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Inhibition of morphine withdrawal by lamotrigine: involvement of nitric oxide

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Abstract

We studied the effects of lamotrigine [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine], a new antiepileptic compound, on naloxone-precipitated morphine withdrawal in mice. Pretreatment with lamotrigine (5–100 mg/kg, s.c.) reversed in a dose-dependent way the withdrawal-induced increase in cerebellar Ca^{2+}-dependent nitric oxide (NO) synthase activity and reduced the number of escape jumps and other motor symptoms of abstinence, at doses that did not modify locomotor activity (25–50 mg/kg). Pretreatment with the NMDA receptor antagonist MK-801 [(+)-5-methyl-10,11-dihydroxy-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine; dizocilpine] (0.1–0.3 mg/kg, s.c.) also reversed the increase in cerebellar Ca^{2+}-dependent NO synthase activity. However, although MK-801 reduced the number of escape jumps and other motor symptoms of abstinence, its effects were not clearly dose-dependent. Furthermore, the highest dose of MK-801 tested (0.3 mg/kg) caused an impairment of the locomotor behaviour in naive mice. Thus, lamotrigine may represent a new and useful agent for the treatment of opiate abstinence.

Keywords: Lamotrigine; Nitric oxide (NO); Dizocilpine (MK-801); Opiate abstinence

1. Introduction

The diversity of drug types capable of attenuating opiate abstinence suggests that the withdrawal syndrome may be modulated at multiple sites involving a variety of neurotransmitter systems (Bhargava, 1994). Excitatory amino acids have been implicated in opiate tolerance and abstinence (Trujillo and Akil, 1991; Marek et al., 1991) and the 'noradrenergic storm' that occurs in opiate abstinence is regulated in the nucleus locus coeruleus and other brain areas by excitatory amino-acidergic fibres (Redmond and Krystal, 1984). This fact is consistent with the increase in the release of excitatory amino acids found during opiate abstinence (Aghajanian et al., 1994) and with the studies demonstrating that excitatory amino acid receptor antagonists attenuate opiate abstinence (Tung et al., 1990; Rasmussen et al., 1991a, b; Tanganelli et al., 1991; Trujillo and Akil, 1991).

It is known that nitric oxide (NO) is produced in response to activation of central excitatory amino acid receptors (Garthwaite et al., 1988; Bredt and Snyder, 1989). NO has also been implicated in opiate abstinence since some NO synthase inhibitors may reduce the symptoms of the withdrawal syndrome (Cappendijk et al., 1993b; Kimes et al., 1993, Kolesnikov et al., 1993). Furthermore, some authors (Cappendijk et al., 1994) have recently demonstrated that there is an increase in the concentrations of total nitrogen oxides (NO_x^-) in brain and cerebrospinal fluid in opiate-withdrawn rats and we have shown that there is an increase of cerebellar NO synthase activity during opiate withdrawal in mice (Leza et al., 1996).

Lamotrigine [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine] is a new antiepileptic compound, chemically unrelated to other anticonvulsant drugs. It has been suggested that the effects of lamotrigine may be due principally to inhibition of glutamate release through blockade of voltage-sensitive Na^+ channels (Leach et al., 1986; Miller et al., 1986). Recently, we have shown that lamotrigine inhibits the release of glutamate and the formation of NO and decreases the levels of cyclic GMP without causing a significant direct inhibition of NO synthase after veratrine depolarization in rat forebrain slices (Lizasoain et al., 1995).

The aim of the present study was then to investigate the use of lamotrigine to diminish the symptoms and signs of

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opiate abstinence. We studied whether the inhibition of NO synthase activity is involved in the mechanism of action of lamotrigine and we compared its effects with those of the non-competitive NMDA receptor antagonist MK-801 [(+)-5-methyl-10,11-dihydroxy-5*H*-diben-zo[*a*,*d*]cyclohepten-5,10-imine; dizocilpine].

2. Materials and methods

Male CD1 mice (Charles-River), aged 5–6 weeks, and weighing 30 ± 3 g at the beginning of the experiments were used. Standard conditions of temperature, humidity and light cycle were used. All experiments were performed between 08:00 and 13:00 h to minimize circadian variations. Immediately after each treatment (see below), brains were rapidly removed and dissected and cerebellum and forebrain were freeze clamped in liquid nitrogen and stored at −80°C until studied. These tissues were selected due to their high levels of NO synthase.

2.1. Induction of morphine abstinence

A pellet containing 75 mg of morphine was implanted in the back of the mice. A placebo-implanted group (with a pellet containing the same amount of saccharose) was also studied. On day 4 post-implantation, the withdrawal syndrome was precipitated by s.c. injection of 1 mg/kg naloxone. Then, mice were kept in a plastic cage to study the main abstinence symptoms: micturition, diarrhoea, stereotyped movements (sniffing and grooming), tremor, jumps (jump off a platform which rests 30 cm above the floor: 'jumping off', and number of escape jumps: 'jumping up') and shaking (Navarro et al., 1991) during a 15 min period, after which the animals were killed. A withdrawal score was calculated based on the proportion of animals that showed each behaviour (10–0; 10: all mice showed this behaviour; 0: none).

On day 4 post-implantation, morphine-dependent mice were treated as follows: (a) naloxone (1 mg/kg), to induce abstinence (withdrawal group); (b) lamotrigine (5, 25, 50 and 100 mg/kg) 45 min before naloxone, and (c) MK-801 (0.001, 0.01, 0.03, 0.1 and 0.3 mg/kg) 45 min before naloxone. This time (45 min) was chosen for pharmacokinetic reasons. All drugs were administered subcutaneously.

2.2. Evaluation of locomotor activity

To determine whether the ability and/or inability to jump during the withdrawal phase induced by different treatments was due to impairment of locomotor activity, we monitored the spontaneous locomotor activity after these treatments in naive mice. The actimetro (Apelab) was located in a sound-proof cubicle. Mice were left in the motility cages ($26 \times 21 \times 9.5$ cm, each with two photocells) for a conditioning period of 5 min before the test time, after which treatments were carried out (saline, lamotrigine or MK-801). Locomotor activity was measured by the number of crossings and recorded every 5 min for a period of 60 min. All of these motor studies were evaluated during the circadian light period. Locomotor activity was also evaluated in a control group during the dark period, but no significant differences were found vs. light period.

2.3. NO synthase activity

Frozen tissues were homogenized at 0°C in 5 volumes of a buffer containing 320 mM sucrose, 50 mM Tris, 1 mM EDTA, 1 mM DL-dithiothreitol, 100 μg/ml phenylmethylsulphonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor and 2 μg/ml aprotinin brought to pH 7.0 at 20°C with HCl. The crude homogenate was centrifuged at 4–6°C at $11\,000 \times g$ for 20 min. Activity of NO synthase was determined in the supernatant by measuring in duplicate the conversion of L-[U^{14}C]arginine to L-[U^{14}C]citrulline as previously described (Salter et al., 1991). Ca^{2+}-dependent NO synthase activity was calculated by subtracting the difference between the samples containing 1 mM EGTA and control samples. Ca^{2+}-independent activity was determined by calculating the difference between samples containing 1 mM EGTA alone and the samples containing both 1 mM EGTA and 1 mM N^{ω}-monomethyl-L-arginine. Measurements are expressed as pmol/min per mg tissue.

2.4. Chemicals

Morphine base was from the Dirección General de Farmacia y productos Sanitarios; MK-801 hydrogen maleate was from Research Biochemicals; lamotrigine (Lamictal) isethionate was from Wellcome Laboratories; L-^{14}C-arginine from Amersham, and all the other chemicals were from Sigma. Drugs were dissolved in distilled water and administered in a volume of 0.5 ml/mouse. The pH of the solutions was corrected when necessary.

2.5. Statistical analysis

Results are means ± standard error of the means (S.E.M.). A $P < 0.05$ or less was considered significant by Fisher's exact test (withdrawal symptoms) and by Student's *t*-test (NO synthase activity and number of crossings).

3. Results

The withdrawal syndrome caused a 2.4-fold increase in the cerebellar Ca^{2+}-dependent NO synthase activity when compared with that of non-morphine-dependent animals treated with 1 mg/kg naloxone (6890 ± 640 and 2902 ± 239, respectively; $n = 16–21$, $P < 0.01$). No significant

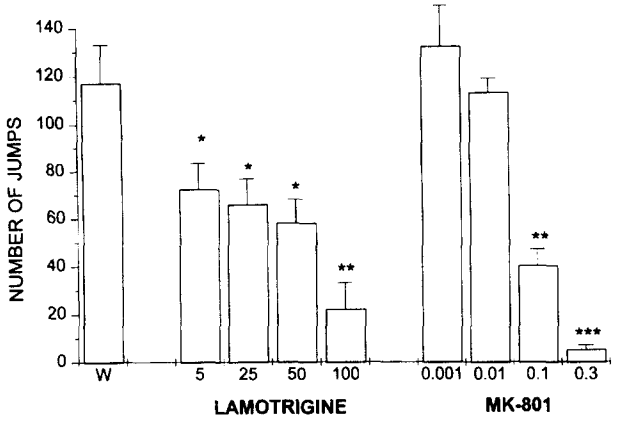

Fig. 1. Effects of administration of lamotrigine (s.c.) and MK-801 (s.c.), 45 min before induction of abstinence by 1 mg/kg of naloxone (s.c.) to morphine-dependent mice on the 4th day of addiction, on the number of escape jumps during the first 15 min of abstinence (mean ± S.E.M., $n = 8$). Withdrawal group (W): saline 45 min before naloxone. * $P < 0.05$ and * * $P < 0.01$ vs. W group (Student's t-test).

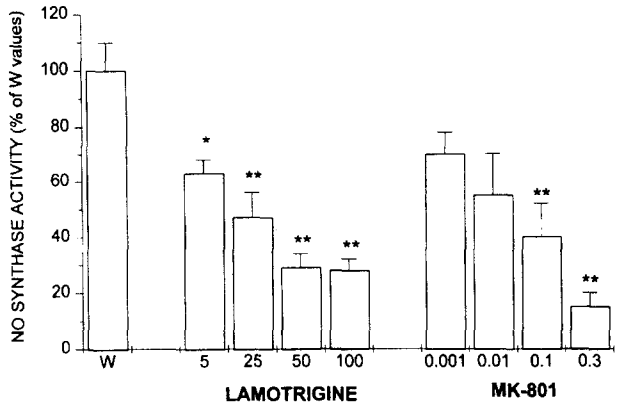

Fig. 2. Effects of administration of lamotrigine (s.c.) and MK-801 (s.c.), 45 min before induction of abstinence by 1 mg/kg of naloxone (s.c.) to morphine-dependent mice on the 4th day of addiction on Ca^{2+}-dependent NO synthase activity in cerebellum (illustrated as a percentage of withdrawal values; means ± S.E.M., $n = 8$). NO synthase activity in morphine-dependent mice after induction of abstinence (withdrawal group, W): 6890 ± 640 pmol of citrulline/min per mg tissue. * $P < 0.05$ and * * $P < 0.01$ vs. W group (Student's t-test).

changes were found in forebrain between naive mice (saline-treated; 5345 ± 931 pmol/min per mg tissue, $n = 10$) and opiate-withdrawn mice (4342 ± 699, $n = 14$). Naloxone (1 mg/kg) alone did not modify NO synthase activity when administered to naive mice. Mice treated with a saccharose pellet did not show any changes in the activity of the brain Ca^{2+}-dependent NO synthase, when compared with naive animals (cerebellum: 2902 ± 239; forebrain: 5345 ± 431 pmol/min per mg tissue, $n = 16$). The inducible, Ca^{2+}-independent form of the enzyme was observed in both tissues, but on the average it was < 10% of the total activity and was not altered by any treatment.

3.1. Effects of lamotrigine on withdrawal symptoms and on NO synthesis

Administration of lamotrigine prior to naloxone on the fourth day of addiction induced a significant and dose-dependent decrease in the number of escape jumps and other motor symptoms of abstinence (stereotyped movements and tremor) (Fig. 1 and Table 1). This effect was parallel to a dose-dependent inhibition of the withdrawal-induced increase in cerebellar NO synthase activity (Fig. 2).

Doses of lamotrigine (25–50 mg/kg) which produced anti-withdrawal effects did not cause any significant change of locomotor behaviour in naive mice (Fig. 3). The highest dose of lamotrigine (100 mg/kg) produced a nearly complete abolition of abstinence (diarrhoea, stereotyped movements and tremor; Table 1). However, the marked inhibition of the number of jumps was not due to an inhibition of the 'jumping off' behaviour, but to an impairment of motor activity caused by this dose (Fig. 3). Subcutaneous co-administration of lamotrigine (25 mg/kg) and L-arginine (300 mg/kg) did not modify either the number of jumps (73 ± 12 jumps) or the NO synthase activity (40 ± 7% of withdrawal value).

3.2. Effects of MK-801 on withdrawal symptoms and on NO synthesis

Administration of MK-801 prior to naloxone on the fourth day of addiction induced an inhibition of the with-

Table 1
Percentage of morphine-dependent mice on the 4th day of addiction that presents each symptom and withdrawal score (10: 100%; 0: 0%) during the first 15 min of abstinence induced by naloxone 1 mg/kg. All treatments were given 45 min before administration of naloxone

Symptoms	Control withdrawal	Lamotrigine (mg/kg)				MK-801 (mg/kg)			
		5	25	50	100	0.001	0.01	0.10	0.30
Micturition	100	100	91	100	80	100	80	70	40 [b]
Diarrhoea	100	100	100	40 [b]	0 [c]	100	80	100	100
Stereotyped movements	100	100	64	0 [c]	0 [c]	100	64	100	80
Tremor	100	100	55 [a]	40 [b]	40 [b]	100	64	55 [a]	55 [a]
Jumping off	100	100	91	100	60	100	100	91	20 [c]
Shaking	100	100	100	100	60	100	100	100	20 [c]
Withdrawal score	10	10	8.3	6.3	4	10	8.1	8.6	5.2

[a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$ vs. saline (control withdrawal). $n = 8$ for all groups (Fisher's exact test).

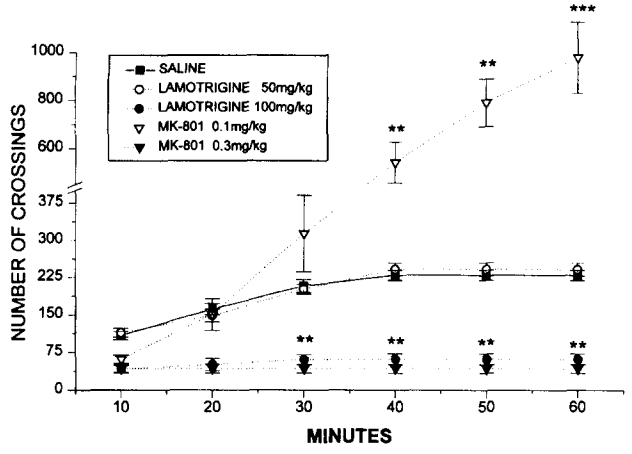

Fig. 3. Effects of lamotrigine and MK-801 on locomotor activity (number of crossings) measured at the times shown in naive mice. Data are presented as cumulative counts (means ± S.E.M.). * $P < 0.05$ and ** $P < 0.01$ vs. saline-treated group (Student's t-test).

drawal-induced increase in NO synthase activity in cerebellum (Fig. 2). MK-801, only at the highest doses tested (0.1–0.3 mg/kg), decreased significantly the number of escape jumps and other symptoms of abstinence (tremor and shaking), during the first 15 min of the withdrawal syndrome induced by naloxone (Fig. 1 and Table 1). The highest dose of MK-801 (0.30 mg/kg) produced a nearly total inhibition of the number of jumps, but this effect was due to an impairment of muscular tone (hindpaws were totally atonic) combined with an inhibition of the 'jumping off' behaviour (Fig. 3). In contrast, lower doses of MK-801 (0.1 mg/kg) induced an increase in motor behaviour (Fig. 3). Subcutaneous coadministration of MK-801 (0.1 mg/kg) and L-arginine (300 mg/kg) did not modify either the number of jumps (32 ± 4 jumps) or the NO synthase activity (40 ± 8% of withdrawal value).

4. Discussion

We have found that lamotrigine produces a dose-dependent inhibition of the symptoms and signs of abstinence. The highest concentration tested (100 mg/kg) produced an almost complete abolition of abstinence, which may be due to inhibition of glutamate release. Indeed, we have previously demonstrated that lamotrigine causes a nearly complete inhibition of excitatory amino acid release in forebrain slices (Lizasoain et al., 1995). It is well known that the release of excitatory amino acids is increased in opiate abstinence (Aghajanian et al., 1994). Therefore lamotrigine, acting presynaptically to reduce glutamate release, is likely to diminish the appearance of the symptoms of abstinence. Thus, the inhibition of glutamate release produced by lamotrigine has two important consequences: a lack of excitatory amino acid receptor stimulation (NMDA and non-NMDA receptors) and an inhibition of the subsequent synthesis of NO. In fact, we have shown that lamotrigine produces a dose-dependent inhibition of NO synthesis that correlates well with the reduction of most of the symptoms of abstinence and which is not due to a direct action on NO synthase (Lizasoain et al., 1995). Additionally, it has been shown that NO synthase inhibitors reduce the symptoms of withdrawal syndrome (Adams et al., 1993; Cappendijk et al., 1993a). Lamotrigine may be beneficial for the treatment of abstinence not only because it inhibits glutamate release but also because it reduces the excessive synthesis of NO found in this condition.

In contrast, we have found that MK-801, a NMDA receptor antagonist, produces an inhibition of the jumping phenomenon and other symptoms but these actions are accompanied by many toxic effects, i.e., the highest dose of MK-801 tested (0.3 mg/kg), which produces anti-withdrawal effects, decreases motor activity, whereas smaller doses may increase motor behaviour (Fig. 3). Thus, the use of MK-801 as anti-withdrawal drug might pose some problems: in addition to our findings, other authors have shown that coadministration of MK-801 with opiates results in severe toxicity in animals (Koek et al., 1988; Bhargava and Matwyshyn, 1993); moreover, NMDA antagonists, especially those that occlude the ion-channel non-competitively (e.g. MK-801), cause pronounced phencyclidine (PCP)-like behaviours, including psychotomimetic, motor and positive reinforcing effects (Koek et al., 1988), that appear to be predictive of PCP-like effects in humans. However, lamotrigine does not induce phencyclidine-like central nervous system effects in rats at doses up to 160 mg/kg (Baxter et al., 1990). Finally, our data and previous work in the literature (Thorat et al., 1994) show that the anti-withdrawal effects of MK-801 are not clearly dose-dependent, since the intermediate dose (0.03 mg/kg) produces an effect much higher than expected when compared with the effects of other doses used. Furthermore, other drugs acting on excitatory amino acid receptors such as DNQX (6,7-dinitroquinoxaline 2,3-dione, a non-NMDA receptor antagonist) and 5,7-DCKA (5,7-dichlorokynurenic acid; an antagonist of the glycine site of NMDA receptors) (Cappendijk et al., 1993a) show a U-shaped dose-effect curve and it is known that high doses of excitatory amino acid receptor antagonists (in contrast to low doses) exert a prominent excitatory and proconvulsant effect in naive animals (Schoepp et al., 1990).

In summary, the use of MK-801 has serious limitations since it produces severe toxicity and only inhibits a limited number of symptoms of abstinence. In contrast, lamotrigine causes dose-dependent anti-withdrawal effects without serious side-effects. It is concluded that the inhibition of glutamate release by lamotrigine may be more beneficial than NMDA and non-NMDA receptor antagonists in managing the symptoms of opiate abstinence.

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