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Decrease of tolerance to, and physical dependence on morphine by, glutamate receptor antagonists

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Abstract

The effects of the non-competitive antagonists of the glutamate complex receptor, dizocilpine (MK 801) and ketamine and of the competitive antagonist CGP 39551 were examined on the induction of tolerance to morphine, the development of physical dependence and the expression of the abstinence syndrome to the opiate in mice. Morphine was administered in a single dose (300 mg/kg) of a slow release preparation. Dizocilpine (0.005 or 0.01 mg/kg given at 3, 12 and 24 h after the priming dose of morphine), ketamine (2, 4 or 8 mg/kg, 30 min before and 3, 6, 9 and 24 h after the priming dose) and DL-(*E*)-2-amino-4-methyl-5-phosphonopentanoate carboxy-ethyl-ester (CGP 39551) (1.5 or 3 mg/kg, but not 6 or 12 mg/kg 30 min before and 12 and 24 h after the priming dose) reduced the intensity of tolerance to, and physical dependence on morphine. The drugs also reduced the intensity of the abstinence behaviour when given in a single dose, 30 min before (s.c.) naloxone (4 mg/kg)-precipitated withdrawal syndrome in mice chronically treated with morphine. Thus, the results of this study indicate that competitive and non-competitive NMDA receptor antagonists prevent morphine tolerance and decrease the development of physical dependence on the opiate in mice. © 1997 Elsevier Science B.V.

Keywords: Morphine tolerance; Morphine dependence; Dizocilpine; Ketamine; CGP 39551 (DL-(*E*)-2-amino-4-methyl-5-phosphonopentanoate carboxy-ethylester)

1. Introduction

The wide distribution of excitatory amino acids in the central nervous system (CNS) has encouraged the study of their involvement in a variety of CNS functions including nociception transmission (Headley and Grillner, 1990; Näsström et al., 1992; Marek et al., 1991; Trujillo and Akil, 1991). In particular, glutamate has received considerable attention and, based on its pharmacological properties, it is now accepted that its effects are produced by acting on three types of ionotropic receptors, named after the selective agonists, 3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate and *N*-methyl-D-aspartate (NMDA) (Watkins et al., 1990; Nakanishi, 1992). Of these, the functions of NMDA have been most clearly defined due to the synthesis of highly specific ligands.

Several transmitters and modulators are involved in CNS adaptive processes to chronic opiate administration (Cox, 1993; Mulder and Schoffelmeer, 1993). Recently, the finding that the non-competitive NMDA receptor antagonist, dizocilpine (MK 801), reduces the induction of physical dependence to opiates (Lufty et al., 1993; Elliot et al., 1994) has focused the interest of investigators in the involvement of glutamate in the induction of tolerance and dependence. However, the possible role of this amino acid has not been wholly clarified since other authors have found that the concomitant administration of morphine and non-competitive NMDA receptor antagonists does not affect the processes induced by chronic opiate treatment (Marquis et al., 1991; Bell and Beglan, 1995). On the other hand, most of the available competitive antagonists of NMDA receptors do not enter the brain: accordingly, studies have been carried out with non-competitive antagonists. Two systemically active competitive NMDA receptor antagonists have recently been synthetised; they are CGP 39551 and CGP 37849 (Fagg et al., 1990). The first of these agents has been reported to decrease the intensity of physical dependence induced in mice by chronic ethanol administration (Ripley and Little, 1995). Therefore, we set out to examine the influence of CGP 39551 and of other compounds reported to non-competitively antagonize glutamate NMDA receptors in the induction of tolerance to and physical dependence on morphine in mice.

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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, maintained on a 12/12 h light/dark cycle at constant room temperature (22 ± 2°C) and allowed 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. All groups consisted of 10 mice and each animal was used for only one experimental condition.

2.2. Drugs

The drugs used were morphine HCl (May and Baker, Dagenham, UK), naloxone (Sigma, St. Louis, MO, USA), ketamine and dizocilpine (MK 801) (both from Research Biochemicals International, Natick, MA, USA), DL-(*E*)-2-amino-4-methyl-5-phosphonopentanoate carboxy-ethylester (CGP 39551; a gift from Ciba-Geigy, Basel, Switzerland). The drugs were dissolved in saline and administered subcutaneously (s.c.) in a volume of 10 ml/kg.

2.3. Analgesic test

The hot-plate test as 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. Reaction times were determined by 30 min intervals over 90 min. To avoid severe animal 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 the antagonists on the analgesic response to morphine, each drug was given 30 min before the s.c. test dose (5 mg/kg) of the opiate.

2.4. Induction of tolerance

To test the effects of glutamate receptor antagonists on the development of morphine tolerance and dependence, for each drug assayed animals were divided into the following groups: one group was injected with vehicle and then with saline at the same schedule as the drug under assay. A second group was injected with vehicle and the drug under assay. A third group received morphine suspension and saline at the same schedule as the drug under assay. The last group of mice was injected with morphine suspension plus the drug under study.

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 a (s.c.) test dose (5 mg/kg) of the analgesic. Control groups were injected s.c. with vehicle. 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 drugs under assay.

The drugs tested for tolerance development were administered during chronic morphine treatment, according to the following schedule: CGP 39551 was given 30 min before and 12 and 24 h after the priming dose of morphine; ketamine was administered 30 min before and 3, 6, 9 and 24 h after the priming dose of the opiate; and dizocilpine at 3, 12, and 24 h after the priming dose. Doses assayed for each antagonist are indicated in Section 3.

2.5. Induction of morphine dependence

To study morphine dependence, a dose of morphine similar to that used for the induction of tolerance was given to mice 30 h before i.p. administration of 4 mg/kg naloxone. The experimental protocol was similar to that described for the induction of tolerance. These groups are referred to as treatment during the course of morphine dependence. Furthermore, all glutamate receptor antagonists were administered in an additional scheme which differed from the former in that mice received a single dose of antagonist 30 min before an abstinence behaviour-precipitating naloxone dose. Results in the latter groups are referred to as changes in intensity of the abstinence behaviour.

In all cases the withdrawal syndrome precipitated by 4 mg/kg naloxone, was characterised by diarrhoea, micturition, body shakes, running, paw tremors, convulsions (auditory evoked by a key-ring), jumping and death. The number of mice presenting with this syndrome was recorded in a 20 min observation period. Comparisons were made by assigning the following withdrawal scores: no appreciable effects, 0; micturition, 1; running, 2; diarrhoea, 3; piloerection, 3; paw tremors, 3; body shakes, 4; jumping, 5; convulsions, 5; death, 10.

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 dose of morphine in tolerance experiments was determined by analysis of variance (ANOVA) and confirmed with the Student–Newman–Keuls test. A

Table 1
Effects of dizocilpine, CGP 39551 and ketamine on the development of tolerance to morphine

Antagonist (mg/kg)	Analgesic response to a test dose of morphine (Area under the time–response curve)	
	Naive mice	Morphine-pretreated mice
Saline	145 ± 12	21 ± 5 [a]
Dizocilpine, 0.005	125 ± 11	63 ± 9 [a]
Dizocilpine, 0.01 [c]	133 ± 9	130 ± 10 [b]
CGP 39551, 0.75	144 ± 12	90 ± 8 [b]
CGP 39551, 1.5 [c]	150 ± 12	120 ± 13 [b]
CGP 39551, 3.0	147 ± 12	53 ± 4 [a]
CGP 39551, 6.0	150 ± 11	31 ± 6 [a]
CGP 39551, 12.0	139 ± 10	32 ± 9 [a]
Ketamine, 2.0	148 ± 9	78 ± 6 [b]
Ketamine, 4.0	144 ± 11	100 ± 16 [b]
Ketamine, 8.0 [d]	149 ± 14	130 ± 18 [b]

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, i.p.). CGP 39551, ketamine and dizocilpine were administered during the course of morphine pretreatment at different time intervals (see text).
[a] Statistically lower with reference to their respective control group.
[b] Statistically higher than the effects observed in mice chronically treated with morphine and receiving saline. $P < 0.05$ (Student–Newman–Keuls test). $n = 14$ mice per group.
[c] One animal died during the treatment.
[d] Two animals died during the treatment.

level of probability of 0.05 was accepted as statistically significant. Statistical analysis of the withdrawal syndrome was made using the Mann–Whitney *U* test based on the scores for individual animals.

3. Results

3.1. Effects of glutamate receptor antagonists on morphine antinociception and on the development of tolerance

The doses of the compounds tested did not induce effects on gross animal behaviour, nor did they affect the responses to thermal stimulation. At all dose levels used glutamate receptor antagonists did not significantly alter the response to test doses of morphine (not shown). Nevertheless, co-chronic treatment with morphine and glutamate receptor antagonists increased toxicity and some animals died during drug administration (see Tables 1 and 2).

Tolerance to morphine resulted following a s.c. dose of 300 mg/kg, administered 30 h before the injection of a test dose. Opiate–induced tolerance was demonstrated by the reduction of the effects of a challenge dose of morphine (Table 1). The analysis of variance of the values found in the control groups for the different compounds assayed did not show statistical differences: therefore controls were considered as only one group for further statistical analysis.

As may be observed from Table 1, ketamine (2, 4 and 8 mg/kg) and dizocilpine (0.005 and 0.01 mg/kg) significantly attenuated morphine tolerance.

The effect of CGP 39551 consisted of a bell-shaped response: small doses (0.75 and 1.5 mg/kg) significantly reduced tolerance, whereas this action was absent at larger doses.

3.2. Effects of glutamate receptor antagonists on morphine dependence

The effects of glutamate receptor antagonists on morphine dependence are shown in Table 2. It is evident that

Table 2
Effects of glutamate receptor antagonists on the development of physical dependence to morphine

Withdrawal sign	Relative frequencies of withdrawal signs as percentage of the maximum									
	Saline	CGP 39551 (mg/kg)				Ketamine (mg/kg)			Dizocilpine (mg/kg)	
		1.5	3.0	6.0	12.0 [a]	2 [a]	4	8	0.005 [b]	0.01 [b]
Micturition	100	60	90	100	100	89	80	60	62	75
Running	70	10	30	20	56	0	0	0	0	0
Diarrhoea	60	30	70	80	78	78	80	40	37	12
Piloerection	100	90	80	60	89	44	70	40	87	75
Paw tremors	100	80	60	80	89	33	40	40	37	12
Body shakes	70	50	20	60	44	44	0	40	12	12
Jumping	40	0	10	0	11	11	0	10	0	0
Convulsions	10	0	10	0	0	0	0	10	0	0
Withdrawal score	13.1	7.9 [c]	8.8 [c]	10.4	11.3	7.4 [c]	7.0 [c]	6.8 [c]	6.0 [c]	4.2 [c]
± S.E.M.	±2.1	±1.0	±1.1	±3.5	±1.5	±1.0	±1.2	±1.6	±2.3	±1.9

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.); CGP 39551, ketamine and dizocilpine were administered during the course of morphine pretreatment at different time intervals (see text). $n = 10$ mice per group.
[a] One animal died during the treatment.
[b] Two animals died during the treatment.
[c] Significantly different from control mice. $P < 0.01$ Mann-Whitney *U* test.

Table 3
Effects of glutamate receptor antagonists on the withdrawal syndrome of morphine treated mice

Withdrawal sign	Relative frequencies of withdrawal signs as percentage of the maximum								
	Saline	CGP 39551 (mg/kg)			Ketamine (mg/kg)			Dizocilpine (mg/kg)	
		1.5	3.0	6.0	2 [a]	4	8 [b]	0.05 [b]	0.1 [a]
Micturition	100	50	90	50	86	90	88	50	44
Running	70	10	30	20	14	0	0	50	0
Diarrhoea	60	10	70	20	57	80	38	25	0
Piloerection	100	50	40	60	86	70	50	88	44
Paw tremors	100	50	40	20	86	90	50	63	33
Body shakes	70	20	30	0	28	30	12	75	33
Jumping	40	10	0	0	14	0	0	38	0
Convulsions	10	0	0	0	0	0	0	13	0
Withdrawal score	13.1	5.0 [c]	6.6 [c]	3.5 [c]	6.9 [c]	9.3 [c]	5.5 [c]	13.5	4.2 [c]
± S.E.M.	±2.1	±1.5	±3.6	±1.0	±1.9	±3.2	±3.7	±4.6	±4.0

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.); CGP 39551, ketamine and dizocilpine were administered 30 min before naloxone. $n = 10$ mice per group.
[a] One animal died during the treatment.
[b] 3 mice died during morphine treatment.
[c] Significantly different from control mice. $P < 0.01$ Mann-Whitney U test.

the agents which attenuated morphine tolerance also decreased physical dependence. CGP 39551 interfered with dependence and tolerance at similar doses. As previously stated, larger doses did not attenuate tolerance development; furthermore they did not alter physical dependence. Physical dependence was also decreased by simultaneous administration of morphine and dizocilpine.

3.3. Effects of glutamate receptor antagonists on the intensity of the abstinence behaviour

Glutamate receptor antagonists were also assayed in single doses administered 30 min before induction of the withdrawal syndrome by naloxone. The results of this treatment are shown in Table 3. All glutamate receptor antagonists decreased the intensity of the withdrawal syndrome. Similarly all doses of CGP 39551 reduced the intensity of the abstinence, an effect differing from that observed when the agent was administered during the course of morphine dependence.

4. Discussion

Evidence has accumulated showing that excitatory aminoacids are involved in neurotransmission processes in the 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 (Kangrga and Randic, 1991; Malmberg and Yaks, 1995). Similarly, it may be reasoned that glutamate receptor antagonists might increase morphine antinocicepton. This latter effect has been reported by Aanonsen and Wilcox (1987) and Bernardi et al. (1996). As the procedure used in the present work to examine tolerance to morphine is algesiometric, we considered it of interest to establish whether the assayed glutamate antagonist increases the reaction time to stimulation in the hot plate test. In this regard, the analysis of morphine-glutamate antagonist interactions revealed some interesting characteristics: the glutamate antagonists per se, at doses used in the present work, did not affect the nociceptive response; furthermore, no synergistic effects were observed by concomitant administration with morphine. The latter results differ from those of other reports; thus, Wong et al. (1996) reported an increase, whereas Lufty et al. (1993) found a decrease of opiate nociception. These inconsistencies could be due to differences in animal species, test procedures for measuring antinociception, doses and route of administration of drugs.

The decreased tolerance to morphine following simultaneous administration of opiate and glutamate receptor antagonists is in line with results reported by a number of authors (Marek et al., 1991; Gustein and Trujillo, 1993; Elliot et al., 1994). In contrast to most investigators, Bell and Beglan (1995) reported that dizocilpine did not decrease the intensity of morphine tolerance in the isolated spinal cord of the neonatal rat. These authors have argued that the antagonism of tolerance described in the literature may be only apparent and related to residual circulating dizocilpine in synergism with opiate antinociception. Our experiments do not support this possibility since administration of NMDA-receptor antagonists to naive mice did not increase the responses to a test dose of the opiate. In contraposition, in morphine pretreated mice the effect of the test dose was significantly higher than in the corresponding control group of mice.

A remarkable parallelism in the effects of tolerance and physical dependence was observed by pretreatment with

NMDA receptor antagonists, i.e., drugs that reduced tolerance also reduced the intensity of physical dependence.

The effects of glutamate receptor antagonists co-administered with morphine or given in a single administration before the induction of the abstinence behaviour differ in several aspects. In this regard, CGP 39551 was more effective when administered in single doses (Table 3) as compared when co-administered with morphine (Table 2); in addition, the bell-shaped response was only present in groups treated during the development of physical dependence. Responses to dizocilpine differed in both groups: whereas repeated doses of 0.005 mg/kg reduced the withdrawal syndrome, a single dose of 0.05 mg/kg given 30 min before naloxone was ineffective. In contrast, only the effects of ketamine did not differ when the drug was administered either in repeated doses or in a single dose.

In reference to the results obtained on administration of increasing doses of NMDA receptor antagonists, it is of interest to note that the effect of CGP 39551 was characterised by a reduction of physical dependence when administered in low doses; larger doses were ineffective. There is a possibility that the increase of opiate toxicity induced by the repeated administration of dizocilpine — two mice died during its concomitant administration with the opiate — could mask the dose–response effect of the glutamate receptor antagonist. However, the drug induced a definite dose–response effect in tolerance development. Ketamine at the dose levels used in the work decreased the intensity of the withdrawal syndrome, but a distinct dose–response curve was not obtained; moreover, the effect reached a maximum which was not overcome by increasing doses.

Other reports agree with our present results on chronic morphine effects: it has recently been demonstrated that acute morphine administration reduces glutamate release in rat brain. Tolerance to this effect is produced by chronic opiate treatment and naloxone administration evokes a pulse of glutamate in rat brain (Sepúlveda et al., 1996). Another direct evidence for the role of glutamate in abstinence behaviour has been reported by Tokuyama et al. (1996) who found that the i.c.v. administration of the amino acid to morphine tolerant rats elicited withdrawal signs similar to those evoked by naloxone. Therefore, it seems reasonable to explain our present results by assuming that the inhibition of glutamate effects at NMDA receptors may account for the decrease of the abstinence behaviour elicited by naloxone in morphine treated mice.

Concerning the results found during morphine tolerance and dependence it is worth-while to point out that Ripley and Little (1995) when analysing the effects of CGP 39551 on chronic ethanol treatment favoured the hypothesis that the protective effects on ethanol withdrawal hyperexcitability were due to the presence of residual amounts of the drug after interruption of treatment. A similar explanation cannot be argued for the present results in morphine dependence, because they differed from ethanol experiments in several aspects. First, results during morphine dependence were consistent with those observed during tolerance, i.e., both processes were either decreased or unaffected by similar doses. Second, antinociception induced by a test dose was increased in tolerant mice but not in naive mice treated with similar doses of the glutamate receptor antagonist. Moreover, the bell shaped effects induced by different doses of the antagonist were not observed after its acute administration (30 min before naloxone). It is conceivable that the bell shaped responses may represent an increment in the depression induced by larger doses of CGP 39551 given during morphine treatment. This increment could lead to an increased degree of dependence and to a more intense withdrawal syndrome following naloxone.

It is concluded that the present findings demonstrate that the co-chronic treatment of mice with morphine and the non-competitive NMDA receptor antagonists, dizocilpine and ketamine, and the competitive antagonist, CGP 39551, reduces the intensity of tolerance to, and physical dependence on the opiate. NMDA receptor antagonists also decrease the intensity of the withdrawal syndrome when given in single doses a few min before naloxone administration to morphine dependent mice.

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