Reduction of Ethanol-Derived Acetaldehyde Induced Motivational Properties by L-Cysteine

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Background: Experimental evidences suggest that acetaldehyde (ACD) contributes to the positive motivational properties of ethanol (EtOH) as assessed by the place conditioning paradigm; indeed, we found that by reducing ACD production and/or by using ACD-sequestrating agents, EtOH is deprived from its motivational properties. Thiol products, such as the amino acid cysteine, are known to be effective ACD-sequestering agents. Cysteine is able to covalently bind ACD thereby forming a stable, nontoxic 2-methyl-thiazolidine-4-carboxylic acid compound. Thus, we treated rats with L-cysteine before intragastric administration of EtOH or ACD.

Methods: Male Wistar rats were pretreated intraperitoneally with saline or L-cysteine (10, 20, or 30 mg/kg), before intragastric administration of saline, EtOH (1 g/kg), or ACD (20 mg/kg). The specificity of L-cysteine effect was addressed using morphine-induced conditioned place preference (cpp) (2.5 mg/kg, i.p.).

Results: L-cysteine dose-dependently prevented both EtOH and ACD-induced cpp but did not interfere with morphine-induced cpp, suggesting that L-cysteine specifically modulates the motivational properties of EtOH.

Conclusion: The present results further underscore the role of EtOH-derived ACD in EtOH-induced motivational properties. L-cysteine, by binding EtOH-derived ACD, would deprive it of its rewarding properties and reduce its abuse liability.

Key Words: Ethanol, Acetaldehyde, Conditioned Place Preference, L-cysteine, Rat, Alcohol Dependence.

ACETALDEHYDE (ACD) IS the primary metabolite of ethanol (EtOH), produced by alcohol dehydrogenase (ADH), cytochrome P4502E1, and catalase. Recently, we showed that a competitive inhibitor of ADH, 4-methylpyrazole (4-MP) and a selective ACD-sequestrating agent, D-penicillamine (DP) administered before the intragastric administration of EtOH reduces EtOH-induced conditioned place preference (cpp), supporting the hypothesis that motivational actions of EtOH ingestion may be mediated by its first metabolite, ACD (Font et al., 2006; Melis et al., 2007; Peana et al., 2008). Thus it seems plausible to suggest that modulation of EtOH-derived ACD, either by reducing its production and/or by using sequestrating agents, may exert a profound influence on the reinforcing and discriminative stimulus effects of EtOH, thereby decreasing the motivational effects associated with EtOH intake. These observations may also bear important theoretical consequences on the therapeutic side of alcoholism. Coherently, other authors have suggested that ACD has positive motivational properties (Hunt, 1996) on its own: ACD is self-administered (Amit, 1977; Brown et al., 1979, 1980; Myers et al., 1984; Rodd-Henricks et al., 2002) and causes cpp (Quertemont and De Witte, 2001; Smith et al., 1984). In line with a primary role of ACD in the positive motivational properties of ETOH, we recently reported that ACD dose-dependently stimulates electrophysiological activity of VTA, DA-containing, neurons whereas ETOH-induced effects are prevented by pharmacological blockade of EtOH metabolism with 4-MP (Foddai et al., 2004) or with DP (Lintas et al., 2007) in the whole animal. Coherently, pretreatment with 4-MP prevented EtOH-induced stimulation of extracellular dopamine (DA) in the mesolimbic system (Melis et al., 2007) while, DP prevented both EtOH- and ACD-induced stimulation of extracellular DA (Mereu et al., 2007; Sirca et al., 2007).

Thiol products, which contain a sulfhydryl (SH) group, such as DP and the amino acid cysteine, are known to be able to protect against ACD effects (Cohen et al., 2000; Nagasawa et al., 1984; Nagasawa et al., 1987; Salaspuro, 2007; Salaspuro et al., 2002, 2006; Sprince et al., 1974; Vasdev et al., 1995). Cysteine, (2R)-2-amino-3-sulfanyl-propanoic acid is a not essential amino acid with a thiol side chain, classified as a hydrophilic amino acid. Because of the high reactivity of this thiol, cysteine is able to bind covalently and efficiently ACD

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by forming stable nontoxic 2-methyl-thiazolidine-4-carboxylic acid compound (Nagasawa et al., 1987; Salaspuro et al., 2006). Cysteine is efficacious to bind ACD derived from alcohol drinking to prevent the possible role of ACD in the pathogenesis of digestive tract cancer (Salaspuro, 2007; Salaspuro et al., 2002, 2006), alcoholic cardiomyopathy, against the chronic toxicity of ACD (Sprince et al., 1974) and also for its antioxidant properties (Shackebaei et al., 2005). Moreover, *N*-acetyl cysteine, an analogue of the dietary amino acid cysteine attenuates EtOH-induced increased blood pressure and adverse renal vascular changes (Vasdev et al., 1995). In rat study, test animals received a LD50 dose of ACD and those receiving cysteine had an 80% survival rate (Sprince et al., 1974). Thus, L-cysteine could also be used to prevent pharmacologic actions of ACD derived from EtOH intake such as to reduce EtOH-induced motivational properties. In line with these evidences, the aim of this study was to study in Wistar rats, both EtOH and ACD given intragastrically (i.g.) by gavage-induced reinforcing properties as measured by cpp method in which cpp was obtained at 1 g/kg of EtOH and at 20 mg/kg of ACD (Melis et al., 2007; Peana et al., 2008) and the relationship between the motivational properties of EtOH and the metabolic production of ACD by determining whether L-cysteine could prevent EtOH- and ACD-induced cpp.

MATERIALS AND METHODS

The study was carried out in accordance with Italian law D.L. 116, 1992, which allows experiments on laboratory animals only after submission and approval of a research project to the competent authorities, and in strict accordance with the "Raccomandazioni della Commissione dell'Unione Europea" (n. 2007/526/CE). All possible efforts were made to minimize animal pain and discomfort and to reduce the number of experimental subjects.

Animals

Male Wistar rats (Harlan, Udine, Italy) weighting between 150 and 250 g were used for cpp procedure. Rats were housed in groups of 3 to 4 per cage and maintained under controlled environmental conditions (temperature 22 ± 2°C; humidity 60% to 65%; 12-h light/dark cycle, light on at 08:00 AM.). All animals were given a standard laboratory diet and tap water *ad libitum*. To minimize stress, subjects were habituated to the experimental procedures (handling, gavage) for at least 3 days before experimental procedures. Experiments were conducted during the light phase of the light/dark cycle.

Conditioned Place Preference

The apparatus consisted of 2 rectangular steel boxes (48 L × 33 W × 30 H cm) separated by a guillotine door. Distinctive visual and tactile cues distinguished the 2 compartments: the wall and floor coloring (1 dark gray and the other clear gray), and the floor texture, smooth or grille. The apparatus was placed in a soundproof room with constant light provided by a 40 W lamp placed above each compartment.

Procedure and Experimental Design

The study used an unbiased procedure. Each experiment consisted of 3 phases. During the first phase (day 1, preconditioning phase), the guillotine door was kept lifted and each rat was placed in the center of the opening, with access to both compartments of the apparatus for 30 minutes (1800 seconds). The time spent by each rat in the compartments was directly recorded using a chronometer, by an experimenter blind to treatment, to indicate the unconditioned preference for each compartment. The spontaneous preference for the 2 compartments was similar, about 900 seconds during 1800 seconds of observation. During the second phase, conditioning phase, (days 2 to 16 for EtOH; days 2 to 9 for ACD and days 2 to 5 for morphine) the rats were administered with the drugs and placed for 30 minutes in the dark gray compartment. On alternate days, the rats were administered with saline and placed in the clear gray compartment. As a result of this conditioning schedule, EtOH, ACD, and morphine with respective saline groups were paired 8 (EtOH), 4 (ACD), and 2 (morphine) times to the dark gray compartment. Doses and schedules for conditioning with EtOH and ACD were selected on the basis of our previous data (Melis et al., 2007; Peana et al., 2008). During the third phase (postconditioning phase), 24 hours after the last treatment, the guillotine door was removed and the time spent by each rat in the drug-paired compartment was recorded during 30 minutes of observation. The conditions of the postconditioning test were identical to those of the preconditioning test. The time spent in the drug-paired compartment during the postconditioning phase with respect to that spent during the preconditioning phase is a measure of the degree of place conditioning induced by the drug (Carr et al., 1989). Thus, a statistically significant difference between the time spent during pre and postconditioning phase as well as the time spent during postconditioning phase with respect to that of saline/saline group indicates the development of cpp, as previously reported by our research group (Melis et al., 2007; Peana et al., 2008).

All experiments were performed between 8:30 AM and 1:00 PM. L-cysteine or saline were administered 30 minutes before the conditioning session with saline, EtOH, ACD or morphine-pairing, only before the drug challenge to the dark gray compartment on alternate days. Care was taken to balance the daily order of treatments as well as the daily order of exposures to each compartment. Control animals (saline/saline) were administered the same volume of saline (vehicle). The number of animals used in each experimental group was: Fig. 1: saline/saline $n = 12$; L-cysteine 10/saline $n = 7$; L-cysteine 20/saline $n = 7$; L-cysteine 30/saline $n = 7$; Fig. 2: saline/saline $n = 12$; saline/EtOH $n = 14$; L-cysteine 10/EtOH $n = 5$; L-cysteine 20/EtOH $n = 6$; L-cysteine 30/EtOH $n = 6$; Fig. 3: saline/saline $n = 16$; saline/ACD $n = 9$; L-cysteine 10/ACD $n = 6$; L-cysteine 20/ACD $n = 7$; L-cysteine 30/ACD $n = 5$; Fig. 4: saline/saline $n = 12$; L-cysteine 30/saline $n = 9$; saline/morphine $n = 10$; L-cysteine 30/morphine $n = 5$.

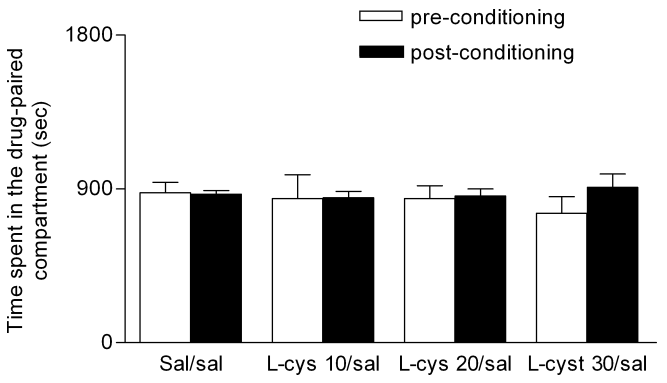

Fig. 1. Effect of different doses of L-cysteine (10, 20, and 30 mg/kg, i.p.) on conditioned place preference, paired with the dark gray compartment. Data are shown as time in seconds (± SEM; $n = 7$ to 12).

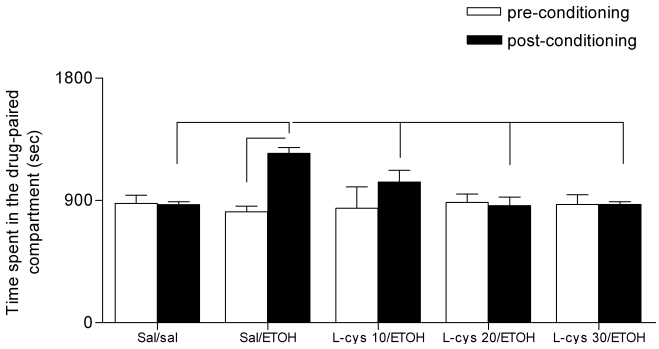

Fig. 2. Effect of L-cisteine-pretreatment (10, 20, and 30 mg/kg, i.p.) on EtOH-induced conditioned place preference (1 g/kg, intragastrically). Data are shown as time in seconds (± SEM; $n = 5$ to 14). Significant differences between time spent during postconditioning phase as compared to preconditioning phase or that of saline/saline or ETOH group are indicated (2 ways ANOVA followed by Newman Keuls, post-hoc test).

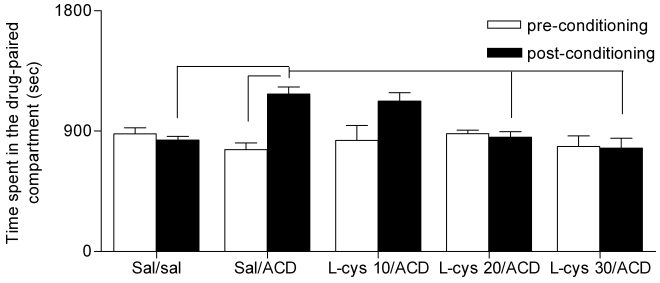

Fig. 3. Effect of L-cysteine pretreatment (10, 20, and 30 mg/kg, i.p.) on acetaldehyde (ACD)-induced compared to preconditioning phase (20 mg/kg, intragastrically). Data are shown as time in seconds (± SEM; $n = 5$ to 16). Significant differences between time spent during postconditioning phase as compared to preconditioning phase or that of saline/saline or ACD group are indicated (2 ways ANOVA followed by Newman Keuls, post-hoc test).

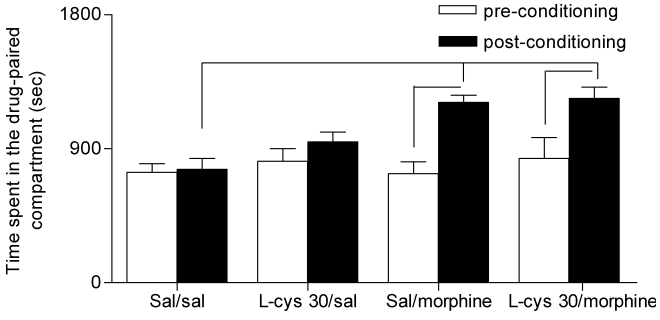

Fig. 4. Effect of L-cysteine-pretreatment (30 mg/kg, i.p.) on morphine-induced compared to preconditioning phase (2.5 mg/kg, i.p.). Data are shown as time in seconds (± SEM; $n = 5$ to 12). Significant differences between time spent during postconditioning phase as compared to preconditioning phase or that of saline/saline group are indicated (2 ways ANOVA followed by Newman Keuls, post-hoc test).

Drugs

EtOH (Zedda-Piras, Alghero, Italy) (1 g/kg) and ACD (Sigma-Aldrich, Milano, Italy) (20 mg/kg) were dissolved in saline (0.9% NaCl) to a final volume of 1 ml and administered by gavage (i.g.). EtOH solutions (20% v/v) were obtained by dilution of ETOH (95%) (Medicamenta, 1991–1992). L-cysteine, (R)-2amino-3-mercaptopropionic acid as hydrochloride (Sigma-Aldrich, Milano, Italy) (10, 20, and 30 mg/kg) was dissolved in saline and administered by i.p. injection. Morphine hydrochloride (morphine) (S.A.L.A.R.S., Camerlata, Como) (2.5 mg/kg) was dissolved in saline and administered by i.p. injection.

All drug dilutions where freshly prepared before every experiment. The gavage infusion rate was rapid (about 5 seconds) and given immediately before each conditioning session.

Statistical Analysis

Data are expressed as mean ± SEM of time in second spent during 1800 seconds of observation in the drug-paired compartment during the postconditioning phase with respect to that spent during the preconditioning phase. To analyze the spontaneous preference during preconditioning phase, data (time spent in each compartment) were analyzed by 1 way analysis of variance (ANOVA). Instead, to determine the effect of saline on saline, EtOH, ACD, or morphine, and L-cysteine on saline, EtOH, ACD, or morphine-induced cpp effect, data were analyzed by repeated measures, 2 ways ANOVA. Post hoc comparisons were undertaken if a significant effect of the interaction was found ($p < 0.05$). The comparisons were carried out using Newman Keuls tests.

RESULTS

Conditioned Place Preference

Preconditioning Phase. During the preconditioning phase, the spontaneous preference for the 2 compartments of the apparatus was unbiased; in fact, the rats spent approximately the same amount of time in both compartments as shown by the absence of statistical differences between the groups [$F(7,54) = 0,255$, $p > 0.99$] (data from saline/saline, L-cysteine/saline, saline/EtOH, and L-cysteine/EtOH groups).

Effect of L-Cysteine Pretreatment on EtOH-Induced cpp

As shown in Fig. 1, pretreatment with L-cysteine did not reveal any cpp effect, in fact, the saline/saline treated group ($n = 12$) versus L-cysteine (10, 20, and 30 mg/kg; $n = 7$ in all cases)/saline groups spent equal time in the pre- and postconditioning session, indicating an absence of motivational effects.

Two-way ANOVA of different doses of L-cysteine (10, 20, and 30 mg/kg, i.p.) on EtOH-induced cpp revealed significant effects of group [$F(4,38) = 4.51$, $p = 0.0004$], conditioning phase [$F(1,38) = 7.16$, $p = 0.001$], and an interaction group × conditioning phase [$F(4,38) = 6.08$, $p = 0.0007$]. L-cysteine prevented EtOH-induced cpp (1 g/kg, i.g.; $n = 17$); its effect is shown in Fig. 2. Rats pretreated with L-cysteine spent less time (10 mg/kg: 1035.00 ± 87.21 seconds, $p = 0.037$; 20 mg/kg: 861.17 ± 62.85 seconds, $p = 0.0037$ and 30 mg/kg: 871.17 ± 19.51, $p = 0.002$, respectively) in the compartment paired with L-cysteine/EtOH, as compared to the group paired with saline/EtOH-induced cpp (1246.29 ± 43.16).

Effect of L-*Cysteine Pretreatment on ACD-Induced cpp*

The effect of pretreatment with L-cysteine (10, 20, and 30 mg/kg, i.p.) was then tested on ACD-induced cpp (20 mg/kg, i.g.), and is shown in Fig. 3. Two-way ANOVA of ACD-induced cpp values in response to L-cysteine pretreatment yielded a significant effect of group [$F(4,38) = 2.86$, $p = 0.036$], conditioning phase [$F(1,38) = 16.14$, $p = 0.00027$], and an interaction group × conditioning phase [$F(4,38) = 11.62$, $p = 0.000003$]. As shown, treatment with saline/ACD induced an increase of time spent in the drug-paired compartment during the postconditioning phase ($n = 9$, 1178.56 ± 52.86 seconds) with respect to its preconditioning phase (761.00 ± 50.12 seconds; $p < 0.00014$) and with respect to postconditioning phase of saline/saline group ($n = 16$, 832.81 ± 29.30 seconds; $p < 0.00015$). L-cysteine prevented ACD-induced cpp (20 mg/kg, i.g.); indeed, rats pretreated with L-cysteine spent less time (20 mg/kg: 853.71 ± 41.30 seconds, $p = 0.00033$ and 30 mg/kg: 773.20 ± 74.20, $p = 0.00022$, respectively) in the compartment paired with L-cysteine/ACD as compared to the group paired with saline/ACD. Pretreatment with 10 mg/kg of L-cysteine, before i.g. ACD, failed to reduce ACD-induced cpp.

Effect of L-*Cysteine Pretreatment on Morphine-Induced cpp*

Two-way ANOVA (group × conditioning phase) yielded a significant effect of group [$F(3,32) = 4.05$, $p = 0.015$], of conditioning phase [$F(1,32) = 30.59$, $p = 0.000004$], and group × conditioning phase interaction [$F(3,32) = 6.52$, $p = 0.0014$]. As shown in Fig. 4 rats conditioned for 4 days with morphine (as hydrochloride, 2.5 mg/kg, i.p) showed cpp for the drug-paired compartment (1210.0 ± 49.06 seconds) with respect to its preconditioning phase (733.40 ± 79.17 seconds, $n = 10$; $p = 0.00018$) and with respect to the postconditioning phase of saline/saline group (763.00 ± 72.33 seconds, $n = 12$; $p < 0.00015$). Pretreatment with the higher dose of L-cysteine (30 mg/kg) before morphine did not interfere with the effect of morphine-induced cpp. Indeed, rats showed preference for the L-cysteine/morphine-paired compartment (1236.60 ± 76.32 seconds) with respect to its preconditioning phase (834.00 ± 142.12 seconds, $n = 5$; $p = 0.0095$) and with respect to the postconditioning phase in saline/saline group (763.00 ± 72.33 seconds, $n = 12$; $p = 0.0006$).

DISCUSSION

The present results further support the contention that EtOH-derived ACD participates in mediating motivational effects of EtOH ingested, as indexed by cpp method (Font et al., 2006; Melis et al., 2007; Peana et al., 2008). The most interesting finding was that pretreatment with L-cysteine, a nonessential amino acid, binding covalently ACD, dose-dependently reduced i.g. EtOH and ACD-induced cpp. In addition, L-cysteine did not interfere with morphine-induced cpp indicating that this amino acid specifically modulates the motivational properties of EtOH and ACD. Thus the ability of L-cysteine to decrease EtOH-induced cpp could be mediated by a reduction of ACD levels formed after i.g. EtOH metabolism. Treatment with L-cysteine (10, 20, and 30 mg/kg) did not produce either rewarding nor aversive effects since when paired with saline, it failed to affect place conditioning. Thus, the observation that L-cysteine failed to produce cpp or conditioned place aversion per se, but reduced EtOH and ACD-induced place preference, suggests that EtOH primary action on cpp can be attributed to EtOH-derived ACD. We have previously shown that i.g (1 g/kg) dose of EtOH achieved similar blood ACD levels than those produced by i.g. ACD treatment (20 mg/kg) (Peana et al., 2008); this fact could well justify the same dose range of L-cysteine used both on EtOH and ACD treatment in reducing motivational effects.

Previous studies, on the effects of EtOH on cpp in rats have generated conflicting results, reporting cpp (Cunningham et al., 2003), cpa (Fidler et al., 2004), or the lack of EtOH-induced cpp (Quertemont and De Witte, 2001). Our cpp studies (Melis et al., 2007; Peana et al., 2008) suggest, however, that an insufficient number of conditioning pairings (4 in the study by Quertemont and De Witte, 2001) might give reason for these differences since in agreement with Bozarth (1990), Bieńkowski et al. (1996) and Biaa and Kotlińska (1999) we could obtain EtOH-induced cpp following 8 conditioning pairings. In this regard, the observation that EtOH can induce cpp, cpa, or have no motivational effects suggests that its motivational properties may result from a complex interaction among a number of variables including genetics, route of administration (Fidler et al., 2004), experimental design (biased vs. unbiased), number and time of conditioning trials, and EtOH doses (Bozarth, 1990; Carr et al., 1989).

The presence of a sulfhydryl group in the L-cysteine molecule may be of importance in the behavioral effects observed in the current study. In fact, the interaction between ACD and some thiol compounds, such as L-cysteine (Salaspuro, 2007; Salaspuro et al., 2002) and DP (Cohen et al., 2000; Font et al., 2005; Kera et al., 1985; Nagasawa et al., 1980, 1984, 1987), have been repeatedly described. L-cysteine has been shown to be able to react covalently with ACD in the oxidation of EtOH *in vivo* (Cederbaum and Rubin, 1976; Nagasawa et al., 1978, 1984) to form a nontoxic 2-methylthiazolidine-4-carboxylic acid compound and thus prevents EtOH-derived ACD from interacting with cellular proteins (Salaspuro et al., 2002; Sprince et al., 1974; Vasdev et al., 1995).

Further, thiol compounds have been evaluated as sequestering agents for metabolically generated ACD associated with heavy EtOH intake. Indeed, buccal L-cysteine-releasing drug formulations markedly decrease the levels of ACD in the saliva after the ingestion of a dose of alcohol (Salaspuro et al., 2002, 2006). Coherently, Vasdev et al. (1995) have shown that *N*-acetyl cysteine, an analogue of the dietary

amino acid cysteine, attenuates EtOH-derived ACD induced hypertension and adverse renal vascular changes induced by chronic EtOH treatment in rats and, importantly, reduced significantly the increase in blood ACD after an oral EtOH treatment in rats without interfering with blood EtOH levels (Escarabajal et al., 2001). On the other hand, in animal studies, exogenous L-cysteine protects from lethal effects of ACD (Nagasawa et al., 1984; O'Neill and Rahwan, 1976). In line with it, Sprince et al. (1974), indicated in L-cysteine a possible protective agent against the chronic toxicity of ACD associated with heavy EtOH intake.

The central mechanism by which L-cysteine reduced EtOH-derived ACD and ACD-induced cpp could be envisaged in the fact that L-cysteine crossing into the brain by excitatory amino acid transporters that are rate limiting factors in neuronal cysteine uptake (Chen and Swanson, 2003) could mediate the reduction of ACD levels formed after peripheral and central EtOH metabolism. On the other hand, there is a strong evidence that red blood cells act as transporters of ACD to other organs. Furthermore, erythrocytes and macrophages, both circulating cells, have a substantial capacity to oxide EtOH to ACD which may then act on peripheral vascular tissue (Baraona et al., 1987; Wickramasinghe, 1987). Our study provides additional support to the hypothesis that EtOH-derived ACD may play a critical role in EtOH-induced positive motivational effects.

Accordingly, we have recently shown that pretreatment with DP, selective ACD-sequestrating agent reduced i.g. EtOH and ACD-induced cpp in rats without interfering with morphine-induced cpp indicating that this functional antagonist specifically modulates the motivational properties of EtOH and ACD (Peana et al., 2008). In fact, DP as well as L-cysteine, interact both in vitro and in vivo preparations, to the high reactivity of the carbonyl carbon atom of the ACD, which reacts nonenzimatically to form stable adducts (Kera et al., 1985; Nagasawa et al., 1980). Coherently, DP is able to reduce ACD blood levels produced by an intraperitoneal injection of EtOH (Nagasawa et al., 1977, 1978, 1980, 1987) as well as *N*-acetyl cysteine in EtOH oral treatment (Vasdev et al., 1995). Nevertheless, blood EtOH levels were not affected by L-cysteine and DP pretreatment in rats (Escarabajal et al., 2001; Nagasawa et al., 1980).

The mechanism underlying the positive effect of L-cysteine on the first metabolic product of EtOH oxidation, ACD-induced cpp in rats also may include the ability of this thiol compound to undergo redox reactions; cysteine has antioxidant properties; in fact, several reports demonstrated antioxidant properties of L-cysteine as precursor of the antioxidant glutathione (Soghier and Brion, 2006) and as direct scavenging of free radicals (Shackebaei et al., 2005).

Cysteine is an important source of sulfide in human metabolism. The sulfide in iron-sulfur clusters is extracted from cysteine, which is converted to alanine in the eukaryotes biogenesis process (Lill and Mühlenhoff, 2006). Beyond the iron-sulfur proteins, many other metal cofactors in enzymes are bound to the thiolate substituent of cysteinyl residues including zinc in ADH (Lippard and Berg, 1994). Thus L-cysteine probably interacting with ADH could reduce EtOH metabolism as 4-MP thereby preventing EtOH-induced motivational effects; even if blood EtOH levels are not affected by L-cysteine pretreatment in rats (Escarabajal et al., 2001). This is in contrast with the inhibitory effect on ADH but it could add at the scavenging and sequestering mechanism of L-cysteine. An alternative/additional explanation could be envisaged in an interaction with the presynaptic group I metabotropic glutamate (mGlu) autoreceptors that mediate a positive modulatory control on synaptic glutamate release both *in vitro* and in vivo. Evidence is also accumulating to suggest that sulphur-containing amino acids are analogues of glutamate (Thompson and Kilpatrick, 1996). Interestingly, low concentrations of L-cysteic acid (1 μM), a similar concentration range to that of L-cysteine tested in our study, inhibited synaptic glutamate release by an interaction with presynaptic group mGlu autoreceptors (Croucher et al., 2001). In line with these results, Blednov and Harris (2008) have shown that group I mGluR5 antagonists decrease alcohol self-administration and suggest that the anticraving medication, acamprosate, may also act to decrease mGluR5 function. Currently, no explanation are available for these reports but it is evident the importance of mGluR5 for several actions of alcohol (Blednov and Harris, 2008; Croucher et al., 2001).

Overall, taking into account the above-mentioned data, we propose that the ability of sequestering ACD by L-cysteine, even being partial, is the clearest and more sustainable argument to explain our results. In conclusion, the present findings provide further evidence for the behavioral relevance of sequestering EtOH-derived ACD, and they give new clues about the implication of ACD on EtOH-induced motivational properties.

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