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Taurine Blocks the Glutamate Increase in the Nucleus Accumbens Microdialysate of Ethanol-Dependent Rats

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DAHCHOUR, A. AND P. DE WITTE. *Taurine blocks the glutamate increase in the nucleus accumbens microdialysate of ethanol-dependent rats.* PHARMACOL BIOCHEM BEHAV **65**(2) 345–350, 2000.—During ethanol withdrawal, dramatic changes in the concentration of many neurotransmitters may be responsible for many of the adverse effects. In the present study, the technique of microdialysis was used to assay the changes in excitatory and inhibitory amino acids after withdrawal from chronic ethanol intoxication. Rats were made physically dependent on ethanol by vapor inhalation for 4 weeks. The basal concentrations of both arginine and GABA were significantly decreased in ethanol-dependent rats, although there were no significant changes in any of the other amino acid basal concentration assayed (i.e., glutamate and taurine). During the first 12 h after withdrawal from ethanol, only glutamate increased significantly ($p < 0.05$) at 6 h, and for the duration of the study period of 12 h. To investigate whether either taurine and ethanol interact with amino acids during ethanol withdrawal, two other ethanol-dependent groups were injected with a single intraperitoneal injection of either taurine or ethanol 5 h after commencement of ethanol withdrawal. The IP injection of ethanol (2 g/kg) significantly increased taurine microdialysate content, and although this dose of ethanol was not able to block completely the increase of glutamate release after ethanol withdrawal, a delayed decrease in glutamate content was observed by the end of the period of the study (i.e., 11–12 h). However, IP injection of taurine (45 mg/kg) significantly blocked the increased glutamate release during ethanol withdrawal. This latter finding suggests that taurine may interact with glutamate, possibly by inducing a blockade of glutamate release during ethanol withdrawal. © 2000 Elsevier Science Inc.

Ethanol Ethanol withdrawal Taurine Nucleus accumbens Glutamate Microdialysis

DRAMATIC changes in many of the brain neurotransmitters occur during chronic alcohol exposure and its withdrawal. Such changes could be responsible for much of the neurotoxicity and the neuronal death observed in brains of alcoholics (4,5) and experimentally ethanol-intoxicated animals (41,44). Although the mechanisms underlying this neuropathology remain unclear, numerous studies have shown that glutamate is implicated in both hyperexcitability and seizures that are often observed during ethanol withdrawal both in vitro (15,38) and in vivo (37).

Glutamic acid (glutamate) is the major excitatory amino acid in the central nervous system. This neurotransmitter has been reported to play an important role in alcoholism (42,43). Recently, particular interest has been directed towards the effect of ethanol on both *N*-methyl-D-aspartate (NMDA) receptors and calcium channels. a synergistic effect of activation of both NMDA and calcium channels would contribute to the hyperexcitability observed in the ethanol withdrawal state (17,46). When administered acutely, ethanol doses of between 5 and 250 mM acted as a potent inhibitor of NMDA receptors (3,18,26,27). Following repeated exposure to ethanol, the number of NMDA receptors increased, caused by a positive feed-back mechanism to the inhibitory effect of acute ethanol (14). Such upregulation of NMDA receptor numbers may be responsible, at least in part, for the increase of glutamate release during the initial periods of ethanol withdrawal syndrome, which can be attenuated by the administration of NMDA receptor antagonists (16,17).

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Numerous studies have reported that acute ethanol inhibits its neuronal calcium channels (31). In contrast, chronic exposure to ethanol upregulates the density of these voltage-operated calcium channels as a consequence of tolerance to the inhibitory effect of ethanol (29,31). Calcium channel antagonists also have been shown to diminish the alcohol withdrawal syndrome (6,24,40).

Taurine, a sulfonated amino acid, plays a neuromodulatory role in combating many of the adverse effects of ethanol, such as decreasing ethanol-induced sleep time (30), and altering ethanol-induced conditioned taste aversion (1). We previously reported an increase of taurine in the nucleus accumbens after acute intraperitoneal injection of ethanol (8), and were, therefore, interested in studying whether acute taurine administration during the ethanol-withdrawal phase might have a modulatory effect on the release of the neuroexcitatory amino acid glutamate.

Changes in both excitatory and inhibitory amino acids were assayed in the nucleus accumbens of freely moving male rats during the first 12 h after withdrawal from chronic ethanol administration by the ethanol vapor technique. The nucleus accumbens receives direct glutamatergic connections from the frontal cortex and hippocampus, and plays an important role in mediating the reinforcing effects of a large number of drugs including ethanol (20,33). The effect of an acute injection of either taurine or ethanol on the amino acid microdialysate content 5 h after the commencement of ethanol withdrawal, when glutamate release is enhanced, was assayed to evaluate the possible interaction between glutamate release and ethanol or taurine to gain an insight into the possible therapeutic use of taurine in the treatment of chronic alcoholism.

METHOD

Animals and Surgery

Four groups of male Wistar rats (250–300 g) were individually housed in plastic cages and kept in a temperature- and light-controlled environment (light/darkness cycle: 12 L:12 D cycle: from 0800 to 2000 h). Three groups were maintained in an isolated plastic chamber (160 × 60 × 60 cm) in an alcohol-containing atmosphere with a mixture of alcohol and air pulsed into the chamber via a mixing system, allowing the quantity of alcohol to be increased every 2 days during the whole experimental procedure for 4 weeks (22). The fourth group, the controls, received only air for the same period while in an isolated plastic chamber. During the third week, the rats were removed from the chamber for surgery. Chloral hydrate (400 mg/kg IP) was used as an anesthetic, and a guide cannula (20-gauge stainless steel; Small Parts, Miami, FL) was implanted 1 mm above the nucleus accumbens (A/P 1.2 mm; M/L 1.2 mm; D/V −5.7 mm) (35), using standard stereotaxic techniques. The cannula was secured to the skull with three steel screws and cranioplastic cement. The rats were allowed to recover, and then returned to the appropriate environment within the chambers for a further week.

After 4 weeks of chronic ethanol administration or air inhalation, the microdialysis experiments commenced by inserting the probe into the nucleus accumbens. During the microdialysis experiments, the first ethanol-dependent group and control group did not receive any treatment, while the second and third ethanol-dependent groups received either an IP injection of ethanol (2 g/kg/ 15% ethanol solution prepared in saline) or an IP injection of taurine (45 mg/kg, prepared in saline) 5 h after the commencement of ethanol withdrawal. Samples were collected each 20 min during the first 12 h after chronic alcohol cessation, and were analyzed by HPLC with electrochemical detection and ophtaldialdehyde β mercaptoethanol (OPA/BME) per column derivatization. These experiments were approved by the Belgian governmental agency under the authorized number LA 1220028 as well as the European Communities Council Directive concerning the Use of Laboratory Animals.

Brain Microdialysis Procedure

The dialysis probes were constructed as described by Robinson and Whishaw (36). Dialysis tubing extended 3 mm beyond the tip of the probe. The probe was connected to a microinfusion pump (Infusion syringe pump 22, Harvard apparatus, Southnatick, MA, continuously perfused at 1 μl/min with a Ringer's solution (145 mM NaCl, 4 mM KCl; 1.3 mM $CaCl_2$, pH 7.2). Perfusates were collected every 20 min in microcentrifuge tubes connected to the outlet cannula. Recovery of each probe was assayed by continuously perfusing the probe with a standard solution containing 5×10^{-6} amino mixture for 5 h. Samples were collected every 20 min, and the amino acid concentration in the outflow was assayed by HPLC analysis and the relative concentration calculated as

$$\text{Recovery in vitro} = C_{out}/C_{int}$$

The mean in vitro recovery for the microdialysis probes for each amino acid was 13.72% ± 1.42 for glutamate, 36.00% ± 4.15 for arginine, 27.10% ± 1.65 for taurine, and 9.34% ± 0.8 for GABA.

Simultaneous Determination of the Excitatory and Inhibitory Amino Acids

The concentration of each amino acid was determined by HPLC-EC following precolumn *o*-phtaldialdehyde (OPA) derivatization. The OPA, 27 mg, was dissolved in 1 ml methanol HPLC grade, to which 10 μl β-mercaptoethanol (BME) was added; this solution was diluted with 9 ml of 0.1 M sodium tetraborate buffer, pH 9.3, and stored at 4° C. The work-

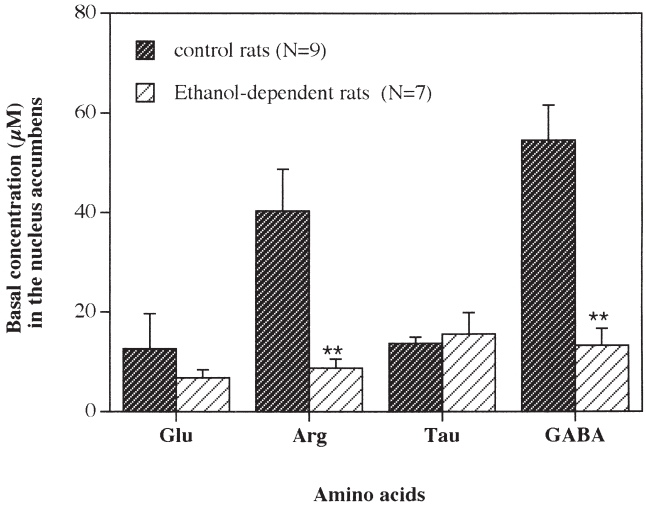

FIG. 1. Comparison between the basal concentrations of glutamate, arginine, taurine, and GABA in the microdialysate from the nucleus accumbens of control and chronically pulmonary ethanol-dependent rats. Data are represented as μM ± SEM, and corrected for in vitro recovery of microdialysis probe. $^{**}p < 0.01$ relative to control group.

ing 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. The derivatization procedure entailed mixing 20 μl of dialysate and 10 μl of 5 μM homoserine as internal standard with 10 μl OPA/BME for 2 min in complete darkness before injection into the HPLC system. This system consisted of a LDC Consta Metric 3200 pump (Riviera Beach, FL) delivering 1 ml/min of the mobile phase (0.1 M Na_2HPO_4; 0.13 mM Na_2EDTA; 32% of methanol HPLC grade; 68% MilliQ H_2O; pH 6.4), which had been filtered through a 0.2-μm cellulose nitrate filter (Gelman Sciences, Ann Arbor, MI) and degassed under vacuum, before use in the HPLC system. Separation of amino acids was achieved with reversed-phase column (100 × 3.2 mm Biophase-II, ODS 3 μm) and detected coulometrically (ESA II, Inc., Bedford, MA) using three electrodes: a guard (0.4 V), preoxidation (−0.4 V) and working (+0.6 V) electrode (Analytical cell ESA Model 5011) (11).

Histology and Statistical Analysis

Upon completion of the experiments, the 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 (35).

Presentation of Data and Statistical Analysis

Data were represented as means ±SEM and analyzed by analysis of variance (ANOVA) with repeated measures on one factor followed by the Fisher least-significant difference test of multiple comparison (Protected *t*-test) to determine statistical significance between treatment and control values (GB-STAT, Dynamic Microsystems, Silver Spring, MD).

RESULTS

Amino Acid Basal Concentration in the Nucleus Accumbens

The basal concentration of each amino acid, following correction for in vitro recovery, is shown in Fig. 1. Both arginine and GABA basal concentrations were significantly decreased after 4 weeks of chronic alcohol intoxication ($p < 0.01$; *t*-test) compared to the control group. No significant changes were detected in the basal concentration of either glutamate of taurine in these two groups.

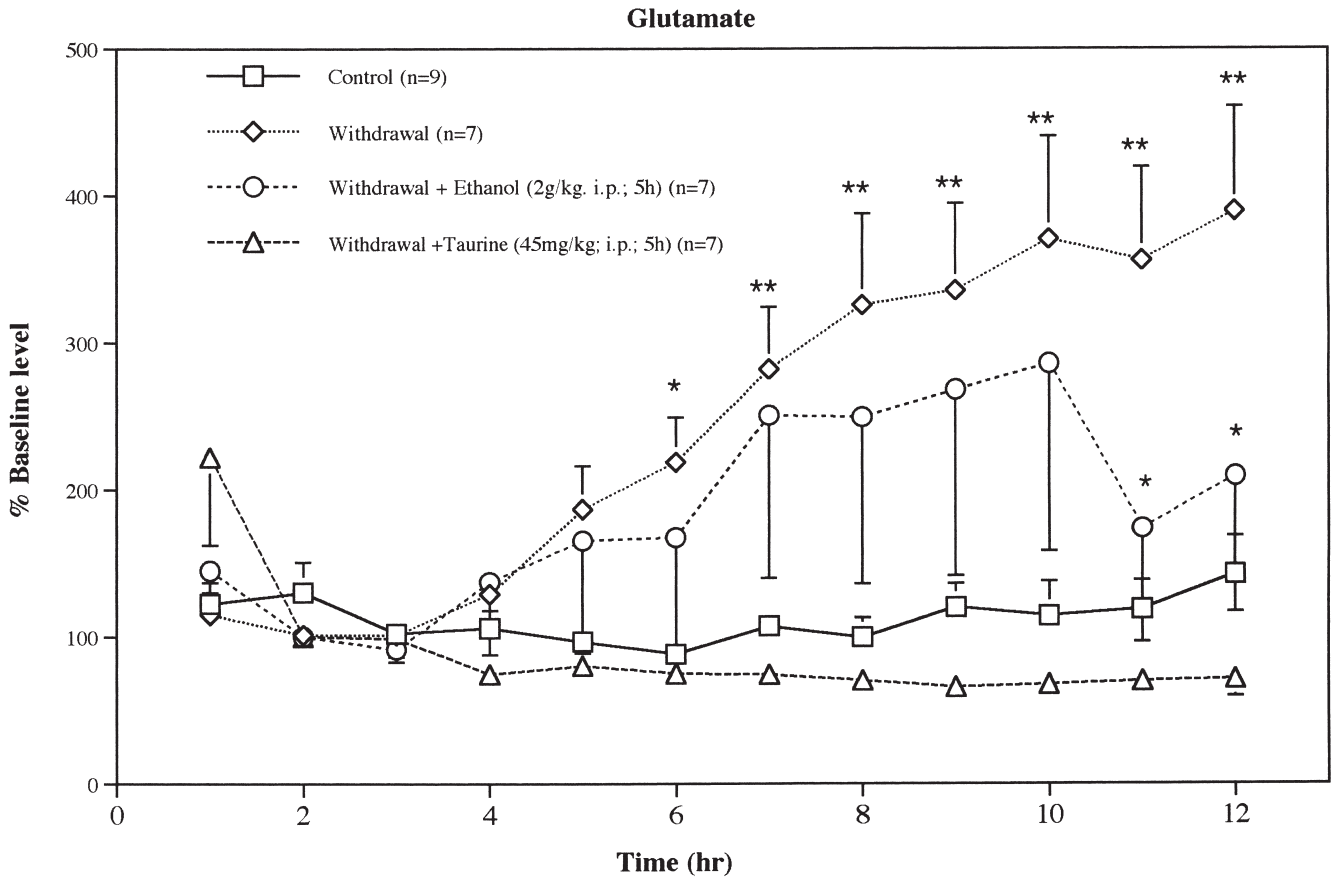

FIG. 2. Changes in nucleus accumbens glutamate microdialysate content expressed as % of the baseline value during the first 12 h after withdrawal from chronic ethanol treatment. The "control" rats received only air for 4 weeks, and during the microdialysis experiment (12 h) received no further treatment. The "withdrawal" rats received ethanol by vapor inhalation for 4 weeks and during the microdialysis experiment (12 h) received no further treatment. The "withdrawal × ethanol" rats received ethanol by vapor inhalation for 4 weeks and during the microdialysis experiment were injected with an IP injection of ethanol (2 g/kg) at 5 h after ethanol withdrawal. The "withdrawal + taurine" rats received ethanol by vapor inhalation for 4 weeks and during the microdialysis experiment (12 h) were injected IP with taurine (45 mg/kg, IP) at 5 h after withdrawal $*p < 0.05$; $**p < 0.01$ relative to control group.

Changes in the Release of Amino Acids in the Microdialysate of the Nucleus Accumbens During the First 12 h After Ethanol Withdrawal

Glutamate, arginine, taurine, and GABA were assayed in the nucleus accumbens microdialysate of the ethanol withdrawn rats ($n = 7$) and the control group ($n = 9$). At 6 h, the glutamate concentration had significantly increased ($p < 0.01$) and continued to increase, approximately fourfold, by the end of the period of the study (i.e., 12 h, Fig. 2). No significant changes in any of the other amino acids assayed were evident.

Effect of an Acute IP Injection of Ethanol (2 g/kg) on Amino Acid Microdialysate Content During Ethanol Withdrawal

Ethanol-dependent rats received an acute IP injection of ethanol 5 h after ethanol withdrawal. Taurine increased significantly 2 h after ethanol injection ($p < 0.01$) and for the duration of the study (Fig. 3). This ethanol dose also reduced the glutamate release during the withdrawal stage, between 20 and 40% between 7 and 10 h after ethanol withdrawal (Fig. 2) and almost completely at 11 and 12 h after ethanol withdrawal. There were no significant changes in any of the other amino acids assayed (i.e. arginine and GABA).

Effect of an Acute IP Injection of Taurine (45 mg/kg) on Amino Acid Microdialysate Content During Ethanol Withdrawal

Ethanol-dependent rats received an acute IP injection of taurine (45 mg/kg) 5 h after ethanol withdrawal. Taurine was able to block completely the glutamate release observed in the ethanol withdrawal phase (Fig. 2). However, no change in taurine microdialysate content was observed after its administration (Fig. 3). There were no significant changes in any of the other amino acid assayed (i.e., arginine and GABA).

DISCUSSION

In the present studies, an in vivo microdialysis technique was utilized to investigate changes in a number of amino acids in the NAC, including excitatory and inhibitory amino acid neurotransmitters, of freely moving male rat during the first 12 h after withdrawal from chronic alcohol treatment by the vapor technique.

The basal concentration of arginine in the nucleus accumbens microdialysate was significantly reduced after chronic ethanol treatment compared to the control group. L-Arginine is the substrate for nitric oxide, which may have many impor-

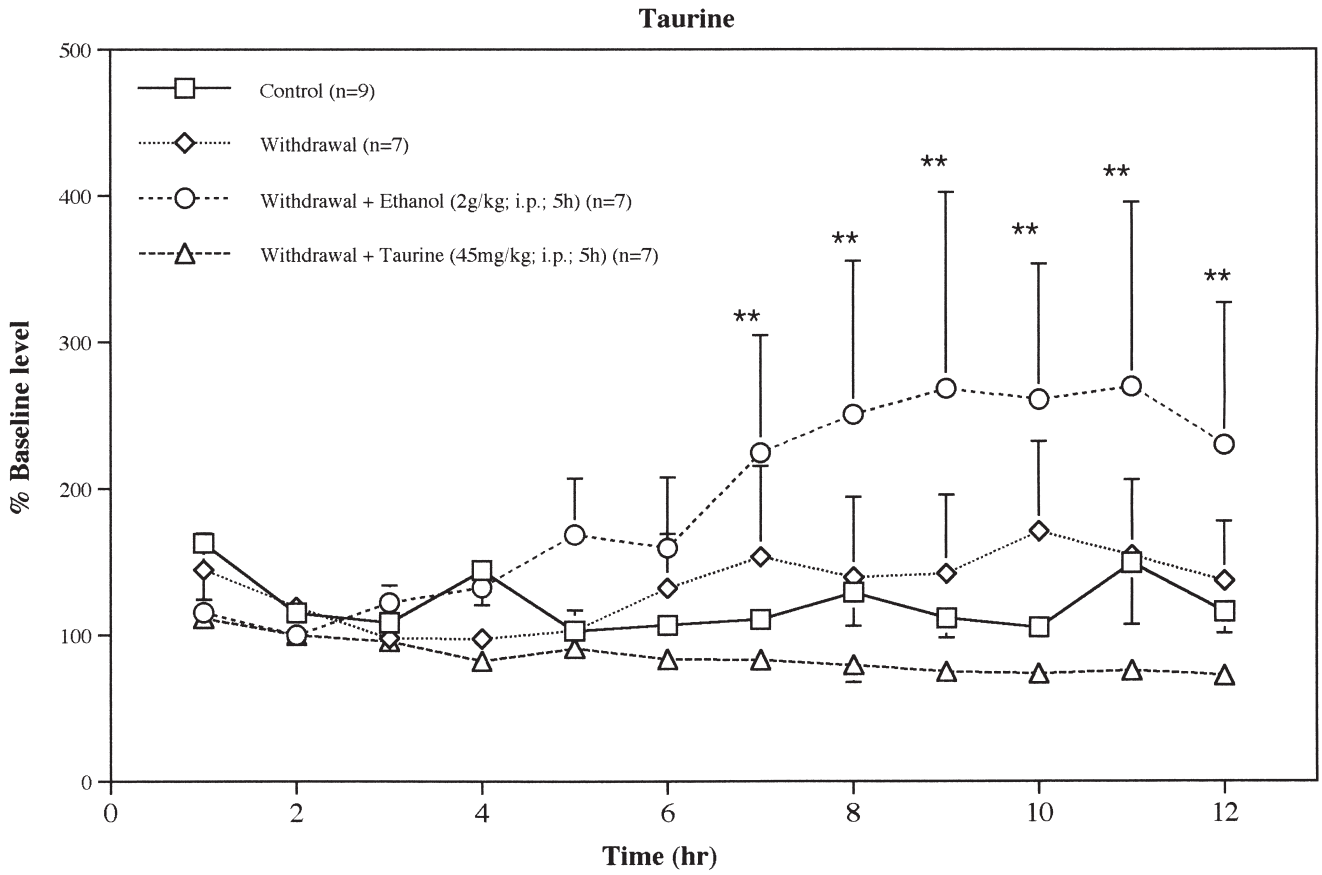

FIG. 3. Changes in nucleus accumbens taurine microdialysate content expressed as % of the baseline value during the first 12 h after withdrawal from chronic ethanol treatment. The "control" rats received only air for 4 weeks, and during the microdialysis experiment (12 h) received no further treatment. The "withdrawal" rats received ethanol by vapor inhalations for 4 weeks and during the microdialysis experiment (12 h) received no further treatment. The "withdrawal + ethanol" rats received ethanol by vapor inhalation for 4 weeks, and during the microdialysis experiment were injected IP with ethanol (2 g/kg) at 5 h after ethanol withdrawal. The "withdrawal + taurine" rats received ethanol by vapor inhalation for 4 weeks, and during the microdialysis experiment (12 h) were injected IP with taurine (45 mg/kg, IP) at 5 h after withdrawal. $^{**}p < 0.01$ relative to control group.

tant neurochemical roles. Its synthesis is controlled by the enzyme nitric oxide synthase together with various cofactors including calcium and calmodulin. Chronic ethanol treatment adversely affects the calcium-independent NOS enzyme (47), and also may influence the two other constituent forms of NOS present in the brain, nNOS and eNOS. Because calcium homeostasis is altered during the period of chronic ethanol treatment, NO production also may be altered (2). Furthermore, NMDA-receptor activation increases nitric oxide formation in vitro via the influx of calcium through NMDA channels (12), which also may be important during the early stages of withdrawal from chronic ethanol treatment, and contribute to excitotoxicity (10).

There is increasing evidence that gamma-aminobutyric acid (GABA) and its receptors are implicated in the development of ethanol dependence in rats. In this study, the GABA basal concentration in the nucleus accumbens microdialysate at the cessation of ethanol intoxication was significantly decreased compared to the control group. This result supports electrophysiological and biochemical data that chronic ethanol administration reduces GABA receptor function (32, 39). Such a decrease in GABA might play a role in the enhanced glutamate release assayed during ethanol withdrawal as well as the various adverse symptoms that are known to occur during this period. In clinical studies, the GABA plasma concentrations were lower in ethanol-dependent patients than control subjects (42), which may indicate a role for this inhibitory amino acid neurotransmitter in the ethanol-withdrawal syndrome and its associated neurotoxicity.

The increase of glutamate release during the initial ethanol withdrawal period can be explained in terms of the known adaptations that occur during chronic ethanol treatment. During chronic ethanol intoxication, the brain shows a multitude of cellular adaptations that include the activation of voltage-sensitive Ca^{2+} (28), as well as changes in the sensitivity of NMDA receptors (9), which causes enhanced release of glutamate from neuronal synapses, upregulation in vivo of both NMDA receptors (14), and dihydropyridine binding sites (13, 31). When chronic ethanol intoxication ceases, the ethanol-withdrawal syndrome commences with symptoms such as hyperexcitability, seizures, and convulsions, which will be present particularly during the initial stages of withdrawal, which corresponds to the increased release of glutamate. The activation of glutamate release may be a consequence of processes related to increased calcium released in neurons, which in turn, cause protein kinase C activation, free radical generation, and NO production. Such processes may activate glutamate release causing neurotoxicity during ethanol withdrawal. The excitatory amino acid plasma levels are increased in alcohol-dependent patients (42, 45) and show positive correlations with hepatic damage (45) and oxidative stress (42).

Acute ethanol administration 5 h after the commencement of withdrawal altered glutamate release during ethanol withdrawal but did not completely block glutamate release until 6 h after its administration. Higher doses of ethanol might be required to completely block the NMDA receptors that are upregulated during ethanol withdrawal. Indeed, Rossetti and Carboni (37) administered a higher acute oral dose of ethanol (5 g/kg) 12 h after withdrawal from chronic ethanol administration, and showed a faster diminution of glutamate release during the proceeding 8 h. However, there was also a reduction in glutamate release in the striatum of ethanol-dependent rats that had not received ethanol during this period. The explanation for these discrepant results may be related to the mode of chronic ethanol treatment (multiple ethanol injections vs. ethanol inhalation), the brain region (striatum vs. NAC), the different doses of ethanol (5 vs. 2 g/kg administered by different routes), and the time of the ethanol challenge (12 vs. 5 h after ethanol withdrawal).

In our previous studies (7, 8) we have shown that acute ethanol administration to naive rats caused a transitory increase in taurine NAC microdialysate content that was dose dependent and could be related to changes in osmolarity caused by ethanol injection and restored by taurine release. It was, therefore, noteworthy that a sustained increase in taurine was observed for 7 h after IP injection of ethanol 2 g/kg. Interestingly, when taurine was administered 5 h after the commencement of ethanol withdrawal, the increased glutamate release was totally modified, no significant change being observed in the concentration of this excitatory amino acid during the period of the study. It has been proposed that taurine may play an important role as an inhibitory neuromodulator in the CNS (25) modulating synaptic activities and stabilizing and regulating cell volume (19, 34). In addition, because taurine inhibits the depolarization of NMDA, kainate and quisqualate receptors (21), a prolonged inhibition of NMDA receptors may occur after its administration that contributed, in this present study, to the lack of change in glutamate content after ethanol withdrawal. Taurine also may act indirectly on glutamate release by stimulating calcium storage (23), in the reticulum endoplasmic and mitochondria, thereby inhibiting the feedback calcium-dependent processes to inhibit the glutamate release.

This study clearly showed that glutamate is directly implicated in ethanol withdrawal. The increased glutamate release after ethanol withdrawal as well as the decreased GABA basal concentration may induce further brain damage by activating the NMDA receptors and increasing the intraneuronal release of calcium. Acute taurine injection during the ethanol withdrawal stage was able to antagonize the glutamate overactivity during ethanol withdrawal. Such an interaction of taurine with glutamate may well be of interest in pursuing, as this sulphonated amino acid may have therapeutic properties that could be of use in the treatment of the adverse effects of glutamate during ethanol withdrawal.

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