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Behavioral consequences of the hypotaurine–ethanol interaction

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Abstract

In order to evaluate the effect of hypotaurine on ethanol-induced locomotion, different groups of mice received an injection of saline or 5.62, 8.45, 11.25, 16.87 or 33.75 mg/kg of hypotaurine 30 min prior to administering ethanol (2.4 g/kg). The duration of the effect of hypotaurine was explored by treating animals with ethanol 0, 30, 60 and 90 min after hypotaurine pretreatment. The effect of hypotaurine on acute stimulating ethanol locomotion was evaluated by pretreating animals with saline or 11.25 mg/kg of hypotaurine 30 or 60 min before ethanol (1.6, 2.4, 3.2 g/kg) or saline injections. Hypotaurine (11.25 mg/kg) required 30 min to boost, specifically ethanol-stimulated locomotion (2.4 g/kg). These results suggest a central locus for the interaction, firstly, because blood ethanol levels were not different between hypotaurine and saline pretreated mice, and, secondly, because a cotreatment with β-alanine (22 mg/kg), a β-amino acid that counteracts the transfer of hypotaurine across the blood–brain barrier (BBB), prevented the enhancement in ethanol-induced locomotion produced by hypotaurine.

Keywords: Hypotaurine; Ethanol; β-Alanine; Locomotor activity; Open field; Blood ethanol levels

1. Introduction

Hypotaurine is synthesized in vivo from L-cysteine in a two-step metabolic process (Brand et al., 1998). Firstly, oxidation of L-cysteine to cysteinesulfinic acid occurs by means of cysteine dioxygenase (Ewetz and Sörbo, 1966; Weinstein et al., 1988). Secondly, cysteinesulfinic acid may decarboxylate by cysteinesulfinic acid decarboxylase to hypotaurine (Kontro and Oja, 1980), which is further oxidized to taurine (Cavallini et al., 1954; Eldjarn et al., 1956; Fujiwara et al., 1995). Although the hypotaurine oxidation has not yet been fully described, both enzymatic and nonenzymatic pathways have been proposed (Fujiwara et al., 1995). In the CNS, taurine is then converted to isethionic acid (Collins, 1974; Wright et al., 1986).

Interestingly, all of these metabolic-related sulfur compounds interact with ethanol. Thus, L-cysteine increased the ethanol-induced locomotion in mice (Miquel and Aragon, 2000), and this effect was also shared by taurine (Aragon et al., 1992) and isethionic acid (Miquel et al., 1999). Besides, isethionic acid potentiated the typical locomotor depression induced by ethanol in rats (Miquel et al., 1999). Moreover, some laboratories have shown that taurine (Boggan et al., 1978; Iida and Hikichi, 1976; McBroom et al., 1986) and isethionic acid (Iida and Hikichi, 1976), in an intraperitoneal injection, decreased the duration of narcosis produced by a high dose of ethanol. L-cysteine, however, was not able to alter this ethanol effect (Messiha, 1979).

In addition, it has been suggested (McBroom et al., 1986) that the intraperitoneal administration of taurine could produce a peripheral rather than a central interaction with ethanol. These authors have shown that pretreating animals intraperitoneally with this amino acid 4 or 5 days before ethanol administration produced an enhancement in ethanol-induced sleeping time. This effect is also observed when animals were treated with taurine intracerebroventricularly (McBroom et al., 1986). Therefore, they suggested that the difference between both administration methods (ip vs. icv) might be due to a slow transport of taurine through the blood–brain barrier (BBB). Nonetheless, a recent study has found taurine in the brain as soon as 10 s following a parenteral infusion through the carotid artery (Benrabh et al., 1995). Similarly, other authors reported taurine uptake across the BBB 30 s after the infusion (Tamai et al., 1995). Moreover, taurine has been detected in the parietal cortex,

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striatum, olfactory bulb and hippocampus 60 s following administration (Benrabh et al., 1995).

Hypotaurine shares a lot of biological characteristics with taurine, such as an amino group in a β-position (Stryer, 1995), a sulfur group in the carbon terminal (Stryer, 1995) and a neuromodulation of the $GABA_A$ receptor through the glycine site (Quinn and Harris, 1995). Despite that, no report has been carried out about a behavioral interaction between ethanol and hypotaurine to date. Therefore, in order to further characterize the complex behavioral interactions between ethanol and these sulfur compounds, and to elucidate whether the locus of such an interaction is a central or peripheral one, we addressed the present research using the amino acid hypotaurine. A study of the behavioral consequences of treating animals with hypotaurine and ethanol at different doses and times was undertaken. Besides, the specificity of this putative interaction was also studied by evaluating the effects of hypotaurine on cocaine- and caffeine-induced behavior. Finally, we tried to discard a peripheral interpretation of the locus of such an interaction in two ways. Firstly, by analyzing differences in blood ethanol levels between animals treated with hypotaurine and control animals. Secondly, by testing the behavioral consequences of the simultaneous β-alanine and hypotaurine administration on ethanol-induced locomotion. The hypotaurine transport across the BBB depends on a sodium and chloride ion-dependent protein (Benrabh et al., 1995; Komura et al., 1996; Tamai et al., 1995; Tayarani et al., 1989). This transport can be blocked by β-alanine, another β-amino acid. β-Alanine, taurine and hypotaurine all share and consequently compete for the same transport protein system (Benrabh et al., 1995; Komura et al., 1996; Tamai et al., 1995; Tayarani et al., 1989).

2. Materials and methods

2.1. Animals

Swiss male albino mice (27–42 g) acquired from Janvier Spain were used in this study. Mice were housed in groups of three or four per cage. The mice were allowed 1 week to adapt to the animal colony prior to experimentation. The colony was maintained in rooms at 22 °C with 12-h light and dark cycles. Access to water and standard laboratory rodent chow were ad libitum. Testing was always carried out during the light cycle. All experimental procedures complied with the European Community Council Directive (86/609/ECC) for the use of laboratory animal subjects.

2.2. Drugs

Hypotaurine obtained from Sigma-Aldrich (Spain) was dissolved at concentrations of 5.62, 8.45, 11.25, 16.87 or 33.75 mg/10 ml. The doses were chosen in accordance with previous studies with other sulfur compounds (Aragon et al., 1992; Miquel and Aragon, 2000; Miquel et al., 1999). β-Alanine purchased from Sigma-Aldrich was prepared at concentrations of 11 or 22 mg/10 ml. Caffeine and cocaine were obtained from Sigma-Aldrich and dissolved at a concentration of 15 mg/10 ml for caffeine and 4 mg/10 ml for cocaine. Ethanol solutions at a concentration of 20% v/v was diluted from ethanol (96%) (Panreac Quimica, Spain). All solutions were freshly prepared in saline. All drugs were administered intraperitoneally.

2.3. Apparatus

The open-field chambers consisted of a clear glass cylinder 25 cm in diameter and 30 cm high. The floor of the cylinder was divided into four equal quadrants by two intersecting lines drawn on the floor. A locomotion score was assigned each time an animal crossed over from one quadrant to another with all four legs. The test room was illuminated with soft white light.

2.4. Procedure

In the first experiment, the effect of different hypotaurine doses on ethanol-stimulated locomotion was explored. Animals received injections of either saline or 5.62, 8.45, 11.25, 16.87 or 33.75 mg/kg of hypotaurine 30 min prior to administering ethanol (2.4 g/kg) ($n = 8$). Animals were placed in open-field chambers for 20 min. For behavioral tests, only the last 10 min was considered. The first 10 min was not taken into account to reduce any effect of handling or novelty of the environment. (Dudek and Tritto, 1994; Kelley, 1993).

To analyze the effect of time interval between hypotaurine pretreatment and ethanol treatment on locomotion, animals were pretreated with hypotaurine (11.25 mg/kg), simultaneously or 30, 60 or 90 min before an ethanol injection (2.4 g/kg) ($n = 8$). Immediately after ethanol treatment, the behavioral test started.

The effect of hypotaurine on ethanol-induced locomotor activity was investigated in two separated studies. In one of them, mice received an injection of saline or 11.25 mg/kg of hypotaurine and 30 min after this treatment, an injection of one of these doses of ethanol (0.0, 1.6, 2.4 or 3.2 g/kg) was administered ($n = 8$). In the other one, mice were injected with saline or 11.25 mg/kg of hypotaurine and 60 min after this pretreatment, animals received an acute injection of one of these doses of ethanol (0.0, 1.6, 2.4 or 3.2 g/kg) ($n = 8$). In order to evaluate locomotor activity, the same procedure as in the abovementioned experiment was carried out.

In another study, the specificity of hypotaurine effects (11.25 mg/kg) was addressed using two drugs that also induce locomotor activity, cocaine and caffeine. Mice received hypotaurine (11.25 mg/kg) or saline. Thirty minutes following this pretreatment, cocaine (4 mg/kg) or caffeine (15 mg/kg) was injected and locomotion was registered.

The consequence of a cotreatment with β-alanine and hypotaurine on ethanol-induced locomotor activity was

evaluated in different groups of mice. Animals were simultaneously injected with hypotaurine (11.25 mg/kg) or saline and β-alanine (0, 11, or 22 mg/kg). Thirty minutes later, all animals received an ethanol (2.4 g/kg) injection, and then, the behavioral test started.

2.5. Blood ethanol levels

Mice were injected with hypotaurine (11.25 mg/kg) or saline. Thirty minutes after this treatment, an ethanol injection (2.4 g/kg) was administered intraperitoneally. Trunk blood was collected 15, 30 and 60 min after ethanol treatment. Each sample of blood was collected in 1.5-ml microcentrifuge tubes and immediately placed in a centrifuge where the samples were spun down for 5 min at 7500 rpm. A micropipette was then used to extract 160 μl of serum and to add it to 1.44 ml of trichloroacetic acid (20%). The mixture was spun down again (5 min at 7500 rpm) to obtain a clear, protein-free supernatant. The supernatant was then placed in cuvettes with optical properties suitable for use with a spectrophotometer set at 340 nm. Blood ethanol content was enzymatically determined with an Alcohol Diagnostic Kit (Sigma-Aldrich).

2.6. Statistical analyses

Data were analyzed by means of one- and two-way ANOVAs. Post hoc comparisons were undertaken if a significant main effect or interaction was found ($p < .05$). These comparisons were carried out using Fisher's Least Significant Difference (LSD) Tests. The statistical computer program, Statistica 5.0, was used in this study.

3. Results

In Fig. 1, the effect of several doses of hypotaurine on the increase in locomotion induced by 2.4 g/kg of ethanol is shown. A one-way ANOVA revealed a significant dose-effect of hypotaurine pretreatment [$F(5,42) = 3.61$, $p < .005$]. Pairwise comparisons demonstrated that 11.25 mg/kg of hypotaurine produced a significant boost of ethanol-induced locomotion ($p < .01$).

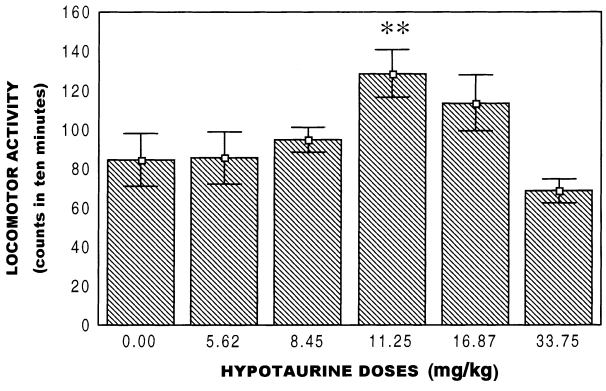

Fig. 1. Effect of hypotaurine doses on ethanol-induced locomotor activity. Mean ± S.E.M. counts in 10 min ($n = 8$). Mice were injected intraperitoneally with hypotaurine (0, 5.62, 8.45, 11.25, 16.87 or 33.75 mg/kg), and 30 min later, ethanol (2.4 g/kg) was injected, and behavioral test took place (** $p < .01$).

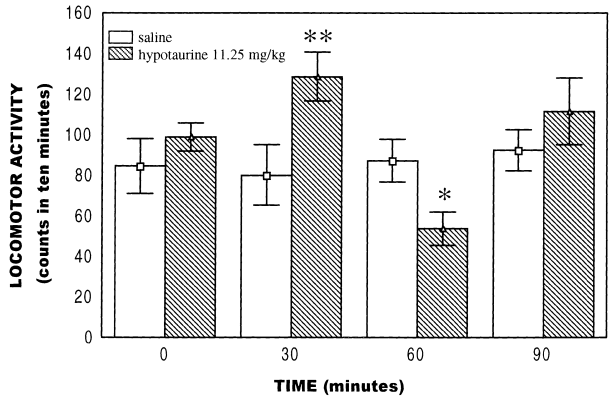

Fig. 2. Time course of the effect of hypotaurine on ethanol induced locomotor activity. Mean ± S.E.M. counts for all treatment groups ($n = 8$). Mice were pretreated with hypotaurine (11.25 mg/kg) and 0, 30, 60 and 90 min later, ethanol (2.4 g/kg) was administered, and locomotor activity was registered (** $p < .01$; * $p < .05$).

The time course of hypotaurine effect on ethanol-induced locomotion is presented in Fig. 2. A two-way ANOVA (Time × Treatment) demonstrated a significant effect of time interval [$F(3,56) = 3.32$, $p < .05$] and a significant effect of time interval–treatment interaction [$F(3,56) = 4.02$, $p = .01$]. As the post hoc test indicated, a significant induction was produced at 30 min ($p < .01$). However, a blockade of ethanol-induced locomotor activity was observed at 60 min following hypotaurine administration ($p = .05$). If the interval between pretreatment and treatment is delayed by 90 min, this sulfonic amino acid loses its stimulating effect on the locomotion produced by ethanol.

Fig. 3 represents the effect of hypotaurine, injected 30 min before ethanol treatment. A two-way ANOVA (Hypo-

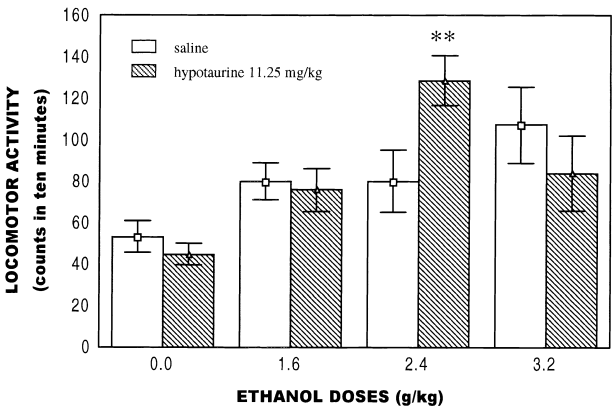

Fig. 3. Effect of a hypotaurine pretreatment 30 min before several doses of ethanol administrations on drug-induced locomotion. Mean ± S.E.M. counts in 10 min ($n = 8$). Mice were pretreated with saline or hypotaurine (11.25 mg/kg), and 30 min later, an ethanol injection was administered (0.0, 1.6, 2.4, 3.2 g/kg), and behavioral test took place (** $p < .01$).

Table 1
Effect of hypotaurine pretreatment (60 min) on ethanol-induced locomotion

	Counts in 10 min			
	0.0 g/kg	1.6 g/kg	2.4 g/kg	3.2 g/kg
Saline	42.87 ± 8.54	84.37 ± 4.71	87.37 ± 10.55	150.37 ± 20.91
Hypotaurine	39.25 ± 3.59	73.75 ± 10.22	53.75 ± 8.20	136.87 ± 40.11

Effect of a hypotaurine pretreatment 60 min before ethanol on alcohol-induced locomotion. Mean ± S.E.M. for all treatment groups ($n = 8$). Mice were pretreated with hypotaurine or saline, and 60 min following this pretreatment, ethanol was injected.

taurine × Ethanol doses) revealed a significant effect of ethanol doses [$F(3,56) = 7.31$, $p < .001$], and a significant hypotaurine–ethanol interaction [$F(3,56) = 3.00$, $p < .05$]. Fisher's LSD comparisons displayed an increase in ethanol-induced locomotion at 2.4 g/kg in the group of animals pretreated with hypotaurine (11.25 mg/kg) as compared with control animals ($p < .01$). Hypotaurine administration did not produce by itself any effect on spontaneous locomotor activity. Table 1 describes the results of deferring time interval between hypotaurine and ethanol administration to 60 min, on ethanol-stimulated locomotion. ANOVA showed an effect of ethanol doses [$F(3,56) = 12.44$, $p < .001$], but neither hypotaurine pretreatment nor hypotaurine–ethanol interaction could be demonstrated. As can be noted, this time interval did not produce any significant change in ethanol-induced locomotion, although a marginal decrease in ethanol-induced locomotion was observed for all ethanol doses.

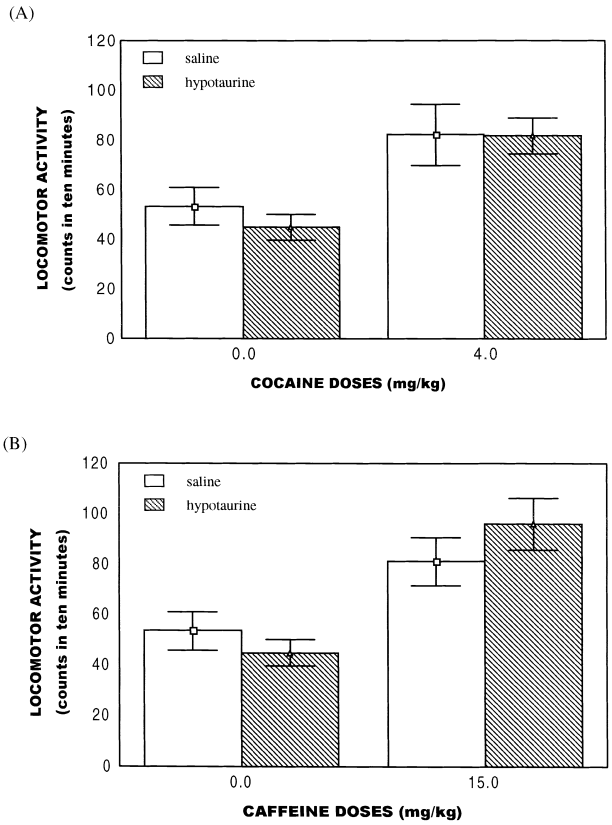

Fig. 4. Effect of hypotaurine on cocaine-(A) or caffeine-induced (B) locomotor activity. Mean ± S.E.M. counts for all treatment groups ($n = 8$). Mice were pretreated with saline or hypotaurine (11.25 mg/kg), and 30 min later, cocaine (4 mg/kg) or caffeine (15 mg/kg) injection was administered.

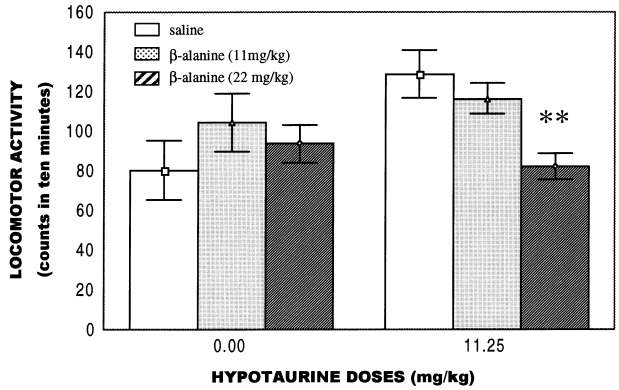

Fig. 5. Effect of the cotreatment with β-alanine and hypotaurine on ethanol-induced locomotor activity. Mean ± S.E.M. counts in 10 min ($n = 8$). Mice were pretreated with saline or hypotaurine (11.25 mg/kg), and then, saline or β-alanine at 11 or 22 mg/kg were administered. Thirty minutes later, all animals were injected with ethanol (2.4 g/kg) (** $p < .01$).

The effect of pretreatment with hypotaurine on (A) cocaine- or (B) caffeine-induced locomotor activity is depicted in Fig. 4. In the case of cocaine, a two-way ANOVA (Hypotaurine × Cocaine treatment) showed a significant effect for cocaine treatment [$F(1,28) = 14.93$, $p < .001$], but neither a significant effect for hypotaurine nor a significant effect for hypotaurine–cocaine interaction was found ($p > .05$). Similarly, regarding the caffeine treatment, a two-way ANOVA (Hypotaurine × Caffeine) revealed a significant effect for caffeine [$F(1,28) = 21.85$, $p < .001$], but neither a significant effect for hypotaurine pretreatment nor a significant effect for hypotaurine–caffeine interaction were shown.

In Fig. 5, the effects of cotreatment with β-alanine and hypotaurine on ethanol-induced locomotor activity are observed. A two-way ANOVA (Hypotaurine × β-Alanine doses) yielded a nonsignificant hypotaurine effect ($p > .05$), and a nonsignificant β-alanine treatment ($p > .05$) effect, but it did show a significant interaction between both factors [$F(2,42) = 3.51$, $p < .05$]. LSD comparisons showed that

Table 2
Effect of hypotaurine on blood ethanol levels

		Blood ethanol levels (mg/dl)		
Group	n	15 min	30 min	60 min
Saline–ethanol	6	382.87 ± 9.41	344.02 ± 20.55	178.58 ± 10.58**
Hypotaurine–ethanol	6	332.26 ± 36.24	329.72 ± 16.44	183.64 ± 17.54**

Effect of hypotaurine on blood ethanol levels. Mean ± S.E.M. blood ethanol levels for all treatments groups ($n = 6$). Mice were pretreated with hypotaurine or saline, and then, they were injected with ethanol. Trunk blood was collected 15, 30 or 60 min after ethanol injection. Data are expressed in mg/dl.

** $p < .01$ for significant differences between the 15-, 30- and 60-min groups.

β-alanine (22 mg/kg) blocked the enhancement in ethanol-induced locomotion produced by hypotaurine ($p < .01$).

Concerning blood ethanol levels (Table 2), a two-way ANOVA showed nonsignificant differences for pretreatment with hypotaurine ($p > .05$) or for time–pretreatment interaction ($p > .05$). However, a time effect was obtained [$F(2,30) = 43.82$, $p < .001$]. Therefore, blood ethanol levels of hypotaurine or saline-pretreated mice were not different at any tested time. Post hoc tests also demonstrated that 60 min following ethanol injection, blood ethanol levels dropped in both treatment groups.

4. Discussion

As has been shown by the current study, hypotaurine interacted with ethanol modifying ethanol-induced stimulating effect. Accordingly, the effect of hypotaurine was related to ethanol dose. Although for the animals used in this study, 1.6 and 2.4 g/kg of ethanol produced similar levels of locomotion, the effect of hypotaurine was totally different between these two doses. Thus, hypotaurine had no effect in animals injected with 1.6 g/kg of ethanol. However, it produced an increase in ethanol-induced locomotion in animals treated with 2.4 g/kg. Furthermore, hypotaurine slightly reduced the stimulating effect of the highest dose of ethanol (3.2 g/kg) used in this study, though this reduction was not statistically significant. These effects were observed clearly when animals were pretreated with hypotaurine (11.25 mg/kg) 30 min before ethanol administration.

The evaluation of the time course of hypotaurine–ethanol interaction revealed a quite complex and paradoxical effect of the time interval between hypotaurine pretreatment and ethanol treatment. Hypotaurine required 30 min to boost ethanol-stimulated locomotion. Furthermore, a blockade of stimulating effects of ethanol was observed 60 min following hypotaurine pretreatment. The recovery to control levels was found after 90 min of the hypotaurine preadministration. Because hypotaurine produced this time-biphasic effect, we further analyzed the consequences of delaying by 60 min the time interval between hypotaurine pretreatment and ethanol injection. However, no significant effect of hypotaurine on ethanol-induced locomotion could be replicated in this new study, although data showed the same tendency towards lowering the ethanol-stimulated locomotion. Currently, it is not possible to offer an adequate explanation for the mechanism used by this sulfonic amino acid to change, in such a time-dependent manner, ethanol-induced locomotion.

Concerning the putative locus of hypotaurine–ethanol interaction, these results suggest a central locus for it. The fact that blood ethanol levels were not different between hypotaurine- and saline-pretreated mice supports the above reasoning. It is interesting to note that blood ethanol levels were not changed by the other metabolic-related sulfur compounds (Aragon et al., 1992; Miquel and Aragon, 2000; Miquel et al., 1999). Moreover, the present study demonstrated that the enhancement in ethanol-induced locomotion carried out by hypotaurine (11.25 mg/kg) was prevented by a cotreatment with β-alanine (22 mg/kg). Previous reports had shown that β-alanine counteracted the transport of β-amino acid taurine and hypotaurine across the BBB, in vitro and in vivo, because β-alanine competed with them for the same sodium- and chloride-dependent transport system (Benrabh et al., 1995; Komura et al., 1996; Tayarani et al., 1989). Therefore, the blockade of hypotaurine effect on ethanol-induced locomotion by β-alanine strongly suggests that the behavioral observed effect is the consequence of a central interaction between hypotaurine and ethanol. Interestingly, it has been described that β-amino acids cross the BBB as soon as 10 s after parenteral administration (Benrabh et al., 1995).

So far, no single explanation can be provided to understand the complex effects of hypotaurine on ethanol-induced behavior. However, we would like to point out some considerations, taking into account the reported common effects that the other metabolic-related sulfur compounds share.

Firstly, L-cysteine, hypotaurine, taurine and isethionic acid produce similar consequences on the stimulating effect of ethanol. Thus, all of them increase the locomotion induced by ethanol in mice (Aragon et al., 1992; Miquel and Aragon, 2000; Miquel et al., 1999). However, both hypotaurine and its immediate precursor L-cysteine required 30 min to increase the effect of ethanol (Miquel and Aragon, 2000), but catabolites taurine and isethionic acid boosted ethanol-induced effects, even if they were administered simultaneously to ethanol (Miquel and Aragon, 2000; Miquel et al., 1999). The present results might well indicate that the effect of hypotaurine could depend on its metabolic transformation.

Secondly, the above-described analogies between these sulfur compounds force us to consider the possibility of a shared neural mechanism of action for all of them. Indeed, taurine behaves as a GABA neuromodulator (Fariello et al., 1975; Quinn and Harris, 1995; Sloley et al., 1992); it inhibits NMDA, kainate and quisqualate receptors (Dahchour and De Witte, 2000; Kurachi et al., 1983). Similarity, L-cysteine can act on NMDA receptor inducing neurotoxicity (Puka-Sundvall et al., 1995) and under some specific conditions, it selectively increases the extracellular concentration of excitatory amino acids glutamate and aspartate in in vitro preparations of rat brain (Abbas et al., 1997). Hypotaurine interacts with the same GABA recognition site as taurine in the GABA receptor complex (Quinn and Harris, 1995), and though no report has been found about hypotaurine inhibitory effect on glutamate function, it might well take place because these amino acids share similar biochemical and pharmacological actions (Wu et al., 1992). On the contrary, although the taurine catabolite isethionic acid alters the ethanol-induced locomotion in the same fashion as the other sulfur compounds, it does not share the abovementioned actions in the CNS (Fariello et al.,

1975; Quinn and Harris, 1995; Sloley et al., 1992). Hence, it is unlikely that a common interaction between ethanol and these sulfur compounds took place in these amino acid neurotransmission systems.

Thirdly, as was reported for other sulfur amino acids, L-cysteine (Miquel and Aragon, 2000) and taurine (Aragon et al., 1992), the effects of hypotaurine on locomotion are specific for ethanol. Thus, neither cocaine- nor caffeine-induced locomotion was affected by this sulfinic amino acid in the current study. The fact that these substances failed to alter the stimulating effect on locomotion of amphetamine (Aragon et al., 1992; Miquel and Aragon, 2000), cocaine and caffeine suggests that the interaction between these sulfur compounds and ethanol could happen in a different central site to that which is functionally responsible for locomotion (for example, the dopaminergic mesolimbic system).

Moreover, the interaction with ethanol could well develop through a process prior to the neural juncture. It is very interesting that all of these compounds present free SO or SH groups bonded to the C terminal, and it has been demonstrated that these groups react with acetaldehyde (the first metabolite originated in ethanol oxidation) to form adducts (Nagasawa et al., 1980; Ogasawara et al., 1994). These acetaldehyde adducts might exert some actions or might simply delay acetaldehyde degradation by the enzyme aldehyde dehydrogenase. Since acetaldehyde seems to mediate some of the psychopharmacological effects of ethanol (Aragon and Amit, 1992, 1993; Aragon et al., 1985, 1989; Smith et al., 1997), it is conceivable to speculate on the possibility that those adducts had some behavioral consequences. Nevertheless, while in vivo interactions between taurine and acetaldehyde (Watanabe et al., 1985a,b) and L-cysteine and acetaldehyde (Nagasawa et al., 1980) have been reported, no study has been published with the other sulfur compounds.

Finally, at present, it is not possible to discard other mechanisms. For example, an osmotic regulation and its putative function on neuronal activity has been a phenomenon demonstrated for taurine, hypotaurine and isethionic acid (Brand et al., 1997; Emery et al., 1991; Englert and Perlman, 1981; Hussy et al., 2000). However, to what extent L-cysteine also shares osmotic functions is unknown. Furthermore, each compound would be able to cause similar behavioral consequences by means of a different and specific mechanism of interaction with ethanol.

In summary, a boost in ethanol-induced locomotor effects carried out by hypotaurine has been demonstrated for the first time in the current report. Furthermore, this effect is limited to ethanol, because no behavioral consequences were observed for cocaine or caffeine in the hypotaurine pretreated mice. Lastly, the present findings also suggest that the effect of hypotaurine could well be central, firstly, because hypotaurine does not change blood ethanol levels, and, secondly, because the hypotaurine effect on ethanol-induced locomotion can be blocked by β-alanine that competed with hypotaurine for the same sodium and chloride ion-dependent transport, reducing the penetration of this amino acid across the BBB.

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