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Short communication

Acamprosate decreases the induction of tolerance and physical dependence in morphine-treated mice

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Abstract

The effects of acamprosate, a drug thought to interact with *N*-methyl-D-aspartate (NMDA) receptors in the central nervous system (CNS), were examined on the antinociceptive action of morphine, induction of tolerance to and physical dependence on morphine, and expression of the abstinence syndrome to the opiate in mice. For the induction of tolerance and dependence, morphine (300 mg/kg) was administered by means of a slow-release preparation. Single doses of acamprosate (50, 100, 200, or 400 mg/kg) administered 30 min before a test dose of morphine did not change the antinociceptive effects of morphine in drug-naive mice. The drug was also administered in repeated doses (50, 100, 200, or 400 mg/kg, 30 min before and 12 and 24 h after the priming dose of morphine) in order to evaluate its effects on the induction of tolerance; all doses assayed, except the 400 mg/kg, did not affect the intensity of tolerance. The acute administration of acamprosate (50, 100, 200, or 400 mg/kg, injected 30 min before naloxone to morphine-pretreated mice) did not affect the intensity of the abstinence behavior. However, the repeated administration of 100 mg/kg of acamprosate (30 min before and 12 and 24 h after the priming dose of morphine) decreased the intensity of physical dependence. The results of these studies suggest that acamprosate may have modulatory effects on glutamatergic neurotransmission participating in the adaptive mechanisms induced by chronic morphine treatment. © 2002 Published by Elsevier Science B.V.

Keywords: Acamprosate; Morphine antinociception; Morphine dependence; Abstinence syndrome

1. Introduction

It is now well established that excitatory amino acid transmission systems are involved in the development of tolerance to opiate actions and in the induction of dependence. It has been shown that glutamate receptor antagonists and antiglutamatergic agents reduce the induction of physical dependence and tolerance to morphine, and decrease the symptoms of naloxone-precipitated withdrawal syndrome in mice (Trujillo and Akil, 1991; González et al., 1997; Sepúlveda et al., 1999). Direct evidence for the role of glutamate in the abstinence behavior has been reported by Tokuyama et al. (1996), who showed that the i.c.v. administration of the amino acid to morphine-tolerant rats elicited withdrawal signs similar to those evoked by naloxone. In addition, Sepúlveda et al. (1998) reported an increased release of glutamate in areas of the limbic system of morphine-tolerant rats following naloxone administration.

Acamprosate (calcium–acetyl homotaurinate) is used therapeutically to prevent relapses in weaned alcoholics (Lhuintre et al., 1985; Sass et al., 1996). The mechanisms by which acamprosate decreases relapse to alcohol use are still poorly understood. It has been demonstrated that acamprosate reduces Ca^{2+} fluxes through voltage-operated channels and that it interacts with NMDA receptor-mediated glutamatergic neurotransmission in various brain regions with functional antagonistic properties (Spanagel and Zieglgänsberger, 1997).

Basic research has provided evidence for the existence of similarities between ethanol and opiate dependence (Nestler and Aghajanian, 1997). In this regard, Kratzer and Schmidt (1998) demonstrated that acamprosate inhibits the conditioned place aversion induced by naloxone-precipitated

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withdrawal in morphine-dependent rats, suggesting that it interferes with the adaptive processes to chronic opiate administration. In addition, Spanagel et al. (1998) have shown that acamprosate suppresses the expression of morphine-induced sensitization in rats.

In view of these data, the present study was undertaken to determine the effects of acamprosate on (a) morphine antinociceptive action, (b) the development of tolerance to and physical dependence on morphine, and (c) the naloxone-precipitated withdrawal syndrome.

2. Materials and methods

2.1. General

Male adult albino Swiss Webster mice 12–15 weeks of age, weighing 26–33 g, from the animal reproduction laboratories of the Department of Pharmacology of the University of Concepción were used in all experiments. Mice were housed in groups of 10 and maintained on a 12:12 h light/dark cycle at constant room temperature (22 ± 2 °C) with free access to food and water. Determinations of antinociceptive responses were carried out in the period between 14.30 and 18.00 h under normal room light and temperature (22 ± 2 °C) conditions. Each animal was used for only one experimental condition.

All experiments were performed in compliance with the institutional guidelines and with National Institutes of Health Guide for the Care and Use of Laboratory Animal.

2.2. Drugs

The drugs used were morphine HCl (May and Baker, Dagenham, England), naloxone (Sigma, St. Louis, MO), and acamprosate (Lipha, Lyon, France). For the induction of chronic morphine effects, the opiate was administered in a suspension of the following composition: morphine, as the base form, 300 mg, 4.2 ml liquid paraffin, and 0.8 ml sorbital sesquioleate mixed with 5 ml saline.

2.3. Analgesic test

The hot-plate test described by Eddy and Leimbach (1953) was used for assessing analgesia (temperature kept at 55 ± 0.5 °C). The end points considered were jumping off the plate or leg kicking. Each mouse was tested twice before drug administration and the values were averaged to obtain a baseline. In order to avoid severe leg burning, a cut-off time of 25 s was used. The total antinociceptive response was obtained as the area under the time–response curve calculated from the experimental values obtained every 30 min. To determine the effects of acamprosate on the analgesic response to morphine, this drug was given 30 min before the s.c. test dose (5 mg/kg) of the opiate.

2.4. Induction of tolerance

To induce opiate tolerance, a single s.c. dose of morphine (300 mg/kg, in a suspension containing 4.2 ml liquid paraffin, and 0.8 ml sorbital sesquioleate mixed with 5 ml saline) was administered 30 h prior to the assay of an s.c. test dose (5 mg/kg) of the analgesic. Control groups were injected s.c. with the suspension without morphine. The criterion for accepting attenuation of tolerance was based on the statistical difference between the effect of the test doses in the primed untreated groups and the effect in the primed mice treated with the drug under assay.

Acamprosate (50, 100, 200, and 400 mg/kg) tested for tolerance development was administered during chronic morphine treatment according to the following schedule: 30 min before and 12 and 24 h after the priming dose of morphine. Control groups were injected with the vehicle instead of acamprosate.

2.5. Induction of morphine dependence

To study morphine dependence, a single s.c. dose of morphine (300 mg/kg, as indicated in Section 2.4) was administered 30 h before the i.p. administration of 4 mg/kg naloxone. To test the effect of acamprosate on the induction of dependence, the drug was administered during chronic morphine treatment according to the following schedule: 30 min before and 12 and 24 h after the priming dose of morphine. These groups are referred to as development of physical dependence. Acamprosate was also administered in an additional scheme, which differed from the former, in which mice received a single dose of the drug 30 min before an abstinence behavior-precipitating naloxone dose. Results in the latter groups are referred to as effects on withdrawal syndrome.

In all cases, the withdrawal syndrome precipitated by 4 mg/kg naloxone was characterized by diarrhea, piloerection, micturition, body shakes, running, paw tremors, and jumping. The number of mice presenting this syndrome was recorded after a 20-min observation period. Comparisons were made by assigning the following withdrawal scores: no appreciable effects, 0; micturition, 1; running, 2; piloerection, 2; diarrhea, 3; paw tremors, 3; body shakes, 4; and jumping, 5. The relative frequencies of withdrawal signs were calculated by adding the number of mice presenting a sign during the observation period. The mean withdrawal scores were also calculated.

2.6. Statistical analysis

The significance of the differences in the mean responses to a test doses of morphine in tolerance experiments was determined by analysis of variance (ANOVA) and confirmed with the Student–Newman–Keuls test. A level of probability of 0.05 was accepted as statistically significant. Statistical analysis of the withdrawal syndrome was made

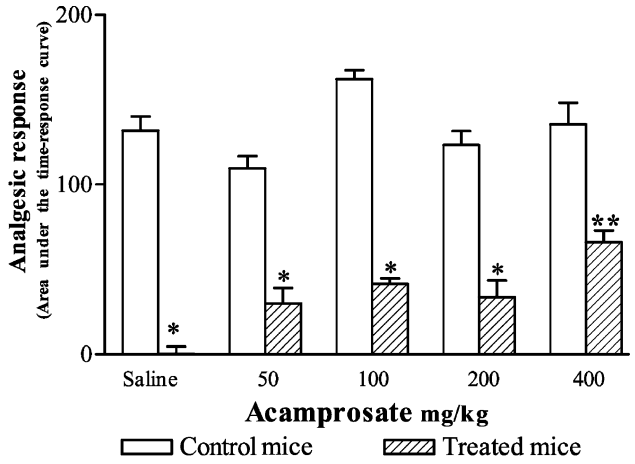

Fig. 1. Mice were treated with a slow release preparation of morphine (300 mg/kg s.c.) 30 h before the administration of a test dose of morphine (5 mg/kg s.c.). Acamprosate (50, 100, 200, and 400 mg/kg) was administered during chronic morphine treatment according to the following schedule: 30 min before and 12 and 24 h after the priming dose of morphine. Control groups were injected with the vehicle instead of acamprosate. Each bar represents the mean ± S.E.M. ($N = 10$ per group). * Statistically lower with reference to the effect of morphine in vehicle-treated mice. ** Statistically higher than the effects observed in mice pretreated with morphine and receiving saline. $P < 0.05$ ANOVA followed by Student–Newman–Keuls test.

using the Mann–Whitney *U*-test based on the scores for individual animals.

3. Results

3.1. Effects of acamprosate on morphine antinociception and on the development of tolerance

A single s.c. dose of morphine (5 mg/kg) elevated the nociceptive threshold. Single doses of acamprosate (50, 100, 200, or 400 mg/kg) had no effects on gross animal behavior, nor did they significantly affect the responses to thermal stimulation (results not shown). The antinociceptive responses of the test dose of morphine were unaffected by acamprosate co-administration (single doses of 50, 100, 200, or 400 mg/kg) (results not shown).

Tolerance to morphine resulted following an s.c. administration (300 mg/kg) given 30 h before the injection of the test dose. Opiate-induced tolerance was demonstrated by the reduction of the effects of a challenge dose of morphine (Fig. 1). Acamprosate (50, 100, 200, or 400 mg/kg, administered during the period of chronic morphine treatment) was tested to evaluate its effects on tolerance development. As shown in the figure, only the highest dose increased the antinociceptive response in morphine-primed mice, which reflects a reduction of the intensity of tolerance.

3.2. Effects of acamprosate on morphine dependence

The effects of acamprosate on the development of morphine physical dependence and on the expression of the withdrawal syndrome are shown in Table 1. A dose of 100 mg/kg significantly decreased the development of physical dependence. The signs most affected by drug administration were tremors, body shakes, running, and jumping.

3.3. Effects of acamprosate on the intensity of the abstinence behavior

Acamprosate was also assayed in single doses (50, 100, 200, and 400 mg/kg) administered 30 min before induction of the withdrawal syndrome by naloxone. The results of this treatment are shown in Table 1. There are no significant effects on the naloxone-precipitated withdrawal signs.

Table 1
Effects of acamprosate on the development of physical dependence and on the withdrawal syndrome of morphine-treated mice

Withdrawal sign	Relative frequencies of withdrawal signs as percentage of the maximum									
	Development of physical dependence					Withdrawal syndrome				
	Saline	Acamprosate (mg/kg)				Saline	Acamprosate (mg/kg)			
		50	100	200	400		50	100	200	400
Micturition	50	60	40	40	30	70	30	30	70	60
Running	60	20	10	10	40	40	60	40	20	40
Piloerection	100	100	90	60	50	100	90	100	100	90
Diarrhea	60	50	20	60	70	40	50	30	40	40
Paw tremors	80	50	40	30	50	90	80	100	80	100
Body shakes	40	0	0	10	20	40	30	10	40	0
Jumping	70	40	0	40	30	40	60	10	50	20
Withdrawal score ± S.E.M.	14 ± 1.0	9.0 ± 1.1	5.6 ± 1.2[a]	9.3 ± 1.3	8.2 ± 1.9	12.7 ± 1.8	13.8 ± 1.7	9.2 ± 1.0	11.6 ± 1.0	8.8 ± 0.9

Mice were treated with a slow release preparation of morphine (300 mg/kg, s.c.) 30 h before the precipitation of the withdrawal syndrome by naloxone (4 mg/kg, i.p.). For the study of effects on the development of physical dependence to morphine, acamprosate was administered during the course of morphine pretreatment at different time intervals (see text). To study the effects on the withdrawal syndrome, the drug under study was administered 30 min before naloxone administration. $N = 10$ mice per group.

[a] Significantly different from control mice. $P < 0.01$, Mann–Whitney *U*-test.

4. Discussion

It is accepted that excitatory amino acids are involved in neurotransmission processes in the central nervous system (CNS) and in the antinociceptive effects of opiate agents (Näsström et al., 1992). Antinociception seems to be induced, at least partly, through inhibition of opiate-evoked amino acid release (Kangrgra and Randic, 1991; Malmberg and Yaksh, 1995).

Although the mechanisms involved in acamprosate affects are not fully understood, it has been shown to affect glutamatergic neurotransmission as a *N*-methyl-D-aspartate (NMDA) receptor modulator with functional antagonistic properties (Spanagel and Zieglgänsberger, 1997). The drug also binds to type B γ-aminobutyric acid ($GABA_B$) receptors (Johnston and Brown, 1983) and its anticraving action seems to be related to this neurotransmitter (Boismare et al., 1992). Both mechanisms may be involved in its interaction with morphine effects. It may be reasoned that the antagonism of glutamate effects might increase morphine antinociception. In the present work, acamprosate per se did not affect the nociceptive response, yet, when administered with morphine, the high dose of acamprosate acted synergistically with the opiate. These results are consistent with earlier findings which show that both the competitive and noncompetitive NMDA receptor antagonists enhance the antinociceptive effect of morphine (Wong et al., 1996).

With regards to the process of tolerance, it has been demonstrated that NMDA receptor antagonists have the ability to inhibit the development of tolerance to opiate analgesia (Trujillo and Akil, 1991; Marek et al., 1991; González et al., 1997; Trujillo, 2000). Our present results demonstrate that a decreased tolerance to morphine was observed only after the simultaneous administration of morphine associated with a high dose of acamprosate, a result compatible with the fact that this drug inhibits NMDA receptor function only at higher concentrations (al Qatari et al., 1998).

The complex effects of acamprosate are also observed in the experiments on physical dependence: the drug, co-administered with morphine, decreased the intensity of the withdrawal syndrome. This effect was observed only with the 100-mg/kg dose. Lower or higher doses (50, 200, and 400 mg/kg) were ineffective. On the other hand, acamprosate, given 30 min before naloxone-induced withdrawal behavior, did not alter the intensity of the syndrome.

A number of studies (Sanjay et al., 1994; González et al., 1997) have demonstrated that glutamate receptor antagonists reduce the intensity of the withdrawal syndrome as well the development of physical dependence, demonstrating that glutamatergic transmission plays an important role in these processes. Therefore, it seems reasonable to postulate that the effects of acamprosate on the development of physical dependence and tolerance may be related to its antiglutamatergic actions. Direct evidence for the role of glutamate in the abstinence behavior has been reported by Tokuyama et al. (1996), who showed that the i.c.v. administration of the amino acid to morphine-tolerant rats elicited withdrawal signs similar to those evoked by naloxone.

On the other hand, the effects of acamprosate on GABAergic mechanisms cannot be excluded since an increase of GABAergic neurotransmission may be influencing the results observed in the experiments with different doses of acamprosate given during the period of chronic morphine treatment. GABA mechanisms seem to be important for the expression of some pharmacological responses to morphine administration. When GABA levels are increased, the responses of acute morphine are reduced and the development of tolerance and dependence are accelerated (Ho et al., 1973). Accordingly, it can be assumed that acamprosate exerts opposite effects in the processes involved in the chronic morphine treatment, depending on the dose administered, which may explain why its effects appear not to be dose-related. Additionally, it is accepted that CNS processes involved in the adaptive mechanisms responsible for opiate tolerance and the withdrawal syndrome are submitted to the influences of practically all known neurotransmitters, and no one of these substances plays a unique role in opiate dependence.

In conclusion, acamprosate may decrease the intensities of morphine tolerance and dependence, but in a non dose-related manner. These results suggest that acamprosate affects both glutamatergic and GABAergic neurotransmission in the CNS resulting in opposite influences in the contraadaptive processes induced by a chronic morphine treatment.

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