Ethanol induces stronger dopamine release in nucleus accumbens (shell) of alcohol-preferring (bibulous) than in alcohol-avoiding (abstainer) rats

Diego Bustamante[1], **Maria Elena Quintanilla**[1], **Lutske Tampier**[1], **Victor Gonzalez**[1], **Yedy Israel**[1,2,3], and **Mario Herrera-Marschitz**[1CA]

1*Programme of Molecular & Clinical Pharmacology, ICBM, Faculty of Medicine, University of Chile, PO Box 70.000 Santiago 7, Chile*

2*Department of Pharmacological & Toxicological Chemistry, Faculty of Chemical & Pharmaceutical Sciences, University of Chile. Santiago, Chile.*

3*Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University. Philadelphia PA, USA.*

Abstract

Several studies on the differences between ethanol-preferring versus non-preferring rat lines suggest an innate deficit in the mesolimbic dopaminergic system as an underlying factor for ethanol volition. Rats would try to overcome such deficit by engaging in a drug-seeking behaviour, when available, to drink an ethanol solution over water. Thus, in the present study we compared the effect of a single dose of ethanol (1g /kg, i.p.) on the extracellular levels of monoamines measured by microdialysis in the shell of nucleus accumbens of University of Chile bibulous (UChB) and University of Chile Abstainer (UChA) rats, bred for 79 and 88 generations to prefer or reject ethanol, respectively. It is reported that under basal conditions extracellular dopamine levels are lower in the bibulous than in the abstainer rats, while ethanol induced a 2-fold greater increase of dopamine release in bibulous than in abstainer rats. The greater effect of ethanol in bibulous rats was not associated to differences in blood ethanol levels, since the concentration and elimination of ethanol were virtually identical in both rat lines, indicating that bibulous rats are more sensitive to the stimulation of dopamine release by ethanol than abstainer rats. No differences were observed in 5-hydroxytryptamine or metabolites measured simultaneously under basal or ethanol-stimulating conditions in bibulous and abstainer rats. Overall, the present results suggest that a low dopaminergic tone and a strong mesolimbic dopamine response to ethanol are concerted neurochemical features associated to an ethanol-seeking behaviour in rats.

Keywords

Dopamine release; Nucleus accumbens; Microdialysis; Ethanol; UChB and UChA rats

CACorresponding author: Prof. Mario Herrera-Marschitz, MD Sci PhD, Programme of Molecular & Clinical Pharmacology, ICBM, Medical Faculty, University of Chile. Santiago, Chile. PO Box 70.000 Santiago 7; Chile, Email: mh-marschitz@med.uchile.cl.

1. Introduction

The behavioural stimulation and the rewarding effects induced by low doses of ethanol on rodents and humans are considered to be mediated by activation of central dopaminergic transmission (Ahlenius et al., 1973; Carlsson et al., 1974; Engel and Lilijequist, 1983). Consisting with this idea, it has been shown that the administration of low doses of systemic (0.5–1g/kg), or intracerebral (20–120 mM) ethanol induce locomotor behaviour (Brodie et al. 1999; see Wise and Bozarth, 1987), concomitantly to an increase of dopamine release in the nucleus accumbens of non-selected (wild type) Wistar and Sprague-Dawley awake rats (Imperato and Di Chiara, 1986; Di Chiara and Imperato, 1988; Blanchard et al., 1993; Yim et al., 1998; 2000; Yim and Gonzales, 2000; Löf et al., 2007).

The involvement of dopamine in ethanol preference is supported by studies on the genetics of alcoholism. Six sets of ethanol-preferring and -nonpreferring rat lines/strains have been developed by selective breeding: (i) the University of Chile Bibulous/Abstainer (UChB/UChA) lines (Mardones et al., 1953; see Mardones and Segovia-Riquelme, 1983; see Quintanilla et al., 2006); (ii) the Alko alcohol/nonalcohol (AA/ANA) lines (Eriksson, 1971); (iii) the alcohol-preferring/-nonpreferring (P/NP) lines (Li et al., 1987); (iv) the high-/low-alcohol drinking (HAD/LAD) lines (Gongwer et al., 1989); (v) the Sardinian alcohol-preferring/-nonpreferring (sP/sNP) lines (Fadda et al., 1989), and (vi) the high (HARF) and low (LARF) alcohol consuming ARF lines (Lê et al., 2001). In the majority of these lines, rats bred to prefer ethanol have innate deficiencies of the mesolimbic dopaminergic system (Murphy et al., 1987; 2002; Gongwer et al., 1989; Stefanini el al., 1992; Zhou et al., 1995; Strother et al., 2005). In agreement, in a recent paper (Quintanilla et al. 2007), we reported that ethanol-naïve UChB rats had lower extracellular dopamine levels in the shell of the nucleus accumbens, as compared to their ethanol-avoiding (UChA) counterparts. Moreover, we have also reported (Quintanilla 1999) that ethanol-naïve UChB rats displayed greater locomotor behaviour than UChA rats after the intraperitoneal (i.p.) administration of ethanol, as reported also for other ethanol-preferring, P (Li et al., 1979) and HAD (Krimer and Schecter, 1992) rats, compared to their corresponding ethanol-avoiding NP and LAD counterparts.

It is proposed here that low doses of ethanol will produce a greater increase of dopamine levels in nucleus accumbens of ethanol-naïve UChB versus UChA rats, indicating that ethanol induces a greater reward in ethanol-preferring than ethanol-avoiding rats. To test this hypothesis, we compared the effect of a single i.p. administration of ethanol on extracellular levels of monoamines monitored by *in vivo* microdialysis (Ungerstedt et al. 1982) in the shell of nucleus accumbens of ethanol naïve UChB and UChA rats.

2. Material and methods

2.1. Animals

Adult (300–350g) ethanol naïve male rats from UChB and UChA lines (generation F_{79} and F_{88}, respectively) were used in the experiments. The rats were housed individually in a temperature- and humidity-controlled environment with a 12/12h light/dark cycle and fed *ad-libitum*.

2.2. Surgical procedure and microdialysis

Rats were anaesthetised with a mixture of air and isoflurane administered via a mask fitted over the nose of the animal and placed in a Kopf stereotaxic frame with the skull oriented according to the atlas of Paxinos and Watson (1998). The rats were chronically implanted with a microdialysis guide cannula (CMA 7, CMA/Microdialysis AB) placed 2 mm above the

vertical target (V-6.2) using the following stereotaxic coordinates: A 1.7, L −0.7, V −8.2. The guide cannula was fixed with dental acrylate anchored by two screws on the skull.

Two to three weeks after the surgery, the stainless steel tubing sealing the guide cannula was removed to allow the insertion of a microdialysis probe (dialysing length, 2 mm; diameter, 0.5 mm; model CMA 12, CMA/Microdialysis AB, Stockholm, Sweden), connected to a perfusion CMA/120 setup (CMA/Microdialysis AB), including a liquid swivel. The microdialysis probe was perfused with a Ringer solution (pH~7) at a flow rate of 2 µl/min using a microinjection pump (CMA 100, CMA/Microdialysis AB). Samples (60 µl) were collected every 30 min using a microfraction collector (CMA 140, CMA/Microdialysis AB) and assayed for monoamines and their metabolites. A two-hour perfusion period elapsed before starting the sampling collection, performed in unanaesthetised animals under basal and pharmacologically stimulated conditions. Drugs or saline were administered systemically (i.p.) or intracerebrally via the microdialysis probe as indicated.

2.4. Analytical procedure

Dopamine and 5-hydroxytryptamine (5-HT) and their acidic metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), as well as 5-hydroxyindoleacetic acid (5-HIAA) were determined by high-performance liquid chromatography (HPLC) coupled to electrochemical detection (ED) as previously described (Bustamante et al., 2002) using a HPLC-pump adjusted to 0.45 ml/min (CMA 250 CMA/Microdialysis AB), an autoinjector (CMA 200, CMA/Microdialysis AB), a Synergy 4-Hydro-RP column (Phenomenex, Torrance, CA) and a carbon electrode adjusted to 700 mV (BAS, Tokyo). The identification and quantification of the substances was achieved by comparison with standard solutions prepared similarly to the samples. Peak integration was performed in a PC with an *ad-hoc* analogous-digital card and a CSW® software (Pronexus, Stockholm); the detection limit was 0.2 nM for DA, DOPAC, 5-HT and 5-HIAA, and 1 nM for HVA.

2.5. Systemic ethanol administration

Immediately after baseline sampling (three 30-min samples), the rats were treated with an equal volume solution of either sterile saline (1 ml/kg, i.p.) or 20% (v/v) ethanol (in saline), to give a dose of 1.0 g of ethanol /kg body weight (i.p.). Three additional 30-min samples were collected and analyzed as described above.

2.6. Intracerebral D-amphetamine administration

D-Amphetamine was administered via the microdialysis probe implanted into the nucleus accumbens (100 µM, diluted in Ringer buffer for a 30 min period), 120 min after the injection of saline or ethanol. Thereafter, three further dialysate samples were collected under perfusion with Ringer alone. Changes of the perfusion medium were performed with a syringe selector (CMA 111, CMA/Microdialysis AB).

2.7. Blood Ethanol Determination

In parallel experiments, blood ethanol concentrations were determined in 100 µl blood samples drawn from the tip of the tail at 30, 60 and 90 min after a single injection of ethanol (1.0 g/kg, i.p.) of ethanol naïve UChB and UChA rats (that had not received y ethanol prior to the study). Ethanol was determined by headspace gas chromatography as described elsewhere (Quintanilla et al., 2007b).

2.8. Histological evaluation

At the end of the experiments, the rats were euthanized by an overdose of isoflurane, and the brain rapidly removed. Serial frozen-, or formalin-fixed coronal sections (50 µm) were cut to

check the location of the microdialysis probe. The probe was inserted into the shell of the nucleus accumbens, apposed to the dorso-medial region of the olfactory tubercle and medial to the anterior limb of the anterior commissure. Only experiments with animals exhibiting a correct microdialysis probe implantation were included in the experimental analysis.

2.9. Chemicals and Drugs

High grade chemicals and ethanol were from Merck-Darmstadt (Darmstadt, Germany). Standard substances for analytical determination (dopamine, DOPAC, HVA, 5-HT and 5-HIAA), and D-amphetamine sulphate were from Sigma-Aldrich (Atlanta, GA, USA).

2.10. Data analysis and statistics

The levels of the assayed substances represent the concentrations found in the perfusates (means ± S.E.M.) without any re-calculation for the recovery of the probe. Basal values refer to those obtained before ethanol administration or before D-amphetamine and are set as 100% for the corresponding comparisons. The results were analysed by a one way analysis of variance (F-ANOVA) and a post-hoc test (Bonferroni`s test for multiple pair-wise comparisons) when required. Differences between lines were tested with a two-way ANOVA (treatment X line) followed by Newman-Keuls' *t* test. A level of $P< 0.05$ was considered statistically significant.

2.11. Ethics guidelines

The protocols were approved by the Ethics Committee for Experiments with Laboratory Animals at the Medical Faculty (Protocol CBA#1054, FMUCH) and by the Chilean Council for Science and Technology Research, (CONICYT), endorsing the principles of laboratory animal care (NIH; N° 86-23; revised 1985).

3. Results

3.1. Basal Extracellular Monoamine Levels

Under basal conditions, extracellular dopamine and 5-HT levels were detected in the low nM range in nucleus accumbens (shell) of both UChA and UChB rats. The extracellular dopamine levels were, however, significantly lower in UChB than in UChA rats (0.75±0.07 nM, n=4; versus 1.09±0.09 nM, n=4, respectively; $P<0.05$). No line differences were observed on the simultaneously monitored 5-HT (1.15 ±0.09 nM, n=4; versus 1.16±0.09 nM, n=4, for UChB and UChA lines, respectively) or for metabolites levels (Table 1).

3.2. Effect of systemic ethanol administration

Fig. 1 shows the effect of saline and ethanol (1.0 g/kg, i.p.) on extracellular dopamine levels assayed in samples collected from nucleus accumbens of UChB and UChA rats. While saline did not produce any effect, ethanol produced a significant increase of extracellular dopamine levels in both rat lines, achieving a maximum during the sample collected immediately after the administration of ethanol (0–30-min sample), returning to the basal levels 90 min after the administration of the drug. No effect was observed on DOPAC, HVA, 5-HT and 5-HIAA levels in any of the rat lines (Table 1). The effect of systemic ethanol on dopamine release was greater in UChB than in UChA rats, whether the peak (157.84±8.91%, n=4; 122.01±4.25%, n=4, for UChB versus UChA rats, respectively), or the AUC (192 ±10%, n=4; 146 ±5%, n=4, for UChB versus UChA rats, respectively) of the effects were used for the comparisons (two-way F-ANOVA for the effect of ethanol $(1,22) = 5.28$, $P<0.05$; F-ANOVA for differences between lines $(1,22)= 25.16$; $P<0.01$).

3.3. Effect of intracerebral D-amphetamine administration

Table 2 shows the effect of D-amphetamine (100 μM) administered via the microdialysis probe 120 min after the administration of ethanol. D-amphetamine increased dopamine levels both in UChA (~4-fold, to 4.26±2.18 nM; n=4)), as well as in UChB (~3.5-fold, to 2.54±0.86 nM, n=4) rats. In contrast, the effect of D-amphetamine on 5-HT levels was significantly greater in UChB (3-fold, to 3.13±0.24 nM; $P<0.05$), than in UChA (2.2-fold, to 1.74±0.14 nM) animals.

3.4. Blood ethanol levels

Table 3 shows the concentration of ethanol in blood samples taken from the rat tail, 30, 60 and 90 min after a single injection of ethanol (1.0 g/kg, i.p.). No significant between-rat lines differences were observed at any of the time periods evaluated after ethanol administration. The ethanol plasma concentration was ~96 mg/dl (21 mM) 30 min after the administration of ethanol to both UChB (96.11±13.82 mg/dl; n=8) or UChA (96.62±12.81 mg/dl; n=8) rats. The elimination rate of ethanol was similar, showing a quasi 0 order kinetic along the three intervals chosen for monitoring.

4. Discussion

The present study shows that ethanol-naive rats of the UChB line (selectively bred for 79 generations to prefer ethanol) have, under basal conditions, lower extracellular dopamine levels in nucleus accumbens (shell), compared to UChA rats (selectively bred for 88 generations to avoid ethanol). A single dose of systemic ethanol (1g/kg, i.p.) increased extracellular dopamine levels in both rat lines, but the effect of ethanol was 2-fold greater in UChB than in UChA rats. The effect was specific for dopamine because no effect was observed on simultaneously monitored 5-HT levels. The greater ethanol effect observed in UChB rats was not associated to differences in blood ethanol levels, since the concentration and kinetics of ethanol elimination were identical in both rat lines. Hence, UChB rats appear to be more sensitive to the ethanol stimulation of dopamine release than UChA rats. When locally administered 120 min after a single dose of ethanol, D-amphetamine increased dopamine and 5-HT extracellular levels. While the effect of D-amphetamine on dopamine levels was similar in both rat lines (although the actual concentration produced by D-amphetamine was significantly lower in UChB than in UChA rats), a greater increase of 5-HT levels was observed in UChB than in UChA rats (also reflected in the actual concentration of 5-HT, i.e. 3.13±0.24, n=4 versus 1.74 ±0.14 nM, n=4; in UChB and UChA rats, respectively). The effect of D-amphetamine was similar following saline or ethanol administration.

The lower basal dopaminergic tone shown in the nucleus accumbens of UChB, compared to that of UChA rats, is in agreement with studies using other rat lines, bred for preferring or avoidance of ethanol. When compared to nonpreferring NP rats, ethanol-naïve P animals, bred to prefer ethanol, showed fewer immunostained dopamine neurons in the ventral tegmental area and fewer dopaminergic fibers in the nucleus accumbens (Zhou et al., 1995). Furthermore, P rats showed lower contents of dopamine and its major metabolites, DOPAC and HVA in the nucleus accumbens (Murphy et al., 1987; 2002). Rats of the HAD (high-alcohol-drinking) line also showed lower dopamine levels in the nucleus accumbens when compared to LAD (low-alcohol drinking) rats (Gongwer et al., 1989; Strother et al., 2005).

The finding that UChB animals exhibited a greater enhancement of dopamine release in nucleus accumbens upon a first exposure to ethanol is also supported by other studies. It was shown that ethanol administration resulted in greater enhancement of dopamine levels in nucleus accumbens of P (alcohol preferring) -rats compared to Wistar- (Weiss et al., 1993) and NP-rats (Smith and Weiss, 1999). A similar result was reported when comparing HAD- versus

LAD-, and AA (Alko Alcohol)- versus ANA (Alko non Alcohol)-rats, respectively (Katner & Weiss, 2001). The effect of ethanol on dopamine release is probably mediated by an increase in dopamine firing, as shown by Gessa and coworkers (Gessa et al. 1985; Mereu and Gessa, 1985; Imperato and Di Chiara 1986), and not due to an increase of dopamine synthesis, since, as shown here, ethanol did not affect the release induced by D-amphetamine, known to stimulate dopamine release from newly synthesised cytosolic pools (Fuxe and Ungerstedt, 1970; Jones et al. 1998).

The stronger effect of ethanol on dopamine release observed in ethanol-preferring, compared to ethanol-non-preferring rats suggests a relative difference in the reinforcing property of ethanol, supporting the idea that predisposition to alcohol-seeking behaviour is associated with a greater dopaminergic response to ethanol (Cloninger, 1987). Low doses of ethanol stimulate locomotion in ethanol-preferring and wild-rats, but rarely in non-preferring rats (Päivarinta and Korpi 1993; Colombo et al. 1998; Quintanilla 1999), in agreement with the idea that stimulation of motor behaviour is an expression of the positive reinforcing properties of drugs of abuse (see Wise and Bozarth 1987).

The mechanism by which ethanol increases dopamine release is still unknown. It has been proposed that the effect of ethanol on dopamine firing and release is indirect, via a polysynaptic des-inhibitory GABAergic loop (Mereu and Gessa, 1985). However, a direct effect on dopamine neurons has also been indicated, via hyperpolarization-activated cation current (I_h) (Okamoto et al. 2006).

The ethanol effect on GABAergic neurotransmission is well documented, although there is no consensus on the specific mechanism underlying that effect. The effect of ethanol on dopamine release may imply a des-inhibitory GABAergic loop. Ethanol can enhance GABAergic transmission, either by a presynaptic mechanism, enhancing GABA release (Carta et al. 2004), via an antagonism of $GABA_B$ receptors (Wan et al. 1996; Kang et al. 1998; Ariwodola and Weiner, 2004; Castelli et al. 2005; Walker and Koob 2007), or by a allosteric postsynaptic mechanism, opening chloride channels modulated by $GABA_A$ receptors (see Koob 2004). Ethanol has also been suggested to directly act on a δ containing subunits of $GABA_A$ receptors (Olsen et al., 2007; Santhakumar et al. 2007). In all cases, the effect of low doses of ethanol on dopamine release necessarily implies polysynaptic GABA-GABA loops, which have been extensively described in many regions of the basal ganglia (Oertel and Mugnaini, 1984; Kita and Kitai 1988; Tepper et al. 1998).

A direct action of ethanol on dopamine neurons has also been proposed (Harris et al. 1992), since ethanol increases neuronal firing even under conditions where synaptic release is blocked (Brodie et al. 1999). Morikawa and co-workers (Okamoto et al. 2006) have proposed a facilitating action of ethanol on voltage gating of I_h channel for explaining a direct action of ethanol on dopamine neurons.

Nevertheless, the above proposed mechanisms are still controversial. So far, δ subunit-containing GABA receptors (β3/δ) have only been reported in cerebellar granule cells (Hanchar et al. 2005), dentate gyrus granule cells (Wei et al. 2004) and hippocampal interneurons (Glyks et al. 2007). No information is available on the expression of δ subunit-in GABA receptors expressed in dopaminergic areas. The idea of a shift in the voltage dependence of the I_h current remains to be clarified, specifying whether the enhancement of I_h by ethanol affects a tonic-pacemaker activity of dopamine neurons (Okamoto et al. 2006), or it reduces the inhibitory influence of GABAergic inputs on tonically firing dopamine neurons, via a des-inhibitory mechanism (Stobbs et al. 2004).

Interestingly, the maximum effect of ethanol on dopamine release was observed here at the first dialysate sample, returning to baseline before 90 min after ethanol administration, despite

the fact that ethanol was still detectable at a significant concentration (~15 mM after 90 min, compared to ~21 and ~18 mM, measured 30 and 60 min after the ethanol administration) in the plasma of both UChB and UChA rats. A similar observation was previously reported by Yim et al. (2000) in Sprague-Dawley rats. The dissociation between the plasma time-course of ethanol and intracerebral dopamine release, in both UChB and UChA rats, suggests that the systemic metabolism of ethanol is not relevant for explaining the differences between UChB and UChA rat lines. However, it should be noted that significant amounts of acetaldehyde (up to 6o μM) can be generated intracerebrally by the action of catalase on ethanol (Zimakin and Buben, 2007; Jamal et al., 2007). Indeed, locally generated acetaldehyde has been proposed to activate the firing of ventral tegmental area dopaminergic neurons (Foddai et al., 2004; Melis et al., 2007). Furthermore, acetaldehyde in micromolar concentrations is self administered into the ventral tegmental area by alcohol-preferring rats (Rodd et al., 2005).

A rapid and transient increase of dopamine release produced upon ethanol administration may be relevant for the development of the reinforcing effects of ethanol, since a rapid stimulation of the dopaminergic pathways is responsible for the positive rewarding effects produced by the majority of drugs of abuse (see Nestler 2001). Previously reported and the present data are in agreement with this notion. It is also noteworthy that the transient effect of ethanol on dopamine release was greater in UChB than in UChA rats.

With respect to 5-HT, neurochemical and neuroanatomical studies have shown that P (ethanol-preferring) rats have lower 5-HT levels in nucleus accumbens tissue, compared to NP (nonpreferring) rats (Murphy et al 1982; 1987; McBride et al., 1990). In the present and previous studies (Quintanilla et al., 2007a), we did not find any significant differences in basal 5-HT and/or 5-HIAA levels between UChB and UChA lines, and ethanol did not increase extracellular levels of 5-HT and/or 5-HIAA in any of the lines. However, intracerebrally administered D-amphetamine produced a greater effect on 5-HT release in UChB than in UChA rats, either following systemic ethanol (by approximately 39%), or saline (by approximately 31%; Quintanilla et al. 2007) administration, suggesting an increased 5-HT synthesis in UChB rats, independently upon any previous systemic ethanol treatment.

There are not clear results concerning the role of 5-HT on the effect of ethanol. While it was reported that extracellular 5-HT levels in nucleus accumbens do not predict ethanol preference (Katner and Weiss, 2001), the same laboratory reported that ethanol-naive ethanol-preferring (P) rats showed a higher concentration of extracellular 5-HT levels in nucleus accumbens than their corresponding control nonpreferring (NP) line (Smith and Weiss, 1999), but repeated ethanol treatment decreased extracellular 5-HT levels in P (ethanol-preferring) rats (Smith and Weiss, 1999, Thielen et al., 2004), while increasing 5-HT levels in nonpreferring NP and wild Wistar rats (Smith and Weiss, 1999). Furthermore, it has been shown that only after high doses of ethanol (>2g/kg, i.p.) 5-HT levels are increased in nucleus accumbens of HAD (High Alcohol Drinking) and LAD (Low Alcohol Drinking) rats (Yoshimoto et al., 1992). So far, the available data are not clear, but possibly indicating that 5-HT neurotransmission is not primarily associated to ethanol preference.

In conclusion, it is shown here, using ethanol-preferring and -nonpreferring rat lines bred for 80 generations for over 50 years, that a strong mesolimbic dopaminergic response to ethanol is the major neurochemical feature associated with high-ethanol preference and/or high-ethanol-seeking behaviour in rats.

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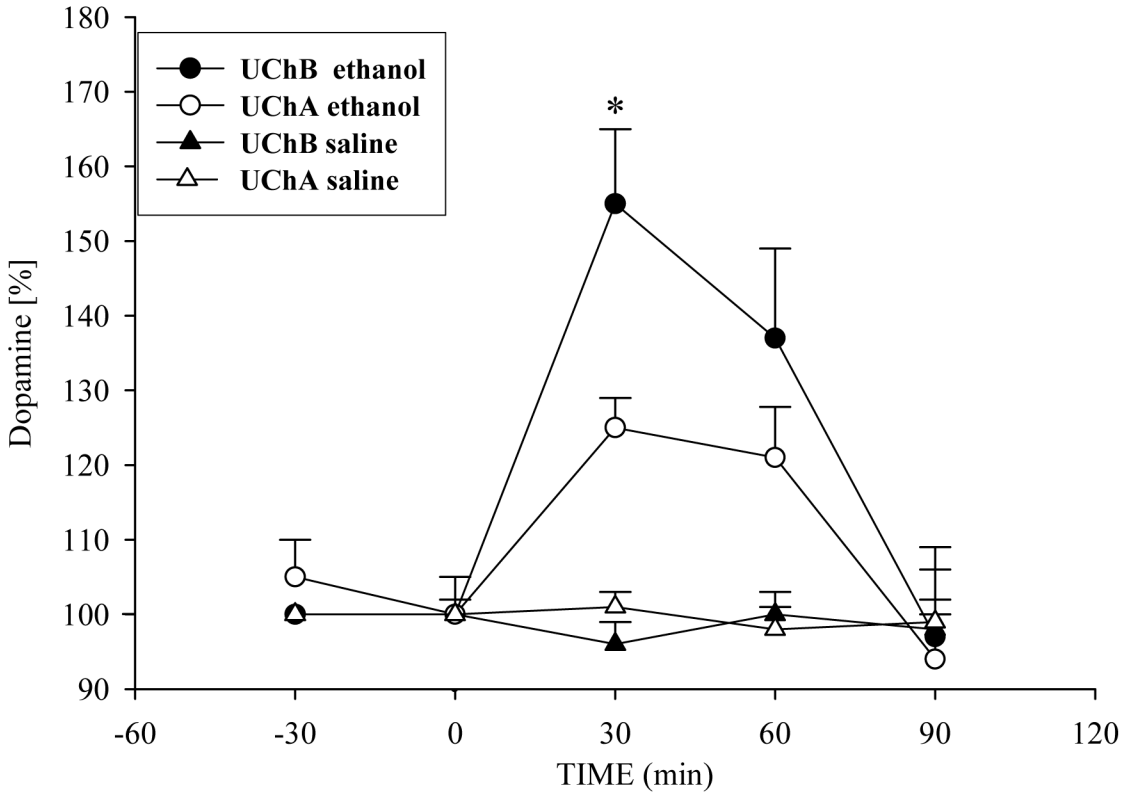

Figure 1.
Effect of systemic saline (1ml/kg, i.p.) or ethanol (1g/kg, i.p.) administration on dopamine release in nucleus accumbens (shell) of ethanol naïve UChA (open triangles and circles for saline and ethanol, respectively) and UChB (filled triangles and circles for saline and ethanol, respectively) monitored by microdialysis in the awake condition. *Abscissa*: time (min) after saline or ethanol administration. Ordinate: changes in dopamine levels (%) compared to the basal condition (30 min sample immediately before drug administration; arrow). *P<0.05, post hoc comparison. The effect of systemic ethanol on dopamine release was stronger in UChB than in UChA rats, whether on the peak, or on the AUC of the effects were used for the comparisons (two-way F-ANOVA for the effect of ethanol (1,22) = 5.28, P<0.05; F-ANOVA for differences between lines (1,22)= 25.16; P<0.01).

Table 1

Dopamine, DOPAC, HVA, 5-HT and 5-HIAA levels measured in microdialysates from the nucleus accumbens (shell) of awake ethanol-naive UChA (low-ethanol-consumer) and UChB (high-ethanol-consumer) rat lines, before and after ethanol (1g/kg, i.p.) treatment.

UChA (n=4)	**Basal (nM)**	**Ethanol (nM)**	**% of basal**
Dopamine	1.09±0.09	**1.33±0.17**[a]	**122.01±4.25**[a]
DOPAC	60.03±26.49	59.88±25.89	99.75±2.73
HVA	72.90±23.95	68.13±23.06	95.62±9.44
5-HT	1.16±0.09	1.28±0.22	110.67±24.53
5-HIAA	76.20±9.48	73.41±10.93	94.88±3.53
UChB (n=4)	**Basal**	**Ethanol**	**% of basal**
Dopamine	**0.75±0.07**[b]	**1.16±0.12**[a]	**157.84±8.91**[a,b]
DOPAC	43.05±12.29	45.14±13.19	104.92±1.01
HVA	76.69±15.77	85.18±26.09	111.07±2.59
5-HT	1.15±0.09	1.15±0.09	100±0
5-HIAA	121.08±15.80	121.91±18.04	99.78±2.91

Values are means ± S.E.M.

[a] ***P<0.05***, significant differences compared to basal levels using t test for paired data

[b] ***P <0.05*** significant differences compared to the corresponding levels in UChA rats.

Table 2

Dopamine, DOPAC, HVA, 5-HT and 5-HIAA levels measured in the microdialysates from the nucleus accumbens (shell) of awake UChA (low-ethanol-consumer) (n=4) and UChB (high-ethanol-consumer) (n=4) rat lines, before and after a 30 min pulse of D-amphetamine (D-Amph) (100 µM) via the microdialysis probe. D-Amph was administered 120 min after a single dose of ethanol (1g/kg, i.p.). Following saline, D-Amph increased dopamine levels in UChA and UChB rats, by 500±130%, n=7, and 370±71, n=6, respectively.

UChA (n=4)	**Basal (nM)**	**D-Amph (nM)**	**% of basal**
Dopamine	1.08±0.36	**4.26±2.18**[a]	**394.44±45.52**[a]
DOPAC	69.48±27.26	47.80±11.23	68.80±9.06
HVA	81.47±20.81	80.93±15.70	100.97±5.12
5-HT	0.81±0.08	**1.74±0.14**[a]	**216.67±11.79**[a]
5-HIAA	70.72±9.82	70.72±8.79	100.33±4.93
UChB (n=4)	**Basal**	**D-Amph**	**% of basal**
Dopamine	0.75±0.06	**2.59±0.86**[a]	**343.99±45.99**[a]
DOPAC	74.73±28.57	68.97±27.18	92.29±1.23
HVA	105.90±18.20	104.62±20.44	96.65±4.87
5-HT	1.05±0.14	**3.13±0.24**[a,b]	**302.22±27.11**[a,b]
5-HIAA	119.11±19.77	116.11±23.18	98.82±2.47

Values are means ± S.E.M.

[a] ***P<0.05***, significant differences compared to basal levels using t test for paired data

[b] ***P <0.05*** significant differences compared to the corresponding levels in UChA rats.

Table 3

Blood ethanol concentrations following a single dose of ethanol (1g/kg, i.p.) in UChB and UChA rats (30, 60 and 90 min after administration).

Rat line	**Blood ethanol concentrations (mg/dl) (mM)**			**Elimination rate (mg/kg/h)**
	30 min	***60 min***	***90 min***	
UChA (n=4)	96.62± 12.81 (21±2.78 mM)	86.03± 9.46 (18.7±2.09 mM)	72.60±10.62 (15.78±2.3 mM)	330.17±18.22
UChB (n=4)	96.11±13.82 (20.89±3.0 mM)	85.50±16.53 (18.58±3.59 mM)	67.22±12.84 (14.61±2.79 mM)	334.08±20.13

Values are means ± S.E.M.