British Journal of Pharmacology (2003) 138, 9–12
www.nature.com/bjp

SPECIAL REPORT

The anti-relapse compound acamprosate inhibits the development of a conditioned place preference to ethanol and cocaine but not morphine

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The effects of the anti-relapse compound acamprosate (calcium acetylhomotaurinate) on the conditioned rewarding effects of ethanol, cocaine and morphine were studied using the conditioned place preference (CPP) paradigm. During 3 days of drug conditioning, mice were pretreated with saline or acamprosate (30, 100 or 300 mg kg^{-1} i.p.) 10 min prior to the administration of ethanol (2 g kg^{-1} i.p.), cocaine (15 mg kg^{-1} i.p.) or morphine (10 mg kg^{-1} i.p.), and subsequently confined to one of two distinct conditioning chambers. On the following day, mice were tested for the expression of CPP. Acamprosate dose-dependently reduced the development of CPP to ethanol and cocaine but not morphine. When tested as the conditioning drug, acamprosate alone produced neither a conditioned place preference nor aversion. These data suggest that acamprosate can suppress the conditioned rewarding effects of ethanol and certain classes of abused substances.
British Journal of Pharmacology (2003) **138,** 9–12. doi:10.1038/sj.bjp.0705059

Keywords: Ethanol; cocaine; morphine; acamprosate; reward; conditioned place preference; glutamate; NMDA

Abbreviations: CPP, conditioned place preference; NMDA, N-methyl-D-aspartate

Introduction Acamprosate (calcium acetylhomotaurinate) is a taurine derivative that is used as a pharmacological adjunct for the treatment of alcoholism. In animals, acamprosate reduces ethanol consumption, ethanol withdrawal severity, and the alcohol deprivation effect, a model of relapse (Spanagel & Zieglgansberger, 1997; Dahchour & De Witte, 2000; Johnson & Ait-Daoud, 2000). In humans, acamprosate is effective in prolonging abstinence and reducing relapse rates (Johnson & Ait-Daoud, 2000; Mason, 2001).

Despite its successful use in the treatment of alcoholism, few studies have examined the effects of acamprosate on ethanol-associated environmental cues (i.e., conditioning factors), which are believed to play a substantial role in relapse (O'Brien *et al*., 1998; Drummond, 2001). In addition, little attention has been given to possible interactions between acamprosate and other drugs of abuse, such as opiates and psychostimulants. The present study utilized the conditioned place preference (CPP) paradigm, a widely-used measure of the rewarding effects of drugs of abuse, to determine if acamprosate alters the conditioned rewarding effects of ethanol, cocaine and morphine.

Methods *Animals* Male C57BL/6J and DBA/2J mice (Jackson Laboratories, Bar Harbor, ME, U.S.A.) weighing 20–30 g and 2–4 months of age at the time of the experiment were used as subjects. Animals were housed four per cage in a ventilated cage rack system under standard laboratory conditions with a 12 : 12 h light–dark cycle (lights on at 0600 h). All experiments were performed during the light phase. Food and water were available *ad libitum* except during conditioning and test sessions. All experimental procedures were performed in accordance with approved institutional protocols and National Institutes of Health guidelines. Based on previously reported strain differences in the ability of ethanol, cocaine and morphine to establish reliable CPP (Seale & Carney, 1991; Cunningham *et al*., 1992), C57BL/6J mice were used for cocaine and morphine CPP procedures, whereas DBA/2J mice were used for ethanol CPP procedures.

Apparatus A three-chambered CPP apparatus was used (Med Associates, St. Albans, VT, U.S.A.), which consisted of two $16.8 \times 12.7 \times 12.7$ cm compartments with distinct visual and tactile cues (one with white coloured walls and a steel rod floor, the other with black coloured walls and a wire mesh floor) connected by a centre $7.2 \times 12.7 \times 12.7$ cm grey compartment. The centre compartment was equipped with two computer-controlled guillotine doors that provided access to one or both of the conditioning compartments. Time spent in each chamber was measured by computer-interfaced infrared photobeams placed on 1.2 cm centres. Each chamber was equipped with a 2.8-watt house light centred above the compartment, and the apparatus was located in a sound-attenuating cubicle equipped with an external exhaust fan that helped to mask external noise.

Conditioned place preference procedures Conditioning sessions were conducted twice daily for 3 days, with a minimum of 5 h between conditioning sessions. Previous studies have demonstrated that plasma levels of ethanol, cocaine or morphine in mice are >80% cleared following this

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time period after a single intraperitoneal (i.p.) injection at similar doses (Benuck *et al*., 1987; Faulkner *et al*., 1990; Pacifici *et al*., 1995).

Animals were randomly assigned to undergo either drug conditioning in the morning and saline conditioning in the afternoon, or vice versa. For drug conditioning, animals ($n=8-12$ per group) were randomly assigned to receive either saline or acamprosate (30, 100 and 300 mg kg^{-1} i.p.) 10 min prior to the administration of the conditioning drug (ethanol 2 g kg^{-1} i.p., cocaine 15 mg kg^{-1} i.p., or morphine 10 mg kg^{-1} i.p.). Immediately following administration of the conditioning drug, animals were confined to one of the two conditioning compartments for 15 min (for cocaine and morphine) or 5 min (for ethanol). The shorter conditioning period for ethanol was used based on previous findings that ethanol CPP is diminished with longer conditioning sessions (Cunningham & Prather, 1992). Saline conditioning sessions were preceded by administration of saline 10 min prior to saline injection. The drug- and saline-paired conditioning compartments and the time of day of the drug or saline conditioning session (morning or afternoon) were randomized and counterbalanced across all groups. On the day following the last conditioning session, animals were tested for CPP by placing them into the centre compartment and allowing free access to both conditioning compartments for 30 min. CPP was determined by subtracting the time spent (in s) in the saline-paired compartment from the time spent in the drug-paired compartment. Data were analysed using a one-way analysis of variance (ANOVA) followed by a Dunnett's *post-hoc* test for individual comparisons, with saline pretreated animals serving as the control group.

To control for possible innate preferences for one of the two conditioning compartments, a separate group of C57BL/6J and DBA/2J mice ($n=12$ per group) underwent conditioning as described above, except that only saline was administered prior to placement in either conditioning compartment (i.e., saline-saline control). Following 3 days of saline conditioning (two sessions per day), animals were tested for place preference for 30 min as described above.

To determine if acamprosate possesses any intrinsic rewarding or aversive properties, a separate set of experiments was conducted in which acamprosate alone (100 and 300 mg kg^{-1} i.p.) was used as the conditioning drug. Both strains of mice were injected with acamprosate immediately prior to placement in one conditioning compartment for 15 min, whereas saline was administered prior to placement in the other conditioning compartment as described above. Following 3 days of conditioning (two sessions per day), animals were tested for place preference for 30 min.

Drugs Morphine sulphate and cocaine hydrochloride were obtained from Sigma Chemicals (St. Louis, MO, U.S.A.), and acamprosate (calcium acetylhomotaurinate) was obtained from EstechPharma (Seoul, South Korea). All drugs were dissolved in sterile physiological saline prior to i.p. injection. Ethanol (95% v v^{-1}) was diluted to 20% v v^{-1} in sterile physiological saline prior to i.p. injection. Cocaine and morphine injections were made in volumes of 1 ml 100 g^{-1}, and ethanol injections were made in volumes of 1.25 ml 100 g^{-1}. Saline injections were made in volumes equal to that of the corresponding drug for each animal.

Results Following conditioning with saline in both conditioning compartments (saline-saline control), both C57BL/6J ($F_{(1,18)}=0.60$, $P>0.05$) and DBA/2J ($F_{(1,18)}=1.706$, $P>0.05$) failed to spend more time in one conditioning compartment over the other, indicating a lack of innate preference for a particular compartment. Mice pretreated with saline (0 mg kg^{-1} acamprosate) displayed a robust preference for the ethanol-, cocaine- and morphine-paired chamber as compared to the saline-paired compartment (Figure 1A–C, $P<0.05$ for all groups). No differences in the degree of preference for the ethanol-, cocaine- or morphine-paired compartment was observed in saline-pretreated mice ($F_{(2,21)}=0.13$, $P>0.05$).

When pretreated with acamprosate (30, 100 or 300 mg kg^{-1} i.p.), a dose-dependent reduction in the development of CPP to ethanol was observed ($F_{(3,40)}=3.094$, $P<0.05$), with the 300 mg kg^{-1} dose significantly reducing ethanol place preference vs saline-pretreated animals (Figure 1A). At this dose, the amount of time spent in the ethanol-paired compartment was not significantly different from the time spent in the saline-paired compartment ($P>0.05$), indicating a lack of development of ethanol CPP.

A similar dose-dependent reduction in the development of cocaine CPP by acamprosate was observed ($F_{(3,35)}=3.218$, $P<0.05$), with the 100 and 300 mg kg^{-1} doses significantly reducing cocaine place preference vs saline-pretreated animals (Figure 1B). At these doses, the amount of time spent in the cocaine-paired compartment was not significantly different from the time spent in the saline-paired compartment ($P>0.05$), indicating a lack of development of cocaine CPP.

Acamprosate had no effect on the development of CPP to morphine (Figure 1C). When used as the conditioning drug, acamprosate (100 and 300 mg kg^{-1}) alone failed to produce a conditioned place preference or aversion in either of the mouse strains used for the present experiments (C57BL/6J or DBA/2J, Figure 1D, $P>0.05$), indicating a lack of intrinsic rewarding or aversive effects of this drug.

Discussion In rodents and humans, acamprosate is effective in reducing excessive ethanol consumption as well as preventing relapse following abstinence (see Spanagel & Zieglgansberger, 1997; Dahchour & De Witte, 2000; Johnson & Ait-Daoud, 2000; Mason, 2001 for reviews). In the present study, we have demonstrated that acamprosate inhibits the development of ethanol CPP, suggesting that the ability of acamprosate to reduce ethanol consumption and relapse rates in humans may be attributable, at least in part, to its ability to reduce the acute rewarding effects of ethanol. Our data are in agreement with several recent studies indicating that acamprosate can also reduce some of the environmentally conditioned motor effects of ethanol (Cole *et al*., 2000; Quertemont *et al*., 2002). Thus, acamprosate may also reduce relapse to alcohol drinking *via* its ability to attenuate the effect of ethanol-conditioned cues on behaviour, which are important factors contributing to relapse (O'Brien *et al*., 1998; Drummond, 2001). Since acamprosate does not alter the pharmacokinetics or pharmacodynamics of ethanol in rodents (Gewiss *et al*., 1991), it is unlikely that acamprosate inhibits the development of ethanol CPP *via* changes in ethanol absorption or clearance.

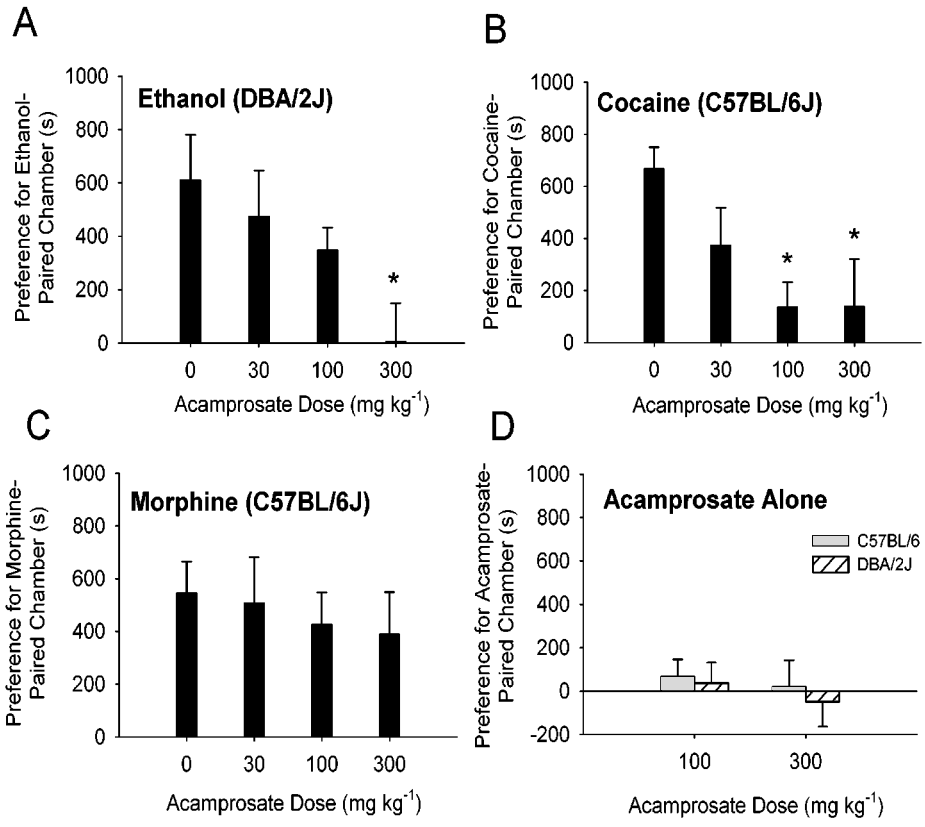

Figure 1 Effect of acamprosate on CPP produced by ethanol (A, 2 g kg^{-1}), cocaine (B, 15 mg kg^{-1}) and morphine (C, 10 mg kg^{-1}). Mouse strains used for each CPP procedure are given in each panel. Acamprosate was given i.p. 10 min prior to the administration of the conditioning drug. (D) Absence of CPP or aversion when acamprosate alone was used as the conditioning drug in C57BL/6J or DBA/2J mice. Data are presented as mean ± s.e. $^*P<0.05$ vs saline pretreated group. $n=8-12$ for all groups.

Surprisingly, we also found that acamprosate inhibits the development of cocaine CPP. To our knowledge, this is the first study of a behavioural interaction between cocaine and acamprosate. Further studies are needed to determine if the reduction in cocaine CPP by acamprosate in mice translates into an ability of this compound to reduce cocaine self-administration and relapse in animals and humans.

However, acamprosate failed to inhibit the development of morphine CPP. These findings are consistent with previous studies that acamprosate does not alter the discriminative stimulus properties of morphine (Pascucci *et al*., 1999) or reduce opiate self-administration and relapse (Spanagel *et al*., 1998). These data also provide further evidence for distinct neural mechanisms underlying the rewarding effects of opiates and cocaine.

When used as the conditioning drug, acamprosate failed to produce a conditioned place preference or aversion, indicating that the inhibition of development of ethanol and cocaine CPP by acamprosate was not due to any intrinsic rewarding or aversive properties of this compound. Consistent with this, acamprosate does not possess discriminative stimulus properties and is not self-administered in primates (Grant & Woolverton, 1989).

Because the CPP paradigm involves repeated pairing of the conditioning drug with a distinct environment, it is possible that any compound that interferes with the development of CPP might do so as a result of inhibition of the formation of associative memories between the conditioning environment and the subjective effects of the conditioning drug, rather than reducing the rewarding properties of the conditioning drug *per se*. However, this explanation for the present results seems unlikely, as acamprosate has been shown to enhance, rather than impair, memory performance in various rodent models at doses similar to those used in the present study (Okulicz-Kozaryn *et al*., 2001; Mikolajczak *et al*., 2002). The observed ability of morphine to establish CPP regardless of acamprosate pretreatment also argues against this possibility.

Although the precise neuropharmacological mechanisms of action of acamprosate are unknown, there is evidence to suggest it may antagonize the function of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors (Littleton, 1995; Spanagel & Zieglgansberger, 1997; Dahchour & De Witte, 2000; Rammes *et al*., 2001). Accordingly, other antagonists of NMDA receptor, such as MK-801, also attenuate ethanol and cocaine CPP (Kim *et al*., 1996; Biala & Kotlinska, 1999). Thus, acamprosate may inhibit the development of ethanol and cocaine CPP *via* antagonism of NMDA receptors. Further studies are needed to investigate this possibility.

This work was supported by funds provided by the State of California for medical research on alcohol and substance abuse through the University of California at San Francisco, and grant AA13276 to M.F. Olive from the National Institute on Alcohol Abuse and Alcoholism.

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(Received August 12, 2002
Revised October 10, 2002
Accepted October 23, 2002)