Increase in Nucleus Accumbens Dopamine Levels Following Local Ethanol Administration Is Not Mediated by Acetaldehyde

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Abstract — Aim: Ethanol (EtOH) activates the mesolimbic dopamine system and increases dopamine levels in the nucleus accumbens (nAc), which is believed to underlie the rewarding effects of alcohol. Accumulating evidence now implicates that acetaldehyde, the first metabolite of EtOH, may play an important role in mediating some of the rewarding properties of its parent compound. The objective of this study was to investigate if the increase in accumbal dopamine output observed when administering EtOH locally in the nAc by reversed microdialysis is mediated by acetaldehyde. Method: Acetaldehyde (1, 10, 100 or 200 μM) or EtOH (300 mM) was administered via reversed microdialysis in the nAc of male Wistar rats. In a separate experiment, animals were administered EtOH (300 mM) in the nAc, following pre-treatment with the acetaldehyde-sequestering agent N-penicillamine (50 mg/kg injected intraperitoneally 60 min before drug challenge). Microdialysates from the nAc were collected every 20 min and dopamine content was quantified using high-performance liquid chromatography. Results: Acetaldehyde administered in the nAc did not influence accumbal dopamine levels at any of the concentrations applied, whereas EtOH induced a significant increase in accumbal dopamine. The dopamine-elevating properties of EtOH were not attenuated by pre-treatment with N-penicillamine. Conclusion: The current results show that EtOH administered in the nAc induces an elevation in accumbal dopamine levels, which is not mimicked by acetaldehyde alone, nor is it influenced by acetaldehyde sequestering. This would suggest that the increase in accumbal dopamine following nAc EtOH administration is not mediated by acetaldehyde.

INTRODUCTION

Many drugs of abuse, including ethanol (EtOH), activate the mesolimbic dopamine system, with an increase in nucleus accumbens (nAc) extracellular dopamine levels as a result (see Spanagel, 2009 and Soderpalm and Ericson, 2013 for review). However, the exact mechanisms by which EtOH produces its dopamine-enhancing effects in the nAc remain to be elucidated. Although traditionally regarded as mediating aversive effects of alcohol in the periphery, accumulating evidence now suggests that acetaldehyde, the first metabolite of EtOH, may play an important role centrally in the rewarding, motivational and addictive effects of alcohol (for extensive reviews, see Quertemont et al., 2005 and Correa et al., 2012).

It appears that acetaldehyde in itself induces several behavioural effects commonly attributed to its parent compound, such as conditioned place preference (Smith et al., 1984; Quertemont and De Witte, 2001; Peana et al., 2008), as well as systemic (Myers et al., 1982; Peana et al., 2010) and central self-administration (Amit et al., 1977; Rodd-Henricks et al., 2002). In regards to effects on the mesolimbic dopamine system, acetaldehyde has been reported to stimulate dopaminergic neurons in the ventral tegmental area (VTA) in vitro (Melis et al., 2007), as well as in vivo (Foddaei et al., 2004; Enrico et al., 2009). More importantly, administration of acetaldehyde has been reported to increase accumbal dopamine output, regardless of whether it is administered intragastrically (Melis et al., 2007; Enrico et al., 2009), or directly into the VTA via microdialysis (Melis et al., 2007) or via microinjections (Deehan et al., 2013). However, it should be noted that one study has reported a decrease in accumbal dopamine levels following intraperitoneal (i.p.) injections of acetaldehyde (Ward et al., 1997).

The question of whether brain acetaldehyde levels produced by physiologically relevant concentrations of EtOH are sufficient to produce any pharmacological or behavioural effects relevant to reward and addiction has been controversial (see Deitrich, 2004 for review); however, studies minimizing the amount of acetaldehyde formed following EtOH administration would suggest that acetaldehyde plays an important role in several of the behavioural effects of its parent compound. Pre-treatment with inhibitors of catalase, the major enzyme responsible for converting EtOH into acetaldehyde in the brain (Zimatkin et al., 2006), attenuates EtOH-induced locomotion (Aragon and Amit, 1993; Sanchis-Segura et al., 1999), as does pre-treatment with N-penicillamine, an acetaldehyde-sequestering agent, regardless of whether EtOH is administered systemically (Font et al., 2005), or directly into the VTA (Martí-Prats et al., 2010). More recently, studies have shown that VTA microinjections with a lentiviral vector encoding anticaatalase shRNA abolish voluntary EtOH consumption in drug-naïve EtOH high-prefering rats (Karahanian et al., 2011; Quintanilla et al., 2012). Regarding dopamine transmission, it has been shown that the stimulatory effects of EtOH on VTA dopaminergic neurons have been abolished in vitro by inhibition of catalase (Melis et al., 2007), as well as in vivo by use of N-penicillamine (Enrico et al., 2009). In addition, the dopamine-enhancing effects of systemic EtOH in the nAc can be prevented by inhibition of peripheral EtOH metabolism with 4-methyl-pyrazole (Melis et al., 2007), by sequestering of centrally formed acetaldehyde (Enrico et al., 2009; Sirca et al., 2011), as well as by microinjections in the VTA with lentiviral vectors encoding anticaatalase shRNA in alcohol-prefering rats (Karahanian et al., 2011).

The findings above indicate an importance of EtOH-derived acetaldehyde in the VTA in EtOH reinforcement; however, research also indicates that the nAc, in addition to being a terminal site of mesolimbic dopamine projection, may play an important role in EtOH reward. EtOH is self-administered in the nAc shell of both Wistar and alcohol-prefering P-rats.
(Engleman et al., 2009) and research performed by our group has repeatedly shown that regardless of whether EtOH is administered systemically, is orally self-administered or perfused locally in the nAc via reversed microdialysis, an increase in extracellular accumbal dopamine is obtained, which can be abolished by local pre-treatment in the nAc with the competitive glycine receptor antagonist strychnine (Molander et al., 2005; Molander and Soderpalm, 2005; Adermark et al., 2011; Jonsson et al., 2014). We hypothesize that glycine receptors in the nAc influence the tone of GABAergic nAc-VTA projecting neurons (Walaas and Fonnum, 1980; Watabe-Uchida et al., 2012), thereby controlling the activity of dopaminergic neurons, and thus creating a nAc-VTA-nAc neuronal circuitry affecting dopamine release in the nAc (see Soderpalm et al., 2009 for review). Supporting the importance of glycine receptors, we recently showed that a concomitant increase in accumbal levels of the glycine-receptor ligand taurine is required for EtOH-induced dopamine elevation (Ericson et al., 2011). Since alpha-1-glycine receptor currents in Xenopus laevis oocytes have been shown to be enhanced by acetaldehyde (Mascia et al., 2001), there is a possibility that acetaldehyde might be involved in modulating dopamine output by interacting with glycine receptors in the nAc. It is therefore possible that the dopamine elevation observed in the nAc after local EtOH administration is in fact mediated by acetaldehyde. However, the effects of acetaldehyde on accumbal dopamine transmission when administered locally in the nAc have, to our knowledge, not been investigated.

The major aim of this study was to investigate if the increase in dopamine levels induced by EtOH administration locally in the nAc could in fact be attributed to acetaldehyde. To this end, we measured accumbal dopamine levels following direct administration in the nAc of acetaldehyde alone (1–200 µM), or of EtOH in combination with pre-treatment with the acetaldehyde-sequestering agent D-penicillamine. D-penicillamine is an inert thiol amino acid that forms a stable adduct with acetaldehyde (Nagasawa et al., 1978; Serrano et al., 2007), thereby inactivating this compound without interfering with EtOH metabolism. Studies were performed using in vivo microdialysis in drug-naïve male Wistar rats.

MATERIALS AND METHODS

All experiments were conducted according to the Declaration of Helsinki and were performed in accordance with protocols approved by the Ethics Committee for Animal Experiments, Gothenburg, Sweden (381/11).

Animals

Male Wistar rats weighing 250–400 g were used in all experiments. Animals were obtained from Taconic (Denmark) and were housed four to a cage under controlled environmental conditions (constant room temperature of 22°C, humidity of 65% and regular light-dark conditions with lights on at 07:00 a.m. and off at 07:00 p.m.). Animals had free access to tap water and standard rat feed (Lantmännen, Sweden) and were allowed to adapt to the novel environment for at least 1 week before any procedures were initiated. Only drug-naïve animals were used and all experiments were performed during the light-phase of the cycle.

Surgery

Animals were anesthetized with isoﬂurane (Baxter, Sweden) and mounted onto a stereotactic instrument (David Kopf Instruments). A heating pad was used to prevent hypothermia during surgery. I-shaped microdialysis probes were custom made in the laboratory. The dialysis membrane was composed of an acrylonitrile and sodium methallyl sulfonate co-polymer, had an inner diameter of 0.2 mm, an outer diameter of 0.3 mm and a molecular cut-off of 20 kDa. The active space of the probes was 2 mm. Probes were lowered monolaterally into the nAc core–shell borderline region (AP +1.85, LM −1.4, DV −7.8, coordinates from Paxinos and Watson, 2007, and relative to the bregma and dura). Probes, as well as two anchoring screws, were fixed to the skull using Harvard cement (Dental AB, Gothenburg, Sweden). Animals were injected s.c. with 2–4 ml of 0.9% NaCl to prevent dehydration and placed in individual cages where they were allowed to recover for 48 h before experiments were initiated.

Drugs and solutions

All drugs were dissolved in Ringer’s solution, with the exception of D-penicillamine (50 mg/kg, Sigma-Aldrich), which was dissolved in physiological NaCl. The Ringer’s solution, consisting of (in mM) 140 NaCl, 1.2 CaCl2, 3.0 KCl and 1.0 MgCl2, was custom made in the laboratory. The pH of acetaldehyde solutions with the highest concentrations (100 and 200 µM) was 0.2 units lower than that of Ringer. The pH of 1 and 10 µM acetaldehyde solutions did not differ from Ringer.

All solutions were freshly prepared on the day of the experiment, and acetaldehyde solutions were prepared and kept under refrigerated conditions to minimize evaporation.

Microdialysis

Animals were perfused at a rate of 2 µl/min, using a microperfusion pump (Univentor-864 Syringe Pump; Agn Tho’s AB, Lidingö, Sweden), connected via a swivel, allowing them to move around freely. Perfusion with Ringer’s solution was initiated 60 min before baseline sampling to allow fluid equilibrium to be established. Samples (40 µl) were collected every 20 min. After a stable baseline had been confirmed, drugs were administered locally in the nAc and dialysates from this region were collected for further analysis. Animals received either acetaldehyde (1, 10, 100 or 200 µM; Sigma-Aldrich, Stockholm, Sweden), EtOH (300 mM; Kemetyl AB, Stockholm, Sweden), the combination of acetaldehyde and EtOH (100 µM and 300 mM, respectively), taurine (50 µM; Sigma-Aldrich) or the combination of taurine and acetaldehyde (50 µM and 100 µM, respectively). One subset of EtOH-treated rats was pre-treated with an i.p. bolus dose of D-penicillamine (50 mg/kg) 60 min prior to EtOH infusion. Animals were sacrificed directly after the experiment and brains were placed in Accustain (Sigma-Aldrich) for 3–7 days before probe placements were verified using a vibroslicer (Campden Instruments Ltd, Leicester, UK; Fig. 1). Only animals with correctly placed probes with no visual defects (e.g. bleeding) were included in the statistical analysis.

Biochemical assays

Microdialysate dopamine content was separated and quantified using high-performance liquid chromatography with
electrochemical detection. Two systems were used simultaneously. The first system utilized a stainless steel ion exchange column of 2 × 150 mm, operated at 32°C and packed with Nucleosil SA (5 µm diameter; pore size 100 Å; Phenomenex Scandinavia, Västra Frölunda, Sweden), with a mobile phase (flow rate 0.3 ml/min) consisting of (in mM) 58 citric acid, 135 NaOH, 0.107 Na₂-EDTA, as well as 20% methanol. The electrochemical detector of this system (Decade, Kovalent AB, Sweden) operated at 400 mV versus the cell (Hy-REF). The second system used a stainless steel reversed phase column of 2 × 50 mm, operated at 30°C and packed with silica (3 µm diameter; pore size 100 Å; Phenomenex Scandinavia, Västra Frölunda, Sweden). The mobile phase for this system (flow rate 0.3 ml/min) consisted of 150 mM NaH₂PO₄, 4.76 mM citric acid, 3 mM sodium dodecyl sulphate, 50 µM EDTA, as well as 10% MeOH and 15% acetonitrile. The electrochemical detector (Dionex, Västra Frölunda, Sweden) operated at 220 mV versus the cell. The limit of detection was calculated to 2.69 pA for the first system and 3.49 pA for the second system. An external standard containing 3.25 fmol/µl of dopamine was used to identify the dopamine peak, as well as to quantify dopamine concentrations in the dialysates. All samples were analyzed on-line. To obtain a stable dopamine baseline (±10%), at least five pre-drug samples were analyzed. The average of the last two stable baseline values was set to 100% for each animal.

**Statistical analysis**

Data are presented as mean values compared with baseline level ± SEM. A two-way ANOVA with repeated measures was used for statistical analysis, followed by a Bonferroni post hoc test. A *P*-value of <0.05 was considered significant. A total of 119 animals were used in this study. Nineteen animals were excluded due to incorrect probe placements, bleeding around the probe or unstable baseline dopamine values.

**RESULTS**

Basal dopamine concentrations did not differ significantly between the experimental groups, and data were therefore pooled. Basal dopamine concentration (mean ± SEM) was 66.4 ± 4.1 fmol/20 µl sample. In the first set of experiments, animals were perfused locally in the nAc with different concentrations of acetaldehyde (1, 10, 100 or 200 µM), or with EtOH alone (300 mM), via reversed microdialysis. The dialysates from the nAc were collected and analyzed for dopamine
content. Statistical analysis using two-way ANOVA with repeated measures (treatment group × time), followed by a Bonferroni post hoc test, revealed no significant elevations in accumbal dopamine in animals treated with acetaldehyde in any of the concentrations applied, as compared with Ringer controls [group effect $F_{(4,44)} = 0.242, P = 0.913$, time effect $F_{(10,440)} = 1.515, P = 0.131$, interaction term $F_{(40,440)} = 0.939, P = 0.581$] (Fig. 2A and B). Animals treated with EtOH showed significant increases in nAc dopamine, as compared with Ringer controls [group effect $F_{(1,14)} = 6.78, P = 0.021$, time effect $F_{(10,140)} = 4.10, P < 0.0001$, interaction term $F_{(10,140)} = 4.53, P < 0.0001$] (Fig. 2C).

In order to exclude the possibility that higher levels of acetaldehyde might influence the extracellular environment in such a way that it would disrupt a possible increase in dopamine, an additional group of rats was perfused with a combination of EtOH and acetaldehyde (300 mM and 100 µM, respectively). These animals showed a significant increase in nAc dopamine output 40 min following drug treatment, when compared with both Ringer controls ($P < 0.01$) and rats perfused with acetaldehyde (100 µM; $P < 0.001$) [group effect $F_{(2,23)} = 2.895, P = 0.0756$, time effect $F_{(10,230)} = 2.350, P = 0.0117$, interaction term $F_{(20,230)} = 2.300, P = 0.0017$] (Fig. 3A).

Since a previous study found that EtOH-induced dopamine elevation requires a concomitant release of taurine (Ericson et al., 2011), we wanted to explore this possibility also with respect to acetaldehyde. However, a co-perfusion of 50 µM taurine and acetaldehyde (100 µM) did not influence dopamine output in the nAc [group effect $F_{(2,26)} = 0.2336, P = 0.7933$, time effect $F_{(10,260)} = 2.042, P = 0.0297$, interaction term $F_{(20,260)} = 2.030, P = 0.0067$; Fig. 3B].

In the last set of experiments, animals were pre-treated with a bolus dose of D-penicillamine (50 mg/kg, i.p.) 60 min before nAc perfusion with EtOH (300 mM) or Ringer. Animals receiving EtOH showed significant increases in accumbal dopamine as compared with Ringer controls [group effect $F_{(1,13)} = 4.747, P = 0.0483$, time effect $F_{(10,130)} = 5.608, P < 0.0001$, interaction term $F_{(10,130)} = 4.340, P < 0.0001$] (Fig. 4A). A two-tailed

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**Fig. 3.** Co-perfusion of acetaldehyde and ethanol did not influence ethanol-induced increases in dopamine. Acetaldehyde (100 µM) and ethanol (300mM) perfused at the same time in the nAc via reversed microdialysis significantly increased accumbal dopamine levels both when compared with Ringer controls and with acetaldehyde perfusion alone (A). Neither taurine alone (50 µM) nor taurine in combination with acetaldehyde (100 µM) induced a significant increase in dopamine output (B). Horizontal bars represent duration of drug treatment. Data are presented as means ± SEM relative to baseline levels. Statistical analysis; repeated measures two-way ANOVA followed by the Bonferroni post-test, significance levels: ***$P < 0.001$ and *$P < 0.05$.**

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**Fig. 4.** D-Penicillamine does not prevent increases in nAc dopamine induced by local ethanol perfusion. Animals pre-treated with acetaldehyde-sequestering agent D-penicillamine (DP; 50 mg/kg, i.p) 60 min prior to local ethanol perfusion in the nAc (300 mM) showed a significant increase in dopamine levels following ethanol treatment, as compared with D-penicillamine/ringer controls. Horizontal bars represent duration of drug treatment. Data are presented as means ± SEM relative to baseline levels. Statistical analysis; repeated measures two-way ANOVA followed by the Bonferroni post-test, significance levels: **$P < 0.01$ and *$P < 0.05$ (A). A two-tailed paired t-test showed a significant increase in accumbal dopamine in ethanol-treated animals following 40 min of ethanol perfusion, as compared with the time-point of ethanol administration (time = 100 min versus time = 60 min, $P = 0.0107$).
paired t-test showed a significant increase in dopamine 40 min after EtOH perfusion, as compared with the time-point of EtOH administration ($P = 0.0107$; Fig. 4B).

**DISCUSSION**

Previous research has shown that EtOH, whether administered systemically or locally in the nAc, or when orally self-ingested, induces an increase in accumbal dopamine levels that can be prevented by administration of glycine-receptor antagonist strychnine in the nAc, indicating an important role for accumbal glycine receptors in mediating the dopamine-elevating effects of alcohol (Molander et al., 2005; Molander and Soderpalm, 2005; Adermark et al., 2011). Because an accumulating amount of evidence now suggests that acetaldehyde might mediate at least some of the rewarding effects of EtOH, and because glycine receptors can be activated by acetaldehyde (1 and 10 µM; Mascia et al., 2001), the primary objective of this study was to investigate whether the increase in extracellular accumbal dopamine induced by local EtOH perfusion could be attributed to acetaldehyde. However, in the present laboratory setting, we were unable to detect any influence of acetaldehyde on nAc dopamine levels. Neither 1, 10, and 100 µM, nor 200 µM acetaldehyde administered locally in the nAc produced any significant elevation in accumbal dopamine levels. Because the dialysis probes in this experimental setup were placed in the nAc core–shell borderline region, suggesting sampling from both accumbal subregions, there is a possibility that different results might have been obtained if the probe had been placed exclusively in the accumbens shell (or core). However, the co-ordinates used in this experimental set-up were able to detect a significant increase in accumbal dopamine following local perfusion of EtOH (300 mM), suggesting that increases in nAc dopamine following local perfusion with acetaldehyde might be absent or less robust than those induced by EtOH perfusion in the nAc.

As the lack of effect of acetaldehyde on accumbal dopamine levels observed in our studies might be attributed to a number of additional factors, we went on to further investigate these results. One possible explanation for the lack of acetaldehyde effect could be that acetaldehyde, when administered in high concentrations, might disrupt the extracellular environment in such a way that increases in dopamine might be hindered. To further investigate this possibility, we co-administered EtOH and acetaldehyde (300 mM and 100 µM, respectively) in the nAc. This combination of drugs produced a dopamine pattern very similar to EtOH alone, indicating that the addition of acetaldehyde did not influence the extracellular environment in such a way that it would disrupt a possible increase in dopamine.

To further explore why we were unable to obtain a dopamine elevation using acetaldehyde, we added a small amount of glycine receptor agonist taurine (50 µM) to the acetaldehyde solution (100 µM). EtOH increases taurine levels in the nAc (Dahchour et al., 1994; Kashkin and De Witte, 2004; Adermark et al., 2011) and taurine itself has been shown to increase accumbal dopamine (Ericson et al., 2006). Thus, a possible mechanism of action of alcohol might involve elevating accumbal taurine, which in turn increases nAc dopamine levels through an interaction with accumbal glycine receptors, perhaps in concert with EtOH. Acetaldehyde may also increase taurine levels (Ward et al., 1997) and has been shown to interact with glycine receptors (Mascia et al., 2001); however, it is possible that the amount of taurine released when administering acetaldehyde alone, without EtOH, might be insufficient to reach the threshold level required for an increase in dopamine to occur. For this reason, taurine was added to the acetaldehyde perfusate in a concentration shown to be sufficient to restore an EtOH-induced dopamine increase during hyperosmotic conditions, when no taurine elevation is produced by EtOH (Ericson et al., 2011). However, the combination of acetaldehyde and taurine did not increase extracellular dopamine levels.

Another possible explanation for the observed lack of effect of acetaldehyde on accumbal dopamine levels could be attributed to the concentrations used in this study. In one previous microdialysis study, administration of 75 µM acetaldehyde in the pVTA induced elevations in nAc dopamine (Melis et al., 2007), and studies in alcohol-preferring rats (Rodd-Henricks et al., 2002) report that acetaldehyde is self-administered via microinjections in the pVTA at concentrations ranging from 6 to 90 µM. Concentrations of acetaldehyde within this same range have also been reported to increase accumbal dopamine when administered via microinjections in the pVTA, with 23–90 µM acetaldehyde inducing the greatest increases in nAc dopamine (Deehan et al., 2013). However, it has yet to be determined how concentrations of acetaldehyde may vary in different brain regions following physiologically relevant EtOH administration, and how these levels correspond to pharmacological importance. Generally, brain acetaldehyde concentrations following EtOH intoxication have been estimated to be in the (low) micromolar range (Deitrich, 2004; Correa et al., 2012). In this study, acetaldehyde concentrations of 1, 10, 100 and 200 µM were used. Although several interfering factors have not been taken into account, and bearing in mind also the difficulties involved when comparing results obtained using different modes of administration, we believe that the concentrations of acetaldehyde used in this experiment are within the range of what would be expected after a dose of EtOH that elevates dopamine levels in the nAc (Blomqvist et al., 1993; Ericson et al., 1998; Molander and Soderpalm, 2005; Lof et al., 2007; Adermark et al., 2011), as well as within the range of what rats self-administer in the VTA (Rodd-Henricks et al., 2002), that have been shown to increase accumbal dopamine when administered in the VTA (Melis et al., 2007; Deehan et al., 2013), and that could activate glycine receptors (Mascia et al., 2001).

However, microinjection studies in the VTA indicate an inverted ‘U-shaped’ dose–response curve for dopamine release following acetaldehyde administration (Deehan et al., 2013), and as acetaldehyde appears to be self-administered within a rather limited concentration span within the VTA, it is possible that the concentrations used in this experiment were insufficient. To this end, in a final experiment, we administered EtOH locally in the nAc via microdialysis following pre-treatment with D-penicillamine (50 mg/kg i.p., 60 min prior to EtOH perfusion), a metabolically inert thiol amino acid, capable of selectively forming adducts with acetaldehyde, thereby inactivating this compound without interfering with EtOH metabolism (Nagasawa et al., 1978, 1980; Serrano et al., 2007). Pre-treatment with D-penicillamine did not attenuate the dopamine-enhancing effects of local EtOH.
administration in the nAc. The dose regimen used in this experimental set-up (or others very similar to it) has proven effective in preventing EtOH-derived activation of VTA dopaminergic neurons and EtOH-induced increases in accumbal dopamine (Enrico et al., 2009), as well as in preventing EtOH-induced conditioned place preference (Peana et al., 2008), and behavioural stimulation induced by systemic (Font et al., 2005) or intra-VTA microinjections of EtOH (Marti-Prats et al., 2010). Yet this dose of D-penicillamine was clearly ineffective in preventing EtOH-induced increases in accumbal dopamine following local administration, further indicating that EtOH-derived acetaldehyde seems to be of little importance to the dopamine-enhancing properties of EtOH when it is administered in the nAc.

In the VTA, the effects of EtOH-derived acetaldehyde have been well documented. Previous research has demonstrated that EtOH-induced increases in VTA neuronal activity can be prevented in vitro by co-application of a pharmacological blocker of local catalase (Melis et al., 2007), or in vivo by systemic administration of D-penicillamine (Enrico et al., 2009). In addition, voluntary EtOH consumption, as well as post-deprivation binge drinking, is greatly reduced in animals treated with lentiviral vectors in the VTA encoding either anticatalase shRNA or aldehyde dehydrogenase-2 (ALDH-2), leading to a decrease in VTA acetaldehyde by either minimizing catalase activity or by increasing acetaldehyde clearance, respectively (Karahanian et al., 2011, 2014; Quintanilla et al., 2012). However, animals that had consumed alcohol chronically for 2–3 months did not reduce their EtOH intake following VTA lentiviral injections encoding anticatalase shRNA (Quintanilla et al., 2012) or ALDH-2 (Karahanian et al., 2014), indicating that factors other than VTA acetaldehyde concentrations may be involved in the perpetuation of alcohol self-administration. As the nAc also appears to play an important role as a point of origin for EtOH activation of the mesolimbic dopamine system (Soderpalm et al., 2009), there is a possibility that the dual importance of the VTA and the nAc may in fact reflect two separate pathways by which EtOH and/or acetaldehyde increase accumbal DA, that are not necessarily mutually exclusive and that may involve separate mechanisms of action.

There appears to be a great variation in local catalase activity in different areas of the brain (Brannan et al., 1981; Moreno et al., 1995), implicating that local acetaldehyde concentrations following EtOH administration may vary greatly in different brain areas. High catalase activity has been found in aminergic neurons, and it has been suggested that this, coupled with a relatively low ALDH activity, might result in local accumulation of acetaldehyde in brain areas rich in such neurons following EtOH intoxication (Zimatkin and Lindros, 1996), which might explain the relative importance of acetaldehyde in the VTA. The nAc, however, is comprised mainly of GABAergic medium spiny neurons (≥95%; Tepper et al., 2004), as well as a small proportion of GABAergic and cholinergic interneurons. In addition, catalase activity in this area has been found to be low/undetectable (Zimatkin and Lindros, 1996), and it is therefore possible that, upon EtOH administration, pharmacologically significant levels of acetaldehyde are normally not generated in the nAc and that the dopamine-elevating effects of EtOH, when applied locally in this region, are dependent on other factors, e.g. an interaction with accumal glycine receptors (see above), which might be mediated via a joint action of EtOH and taurine directly at the receptor (Ericson et al., 2011) and which is dependent on the presence of zinc (Morud et al., 2013).

In conclusion, this study shows that acetaldehyde perfused locally in the nAc via reversed microdialysis does not increase accumbal dopamine levels and that pre-treatment with acetaldehyde-sequestering agent D-penicillamine does not prevent the increase in nAc dopamine observed after local perfusion of EtOH. This would suggest that acetaldehyde is of little importance to the dopamine-enhancing properties of EtOH that are seen upon accumbal administration of this drug. This would suggest that acetaldehyde is of little importance to the dopamine-enhancing effect instigated by EtOH when present in the nAc, which is the case not only after local perfusion but most likely also after voluntary oral alcohol intake (Nurmi et al., 1999).

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