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Effects of ethanol on the accumbal output of dopamine, GABA and glutamate in alcohol-tolerant and alcohol-nontolerant rats

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Abstract

Effects of ethanol on the accumbal extracellular concentrations of dopamine, as well as of the amino acid transmitters γ-amino butyric acid (GABA), glutamate and taurine, were studied in the alcohol-insensitive (alcohol-tolerant, AT) and alcohol-sensitive (alcohol-nontolerant, ANT) rats selected for low and high sensitivity to ethanol-induced motor impairment. Ethanol (2 or 3 g/kg ip) enhanced the output of dopamine and its metabolites in freely moving rats of both lines as measured by in vivo microdialysis. The effect of ethanol on the metabolites of dopamine tended to be stronger in the ANT rats. The smaller dose of ethanol decreased the output of GABA only in the AT rats, whereas the larger dose of ethanol decreased the output of GABA in rats of both lines to a similar degree. Ethanol at the dose of 2 g/kg slightly, but statistically, significantly decreased the output of glutamate in rats of both lines, but the larger dose of ethanol decreased the output of glutamate only in the AT rats. Ethanol at the dose of 2 g/kg induced a small transient increase in the output of taurine within 2 h after its administration in rats of both lines, but the larger dose of ethanol was without significant effect. These results confirm the previous findings that ethanol suppresses the release of GABA more in the AT than ANT rats. Thus, among the neurotransmitter systems we studied, the effects of ethanol might be the most relevant on GABAergic transmission regarding the sensitivity towards ethanol. However, our findings suggest that glutamate is also involved in this respect.

Keywords: γ-Aminobutyric acid (GABA); Dopamine; Amino acid transmitters; Glutamate; Taurine; Nucleus accumbens; In vivo microdialysis; Alcohol-sensitivity

1. Introduction

It is evident that the inhibitory and excitatory amino acid neurotransmitters and their receptors are involved in behavioural effects of ethanol. For instance, antagonists of inhibitory $GABA_A$ receptors such as picrotoxin and bicuculline have been shown to enhance the stimulatory effects of ethanol and to antagonize motor impairing effects of ethanol (Frye and Breese, 1982; Liljequist and Engel, 1982; Martz et al., 1983). At biochemical level, ethanol has been shown to enhance the functional responsiveness of the $GABA_A$ receptors and to decrease that of the *N*-methyl-D-aspartate (NMDA) type of excitatory glutamate receptors (Harris and Allan, 1989; Lovinger, 1996; Lovinger et al., 1989; Mihic et al., 1997). Acute administration of ethanol has also been shown to decrease the output of glutamate in the hippocampus (Shimizu et al., 1998) and nucleus accumbens (Dahchour et al., 2000; Moghaddam and Bolinao, 1994; Yan et al., 1998) of rat brain. Moreover, ethanol has been shown to attenuate increased glutamate output during withdrawal from chronic ethanol administration (Dahchour and De Witte, 2000; Rossetti and Carboni, 1995). Thus, decrease in the output of glutamate might contribute to the depressant effects of ethanol.

Rodents belonging to lines differing in their sensitivity to ethanol can be used as a model to study of the mechanisms of action of ethanol. Alcohol tolerant (AT) and alcohol nontolerant (ANT) lines have been selected for their low and high sensitivity to the motor impairing effects of ethanol (2 g/kg ip), respectively (Eriksson, 1990; Eriksson and Rusi, 1981). This selection appears to be relatively

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specific for motor impairing effects of ethanol, since rats of these lines do not differ significantly in respect of hypothermic and hypnotic effects of ethanol. AT rats are rather similar to nonselected heterogeneous rats and serve as alcohol-insensitive controls for the alcohol-sensitive ANT rats. ANT rats also show enhanced sensitivity to benzodiazepine agonists and sodium barbital, which suggests a role for $GABA_A$ receptors in ethanol sensitivity (Hellevuo et al., 1987; Wong et al., 1996). Indeed, a point mutation in the cerebellar $GABA_A$ receptor α_6-subunit was discovered in ANT rats (Korpi et al., 1993). This mutation alone, however, cannot explain the enhanced sensitivity of the ANT rats to ethanol. It has also been found that the basal tissue concentration of GABA in the striatum is higher in the ANT than in the AT rats and ethanol suppresses the turnover of GABA more in the cerebral cortex and cerebellum of AT rats than in those of ANT rats (Hellevuo and Kiianmaa, 1989). Thus, the regulation of GABAergic neurons in the brain of AT rats appears to be more sensitive to the effects of ethanol than that of ANT rats. In contrast, ethanol was found to increase the utilization of limbic dopamine more in the ANT than in the AT rats (Hellevuo et al., 1990). Concerning glutamatergic transmission, the ANT rats were shown to be more sensitive to the behavioural effects of an NMDA receptor antagonist than the AT rats (Toropainen et al., 1997; Vekovischeva et al., 2000).

The purpose of this study was to further explore the effects of ethanol on dopamine and GABA in the nucleus accumbens of AT and ANT rats by means of in vivo microdialysis. Nucleus accumbens is a highly important part of the limbic forebrain, which is thought to integrate limbic reward information with motor output. Furthermore, previously it was found that the differences in dopamine turnover between the AT and ANT rats were most prominent in the limbic forebrain among four brain areas studied (Hellevuo et al., 1990). Also, the ethanol-induced differences in GABA turnover between the rat lines were pronounced in this brain area (Hellevuo and Kiianmaa, 1989). For comparison, we also measured the concentrations of glutamate and taurine. Intermediate doses of 2 and 3 g/kg of ethanol were used. A total of 2 g/kg of ethanol impairs the motor performance in the tilting plane only in ANT rats and, in fact, this test was used as a selection criterion in the selection of the rat lines (Eriksson and Rusi, 1981). A total of 3 g/kg of ethanol, however, impairs the motor performance in both rat lines (Hellevuo et al., 1989; Hellevuo and Korpi, 1988).

2. Materials and methods

2.1. Animals

The adult male ANT and AT rats weighing 250–320 g were used in the experiments. Before surgery, the rats were housed in groups of four to five rats of each line per cage under 12/12-h light/dark cycle (lights on at 6 a.m.) at an ambient temperature of 22–23 °C. Tap water and standard laboratory food were available ad libitum. The animal experiments were approved by the local institutional animal care and use committee and the chief veterinarian of the county administrative board, and were conducted according to the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes." All animals were naive to ethanol before experiments.

2.2. Surgery

The rats were implanted with guide cannulae (BAS MD-2250, Bioanalytical Systems, IN, USA) under halothane anaesthesia (3.5% during induction for 5 min and then 2.5–1% during surgery). The placement of guide cannulae was calculated relative to bregma and was aimed at the point above the nucleus accumbens (NAC; A/P = + 1.7, L/M = 1.4, D/V = 6.8) according to the atlas by Paxinos and Watson (1986). The cannula was fastened to the skull with dental cement (Aqualox, Voco, Germany) and three stainless steel screws. After the surgery, the rats were placed into individual test cages ($30 \times 30 \times 40$ cm) and allowed to recover at least for 4 days before the experiment. The rats were weighed and handled for at least 2 days before the beginning of the microdialysis experiments.

2.3. Microdialysis

In the evening of the day preceding the microdialysis experiments, a used microdialysis probe (dummy probe) was inserted into the guide cannula and left there overnight without dialysis. Dummy probes were used because in preliminary experiments we found that the levels of glutamate did not stabilize when the probe was inserted for the first time. Concerning striatal dopamine, it has been shown previously that the puncture of the striatal tissue before the insertion of the microdialysis probes facilitates early sampling and improves the impulse-flow sensitivity of dopamine release (Devine et al., 1993). In the next morning, the dummy probe was removed and replaced by the actual microdialysis probe (BAS, MD-2200, 2-mm membrane). Modified Ringer solution (147 mM NaCl, 1.2 mM $CaCl_2$, 2.7 mM KCl, 1.0 mM $MgCl_2$ and 0.04 mM ascorbic acid) was infused through the probe at a flow rate of 2 μl/min. The collection of microdialysis samples (every 30 min, 60 μl/sample) was started 2.5–3 h after the probe insertion. The samples were discarded until a stable baseline was achieved; the average concentration of the first three to four stable samples was used as basal level. Thereafter, the rats were intraperitoneally administered saline or ethanol (2 or 3 g/kg ip, in 20% w/v saline) and the samples were collected for the next 4 h.

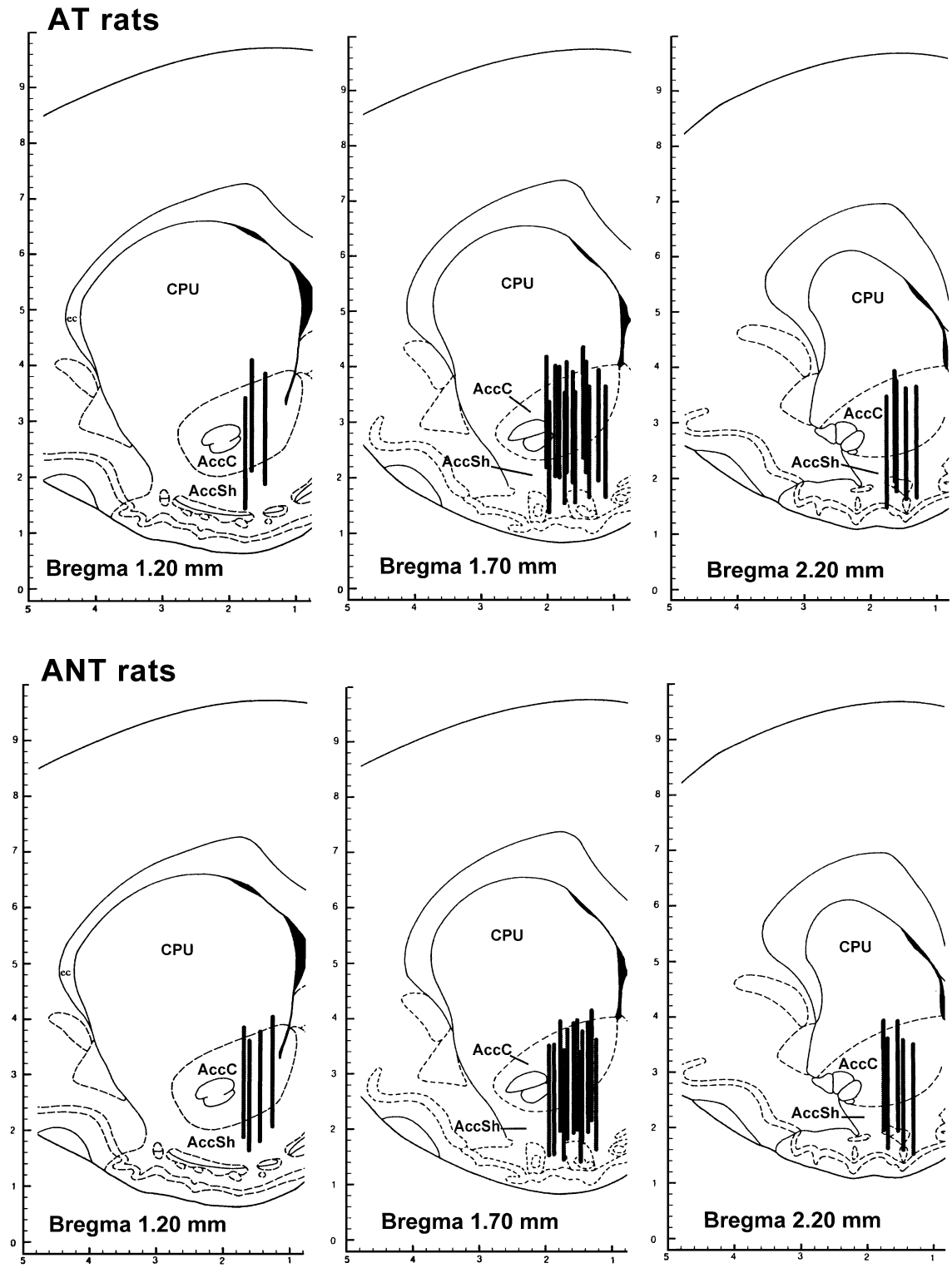

Fig. 1. The placements of the probes implanted in the nucleus accumbens of AT and ANT rats. For clarity, the diameters of the probes are reduced (actual OD: 0.32 mm). The locations are drawn to the nearest corresponding slice. The figure is modified from the atlas of Paxinos and Watson (1986). AccC, nucleus accumbens core; AccSh, nucleus accumbens shell; CPU, caudate–putamen.

2.4. Determination of dopamine, DOPAC and HVA

Dopamine, DOPAC and HVA were analyzed from a fraction of the collected sample with HPLC immediately after collection. The system used for determination of the extracellular concentrations of DA, DOPAC and HVA consisted of an ESA Coulochem II detector (ESA, MA, USA) equipped with a model 5014A microdialysis cell, a Pharmacia LKB model 2248 HPLC pump (Pharmacia LKB, Sweden) and a SSI model LP-21 pulse damper (Scientific Systems, PA, USA). The column (Spherisorb ODS2, 3 μm, 4.6 × 100 mm) was kept at 40 °C with a column heater (model CROCO CIL, Cluzeau Info-Labo, France). The mobile phase used consisted of 0.1 M NaH_2PO4 buffer, pH 4.0 (adjusted with 1.0 mM citric acid), 0.8–1.2 mM octane sulfonic acid, 16% methanol and 1.2 mM EDTA. The flow rate of the mobile phase was set at 1.0 ml/min. Thirty microliters of the dialysate sample was injected with a CMA/200 autoinjector (CMA, Stockholm, Sweden). Dopamine was reduced with an amperometric detector (potential −80 mV) and DOPAC and HVA were oxidized with a coulometric detector (+300 mV). Chromatograms were processed with a Hitachi D-2000 chromato-integrator.

2.5. Determination of glutamate and taurine

Glutamate and taurine were analyzed from a fraction of the microdialysates as described previously (Piepponen and Skujins, 2001). The HPLC system consisted of a solvent delivery pump (Pharmacia LKB Gradient pump 2249, connected to Pharmacia Biotech low pressure mixer, Pharmacia Biotech, Sweden), a refrigerated microsampler (Model CMA/200, CMA/Microdialysis, Stockholm, Sweden), an analytical column (Micra NPS ODS-II, 100 × 4.6 mm, 3 μm particle size, Micra Scientific, IL, USA) protected by a 0.5-μm inlet filter and thermostated by a column heater (model CROCO CIL, Cluzeau Info-Labo, France), and a fluorescence detector (model CMA/280, CMA/Microdialysis). Automated sample derivatization was carried out using a CMA/200 refrigerated autosampler at 4 °C. Five microliters of a striatal microdialysis sample was diluted with 10 μl 1.5 mM β-alanine (internal standard) in Ringer solution. The autosampler was programmed to add 6 μl of the derivatizing reagent [OPA/mercaptoethanol; prepared daily by mixing 1 ml OPA borate buffer solution (OPA incomplete, Sigma, MO, USA) with 3 μl 2-mercaptoethanol solution (Sigma)] to the sample, to mix two times and to inject 20 μl onto the column after a reaction time of 1 min. The mobile phase A [0.05 M disodiumhydrogen phosphate, pH 6.1 (adjusted with 85% phosphoric acid), acetonitrile 0.5% (v/v), tetrahydrofuran 1% (v/v)] was pumped at a flow rate of 1 ml/min, column temperature was maintained at 37 °C. The external low pressure mixer was programmed to switch to the mobile phase C [acetonitrile 70% (v/v), tetrahydrofuran 1% (v/v), water 29%] after 3 min of the beginning of the run and to switch back to the mobile phase A after 2 min (washout-step). The detector gain was set to 10 times signal amplification. The chromatograms were recorded on an integrator (model C-R 4A Chromatopac, Shimadzu, Kyoto, Japan).

2.6. Determination of GABA

GABA was analyzed similarly as glutamate and taurine in exception that 15 μl of undiluted microdialysate (without internal standard) was derivatized, mobile phase B [0.05 M disodiumhydrogen phosphate, pH 4.8 (adjusted with 85% phosphoric acid), acetonitrile 7% (v/v), tetrahydrofuran 1%] was used instead of mobile phase A and detector gain was set to 100 times signal amplification.

2.7. Histology

After completion of the experiments, the brains were removed from the skull and frozen (−80 °C) until histological examination. The positions of the probes were verified by slicing the frozen brain tissue into 80-μm coronal sections, which were then stained with thionine. The placement of the probes was examined microscopically and was shown in the Fig. 1.

2.8. Statistical analysis

Statistical analysis was carried out on data normalized to percentage of the preinjection baseline values for each rat using two-way analysis of variance (ANOVA) for repeated measures. Between-factors were the rat line (AT or ANT) and treatment (saline or ethanol), and within-factor the time interval where the changes were evident. The basal extracellular concentrations of estimated compound were compared with Student's *t*-test.

3. Results

3.1. Basal accumbal concentrations of dopamine, DOPAC, HVA, GABA, glutamate and taurine

The basal accumbal concentrations of dopamine, DOPAC, HVA, GABA, glutamate and taurine in the AT and ANT rats (not corrected with in vitro recovery) are

Table 1
Basal extracellular concentrations (not corrected for in vitro recovery) of dopamine, DOPAC, HVA, GABA, glutamate and taurine in the nucleus accumbens of the AT and ANT rats

Compound	AT rats	*n*	ANT rats	*n*
Dopamine (pM)	407 ± 65	21	266 ± 51	20
DOPAC (nM)	101 ± 16	21	120 ± 14	20
HVA (nM)	59 ± 8	21	59 ± 6	20
GABA (nM)	57 ± 11	19	46 ± 7	19
Glutamate (μM)	0.97 ± 0.12	20	1.21 ± 0.23	21
Taurine (μM)	4.34 ± 0.43	21	4.77 ± 0.62	21

Given are means ± S.E.

shown in Table 1. No significant differences were found in these basal values between the rat lines.

3.2. Effects of ethanol on the output of dopamine, DOPAC and HVA

Both doses of ethanol (2 and 3 g/kg) slightly but significantly elevated the extracellular concentration of dopamine in the nucleus accumbens [treatment effects, $F(1,17)=4.65$, $P<.05$ and $F(1,16)=5.56$, $P<.05$, respectively (two-way ANOVA); Fig. 2]. The effect of ethanol did not differ statistically significantly between the rat lines [Treatment × Rat Line interactions, $F(1,17)=0.17$, $P>.5$ and $F(1,16)=0.06$, $P>.5$, respectively, for 2 and 3 g/kg of ethanol]. However, the smaller dose of ethanol appeared to elevate the output of dopamine somewhat more in the

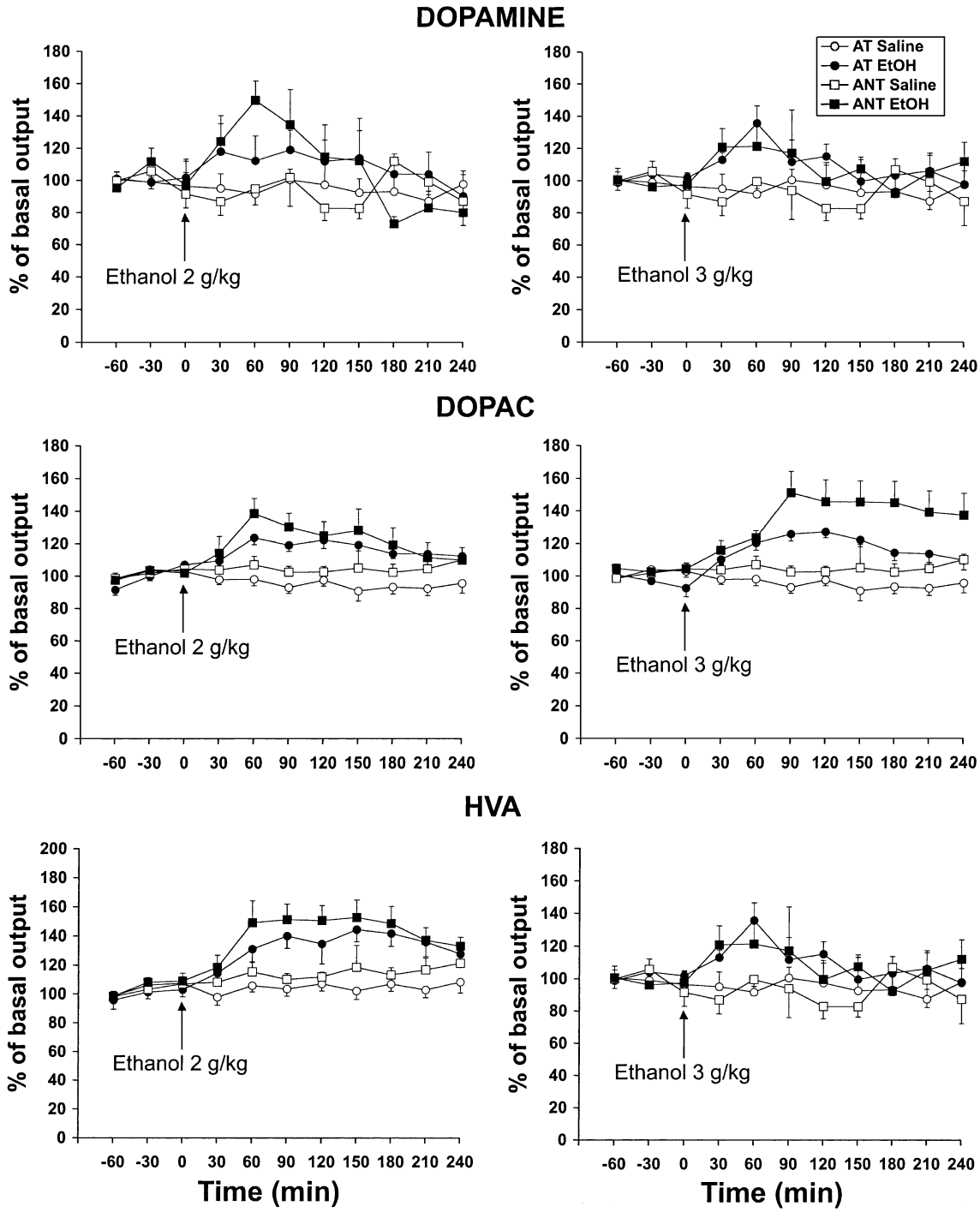

Fig. 2. Effects of ethanol (2 or 3 g/kg ip) on the extracellular concentrations of dopamine and its metabolites, DOPAC and HVA, in the nucleus accumbens of AT and ANT rats. Ethanol or saline was given at the time point indicated by the arrow. All results are shown as means ± S.E. ($n=5-6$).

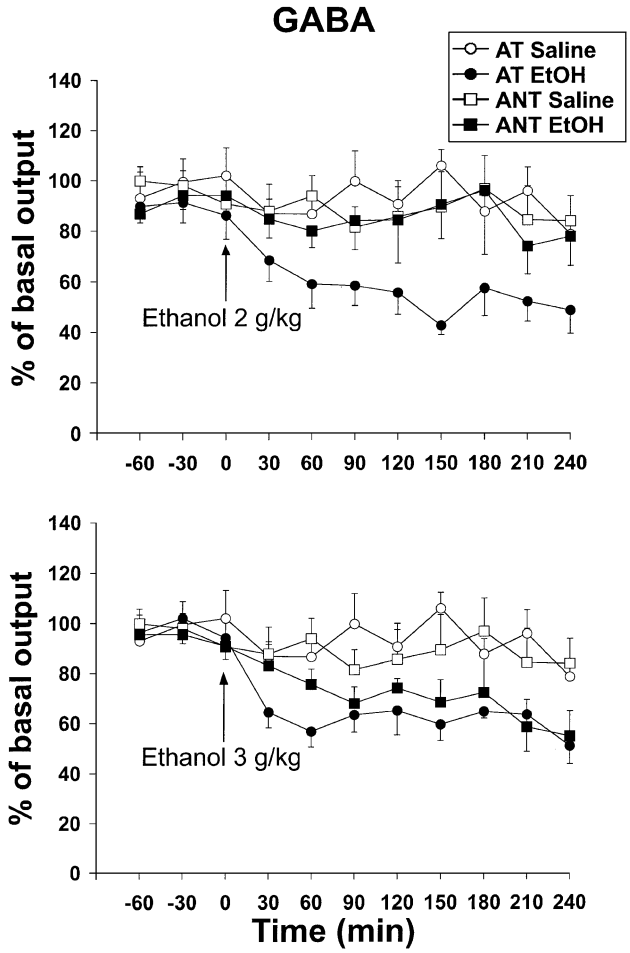

Fig. 3. Effects of ethanol (2 or 3 g/kg ip) on the extracellular concentration of GABA in the nucleus accumbens of AT and ANT rats. Ethanol or saline was given at the time point indicated by the arrow. All results are shown as means ± S.E. ($n = 4–6$).

ANT rats than in AT rats during 60–90 min after ethanol administration. Both doses of ethanol also increased the output of dopamine metabolites, DOPAC and HVA. The *F*-values of treatment effects (1,16–17) varied between 13.91 and 36.43, *P*-values being less than .01. Again, there were no significant rat line × treatment interactions [$F(1,16–17) = 0.01–0.41$, $P > .5$]. Still, there was some tendency that the metabolites of dopamine were increased more in the ANT than in the AT rats after administration of ethanol (Fig. 2).

3.3. Effects of ethanol on the output of GABA

The smaller dose of ethanol (2 g/kg) significantly decreased the output of GABA [treatment effect, $F(1,16) = 6.84$, $P < .05$], this effect occurring only in the AT rats [Treatment × Rat Line interaction, $F(1,16) = 4.72$, $P < .05$; Fig. 3]. The larger dose of ethanol decreased the output of GABA in rats of both lines [treatment effect, $F(1,14) = 8.10$, $P < .05$] and the decreases were of the same magnitude in both rat lines [Treatment × Rat Line interaction, $F(1,14) = 0.68$, $P > .5$].

3.4. Effects of ethanol on the output of glutamate

The smaller dose of ethanol (2 g/kg) slightly but statistically significantly decreased the output of glutamate [treatment effect, $F(1,17) = 6.23$, $P < .05$; Fig. 4]. The effect was similar in both rat lines (Treatment × Rat Line interaction, $P > .5$). However, the larger dose of ethanol decreased the output of glutamate only in the AT rats [Treatment × Rat Line interaction, $F(1,16) = 8.23$, $P < .05$], whereas the output of glutamate was not decreased at all in the ANT rats after administration of 3 g/kg ethanol. Profound decrease in the output glutamate occurring only in the AT rats brought about also significant rat line effect [$F(1,16) = 8.83$, $P < .01$].

3.5. Effects of ethanol on the output of taurine

Ethanol at the dose of 2 g/kg induced a small transient increase in taurine output, which peaked at 30 min after

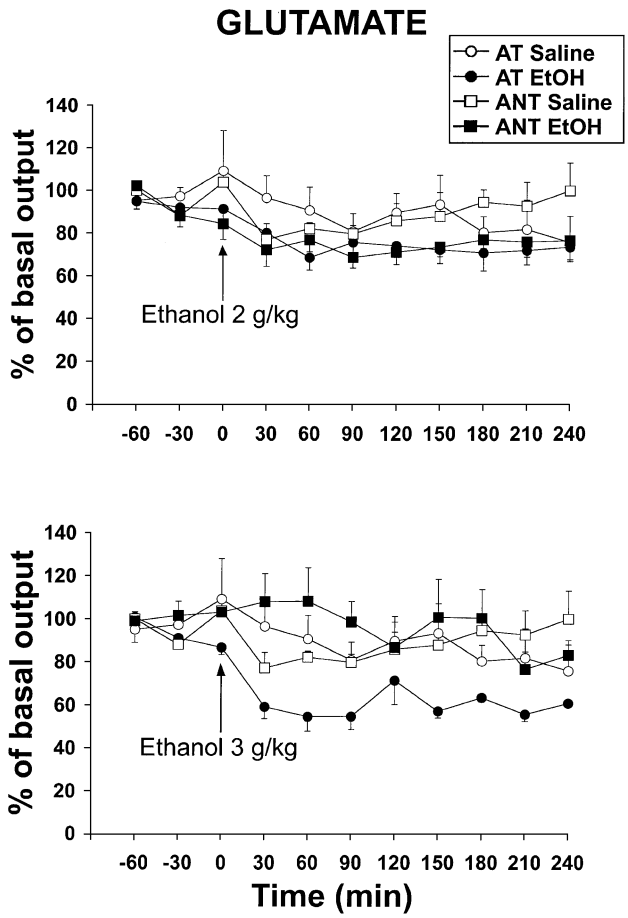

Fig. 4. Effects of ethanol (2 or 3 g/kg ip) on the extracellular concentration of glutamate in the nucleus accumbens of AT and ANT rats. Ethanol or saline was given at the time point indicated by the arrow. All results are shown as means ± S.E. ($n = 5–6$).

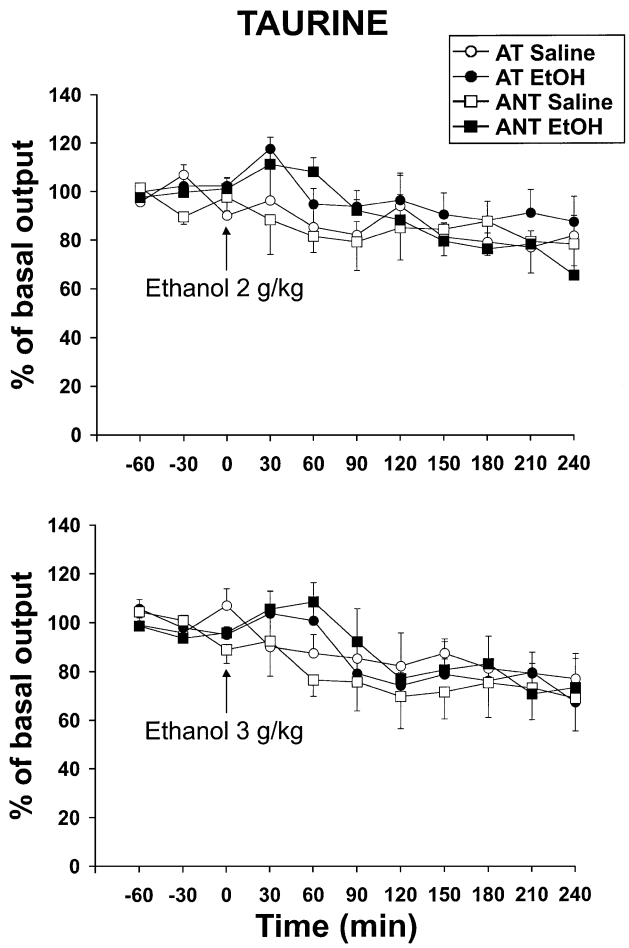

Fig. 5. Effects of ethanol (2 or 3 g/kg ip) on the extracellular concentration of taurine in the nucleus accumbens of AT and ANT rats. Ethanol or saline was given at the time point indicated by the arrow. All results are shown as means ± S.E. ($n = 5–6$).

ethanol administration (Fig. 5). Two-way ANOVA revealed a significant treatment effect when the analysis was performed on the time interval 30–120 min after administration of ethanol [treatment effect, $F(1,19) = 4.61$, $P < .05$]. The effect was similar in both rat lines [Treatment × Rat Line interaction, $F(1,19) = 0.001$, $P > .5$]. However, the larger dose of ethanol was without significant effect on taurine output [treatment effect, $F(1,16) = 0.51$, $P > .05$].

4. Discussion

In the present experiments, acutely administered ethanol (2–3 g/kg) elevated the extracellular concentrations of dopamine and its metabolites, but reduced the levels of amino acid transmitters, GABA and glutamate, in the nucleus accumbens of rats of both lines. However, there were dose-dependent differences in the responses of the alcohol-insensitive AT and alcohol-sensitive ANT rats to ethanol. Thus, 2 g/kg of ethanol decreased the output of GABA more in the AT rats than in the ANT rats, and tended to enhance the output of dopamine and its metabolites more in the ANT rats than in the AT rats. In addition, 3 g/kg of ethanol was found to decrease the efflux of glutamate only in the AT rats. No significant differences were found in the basal extracellular concentrations of the neurotransmitters measured.

The main finding of this study was that ethanol at the dose (2 g/kg), which induces the most obvious behavioural differences between the rat lines and which was used in the selection of the lines, reduced the output of GABA significantly more in the nucleus accumbens of the AT rats than in that of the ANT rats. This finding complements previous studies where by using indirect measurements ethanol was found to decrease the turnover of limbic GABA more in the AT rats than in the ANT rats (Hellevuo and Kiianmaa, 1989). As an explanation for this difference, Hellevuo and Kiianmaa (1989) suggested that the AT rats would have more GABA-autoreceptors than ANT rats and activation of these autoreceptors after ethanol administration would reduce the release of GABA. This assumption is supported by the fact that there are more $GABA_A$ binding sites in the cerebellum of the AT rats than the ANT rats (Malminen and Korpi, 1988).

Besides motor impairing effects of ethanol, the alcohol-sensitive ANT rats are also in some other respects more vulnerable to the effects of ethanol than the AT rats. For instance, a low dose of alcohol (1 g/kg) produces an anxiolytic response only in the ANT rats (Tuominen et al., 1990). In addition, diazepam produced a greater anxiolytic response in the ANT rats than in the AT rats (Vekovischeva et al., 2000). ANT rat line has a pharmacologically critical point mutation in the α_6-subunit of their cerebellar $GABA_A$ receptors, which alters these normally diazepam-insensitive receptors to diazepam-sensitive ones (Korpi et al., 1993). This subunit is selectively expressed in the cerebellum (Luddens et al., 1990). Indeed, no other significant biochemical differences in $GABA_A$ receptor function have been detected between the rat lines (Uusi-Oukari and Korpi, 1992). Thus, it was suggested that the enhanced anxiolytic response of diazepam in the ANT rats as compared to the AT rats is also mediated by cerebellar mechanisms (Vekovischeva et al., 2000). Our results, however, support the view that there are functional differences in the GABAergic mechanisms between the rat lines occurring also in other brain areas besides cerebellum.

Previous studies have shown that ANT rats have higher concentration of dopamine in the limbic forebrain than AT rats, but there is no difference in the basal turnover rate of limbic dopamine between the rat lines (Ahtee et al., 1980; Hellevuo et al., 1990). However, it appears that ethanol increases the utilization of cerebral monoamines more in the ANT than in AT rats and this difference might be involved in their differential sensitivity to motor impairing effects of ethanol (Hellevuo et al., 1990). In this study, we found no statistically significant differences in the output of dopamine or its metabolites between the rat lines. Still, there was

tendency towards augmented response to the effect of ethanol in the ANT rats, especially concerning the metabolites of dopamine. Thus, the involvement of central dopaminergic mechanisms in the motor impairing effects of ethanol can not be ruled out.

In line with previous studies, we found that ethanol decreased the output of accumbal glutamate. The smaller dose of ethanol used induced a similar small decrease in the output of glutamate in rats of both lines, but, surprisingly, the larger dose decreased the output of glutamate only in the AT rats. In contrast, in a recent study ethanol at the dose of 2 g/kg decreased the output of accumbal glutamate in the high-alcohol sensitive (HAS) rats, but not in the low-alcohol sensitive (LAS) rats (Dahchour et al., 2000). This finding, however, was not reproduced in a subsequent study (Quertemont et al., 2002). The discrepancy in the effect of ethanol on glutamate may be due to the fact that there appears to be no linear dose–response effect of ethanol on the overflow of glutamate. Thus, ethanol at small doses (0.5–1 g/kg) has been shown to enhance the overflow of accumbal glutamate (Moghaddam and Bolinao, 1994; Selim and Bradberry, 1996), whereas the larger dose of 2 g/kg of ethanol was found either to decrease (Moghaddam and Bolinao, 1994; Yan et al., 1998) or to have no effect (Dahchour et al., 1994; Selim and Bradberry, 1996) on accumbal glutamate output. Therefore, it is possible that there are differences in the responses to ethanol-induced changes in accumbal glutamate between rats belonging to different lines or strains, i.e., the peak effect of ethanol on the extracellular glutamate output might occur at different ethanol doses. As the difference was found only with the larger dose of ethanol, which impairs the motor function in both lines, it might not be involved in the differential sensitivity of these rat lines to ethanol. However, involvement of glutamatergic transmission cannot be ruled out in the differential effect of ethanol between the rat lines, especially because ANT rats have been shown to be more vulnerable to the motor impairing effects of an NMDA receptor antagonist (Toropainen et al., 1997).

The neuromodulatory amino acid taurine might be involved in the modulation of the effects of ethanol. Intracerebroventricularly administered taurine has been shown to enhance the depressant effects of ethanol in mice and rats (Ferko, 1987; Ferko and Bobyock, 1988; Mattucci-Schiavone and Ferko, 1985). In addition, taurine has been suggested to modulate some of the aversive or rewarding effects of ethanol (Quertemont et al., 1998). Ethanol has also been shown to increase the output of taurine (Dahchour et al., 1994, 1996; Peinado et al., 1987; Quertemont et al., 2000, 2002) and administration of taurine has been shown to normalize the augmented glutamate release during withdrawal from chronic ethanol exposure (Dahchour and De Witte, 2000). Accordingly, in the present study, we found a small transient increase in the output of accumbal taurine. Interestingly, this effect occurred only with the smaller dose of ethanol (2 g/kg), whereas the larger dose was without significant effect. This is in contrast with a recent study, where the doses of 2 and 3 g/kg of ethanol induced a similar increase in the output of taurine in LAS rats and in the HAS rats the increase was larger with the dose of 3 g/kg as compared to 2 g/kg (Quertemont et al., 2002). Thus, the increase in the output of taurine induced by 2 g/kg of ethanol was larger in the LAS rats than in the HAS rats and the authors concluded that larger increase in the output of taurine in the LAS rats might contribute to their lower ethanol sensitivity. Our results, however, do not support the role for taurine in the sensitivity to ethanol, since no differences in the output of taurine was found between the rat lines.

It is not clear to what extent the extracellular concentrations of glutamate and GABA as measured by microdialysis are of neuronal origin, thus, reflecting the vesicular release of them. It is well known that glutamate is the precursor in the synthesis of GABA and released GABA can be further metabolized, via the Krebs cycle and the GABA-transaminase enzyme, back to glutamate. These effects can occur both in neuronal and extraneuronal compartments. Thus, interconnection between glutamate and GABA makes it difficult to interpret the functional roles of these transmitters as measured by microdialysis. Indeed, the validity of microdialysis in the measurement of amino acid has been questioned (Timmerman and Westerink, 1997). Inhibition of neural activity by tetrodotoxin or removal of calcium from the dialysis fluid has been the most frequently used methods for the verification of the neuronal origin of the transmitters measured by microdialysis. Basal extracellular glutamate appears to poorly respond to these manipulations (Abarca et al., 1995; Hashimoto et al., 1995; Herrera-Marschitz et al., 1996; Miele et al., 1996; Shiraishi et al., 1997), indicating that major part of the extracellular glutamate is derived from other sources than vesicular release. In contrast, in most cases, the output of GABA has been shown to be sensitive to these manipulations (Biggs et al., 1995; Campbell et al., 1993; Herrera-Marschitz et al., 1996; Osborne et al., 1990, 1991; Smith and Sharp, 1994). Interestingly, in the anaesthetized rats, the output of GABA appears not to be calcium- or tetrodotoxin-sensitive (Biggs et al., 1995; Campbell et al., 1993; Drew et al., 1989; Osborne et al., 1990), which may explain the discrepancy concerning the validity of GABA measurements. Taken together, changes in the outflow of GABA appear, at least partly, to reflect changes in the GABAergic transmission, whereas the results concerning the glutamate should be interpreted with caution.

In conclusion, regardless of the precise mechanism involved, the present results suggest that AT rats can apparently adapt more easily to the motor impairing effect of ethanol than ANT rats by adjusting their cerebral GABAergic tone. This difference seen between the rats is probably the most relevant concerning the motor impairing effects of ethanol; however, the involvement of dopaminergic and glutamatergic mechanisms cannot be ruled out in this respect. In addition, our results support the view that the

GABAergic systems of the two lines differ also in other brain areas besides cerebellum.

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