdialysis.

The mechanisms of action for psychostimulants, opiates and nicotine in their activation of the mesocorticolimbic dopamine system are fairly well-established, whereas for ethanol it remains unknown. However, based on a series of investigations in mice and rats we have recently suggested that ethanol-induced activation of the mesocorticolimbic dopamine system involves central nicotinic acetylcholine receptors (Blomqvist et al., 1992, 1993; Söderpalm et al., 1993; Johnson et al., 1995; Blomqvist et al., 1996, 1997).

* Corresponding author. Tel.: +46-31-773-3400; Fax: +46-31-821-795.

0014-2999/98/$ - see front matter
PII: S0014-2999(98)00602-5

The most important evidence for such an involvement is that ethanol-induced dopamine overflow in the rat nucleus accumbens is counteracted by systemic administration of mecamylamine, a blood brain barrier penetrating nicotinic acetylcholine receptor antagonist, but not by the peripheral nicotinic antagonist hexamethonium (Blomqvist et al., 1993, 1997). Moreover, since local application of mecamylamine or hexamethonium into the ventral tegmental area, but not into the nucleus accumbens, prevents ethanol-induced dopamine overflow after systemic administration, ventral tegmental nicotinic acetylcholine receptors appear to be the most important in this respect (Blomqvist et al., 1997). That this interaction may be important also for ethanol reinforcement is illustrated by the finding that systemic mecamylamine, but not hexamethonium, reduces ethanol intake in ethanol high-preferring Wistar rats (Blomqvist et al., 1996).

The second part of the present study was aimed at determining in the same animal whether nicotinic receptor blockade in the ventral tegmental area by means of mecamylamine is associated with reduced voluntary ethanol intake and preference as well as a reduction of ethanol-induced accumbal dopamine overflow. This part of the study represents the first attempt to monitor neurochemical events believed to be associated with ethanol reward concomitantly with measuring the reinforced behavior and applying a treatment that is hypothesized to prevent it.

2. Materials and methods

2.1. Animals

Male Wistar rats, about 100 days old, were supplied by Beekay (Stockholm, Sweden). Upon arrival in the laboratory, the animals were housed in groups of 5 per cage ($55 \times 35 \times 20$ cm^3) 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 0900 a.m. and off at 0900 p.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; $40 \times 24 \times 15$ cm^3). 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 were measured over a 5-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- ($< 20\%$ ethanol), medium- (20–60% ethanol) or high- ($> 60\%$ ethanol) preferring based on their ethanol preference. Approximately 8% of the animals were classified as high-preferring rats.

According to previous tests in our laboratories most rats (90%) do not show side preference that affects their ethanol preference (unpublished). In these tests some animals (25–30%), appeared disturbed by the side switch, but returned to their baseline ethanol preference after 1–2 days. In the present study the animals were not tested for side preference in order to avoid such disturbances.

2.3. *In vivo* microdialysis

Brain microdialysis experiments were performed in awake, freely moving animals as described by Waters et al. (1993). Briefly; the rats were anaesthetized with a 1:2 v/v mixture of xylazine (13 mg/kg i.p., Bayer Leverkusen) and ketamine (67 mg/kg i.p., Parke-Davis). The animals then were mounted into a Kopf stereotaxic instrument. The probe co-ordinates relative to the bregma and according to Paxinos and Watson (Paxinos and Watson, 1986) were A/P +1.85, L/M −1.4, V/D −7.8 for the nucleus accumbens and A/P −5.2, L/M −0.7, V/D −8.4 for the ventral tegmental area. The probes were slowly lowered into the brain monolaterally and cemented to the skull with Phosphatine cement (Dentalhuset, Sweden). The rats were then housed in spherical cages and were allowed to recover for 48 h before the dialysis experiments.

On the day of the experiment the rats were connected to a microperfusion pump (CMA/100, Carnegie Medicine, Sweden), and were replaced in their home cages where they could move freely. The pump was set to 2 μl/min. A total of 40 μl samples was collected every 20 min and injected to the chromatographic system. The perfusion medium was a Ringer solution containing in mmol/l: NaCl, 140; $CaCl_2$, 1.2; KCl, 3.0; and $MgCl_2$, 1.0. To analyze dopamine in the fractions a high pressure liquid chromatography system with electrochemical detection was used (Waters et al., 1993).

After the completion of the studies the animals were sacrificed by decapitation, the brains were sliced on a cryotome and the positions of the microdialysis probes were inspected. Only results from animals with correctly placed probes are included in the presented figures.

2.4. Experimental procedure

In order to adapt to the experimental circumstances ethanol high-preferring rats were limited to drink only 1 h

a day for 1 week in the test cage, whereas food was available ad libitum. On the days of the experiments the rats were thus both water and ethanol deprived when they were connected to the microdialysis system and the sampling started. The experiments were performed between 9 a.m. and 3 p.m. in a dark room. Baseline samples were collected until at least four consistent values (±5%) of dopamine levels were obtained. Mecamylamine (100 μM) or vehicle was then perfused in the ventral tegmental area for 40 min before the rats were presented with a bottle of water and a bottle of 6% ethanol solution, which were weighed before and after the experiment. The next day the rats were handled in the same way as on the day before but animals that received mecamylamine on day 1 now received vehicle instead and vice versa. The order of treatments was balanced between the different animals. In some animals ($n = 4$) the time spent drinking ethanol or water, as well as at what time-points drinking occurred, was recorded manually during the first 20 min of the drinking period.

2.5. Drugs

Ethanol (AB Svensk Sprit) was diluted (2–4–6% v/v) with regular tap water and presented in regular plastic 300 ml bottles. Mecamylamine HCl (2-[methylamino]isocamphane hydrochloride purchased from Sigma) was dissolved in Ringer solution. The concentration of mecamylamine used (100 μM) was chosen based on previous studies indicating that when applied in the ventral tegmental area it prevents ethanol-induced activation of the mesolimbic dopamine system (Blomqvist et al., 1997).

2.6. Statistics

The in vivo microdialysis data were statistically evaluated by using a two-factor analysis of variance (ANOVA) with repeated measures or paired *t*-test. Ethanol intake and preference data were statistically evaluated using Wilcoxons test. All values are expressed as means ± S.E.M. A probability value (P) less than 0.05 was considered statistically significant.

3. Results

3.1. Methodological considerations

Approximately one third of the high-preferring animals was excluded from the study due to technical malfunctions (malfunctioning probes or misimplanted probes), or due to an inability to maintain ethanol high-preference (according to the above definition) after surgery. Thus, in order to collect the results from the number of animals reported in this study 150 animals were initially screened for ethanol preference.

3.2. Ethanol intake and preference

When subjected to a free-choice between ethanol and water in a limited access paradigm after receiving vehicle via reversed dialysis in the ventral tegmental area, ethanol high-preferring rats maintained their ethanol preference

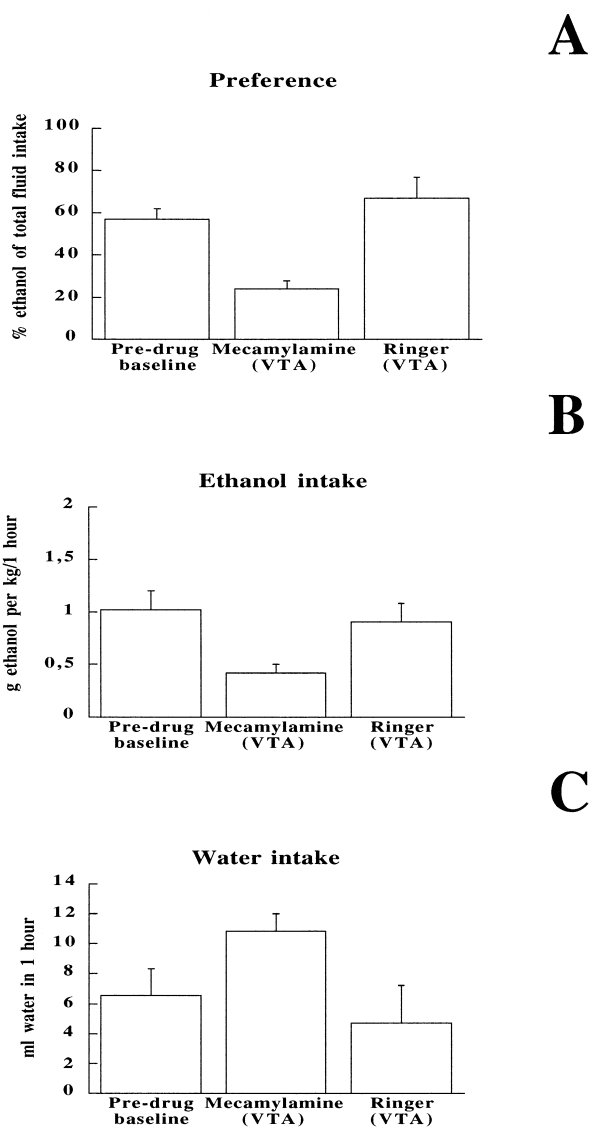

Fig. 1. (A) Effect of mecamylamine (100 μM) or Ringer solution in the ventral tegmental area (VTA) on ethanol preference after voluntary ethanol/water intake. Statistics: Wilcoxons test—baseline vs. mecamylamine $P = 0.043$, mecamylamine vs. Ringer $P = 0.046$, baseline vs. Ringer $P = 0.249$. (B) Effect of mecamylamine (100 μM) or Ringer solution in the ventral tegmental area on ethanol intake after voluntary ethanol/water intake. Statistics: Wilcoxons test—baseline vs. mecamylamine $P = 0.028$, mecamylamine vs. Ringer $P = 0.080$, baseline vs. Ringer $P = 0.916$. (C) Effect of mecamylamine (100 μM) or Ringer solution in the ventral tegmental area on water intake after voluntary ethanol/water intake. Statistics: Wilcoxons test—baseline vs. mecamylamine $P = 0.173$, mecamylamine vs. Ringer $P = 0.043$, baseline vs. Ringer $P = 0.138$. All values are expressed as means ± S.E.M., $n = 6$.

and ethanol intake, as compared to their baseline levels determined in the limited access paradigm before operation. The water intake, however, tended to decrease slightly during the 1-h experimental period (Fig. 1). The total liquid intake (i.e., both water and ethanol solution intake) did not differ between the experimental groups. All groups consumed approximately 11 ± 2 ml fluid in total (data not shown).

Application of mecamylamine in the ventral tegmental area via reversed microdialysis significantly decreased ethanol preference ($P = 0.046$) and tended to decrease ethanol intake ($P = 0.079$), as compared to the behavioral response observed after vehicle application in the ventral tegmental area. When compared to baseline responding mecamylamine significantly reduced both ethanol preference ($P = 0.043$) and ethanol intake ($P = 0.028$). The water intake was significantly increased ($P = 0.043$) after perfusion of mecamylamine in the ventral tegmental area, as compared to after vehicle treatment (Fig. 1).

As evident from Fig. 2, most of the drinking both after vehicle and mecamylamine in the ventral tegmental area occurred during the first 10 min after presentation of the water and ethanol bottles. This figure also demonstrates that ethanol consumption dominated over water consumption after vehicle in the ventral tegmental area and vice versa after mecamylamine.

3.3. Extracellular dopamine in the nucleus accumbens

Voluntary ethanol intake in a limited access paradigm invoking free-choice between ethanol and water resulted in a significant elevation of extracellular dopamine levels in the nucleus accumbens ($P = 0.030$). Mecamylamine in the ventral tegmental area did not by itself alter basal extracellular dopamine levels. However, with mecamylamine present no significant alteration of dopamine levels was observed after ethanol or water consumption (Fig. 3).

Fig. 4 shows the drinking pattern and the extracellular dopamine levels in the nucleus accumbens in a single animal. With vehicle in the ventral tegmental area ethanol

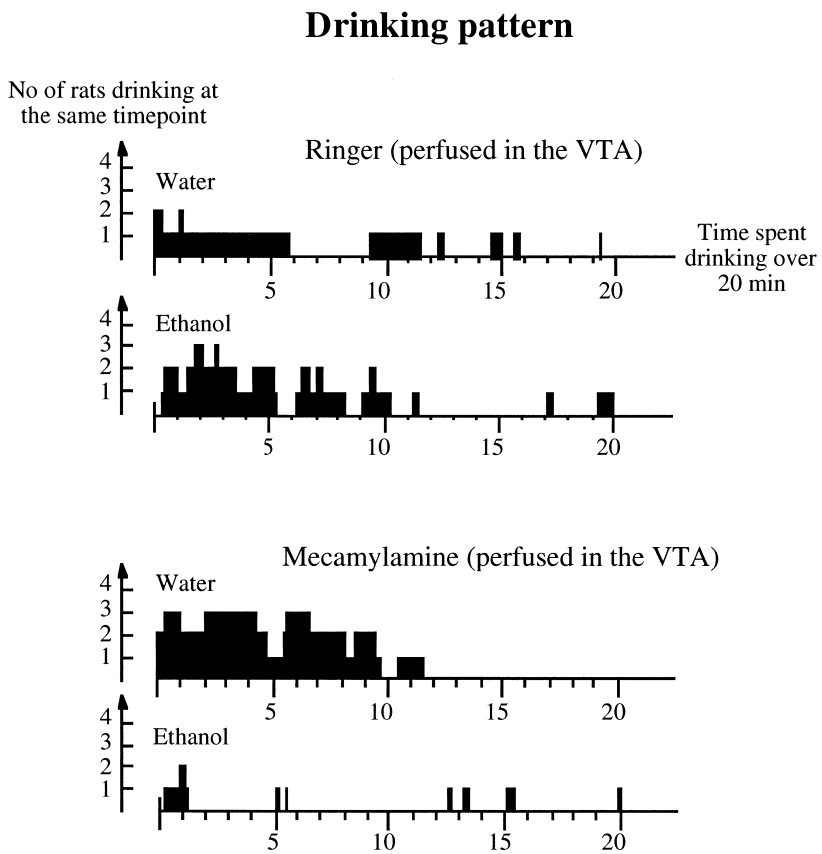

Fig. 2. Effect of mecamylamine (100 μM) or Ringer solution in the ventral tegmental area (VTA) on the drinking pattern of water and ethanol. Data recorded in seconds during the first 20 min of the drinking period, $n = 4$. The *Y*-axis represents number of animals drinking at the same time.

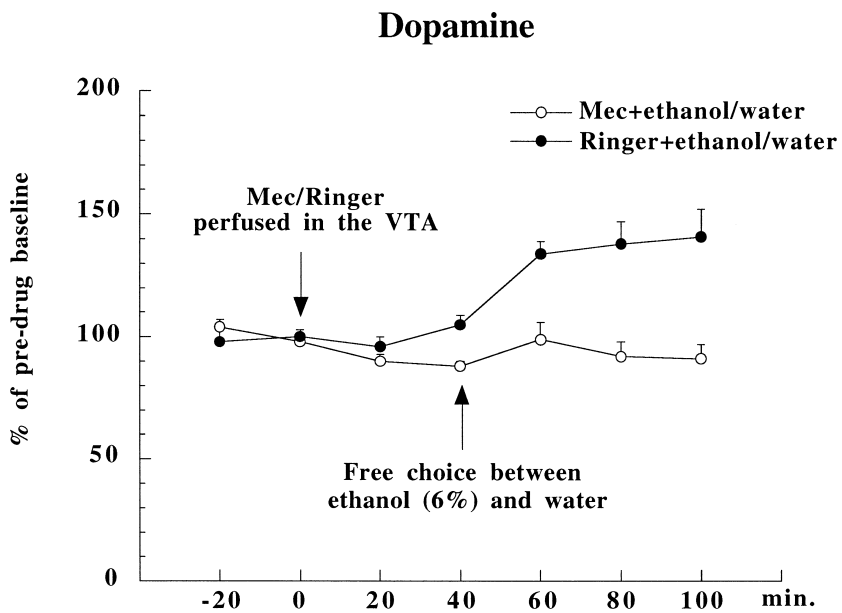

Fig. 3. Effect of mecamylamine (100 μM) or Ringer solution in the ventral tegmental area (VTA) on accumbal dopamine after voluntary ethanol intake as measured by in vivo microdialysis in freely moving animals. All values are expressed as means ± S.E.M., $n = 7$. Statistics: ANOVA with repeated measures $P = 0.030$.

consumption dominated over water consumption, and the extracellular dopamine levels in the nucleus accumbens peaked in the microdialysis sample obtained after ethanol consumption (at time-point 60′). With mecamylamine in the ventral tegmental area this particular animal first ingested ethanol for approximately 1 min and then switched to water consumption, and the extracellular dopamine levels in the nucleus accumbens were not altered.

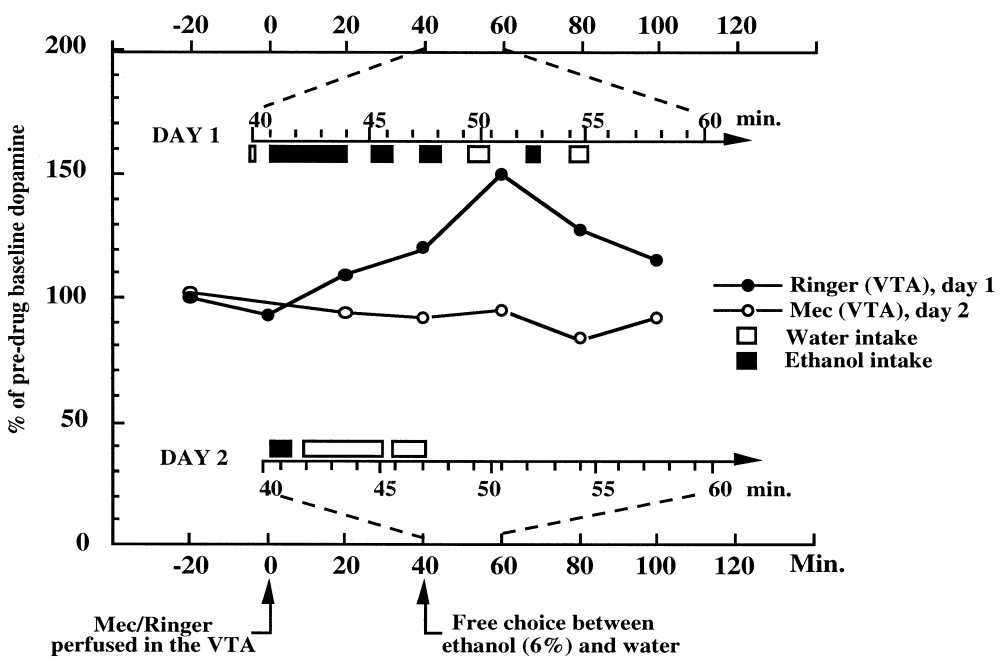

Fig. 4. A representative example of drinking pattern and accumbal dopamine after voluntary ethanol/water intake, as measured by in vivo microdialysis in freely moving animals. Ringer solution was perfused in the ventral tegmental area (VTA) on day 1 and mecamylamine (Mec) (100 μM) on day 2, the animal was allowed to choose to drink between a bottle of ethanol solution (6%) and a bottle of water. The drinking pattern was recorded during the first 20 min of the drinking period.

4. Discussion

The present study demonstrates that ethanol intake in ethanol high-preferring Wistar rats in a limited access paradigm invoking a free choice between ethanol and water is associated with increased extracellular dopamine levels in the nucleus accumbens. This finding confirms that of Weiss et al. (1993), who reported that ethanol-reinforced lever-pressing behavior in the rat is associated with elevated accumbal dopamine levels. Furthermore, the present results extend these findings by showing that oral ethanol intake in a paradigm not invoking complex motor behavior (lever-pressing) also results in increased dopamine levels in the nucleus accumbens.

Interestingly, Weiss et al. (1993) demonstrated that accumbal dopamine levels were elevated already in anticipation of ethanol self-administration. No such anticipatory elevation of dopamine levels could be observed in the present study. It is possible that such an effect can be obtained only in extensively trained animals that associate ethanol intake with a discrete environment only (e.g., the box in which they have been trained). The rats used in the present study were exposed to ethanol in their home-cages during a long selection period before the relatively short period of training in the limited access paradigm, and hence could not exclusively pair ethanol intake with environmental factors associated with the microdialysis experiment. Alternatively, in our animals the most important cue signalling ethanol availability and triggering approach behavior towards the ethanol bottle may be the smell/taste rather than other environmental factors. The increase in dopamine levels here observed after ethanol intake could thus represent a combination of conditioned smell-induced dopamine release and ethanol-induced dopamine release. Arguing against the enhanced dopamine levels being due solely to conditioning is the fairly long-lasting effect observed, as well as the facts that the amount of ethanol consumed is pharmacologically significant and that systemic ethanol injections increase dopamine to similar levels (e.g., Blomqvist et al., 1993, 1997).

With the sampling times here applied it is not possible to firmly determine whether the dopamine overflow in the nucleus accumbens after ethanol intake is time-locked with the drinking behavior, although the drinking pattern observed, just as in the study by Weiss et al. (1993), indicates that this may be the case. The present experiments further indicate that the dopamine elevation is related to ethanol intake and not to drinking behavior in general. Thus, no accumbal dopamine increase was observed in animals that received mecamylamine in the ventral tegmental area and that apparently consumed water instead of ethanol.

The finding that mecamylamine applied locally in the ventral tegmental area decreased ethanol intake is in line with our previous finding that systemic mecamylamine lowers ethanol intake in ethanol high-preferring Wistar rats (Blomqvist et al., 1996). In addition, a significant reduction of ethanol preference after tegmental application of mecamylamine was observed. This observation is interesting when related to our previous findings. Thus, we have reported that systemic mecamylamine concomitantly lowers ethanol and water intake, leaving ethanol preference unaltered. Since a similar reduction of water but not ethanol intake was observed after hexamethonium, we suggested that peripheral blockade of nicotinic acetylcholine receptors unspecifically lowers water intake, whereas central antagonism reduces ethanol intake (Blomqvist et al., 1996). The present results support this interpretation, since local, central administration of mecamylamine reduced ethanol intake and concomitantly increased water intake.

The most important finding in the present study is that local mecamylamine in the ventral tegmental area, a manipulation that blocks ethanol-induced dopamine release after systemic injection (Blomqvist et al., 1997), reduced ethanol intake, and that, at the same time, no dopamine elevation could be observed. Even though a causal relationship between these two events is difficult to firmly establish, it appears likely. Indeed, it would be much of a coincidence if they were not, given the well established role for the mesolimbic dopamine system in maintaining self-administration of various drugs of abuse in experimental animals (cf. Wise and Rompre, 1989, Koob, 1992), and the fact that mecamylamine was administered into a restricted, important area along this system. Thus, it may be suggested that with mecamylamine present in the ventral tegmental area, ethanol intake and/or cues associated with ethanol intake (see below) in ethanol high-preferring Wistar rats fail to produce a reinforcing dopamine liberating effect, and hence ethanol intake and preference are reduced. The increased water intake observed would then be a compensatory phenomenon in these ethanol and water deprived rats. The alternative interpretation would be that ventral tegmental mecamylamine primarily increases water intake and that the reduced ethanol intake is but a consequence of this increased water intake. However, considering that systemic mecamylamine lowers ethanol intake and, if anything, lowers also water intake (Blomqvist et al., 1996), and the fact that the total fluid intake was unaltered in the present experiments, this explanation, although it has not been experimentally challenged, appears less likely.

As indicated above some observations call for a complementary interpretation of the role of dopamine, and of ventral tegmental mecamylamine, in the present ethanol drinking behavior. Although some mecamylamine-treated animals indeed tasted ethanol before switching to water consumption, this did not apply to all. This finding could indicate that ventral tegmental mecamylamine not only blocks ethanol-induced accumbal dopamine overflow but also a tentative conditioned smell/taste-induced dopamine release. Since enhanced accumbal dopamine may be an important signal to elicit consumption of different 'rewards' (Robinson and Berridge, 1993; Mirenowicz and

Schultz, 1996; Schultz et al., 1997) this could explain why ethanol intake was not elicited. Notably, water consumption substituted for ethanol, indicating that intake of this natural 'reward' apparently does not require an elevation of accumbal dopamine. Thus, elicitation of water consumption is most likely governed also by mechanisms other than activation of mesolimbic dopamine neurons. It should also be noted that after mecamylamine in the ventral tegmental area ethanol was actually avoided (ethanol preference approximately only 20%), indicating that an aversive signal dominates in this situation. Thus, mecamylamine may have removed the approach signal leaving a competitive aversive signal untouched. Alternatively, ventral tegmental mecamylamine may directly have enhanced an aversive signal.

Some previous studies have demonstrated a decrease in voluntary ethanol intake also after other manipulations that presumably prevent conditioned as well as ethanol-induced activation of the mesolimbic dopamine system. Thus, both administration of 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) reduce ethanol intake. However, evidence for an increase in (Gauvin et al., 1993) or no effect on ethanol consumption after dopamine receptor antagonists or lesions have also been reported (Brown et al., 1982; Gauvin et al., 1993; Rassnick et al., 1993). Although strain differences and incomplete dopaminergic lesions may explain some of these contradictory results, it is difficult to conclusively determine the role for mesolimbic dopamine in ethanol reward from these studies. However, the results of the present study concomitantly measuring the effect of a pharmacological manipulation on ethanol intake and extracellular dopamine levels in the nucleus accumbens, and performed in neuronally intact, non-lesioned animals, strongly support the notion of an important role for accumbal dopamine in ethanol reinforcement.

Although mecamylamine has been considered a selective antagonist at central as well as peripheral nicotinic acetylcholine receptors, this compound may inhibit also *N*-methyl-D-aspartate (NMDA)-stimulated currents (patch clamp studies by O'Dell and Christensen, 1988) and NMDA-induced noradrenaline release in the rat hippocampus (Snell and Johnson, 1989). These effects have been suggested to be due to non-competitive blockade of the NMDA receptor ion channel via an action at the phencyclidine (PCP) site. However, whereas the non-competitive NMDA antagonists PCP and MK-801 may increase dopamine turnover and release by themselves (Imperato et al., 1990; Rao et al., 1990a,b; Svensson et al., 1991), neither systemic nor ventral tegmental mecamylamine in the present concentration alters dopamine overflow, as measured by in vivo microdialysis (Blomqvist et al., 1993, 1997). Furthermore, in contrast to mecamylamine (present study and Blomqvist et al., 1996), MK-801 fails to reduce ethanol intake in the rat (Danysz et al., 1992). Moreover, since ethanol itself is a functional antagonist at NMDA receptors (e.g., Lovinger et al., 1989) it appears unlikely that another NMDA antagonist would counteract its acute neurochemical effects. These circumstances argue against, but do not rule out, the possibility of the behavioral and neurochemical effects of mecamylamine observed here being due to its NMDA blocking properties. It appears more likely that the obtained results are indeed due to an antagonism of nicotinic acetylcholine receptors. Needless to say, only further experimentation can firmly resolve this issue.

Taken together, the present findings in rats provide support for a mechanistic relation between ethanol and nicotinic acetylcholine receptors in the dopamine-activating and reinforcing properties of ethanol. As discussed previously (see Blomqvist et al., 1997), this interaction between ethanol and nicotinic acetylcholine receptors may be direct or indirect, an issue that is currently being investigated in our laboratories. Regardless of the exact nature of this interaction it may provide a neurochemical background to the well known fact that most alcoholics smoke, and that alcoholism may be 10 times more common among smokers than non-smokers (DiFranza and Guerrera, 1990). Furthermore, the present results suggest that nicotinic acetylcholine receptors in the ventral tegmental area may not be involved only in mediating the dopamine activating effects of ethanol (Blomqvist et al., 1997) but also in mediating conditioned activation of the mesolimbic dopamine system, indicating that these receptors could be more generally involved in mediating 'craving' (cf. Robinson and Berridge, 1993).

Finally, the animal model used in the present study has 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 both after systemic (Blomqvist et al., 1996) and local ventral tegmental administration (present study) reduces ethanol consumption in the rat, it is suggested that mecamylamine or specific antagonists aimed at ventral tegmental nicotinic acetylcholine receptors represent a new pharmacological treatment principle, the efficacy of which should be explored in high-scale alcohol consumers or alcoholics.

Acknowledgements

This study was financially supported by grants from the Swedish Medical Research Council (nos. 11583 and 4247), the Swedish Alcohol Monopoly Foundation for Alcohol Research, Swedish Society for Medical Research, Swedish Match, Orion Pharma neurology, Lundbecks Fond för Psykofarmakologisk Forskning, Wilhelm och Martina

Lundgrens vetenskapsfond, Fredrik och Ingrid Thurings Stiftelse, Stiftelsen Sigurd och Elsa Goljes minne and National Institute on Drug Abuse Grant (IR 01 DA 10765-01A1).

References

Blomqvist, O., Söderpalm, B., Engel, J.A., 1992. Ethanol-induced locomotor activity: involvement of central nicotinic acetylcholine receptors?. Brain Res. Bull. 29, 173–178.

Blomqvist, O., Engel, J.A., Nissbrandt, H., Söderpalm, B., 1993. The mesolimbic dopamine-activating properties of ethanol are antagonized by mecamylamine. Eur. J. Pharmacol. 249, 207–213.

Blomqvist, O., Ericson, M., Johnson, D.H., Engel, J.A., Söderpalm, B., 1996. Voluntary ethanol intake in the rat: effects of nicotinic acetylcholine receptor blockade and nicotine sensitization. Eur. J. Pharmacol. 314, 257–267.

Blomqvist, O., Ericson, M., Engel, J.A., Söderpalm, B., 1997. Accumbal dopamine-overflow after ethanol; localization of the antagonizing effect of mecamylamine. Eur. J. Pharmacol. 334, 149–156.

Brown, Z.W., Gill, K., Abitbol, M., Amit, A., 1982. Lack of effect of dopamine receptor blockade on voluntary ethanol consumption in rats. Behav. Neural Biol. 36, 291–294.

Danysz, W., Dyr, W., Jankowska, E., Glazewski, S., Kostowski, W., 1992. The involvement of NMDA receptors in acute and chronic effects of ethanol. Alcohol. Clin. Exp. Res. 16, 499–504.

DiFranza, J.R., Guerrera, M.P., 1990. Alcoholism and smoking. J. Stud. Alcohol 51 (2), 130–135.

Engel, J.A., Carlsson, A., 1977. Catecholamines and behaviour. In: Valzelli, L., Essman, W.B. (Eds.), Current Developments in Psychopharmacology, Vol. 4. Spectrum Publishing, New York, NY, p. 1.

Fahlke, C., 1994. Alcohol consumption in the rat: modulation by adrenal steroids and mesotelencephalic dopamine. Thesis, Göteborg University, Göteborg.

Gauvin, D.V., Moore, K.R., Holloway, F.A., 1993. Do rat strain differences in ethanol consumption reflect differences in ethanol sensitivity or the preparedness to learn?. Alcohol 10, 37–43.

Imperato, A., Di Chiara, G., 1986. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J. Pharmacol. Exp. Ther. 239, 219–228.

Imperato, A., Scrocco, M.G., Bacchi, S., Angelucci, L., 1990. NMDA receptors and in vivo dopamine release in the nucleus accumbens and caudatus. Eur. J. Pharmacol. 187, 555–556.

Johnson, D.H., Blomqvist, O., Engel, J.A., Söderpalm, B., 1995. Subchronic intermittent nicotine treatment enhances ethanol-induced locomotor stimulation and dopamine turnover in mice. Behav. Pharmacol. 6, 203–207.

Koob, G.F., 1992. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol. Sci. 13, 177.

Koob, G.F., Bloom, F.E., 1988. Cellular and molecular mechanisms of drug dependence. Science 242, 715–723.

Lovinger, D.M., White, G., Weight, F.F., 1989. Ethanol inhibits NMDA-mediated ion current in hippocampal neurons. Science 243, 1721–1724.

Mereu, G., Yoon, K.-W.P., Boi, V., Gessa, G.L., Naes, L., Westfall, T.C., 1987. Preferential stimulation of ventral tegmental area dopaminergic neurons by nicotine. Eur. J. Pharmacol. 141, 395–399.

Mirenowicz, J., Schultz, W., 1996. Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. Nature 379, 449–451.

Myers, R.D., Melchior, C.L., 1975. Alcohol drinking in the rat after destruction of serotonergic and catecholaminergic neurons in the rat. Res. Commun. Chem. Pathol. Pharmacol. 10, 363–378.

Naranjo, C.A., Sellers, E.M., 1989. Serotonin uptake inhibitors attenuate ethanol intake in problem drinkers. Rec. Dev. Alcohol 7, 255–266.

O'Dell, T.J., Christensen, B.J., 1988. Mecamylamine is a selective non-competitive antagonist of *N*-methyl-D-aspartate- and aspartata-induced currents in horizontal cells dissociated from the catfish retina. Neurosci. Lett. 94, 93–98.

O'Malley, S.S., Jaffe, A.J., Chang, G., Schottenfeld, R.S., Meyer, R.E., Rounsaville, B., 1992. Naltrexone and coping skills therapy for alcohol dependence: a controlled study. Arch. Gen. Psychiatry 49 (11), 881–887.

Panocka, I., Pompei, P., Massi, M., 1993. Suppression of alcohol preference in rats induced by risperidone, a serotonin 5-HT_2 and dopamine D_2 receptor antagonist. Brain Res. Bull. 31, 595–599.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, Sidney.

Rao, T.S., Kim, H.S., Lehmann, J., Martin, L.L., Wood, P.L., 1990a. Selective activation of dopaminergic pathways in the mesocortex by compounds that act at the phencyclidine (PCP) binding site: a tentative evidence for PCP recognition sites not coupled to *N*-methyl-D-aspartate (NMDA) receptors. Neuropharmacology 29, 225–230.

Rao, T.S., Kim, H.S., Lehmann, J., Martin, L.L., Wood, P.L., 1990b. Interactions of phencyclidine receptor agonist MK-801 with dopaminergic system: regional studies in the rat. J. Neurochem. 54, 1157–1162.

Rassnick, S., Stinus, L., Koob, G.F., 1993. The effects of 6-hydroxydopamine lesions of the nucleus accumbens and the mesolimbic dopamine system on oral self-administration of ethanol in the rat. Brain Res. 623, 16–24.

Robinson, T.E., Berridge, K.C., 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res. Rev. 18, 247–291.

Schultz, W., Dayan, P., Montague, P.R., 1997. A neural substrate of prediction and reward. Science 275, 1593–1599.

Snell, J., Johnson, K.M., 1989. Effects of nicotinic agonists and antagonists on *N*-methyl-D-aspartate-induced 3H-norepinephrine release and 3H-(1[1-(2-thielyl)cyclohexyl]-piperidine) binding in rat hippocampus. Synapse 3, 129–135.

Svensson, A., Pileblad, E., Carlsson, M., 1991. A comparison between the non-competitive NMDA antagonist dizocilpine (MK-801) and the competitive NMDA antagonist D-CPPene with regard to dopamine turnover and locomotor-stimulatory properties in mice. J. Neural Transm. 85 (GenSect), 117–129.

Söderpalm, B., Blomqvist, O., Johnson, D., Engel, J.A., 1993. Involvement of central nicotinic acetylcholine receptors in the mesolimbic DA-activating and reinforcing properties of ethanol. Soc. Neurosci. Abstr. 254.6.

Volpicelli, J.R., Alterman, A.I., Hayashida, M., O'Brien, C.P., 1992. Naltrexone in the treatment of alcohol dependence. Arch. Gen. Psychiatry 49, 876–880.

Waters, N., Lagerkvist, S., Löfberg, L., Piercey, M., Carlsson, A., 1993. The dopamine D_3 receptor and autoreceptor preferring antagonists (+)-AJ76 and (+)-UH232; a microdialysis study. Eur. J. Pharmacol. 242, 151–163.

Weiss, F., Lorang, M.T., Bloom, F.E., Koob, G.F., 1993. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. J. Pharmacol. Exp. Ther. 267, 250–258.

Wise, R.A., Bozarth, M.A., 1987. A psychomotor stimulant theory of addiction. Psychol. Rev. 94, 469–492.

Wise, R.A., Rompre, P.-P., 1989. Brian dopamine and reward. Annu. Rev. Psychol. 40, 191.