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European Journal of Pharmacology 444 (2002) 143–150

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Differential taurine responsiveness to ethanol in high- and low-alcohol sensitive rats: a brain microdialysis study

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Received 10 December 2001; received in revised form 8 April 2002; accepted 19 April 2002

Abstract

Several microdialysis studies have investigated the effects of acute ethanol on extracellular amino acids in various rat brain regions. However, these studies led to conflicting results, suggesting that individual differences between rat strains and lines may play an important role. In the present study, high-alcohol sensitive (HAS) and low-alcohol sensitive (LAS) rats were used to investigate the possible relationship between ethanol sensitivity and the concentrations of extracellular amino acids in the nucleus accumbens. Several groups of HAS and LAS rats were injected with either saline or ethanol (1.0, 2.0 or 3.0 g/kg, i.p.) and the concentrations of amino acids in the nucleus accumbens microdialysates were assayed by electrochemical detection. Acute ethanol induced a dose-dependent increase in extracellular taurine concentrations. However, this increase was significantly reduced at 2.0 and 3.0 g/kg ethanol in HAS rats relative to LAS rats. Since the biological functions of taurine suggest its implication in the reduction of ethanol adverse effects, a higher increase in taurine concentrations may contribute to the lower ethanol sensitivity of LAS rats. Although 2.0 and 3.0 g/kg ethanol did not affect extracellular glutamate concentrations, a significant increase in glutamate was observed after 1.0 g/kg ethanol to HAS rats but not to LAS rats. Such an effect remains unexplained but suggests that discrepancies between the results of previous microdialysate studies may be related to differences in the ethanol sensitivities of various rat strains.

Keywords: Ethanol; Taurine; Glutamate; Microdialysis; Alcohol sensitivity, high; Alcohol sensitivity, low

1. Introduction

Ethanol, one of the most abused drugs, exerts its action by affecting multiple targets in the central nervous system. It has been shown to interact with almost all identified neurotransmitter systems (see review in Deitrich and Erwin, 1996), although its effects on excitatory and inhibitory amino acid neurotransmissions are particularly important in the mediation of its behavioral effects. Indeed, its positive modulatory action on γ-aminobutyric acid ($GABA_A$) receptors and its inhibitory effects on glutamatergic NMDA receptors have been demonstrated up to the molecular level (Grobin et al., 1998; Woodward, 2000). While ethanol's effects on these receptors were extensively studied, ethanol also affects amino acid neurotransmission by altering directly or indirectly the brain extracellular concentrations of these amino acids.

Several in vivo microdialysis studies investigated the effects of an acute ethanol administration on extracellular glutamate concentrations in various rat brain regions, but these experiments led to conflicting results. While the majority of these studies failed to show any effect of ethanol administration on glutamate microdialysate contents (Dahchour et al., 1994, 1996; Selim and Bradberry, 1996; Quertemont et al., 1998a, 1999, 2000), high ethanol doses were found to inhibit the release of glutamate in several other studies (Shimizu et al., 1998; Yan et al., 1998). In a particular study, ethanol was also shown to induce a biphasic effect on extracellular glutamate concentrations (Moghaddam and Bolinao, 1994). At 0.5 g/kg, ethanol increased the extracellular concentrations of glutamate within the nucleus accumbens and the hippocambus, while ethanol doses higher than 1.0 g/kg reduced extracellular glutamate concentrations. Although these conflicting results remain unresolved, these differences in ethanol-induced changes in extracellular glutamate concentrations could be

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0014-2999/02/$ - see front matter
PII: S0014-2999(02)01648-5

related to differential sensitivities of various rat brain regions and/or to differences between strains of rats.

While there is no clear evidence that ethanol alters extracellular γ-aminobutyric acid (GABA) concentrations, recent studies show that ethanol increases the release of taurine, another inhibitory amino acid, in various rat brain regions (Dahchour et al., 1994, 1996; Quertemont et al., 1999, 2000). Although taurine release after ethanol administration seems to be part of an osmoregulatory process (Quertemont et al., 2001), the effects of the released taurine might be greater than the sole regulation of cell volume. Indeed, taurine has been shown to exert a neuromodulatory action by inhibiting neuronal cell excitability (Billard, 1990; Wang et al., 1998). Although a specific taurine receptor has not been identified in the brain, there is evidence that taurine modulates the activity of the GABA–benzodiazepine receptor complex (Bureau and Olsen, 1991; Malminen and Kontro, 1987). Therefore, the increase of brain extracellular taurine concentrations after ethanol administration may contribute to the neurochemical and behavioral effects of ethanol. In agreement with this hypothesis, taurine administration was shown to alter several pharmacological and behavioral effects of ethanol (Aragon et al., 1992; Aragon and Amit, 1993; Boggan et al., 1978; Ferko, 1987; McBroom et al., 1986; Messiha, 1979). Particularly, taurine modulates ethanol-reinforcing and aversive effects (Quertemont et al., 1998b), suggesting a possible implication of taurine in ethanol consumption. A previous microdialysis study also shows that the magnitude and duration of ethanol-induced taurine release is related to genetic differences in ethanol preference and aversion (Quertemont et al., 2000). Sardinian ethanol-preferring rats genetically selected for ethanol preference showed a reduced ethanol-induced taurine release in comparison to ethanol nonpreferring rats. However, the Sardinian ethanol-preferring and nonpreferring rats that were used in this later study, display differential sensitivities to ethanol in addition to their differences in ethanol preference (Colombo et al., 2000). Therefore, it remains uncertain whether ethanol-induced taurine release is primarily related to either ethanol preference or sensitivity.

In the present study, high-alcohol sensitive (HAS) and low-alcohol sensitive (LAS) rats were used to test whether the effects of acute ethanol on brain extracellular concentrations of both glutamate and taurine might be related to genetic differences in ethanol sensitivities. HAS and LAS rats from the University of Colorado Health Sciences Center were genetically selected according to their differential sensitivities to the hypnotic effects of acute ethanol. Indeed, the administration of high ethanol doses induces a significantly longer sleep time in HAS rats than in LAS rats (Hansen and Spuhler, 1984; Draski et al., 1992). In the present experiment, an in vivo brain microdialysis was performed in the nucleus accumbens of nonanaesthetized HAS and LAS rats and a range of acute ethanol doses (1.0, 2.0 and 3.0 g/kg) were tested. The nucleus accumbens was chosen for two reasons. Firstly, biphasic effects of ethanol on glutamate concentrations were reported in a previous microdialysis experiment within the nucleus accumbens (Moghaddam and Bolinao, 1994). Secondly, although increases in extracellular taurine concentrations after acute ethanol injections were shown in various rat brain regions (Dahchour and De Witte, 2000), this ethanol effect was mainly studied within the nucleus accumbens (Dahchour et al., 1994, 1996, 2000; Quertemont et al., 2000, 2001), allowing reliable comparisons with other rat lines and strains.

2. Materials and methods

2.1. Animals

From two replicated lines, 28 HAS and 28 LAS male rats, weighing 250–300 g at the time of the study, were randomly divided into four groups of seven rats from each line. All animals were housed in standard individual plastic cages in a temperature- and light-controlled environment (light/dark cycle: 12 h light:12 h dark) with food and water available ad libitum.

All experiments and procedures were carried out according to the European Communities Council Directive (86/609/EEC) for care and use of laboratory animals.

2.2. Surgery

The rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic apparatus. A guide cannula (20 gauge stainless steel; Small Parts, Miami, FL) was implanted with the lower end above the left nucleus accumbens, 1.2 mm anterior to bregma, 1.2 mm lateral to bregma and 5.7 mm ventral from the skull. The cannula was secured to the skull with stainless steel screws and dental cement. The guide cannula was kept patent with a cannula obturator. After the surgery, the rats were allowed a post-operation recovery period for at least 3 days.

2.3. Brain microdialysis

Microdialysis probes were constructed according to the method of Robinson and Whishaw (1988). When inserted into the guide cannula, the dialysis membrane (200 μm i.d., 13 000 MW cutoff; Spectrum, CA) extended 2 mm beyond the tip of the guide cannula. The probe was connected to a microinfusion pump (Infusion syringe pump 22, Harvard apparatus), continuously perfused at 1.0 μl/min with artificial cerebrospinal fluid (147 mM NaCl, 4.0 mM KCl, 1.2 mM $CaCl_2$, pH 7.0).

Perfusates were collected every 20 min in microcentrifuge tubes connected to the outlet cannula. After a recovery period of 30 min following the probe insertion, six consecutive samples were collected at 20-min intervals. Rats were then injected i.p. with either 0.9% saline or ethanol (1.0, 2.0

or 3.0 g/kg body weight). Ethanol solutions, 15% v/v, were prepared by mixing ethanol (Merck, Darmstadt, Germany) with 0.9% saline. Immediately after ethanol administration, dialysis samples were collected at 20-min intervals for a further 240-min period. Each microdialysis procedure was begun at about 10 a.m.

2.4. Simultaneous determination of amino acids

The concentration of amino acids was determined using a high performance liquid chromatography (HPLC) with electrochemical detection following precolumn *O*-phtaldialdehyde derivatisation. *O*-phtaldialdehyde, 27 mg, was dissolved in 1-ml methanol HPLC-grade with 10 μl ß-mercaptoethanol and then diluted with 9 ml of 0.1 M sodium tetraborate buffer, pH 9.3, and stored at 4 °C. The working solution was prepared each day, 24 h before use, by diluting 1 ml of the above solution in 3 ml of 0.1 M sodium tetraborate. For the analysis, 15 μl of the extracellular perfusate was pipetted into a glass vial, sealed and placed within the autosampler. The internal standard, homoserine, 10^{-5} M, 10 μl, was added to each sample immediately prior to derivatisation by the autosampler, left for 2 min, before automatic injection onto the HPLC column. The HPLC system consisted of a LDC Consta Metric 3200 pump delivering 0.75 ml/min of the mobile phase at a pressure of 4500 psi. Separation of the amino acids was achieved by reversed phase column (100 × 3.2 mm Biophase-II, ODS 3 μm) and detected coulometrically (ESA, Bedford, MA, USA) using three electrodes, guard (0.4 V), preoxidation (−0.4 V) and working (+0.6 V) electrodes (analytical cell ESA Model 5011). The mobile phase (0.1 M Na_2HPO_4; 0.134 mM EDTA; 32% of methanol, HPLC grade, 68% Millipore H_2O, pH 6.4) was filtered through 0.2 μm cellulose acetate filter (Gelman Sciences, Ann Arbor, MI, USA) and degassed under vacuum before use in the HPLC system.

The position and height of peaks of the endogenous components were compared with a standard solution prepared from 10^{-3} M concentration of amino acids aspartate, glutamate, arginine, taurine and alanine in a solution of Millipore water and HPLC grade methanol (50:50 v/v). The working solution was prepared each day by diluting 10^{-3} to 10^{-6} M in Ringer's solution and 15 μl samples of this solution was injected and quantified. All reagents were analytic grade and obtained from Sigma (St Louis, MO). The heights and areas of the peaks were quantified by PC Integration Pack (Kontron Instruments).

2.5. Histology and statistics

Upon completion of experiments, rats were killed and the brain fixed with 10% formalin. Coronal sections through the extent of the cannula tracks were cut (100 μm) with a vibratome (Polaron H 1200, BioRad, Cambridge, MA) and stained with 0.5% cresyl violet. Dialysis probe placement was localized according to the atlas of Paxinos and Watson (1982). Of the 56 rats used in the study, two were eliminated from the data analysis due to histological visualization of the microdialysis probe outside the nucleus accumbens.

For the presentation of the results and the calculation of statistical significances, the mean baseline level for each of the extracellular amino acids in the nucleus accumbens was calculated for each animal by averaging the concentration of the three sample values before injection. The variations in their extracellular concentrations in each perfusate sample were then expressed as percentages of baseline level and then analyzed by a three-way analysis of variance (ANOVA) (rat line × Dose × Time point) with repeated measures on the last factor (Time). When necessary, post-hoc analyses

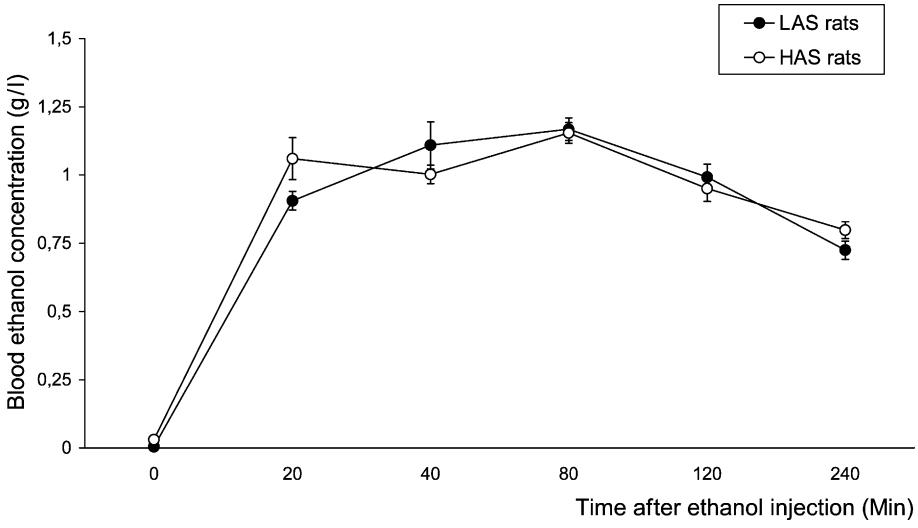

Fig. 1. Time course of the tail blood alcohol concentrations after the i.p. injection of 2.0 g/kg ethanol in LAS and HAS rats. Data are expressed as mean (± S.E.M.) in g/l.

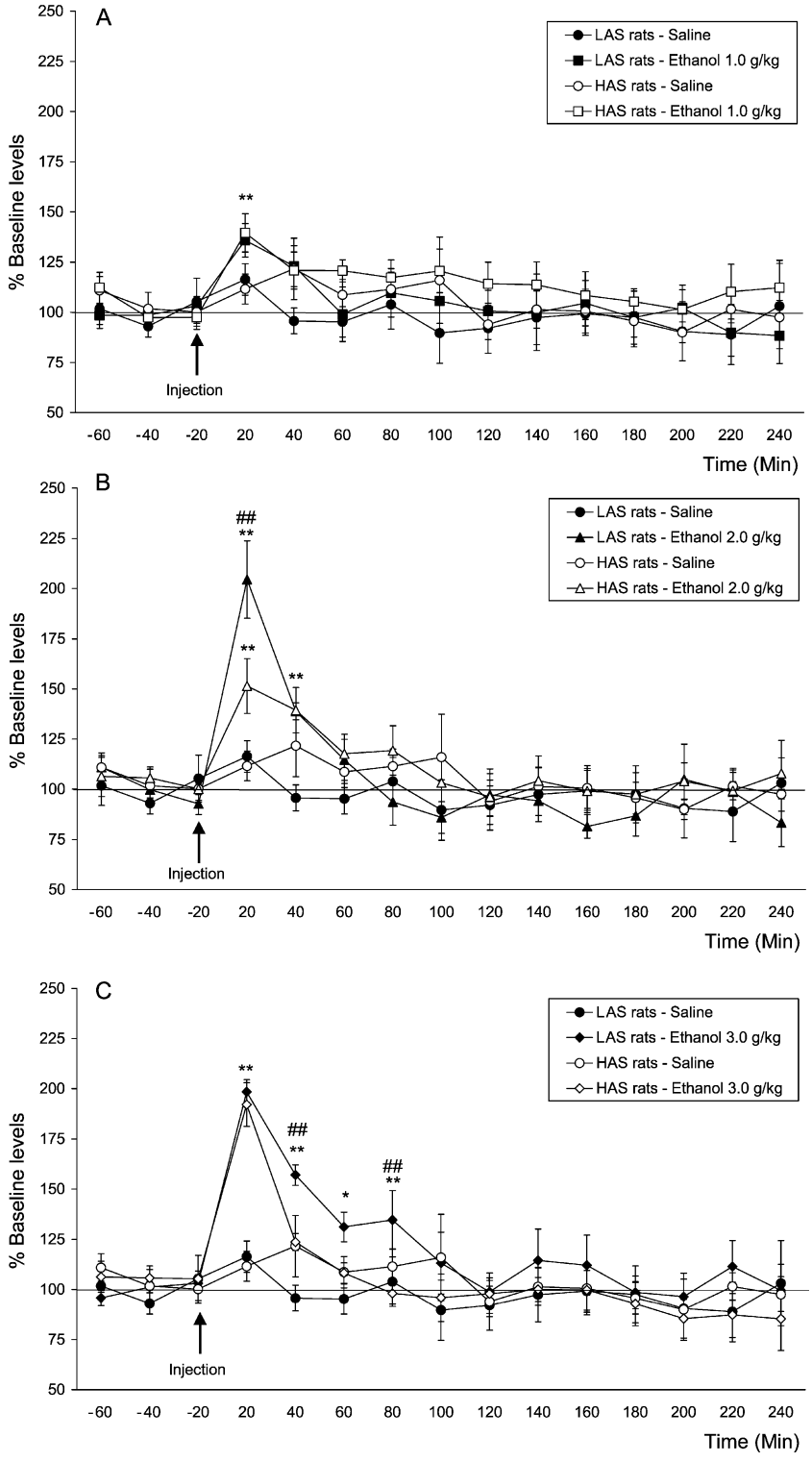

Fig. 2. Time course of extracellular taurine content of the microdialysate from the nucleus accumbens before and after an acute intraperitoneal injection of either saline or ethanol in LAS and HAS rats. The rats were injected with 1.0 g/kg (panel A), 2.0 g/kg (panel B) or 3.0 g/kg (panel C) ethanol. Data are expressed as mean (± S.E.M.) percentage of baseline level that was calculated for each rat by averaging the concentration of the three sample values before injection. * $P<0.05$ relative to the respective saline control group. ** $P<0.01$ relative to the respective saline control group. $^{\#\#}P<0.01$ relative to HAS rats injected with the same ethanol dose.

were conducted using Fisher least significant difference protected *t* test (GB-STAT, Dynamic Microsystems, Silver Spring, MD).

2.6. Blood ethanol level

In a separate experiment, 6 HAS and 6 LAS rats were injected i.p. with 2.0 g/kg ethanol. At 0, 20, 40, 80, 120 and 240 min, blood was collected from the caudal portion of each rat tail. The concentration of ethanol in the blood samples was assayed by the alcohol-dehydrogenase-based method of Bucher and Raz (Boerhinger Mannheim, Germany).

3. Results

3.1. Blood ethanol concentrations

After i.p. 2.0 g/kg ethanol injections, there was a rapid increase in blood ethanol concentration that reached a maximal level between 40 and 80 min (Fig. 1). The two-way ANOVA indicated no significant differences ($P > 0.05$) with regard to absorption, redistribution and elimination phases of ethanol metabolism between HAS and LAS rats throughout the 240-min experimental period.

3.2. Basal amino acid concentrations

Similar mean basal concentrations of alanine, arginine, aspartate, glutamate and taurine were found in the nucleus accumbens microdialysates from HAS and LAS rats ($P>0.05$). The concentrations for each assayed amino acid in the microdialysates were: 1.78 ± 0.25 and 1.48 ± 0.20 μM alanine, 0.33 ± 0.09 and 0.26 ± 0.04 μM arginine, 0.11 ± 0.03 and 0.10 ± 0.02 μM aspartate, 0.40 ± 0.09 and 0.33 ± 0.04 μM glutamate, 1.05 ± 0.16 and 0.93 ± 0.15 μM taurine for HAS and LAS rats, respectively. These values are the mean ± S.E.M. of the basal concentrations in the microdialysates (mean of the three samples collected before injections) of all animals.

3.3. Effects of acute ethanol injections on extracellular taurine concentrations

The three-way ANOVA calculated from the percentage changes from taurine baseline levels showed no significant main effects for either rat line [$F(1,52) = 0.16$, $P>0.05$] or ethanol dose [$F(3,52) = 0.95$, $P>0.05$]. However, there was a significant main effect for time [$F(14,728) = 34.13$, $P < 0.0001$] and significant interactions Dose × Time [$F(42,728) = 4.12$, $P < 0.0001$] and Line × Dose × Time [$F(42,728) = 1.64$, $P < 0.01$].

After saline administration, there were no significant ($P>0.05$) changes in taurine microdialysate content in either HAS or LAS rats. However, acute ethanol injections induced significant dose-dependent increases in extracellular taurine concentrations in both rat lines. After 1.0 g/kg ethanol, there was a significant ($P < 0.01$) but transient increase in taurine microdialysate content in both rat lines (Fig. 2A). At 2.0 g/kg, ethanol induced a higher taurine increase that remained significant by comparison to the saline control groups for 40 min after injection (Fig. 2B). Furthermore, this increase in taurine microdialysate content was significantly higher in LAS rats than in HAS rats at 20 min after injection. After 3.0 g/kg ethanol, there was a rapid

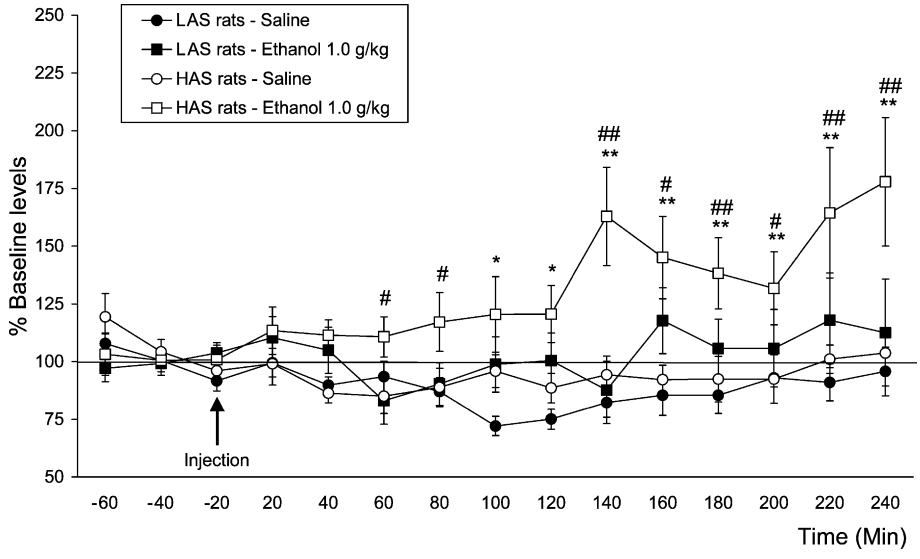

Fig. 3. Time course of extracellular glutamate content of the microdialysate from the nucleus accumbens before and after an acute intraperitoneal injection of either saline or 1.0 g/kg ethanol in LAS and HAS rats. Data are expressed as mean (± S.E.M.) percentage of baseline level that was calculated for each rat by averaging the concentration of the three sample values before injection. * $P < 0.05$ relative to saline control group. ** $P < 0.01$ relative to saline control group. $^{\#}P < 0.05$ relative to LAS 1.0 g/kg ethanol group. $^{\#\#}P < 0.01$ relative to LAS 1.0 g/kg ethanol group.

increase in taurine microdialysate content in both rat lines (Fig. 2C). While taurine quickly returned to baseline levels in HAS rats, it remained significantly elevated for 80 min in LAS rats. The concentration of taurine was significantly ($P < 0.01$) higher in LAS rats relative to HAS rats at 40 and 80 min.

3.4. Effects of acute ethanol injections on extracellular glutamate concentrations

The three-way ANOVA calculated from the percentage changes from glutamate baseline levels showed no significant main effect for rat line [$F(1,52) = 2.62$, $P > 0.05$], but significant main effects for both ethanol dose [$F(3,52) = 5.24$, $P < 0.01$] and time [$F(14,728) = 7.28$, $P < 0.0001$]. In addition, significant interactions were found for both Line × Time [$F(14,728) = 2.04$, $P < 0.05$] and Dose × Time [$F(42,728) = 2.54$, $P < 0.0001$]. No other interaction was significant at the level $P < 0.05$.

While 2.0 and 3.0 g/kg ethanol administrations failed to alter glutamate concentrations in either LAS or HAS rats (data not shown), the injection of 1.0 g/kg ethanol induced a significant increase in glutamate microdialysate contents from HAS rats (Fig. 3). The glutamate microdialysate contents from the nucleus accumbens of HAS rats were significantly elevated by comparison to the saline control group from 100 to 240 min. By contrast, 1.0 g/kg ethanol failed to alter glutamate concentrations in LAS rats. As a result, glutamate microdialysate contents after the injection of 1.0 g/kg ethanol were significantly higher in HAS rats relative to LAS rats at 60, 80, 140, 160, 180, 200, 220 and 240 min.

3.5. Effects of acute ethanol injections on the extracellular concentrations of other amino acids

The acute i.p. injection of either saline or ethanol (1.0, 2.0 or 3.0 g/kg) failed to modify the extracellular concentrations of aspartate, arginine and alanine in the microdialysate from the nucleus accumbens of either HAS or LAS rats (data not shown).

4. Discussion

The results of the present study confirm the effects of acute ethanol on brain extracellular taurine concentrations that were reported in previous microdialysis experiments (Dahchour et al., 1994, 1996; Quertemont et al., 1999, 2000). Acute ethanol at high doses induced an immediate and significant increase in extracellular taurine concentrations from various rat brain regions. This effect required ethanol doses of at least 1.0 g/kg. Indeed, smaller ethanol doses (e.g. 0.5 g/kg) usually failed to alter taurine concentrations. With increasing ethanol doses, the effects on taurine concentrations increased in magnitude until a maximal effect was generally found around 200% of the pre-injection basal levels (Quertemont et al., 1999). Once this threshold is reached, further increases in ethanol doses resulted in a prolonged effect with elevated taurine concentrations for up to 80 min after the injection of 3.0 g/kg ethanol. In addition, the results of the present study together with those of previous experiments (Dahchour et al., 1994, 1996; Quertemont et al., 1999, 2000) clearly showed the specificity of ethanol effects on brain extracellular taurine concentrations, since the concentrations of other amino acids, such as aspartate, glutamate, GABA and alanine, were not altered by acute ethanol injections.

Interestingly, the present study showed that HAS rats displayed a reduced ethanol-induced taurine release in comparison to LAS rats. At 2.0 g/kg ethanol, the magnitude of taurine response to ethanol was significantly reduced in HAS rats (151% of the basal levels) relative to LAS rats (204% of the basal levels). The levels of taurine in HAS rats barely reached the threshold of 200% of basal levels after 3.0 g/kg ethanol but quickly returned to baseline values at 40 min while taurine concentrations in LAS rats remained elevated for 80 min. Taurine responsiveness to ethanol was therefore inversely related to initial ethanol sensitivity in these genetically selected rat lines. This result is unlikely to be due to pharmacokinetic differences between HAS and LAS rats in ethanol absorption, distribution and elimination. Indeed, the present experiment together with previous published data showed no differences in blood ethanol concentrations in these two lines of rats (Dahchour et al., 2000; Draski et al., 1992).

Several biological functions have been attributed to taurine within the brain. Taurine is believed to play a role in various processes such as osmoregulation (Solis et al., 1988), neuromodulation (Billard, 1990; Wang et al., 1998), modulation of ion permeability (El Idrissi and Trenkner, 1999) and antioxidation (Wright et al., 1986). The common feature of all these functions resides in the regulation of adverse effects to brain cells and, indeed, taurine was shown to be released by brain cells subsequent to various cellular stresses (Huxtable, 1989). In a similar way, taurine may contribute to the reduction of several ethanol adverse effects and this may explain why high-alcohol sensitive rats show a reduced release of this regulatory amino acid following ethanol administration. On the contrary, in low-alcohol sensitive rats, a higher taurine release may oppose some of the adverse effects of ethanol, thereby contributing to their overall lower sensitivity to ethanol. In agreement with this explanation, taurine administration was shown to reduce several behavioral effects of ethanol (Aragon et al., 1992; Aragon and Amit, 1993; Quertemont et al., 1998b), including its hypnotic effects (Boggan et al., 1978). However, there are considerable discrepancies between the behavioral studies on the relationship between taurine and ethanol effects. Some of these experiments even showed that taurine administration enhanced ethanol effects (Ferko, 1987). To date, these discrepancies remain unexplained, although both

the interval between taurine and ethanol administrations and the route of taurine administration seem to play an important role (McBroom et al., 1986). Furthermore, taurine seemed to antagonize specifically the adverse effects of high ethanol doses, while it tended to enhance the effects of small ethanol doses (Aragon et al., 1992; Aragon and Amit, 1993; Quertemont et al., 1998b).

The effects of ethanol on brain extracellular taurine concentrations were previously investigated in two microdialysis studies with genetically selected lines of rats. In the first study, a similar intracerebral microdialysis procedure was undertaken on anaesthetized HAS and LAS rats, but quite different results from the present experiment were obtained (Dahchour et al., 2000). However, the use of anaesthetics during a microdialysis procedure was shown to alter both the basal levels and the drug responsiveness of various neurotransmitters, including amino acids (Rozza et al., 2000). Furthermore, LAS and HAS rats display differential sensitivities to many anaesthetics (e.g. Deitrich et al., 1994). It is therefore very difficult to draw any conclusion from a microdialysis experiment undertaken on anaesthetized LAS and HAS rats. Therefore, the present results are physiologically more valid for the study of ethanol effects on brain extracellular amino acids in HAS and LAS rats. In another brain microdialysis study, Sardinian ethanol-preferring (sP) and nonpreferring (sNP) rats displayed similar differences in extracellular taurine responsiveness to ethanol (Quertemont et al., 2000). Indeed, acute ethanol induced a higher increase in extracellular taurine concentrations in sNP rats relative to sP rats. However, since sP rats also showed a higher initial sensitivity to the motor impairing and sedative effects of ethanol (Colombo et al., 2000), it remained unknown whether ethanol's effects on taurine were primarily correlated with either ethanol aversion or ethanol resistance. Altogether, the results of this later study and the present experiment suggest that ethanol-induced taurine release is ultimately related to ethanol resistance. Indeed, in both studies, the less ethanol sensitive rats—sNP and LAS rats—showed the higher increases in brain extracellular taurine concentrations after acute ethanol administrations.

There were considerable discrepancies between microdialysis studies about the effects of ethanol on extracellular glutamate concentrations, although the majority of these studies failed to show any effect of ethanol on glutamate concentrations (Dahchour et al., 1994, 1996; Selim and Bradberry, 1996; Quertemont et al., 1998a, 1999, 2000). This lack of effect was confirmed in the present study with high ethanol doses (2.0 and 3.0 g/kg). However, 1.0 g/kg ethanol injections induced significant increases in extracellular glutamate concentrations in HAS rats but not in LAS rats. A similar effect on extracellular glutamate concentrations was observed in a previous microdialysis experiment with nonselected rats, but with a smaller ethanol dose (Moghaddam and Bolinao, 1994). The present results suggest that these changes in glutamate concentrations after ethanol administration may be related to the initial ethanol sensitivities of various rat strains and lines although the mechanisms explaining such an effect remain undefined. Clearly, further studies will be required to confirm the relationship between ethanol sensitivity and changes in extracellular glutamate concentrations and to investigate the role of extracellular glutamate in the mediation of ethanol behavioral effects.

Acknowledgements

This work was supported by the Fonds de la Recherche Scientifique et Médicale (1997–2001), the Belgian National Funds for Scientific Research (to Etienne Quertemont), l'Institut de Recherches Economiques sur les Boissons (IREB) and sponsored by LIPHA. The authors wish to thank Dr. Françoise Beaugé for providing the rats.

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