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Local infusion of low, but not high, doses of alcohol into the anterior ventral tegmental area causes release of accumbal dopamine

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ABSTRACT

The mesolimbic dopamine system consisting of dopaminergic neurons projecting from the ventral tegmental area (VTA) to the nucleus accumbens (N.Acc.) mediates the reinforcing effects of addictive drugs including alcohol. Given that VTA is a heterogeneous area and that alcohol, in rather low doses, interacts directly with ligand-gated ion channels, we hypothesised that low, rather than high, doses of alcohol into the VTA activate the mesolimbic dopamine system and that alcohol may have different effects in the anterior and posterior parts of the VTA. The present study was undertaken to investigate this hypothesis. The present series of experiment show that infusion of a low dose of alcohol (20 mM) into the anterior, but not posterior, part of the VTA increases accumbal dopamine release in rats. In addition, higher doses of alcohol (100 or 300 mM) into the anterior or posterior part of the VTA do not affect the release of dopamine in the N.Acc., suggesting that low doses of alcohol can activate the mesolimbic dopamine system via mechanisms in the VTA. These data contribute to understanding the neuronal mechanisms underlying the dependence-producing properties of alcohol and could tentatively contribute to that new treatment strategies for alcohol use disorder can be developed.

KEYWORDS

Alcohol; Ventral Tegmental Area; Nucleus Accumbens; Dopamine; Rats

1. INTRODUCTION

The mesolimbic dopamine system, a common neurochemical dominator of the reward system, consists of dopaminergic neurons projecting from the ventral tegmental area (VTA) to the nucleus accumbens (N.Acc.) [1-4]. The first evidence demonstrating a causal relationship between alcohol-induced stimulation of behaviour and dopamine activity was that a catecholamine-synthesis inhibitor antagonizes the alcohol-induced euphoria in humans [5] and locomotor stimulation in rats [6]. Since then, microdialysis studies have shown that addictive drugs, including alcohol, as well as natural rewards, such as food, activate this reward link causing a release of accumbal dopamine *in vivo* in rats (see e.g. [7-12]). Additionally, voluntary alcohol consumption increases the extracellular concentrations of dopamine in rats [13-15] and in humans [16]. The accumbal dopamine release mediates the reinforcing effects of addictive drugs and palatable foods (for review see [17-19]), and a dysfunction in this system may cause addictive behaviours such as alcohol dependence (for review see [20]). Moreover, a role for accumbal dopamine in incentive salience, a motivated behaviour for reward has been implied (for review see [21]).

The neuronal mechanisms underlying the dependence-producing properties of drugs of abuse, such as increased accumbal dopamine release, are not clearly understood and need to be further elucidated. To date, both VTA and N.Acc. have been shown to have important roles for the ability of alcohol to activate the mesolimbic dopamine system. Thus, infusing alcohol into the N.Acc. causes a release of dopamine in the N.Acc. [22-26] and intravenous administration of low doses of alcohol produces a increase of dose dependent in firing rate of dopamine

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neurons in the VTA projecting to N.Acc. [27]. Given that VTA is a heterogeneous area and that alcohol, in rather low doses, interacts directly with ligand-gated ion channels (for review see [28,29]), it was hypothesised that low, rather than high, doses of alcohol into the VTA indirectly activate the mesolimbic dopamine system and that alcohol may have different effects in the anterior and posterior parts of the VTA. The present series of experiments were undertaken to directly test this hypothesis by investigating the effect of alcohol, low or high doses, infused directly into the anterior or posterior part of the VTA on accumbal dopamine release in freely moving rat by using *in vivo* microdialysis.

2. MATERIALS AND METHODS

2.1. Animals

Adult post-pubertal age-matched male Wistar rats (250 - 300 g body weight; Charles River, Sulzfeld, Germany) were used as asimilar study is documented in this strain (Larsson *et al.*, 2005). All rats were maintained at 20˚C with 50% humidity and a 12/12 hour light/dark cycle (lights on at seven a.m.) and were allowed to habituate to the animal facilities for at least one week before initiation of the experiment. Tap water and food (Normal chow; Harlan Teklad, Norfolk, England) were supplied *ad libitum*, except during the microdialysis experiment. The Ethics Committee for Animal Experiments in Gothenburg, Sweden has approved the experiments.

2.2. Drugs

Alcohol (VWR International, Stockholm, Sweden) was dissolved in Ringer solution (140 mM NaCl, 1.2 mM $CaCl_2$, 3.0 mM KCl and 1.0 $MgCl_2$ (Merck KGaA, Darmstadt, Germany)) to the correct concentrations (20, 100, 300 mM) and was administered locally into the VTA via reversed microdialysis. All drug challenges were part of a balanced design with regard to both the treatment order and the number of subjects per treatment. Each rat was only included in one microdialysis experiment.

2.3. *In Vivo* Microdialysis

For measurements of extracellular levels of dopamine in the N.Acc. (that reflects the release of the neurotransmitter) rats were implanted unilaterally with microdialysis probes positioned in the N.Acc.. For local infusion of alcohol or vehicle rats were also implanted with a microdialysis probe ipsilaterlly into the VTA. Only rats with correct probe positions in the VTA as well as N.Acc. were included in the statistical analysis. The surgery was performed as described thoroughly elsewhere [14]. In brief, the rats were anesthetized with isofluran (Isofluran Baxter; Univentor 400 Anaesthesia Unit, Univentor Ltd., Zejtun, Malta), placed in a stereotaxic frame (David Kopf Instruments; Tujunga, CA, USA) and kept on a heating pad to prevent hypothermia. The skull bone was exposed and two holes for the probes and one for the anchoring screw were drilled. The probes were randomly alternated to either the left or right side and were always positioned ipsilateral. The following coordinates were used for N.Acc.: 1.85 mm anterior to the bregma, ±1.0 mm lateral to the midline and 7.8 mm below the surface of the brain surface, and for VTA: 5.00 mm (anterior part of VTA) or 6.0 mm (posterior part of the VTA) posterior to the bregma, ±0.6 mm lateral to the midline and 8.5 mm below the surface of the brain surface [30]. The choice of coordinates for anterior and posterior VTA was based on previous studies with local administrations of alcohol into the VTA [22,31] in rats. The probes were attached to the skull with dental cement (Agntho's AB, Lidingö, Sweden). The exposed tip of the dialysis membrane (20,000 kDa cut off with an o.d./i.d. of 310/220 µm, HOSPAL, Gambro, Lund, Sweden) of the probe was 2 mm for N.Acc. and 1.5 mm for VTA. All probes were surgically implanted two days prior to the experiment. After surgery the rats were kept in individual cages (Macrolon III). All microdialysis experiments were conducted during the light phase.

The microdialysis technique enables measurements of neurotransmitters in awake, freely moving animals. On the day of the experiment the probe was connected to a microperfusion pump (U-864 Syringe Pump; AgnThós AB) and perfused with Ringer solution at a rate of 1.6 µl/minute. After one hour of habituation to the microdialysis set-up, perfusion samples were collected every 20 minutes.

In the first series of experiments the effect of two higher doses of alcohol (100 mM or 300 mM) compared to vehicle (Ringer's solution) on accumbal dopamine in rats were investigated. The baseline dopamine level was defined as the average of three consecutive samples before the drug challenge, and the increase in dopamine was calculated as the percent increase from baseline. After the baseline samples, rats were locally perfused with either alcohol (100 mM or 300 mM) or vehicle (Ringer's solution) locally into the anterior (−5.0 mm) or posterior (−6.0 mm) part of the VTA and the six subsequent samples were collected and analyzed. Alcohol or vehicle was perfused until termination of the experiment at a rate of 1.6 µl/minute.

In subsequent experiments, in different rats, the effects of a low dose of alcohol (20 mM) compared to vehicle (Ringer's solution) on accumbal dopamine in rats were investigated. The baseline dopamine level was defined as the average of three consecutive samples before the drug challenge, and the increase in dopamine was calculated

as the percent increase from baseline. After the baseline samples, rats were locally perfused with either alcohol (20 mM) or vehicle (Ringer's solution) locally into the anterior (−5.0 mm) or posterior (−6.0 mm) part of the VTA. Alcohol or vehicle was administered until termination of the experiment. Six subsequent samples were collected and analyzed. Alcohol or vehicle was perfused until termination of the experiment at a rate of 1.6 µl/minute.

In both these series of experiments Ringer's solution was switched to alcohol solution. This was done by changing the syringe and inlet tube into the swivel, which allows a rapid change of solution into the VTA.

2.4. Verification of Probe and Cannula Placement

After the microdialysis experiments were completed, the location of the probes was verified. The rats were decapitated, probes were perfused with pontamine sky blue 6BX to facilitate probe localization, and the brains were mounted on a vibroslice device (752M Vibroslice; Campden Instruments Ltd., Loughborough, UK). The brains were cut in 50 µm sections and the location of the probe was determined by gross observation using light microscopy. The exact position of the probes was verified [30].

2.5. Dopamine Analysis

The dopamine levels in the dialysates were determined by means of HPLC with electrochemical detection (HPLC-EC). The analysis was performed as described thoroughly elsewhere [14,32]. In brief, a pump (Gyncotec P580A; Kovalent AB), an ion exchange column (2.0 × 100 mm, Prodigy 3 µm SA; Skandinaviska GeneTec AB; Kungsbacka, Sweden) and a detector (Antec Decade; Antec Leyden, Zoeterwoude, The Netherlands) equipped with a VT-03 flow cell (Antec Leyden) was used. The mobile phase (pH 5.6), consisting of sulfonic acid 10 mM, citric acid 200 mM, sodium citrate 200 mM, 10% EDTA, 30% MeOH, was vacuum filtered by using a 0.2 µm membrane filter (GH Polypro; PALL Gelman Laboratory, Lund, Sweden). The mobile phase was delivered at a flow rate of 0.2 ml/min passing a degasser (Kovalent AB), and the analyte was oxidized at +0.4 V.

2.6. Statistical Analyses

All microdialysis experiments were evaluated by a two-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test for comparisons between different treatments and specifically at given time points. Data are presented as mean ± SEM. A probability value of $P < 0.0167$ was considered as statistically significant.

3. RESULTS

Effects of Local Administration of Alcohol into the VTA on Accumbal Dopamine Release in Rats

Here we initially showed that local infusion of alcohol (100 mM or 300 mM), either into the anterior (−5.0 mm) or posterior (−6.00 mm) part of the VTA, did not affect the extracellular levels of dopamine in N.Acc. in rats (data not shown) (treatment F(5, 35) = 1.43, $P = 0.2375$; time F(8280) = 2.76, $P = 0.0060$; treatment × time interaction F(8280) = 1.68, $P = 0.0092$). No statistically difference was observed between alcohol or vehicle (Ringer) perfusion into the anterior or posterior part of the VTA.

Subsequently we showed that a low dose of alcohol (20 mM) into the anterior (−5.0 mm), but not posterior (−6.0 mm) part of the VTA, increased the levels of dopamine in N.Acc. in rats (Figure 1).

Alcohol (20 mM) to the anterior, but not posterior, part of the VTA increased accumbal dopamine release relative to vehicle treatment ($P = 0.0002$ and $P = 0.2013$ respectively) (treatment F(3, 37) = 7.577, $P = 0.0005$; time F(8296) = 2.804, $P = 0.0052$; treatment × time interaction F(8296) = 3.511, $P < 0.0001$; n = 8 for vehicle −5.0 and vehicle −6.0, n = 11 for alcohol −5.0 and n = 14 for alcohol −6.0). This increase was evident at time interval 40 minutes ($P = 0.0034$), 60 minutes ($P < 0.0001$) and 80 minutes ($P = 0.0049$) (for alcohol-5.0 versus vehicle-5.0). No statistical significant difference was observed between vehicle (Ringer) perfusion into the anterior or posterior part of the VTA ($P = 0.7558$).

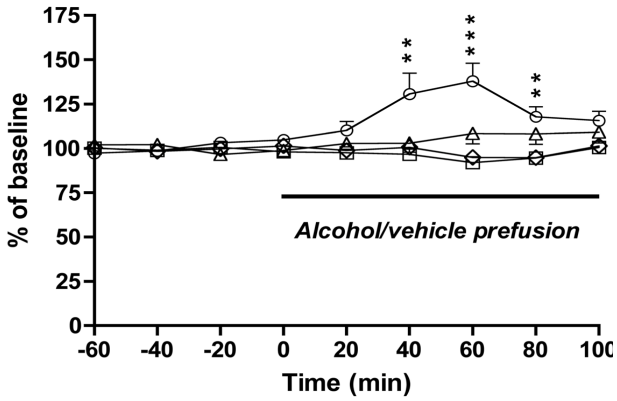

Figure 1. Effects of local administration of alcohol into the ventral tegmental area on accumbal dopamine release in rats. A low dose of alcohol (20 mM) into the anterior (−5.0 mm), but not posterior (−6.0 mm) part of the VTA, increased the levels of dopamine in N.Acc. in rats. No significant difference was observed between the vehicle treatment into the anterior (−5.0 mm) posterior part of VTA (−6.0 mm) (n = 8 for vehicle −5.0 mm (square), n = 8 for vehicle -6.0 mm (rhomb), n = 11 for alcohol −5.0 mm (circle) and n = 14 for alcohol −6.0 mm (triangle). All values represent mean ± SEM ($^{***}P < 0.001$ and $^{**}P < 0.01$).

It should be emphasized that only rats with correct probe positions in the VTA, *i.e.* alcohol/vehicle administration, as well as N.Acc., *i.e.* dopamine measurements, were included in the statistical analysis.

4. DISCUSSION

The present series of experiments show that local perfusion of a low dose of alcohol into the anterior, but not posterior, part of the VTA increases accumbal dopamine releasein rats. Moreover, high doses of alcohol into either part of the VTA, does not affect the extracellular concentrations of dopamine in N.Acc.; suggesting that low, and not high, doses of alcohol activate the mesolimbic dopamine system via mechanisms in the anterior part of the VTA.

VTA is a heterogeneous brain structure and the anterior versus the posterior part of the VTA differ in dopaminergic cell morphology, topography of their efferent projections, and presumably in function (for review see [33]). Supportively, caudoventral dopamine neurons are more active during the rat's active dark period, whereas rostrodorsal DA neurons are active during the light period [34]. Given that we here showed that low dose of alcohol in the anterior, but not posterior, part of the VTA increases accumbal dopamine release and that rats self-administer nicotine in the anterior part of the VTA [35], an important role for anterior VTA in drug reward may be suggested. However, dopamine neurons in the posterior part of the VTA may also be involved in the reinforcing properties of addictive drugs. Thus rats voluntarily self-administer alcohol, as well ascholinergic agonists, muscimol or acetaldehyde, into the posterior, but not anterior, part of the VTA [36-41]. Moreover, co-administration of alcohol with a dopamine D_2 receptor agonist into the posterior, but not anterior, VTA extinguished the maintenance of alcohol self-infusion [40].

The present data confirm previous results showing that high doses of alcohol, neither into the anterior nor posterior part of the VTA, affect the extracellular levels of dopamine in the N.Acc. in Wistar [22] and in alcohol preferring AA [31] rats. Alcohol, in rather low doses, activates ligand-gated ion channels including nicotinic acetylcholine, and $GABA_A$ receptors (for review see [28,29]). Previously, alcohol-induced reward, as measured by alcohol-induced locomotor stimulation, accumbal dopamine release, and alcohol intake were shown to be mediated via nicotinic acetylcholine receptors, especially those located in the VTA [10,12,13,23,42-47]. Additionally, anterior ventral tegmental acetylcholine receptors mediate the ability of accumbal alcohol to increase dopamine in the N.Acc. [22]. GABA on the other hand, mediates the sedative, anxiolytic and muscle relaxant effects of alcohol [48] and local administration of a $GABA_A$ antagonist into the anterior part of VTA increases accumbal dopamine [49]. It may therefore be suggested that low doses of alcohol into the anterior part of the VTA activate nicotinic acetylcholine receptors, which causes an activation of the mesolimbic dopamine system, *i.e.* accumbal dopamine release. Higher doses of alcohol into the VTA may on the other hand activate $GABA_A$ receptors and therefore not cause a release of dopamine in N.Acc.. However this needs to be further elucidated.

Our present findings are in consensus with previous suggestions on alcohol interfering with VTA-dopamine neurons. Thus, a role for the VTA was initially suggested since intravenous administration of low doses of alcohol produces a dose dependent increase in firing rate of dopamine neurons in the VTA projecting to N.Acc. *in vivo* [27] and *in vitro* [50-54]. Furthermore, alcohol increases the firing of acutely dissociated VTA dopamine neurons [53] and that this effect persists albeit blockade of synaptic transmission by high magnesium/low calcium [50], suggesting that alcohol activates dopamine neurons directly in absence of input from surrounding neurons. However, additional sites of actions are most likely since alcohol is a small hydrophilic/lipophilic molecule and following alcohol consumption alcohol may in all probability effects several brain regions. Indeed, reverse microdialysis of alcohol into the N.Acc. has previously been shown to increases accumbal dopamine [22-26] involving nicotinic acetylcholine receptors in the anterior part of the VTA [22]. Growing evidence has collectively implied that the cholinergic-dopaminergic reward link, encompassing the cholinergic afferent projection from the laterodorsal tegmental area (LDTg) onto the VTA dopamine cells together with the mesolimbic dopamine neurons from the VTA to N.Acc., mediates the reinforcing properties of natural as well as artificial rewards [3,55] (for review see [28,29]). Further support for an important role for nicotinic receptor in the VTA is given by the findings that rats self-administer nicotine in the anterior part of the VTA [35]. The data showing that alcohol intake in high alcohol-preferring rats causes a concomitant increase in ventral tegmental acetylcholine and accumbal dopamine [14], further strengthen an important role of the cholinergic-dopaminergic reward link and indirectly imply that alcohol also may have local effects in the LDTg. Although, other sites of action for alcohol should also be taken into consideration.

5. CONCLUSION

In summary, the present study shows that low, but not high, dose of alcohol activates the mesolimbic dopamine system via the anterior, but not posterior, part of the VTA. These data contribute to understanding the neuronal mechanisms underlying the dependence-producing properties of drugs of abuse, such as increased accumbal do-

pamine release. This additional knowledge in how alcohol activates the mesolimbic dopamine system could tentatively contribute to that new treatment strategies for alcohol use disorder can be developed.

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