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Short communication

Involvement of locus coeruleus projections in opiate withdrawal but not in opiate tolerance in mice

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Abstract

The ability of *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4), a potent and selective noradrenergic neurotoxic compound, to modify morphine tolerance and dependence was investigated in mice. DSP-4 pretreatment, either 50 or 100 mg/kg i.p., had no effect on the development of tolerance to the analgesic effect of morphine evaluated by the tail-flick test. On the contrary, the higher dose of DSP-4 prevented repetitive vertical jumping, a major naloxone-precipitated withdrawal symptom in mice. These results demonstrate that coerulean neuronal projections are not necessary for the development of tolerance but are clearly involved in the expression of withdrawal-induced jumping in mice.

Keywords: Opiate; Tolerance; Withdrawal; DSP-4 (*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine); Locus ceruleus; (Mouse)

1. Introduction

The noradrenergic system plays an important role in many opiate pharmacological actions. In particular, central catecholamines are likely to be involved in the development of tolerance and physical dependence to narcotic analgesics. Clonidine, an α_2-adrenoceptor agonist, clearly reduced opiate withdrawal manifestations in rat (Taylor et al., 1988). A substantial body of evidence indicates that the locus coeruleus, which is a major noradrenergic nucleus, could be considered as a critical site of regulation of the expression of opiate withdrawal syndrome. Electrophysiological data revealed that the opiate withdrawal syndrome was associated with an increased firing rate of locus coeruleus neurones in vivo (Aghajanian, 1978) and in vitro (Kogan et al., 1992). Moreover, microinjections of clonidine into the locus coeruleus prevented this increase of firing rate (Aghajanian, 1978). The important role of the locus coeruleus in opiate withdrawal is also supported by the fact that microinjections in the locus coeruleus of methylnaloxonium (a hydrophilic opioid receptor antagonist) induced withdrawal symptoms in morphine-dependent rats (Maldonaldo et al., 1992).

The noradrenergic system has also been implicated in the development of opiate tolerance. Chronic administration of morphine in vivo resulted in the development of tolerance to the inhibitory actions of morphine on locus coeruleus firing rate (Aghajanian, 1978). In addition, morphine tolerance was associated with an upregulation of adenylate cyclase and cAMP-dependent protein kinase in rat locus coeruleus cells (Rasmussen et al., 1990). Moreover, intracerebroventricular administration of α_2-adrenoceptor antagonists prevented morphine tolerance in mice (Kaneto and Inoue, 1990).

Extrinsic mechanisms have been implicated in the activation of locus coeruleus neurones. In particular, increased firing of locus coeruleus neurones in opiate withdrawal could be secondary to an increase in excitatory amino acid input from excitatory afferents (Akoaka and Aston-Jones, 1991). This implies that locus coeruleus neurones are not necessary for the development of both opiate tolerance and withdrawal symptoms.

In order to further characterize the role of locus coeruleus projections in these phenomena, we have studied the effects of *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) pretreatment on the development of morphine tolerance and withdrawal manifestations in mice. DSP-4 is a potent noradrenergic neurotoxic agent which has been shown to be highly specific for locus coeruleus

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projections since it induces a long lasting degeneration of almost all coerulean efferents (Fritschy and Grzanna, 1989).

2. Materials and methods

2.1. Animals

Adult male CDF 1 mice (20–25 g) were housed at 10–12 per cage at 22 (±0.5)°C and maintained in a 12 h-12 h light-dark cycle. Food and water were available ad libitum.

2.2. Drugs

Morphine hydrochloride (Francopia, France), naloxone hydrochloride (Sigma, France), *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP-4) (RBI, France) were dissolved in 150 mM NaCl (Prolabo, France). DSP-4 solutions were prepared extemporaneously.

2.3. Treatments

Mice were allotted to 6 groups of 20–24 animals. All drugs were injected intraperitoneally (i.p.) in a volume of 0.1 ml/10 g body weight. Mice were pretreated with one injection of 150 mM NaCl for 2 groups, or of 50 mg/kg DSP-4 for 2 other groups and of 100 mg/kg DSP-4 for the last 2 groups. After 7 days, mice of each group were treated with 12 mg/kg morphine or 150 mM NaCl every day for 17 days. On the 17th day, mice of each group were treated with 10 mg/kg naloxone 3 h after the last administration of morphine or 150 mM NaCl. Injections were done between 8:00 and 9:30 a.m.

2.4. Analgesic test

Analgesic effects were assessed by the tail-flick test performed every other day during the 17 days of experimentation. A cut-off time of 6 s was arbitrarily chosen. The test was performed 30 min after injection of either morphine or NaCl. Mice were adapted to the tail-flick boxes the day before the beginning of chronic treatment.

2.5. Dependence test

On the 17th day of chronic treatment, immediately after the administration of naloxone, mice were individually placed in a cylinder of 0.18 m diameter and 0.60 m height. The degree of dependence was quantified as the number of jumps in 15 min.

2.6. Catecholamine dosage in brain tissue

In order to evaluate the effect of DSP-4, norepinephrine and dopamine were measured in brain tissue. 6 mice per group were decapitated after the last injection of either morphine or 150 mM NaCl, or naloxone. The brain was rapidly removed and dissected in 3 regions (telencephalon, diencephalon and pons plus medulla oblongata) on an ice cold glass plate. Tissues were weighed, frozen at −80°C and stored until analysis. Catecholamine content was measured as described previously (Atgie et al., 1990). Briefly, brain regions were homogenized with a Potter in 2.7 mM EDTA, 0.4 mM sodium metabisulfite and 0.1 M perchloric acid and then centrifuged at 4°C, 10000 × *g* for 10 min. 500 μl of supernatant was added to 20 mg of acid washed activated alumina (Biochrom, France) with dihydroxybenzylamine as an internal standard. Then extraction was performed with acetic acid 0.6 N. 20 μl of eluted solution was directly injected into a high pressure liquid chromatography system coupled with electrochemical detection (BAS-L4C); 50 mM sodium acetate, 20 mM citrate, 0.04 mM sodium octyl sulfate and 5% methanol, pH 4.6 was used as mobile phase. A detection potential of 0.680 V and a flow rate of 1 ml/min were selected. The standards were similarly processed.

2.7. Statistical analysis

Analgesia measured as tail-flick latencies and withdrawal evaluated as number of jumps were compared with control data with one-way analysis of variance followed by post-hoc Dunnett or Scheffé's test (StatView, Macintosh). Biochemical data were statistically evaluated using the Mann-Whitney U-test (StatView, Macintosh). All data are given as means ± S.E.M.

3. Results

3.1. Effect of DSP-4 pretreatment on morphine tolerance

As shown in Fig. 1, DSP-4 pretreatment by intraperitoneal route had no effect on the development of tolerance to the analgesic effect of morphine. In either group pretreated with saline or DSP-4 (50 or 100 mg/kg) before chronic morphine treatment, animals became tolerant to the analgesic effects of morphine. From the 5th day of treatment onwards tail-flick latencies were significantly shorter than those determined on the first day of treatment ($P < 0.05$) for each kind of treatment. Moreover, DSP-4 pretreatment had no effect on the analgesic effect of morphine.

3.2. Effect of DSP-4 pretreatment on morphine dependence

DSP-4 pretreatment at 100 mg/kg reduced the number of jumps induced by naloxone in morphine-tolerant animals (Fig. 2). The number of jumps in 100 mg/kg DSP-4-pretreated animals (29.2 ± 5.7) was significantly lower ($P < 0.001$) than that observed in saline-pretreated animals

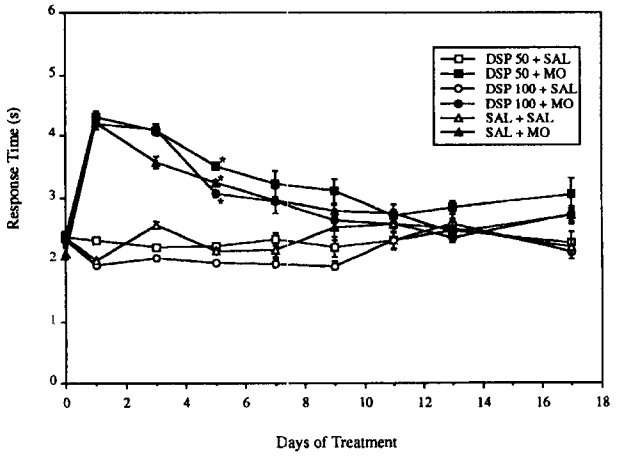

Fig. 1. Effect of DSP-4 pretreatment on tolerance to morphine analgesia. The tail-flick test was performed 30 min after 12 mg/kg morphine or 150 mM NaCl i.p. injections. Mice were pretreated i.p. with DSP-4 50 or 100 mg/kg (DSP 50 or DSP 100, respectively) 7 days before the beginning of chronic treatment. $n = 10–12$ mice per group. * $P < 0.05$ vs. tail-flick response time on the first day of chronic treatment in the same group. After the 5th day of treatment tail-flick response times were significantly different from those on the first day in all treated groups.

(65.5 ± 7.7). At the dose of 50 mg/kg, DSP-4 (51.4 ± 8.2) did not reduce significantly the number of jumps (51.4 ± 8.2 vs. 65.5 ± 7.7 in control group).

3.3. Effect of DSP-4 pretreatment on catecholamine contents in various brain regions

Norepinephrine and dopamine contents were measured in three brain regions: telencephalon, diencephalon and pons plus medulla oblongata. DSP-4 pretreatment (50 mg/kg or 100 mg/kg) had no effect on the dopamine content of these regions. In contrast, DSP-4 pretreatment decreased significantly norepinephrine content. In diencephalon, norepinephrine content was similar in 50 or 100 mg/kg DSP-4-pretreated mice (494.8 ± 36.9 ng/g and 437.7 ± 42.7 ng/g, respectively) and was significantly lower ($P < 0.05$) than in control mice (640.3 ± 38.2 ng/g). In pons plus medulla oblongata, norepinephrine content was not different neither in 50 or 100 mg/kg DSP-4-pretreated mice (259.8 ± 43.5 ng/g and 288.4 ± 18.51 ng/g, respectively) but was significantly lower ($P < 0.01$) than in control mice (609.8 ± 20.7 ng/g). In telencephalon, norepinephrine content was lower in 100 mg/kg than in 50 mg/kg DSP-4-pretreated mice (62 ± 11.1 ng/g vs. 108.75 ± 4.2 ng/g, $P < 0.05$) and in both groups norepinephrine content was lower than in the control group (319.5 ± 13.9 ng/g, $P < 0.01$ for the 2 groups). Moreover, naloxone-induced withdrawal (dependent mice) had no effect on norepinephrine or dopamine content in any of the three brain regions when compared with chronic morphine treatment (tolerant mice). In 100 mg/kg DSP-4-pretreated mice, the norepinephrine content was 73 ± 10.7 ng/g for dependent mice vs. 62 ± 11 ng/g for tolerant mice in telencephalon; 437.7 ± 42.7 ng/g for dependent mice vs. 385.6 ± 62.7 ng/g for tolerant mice in diencephalon; 290.7 ± 18.3 ng/g for dependent mice vs. 288.4 ± 18.5 ng/g for tolerant mice in pons plus medulla oblongata. Similarly, the norepinephrine content in these regions was identical in tolerant and dependent mice after treatment with 50 mg/kg DSP-4 or saline.

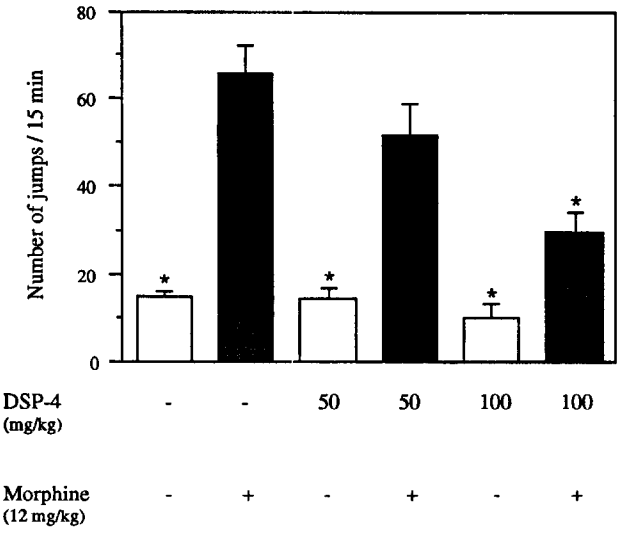

Fig. 2. Effect of DSP-4 pretreatment on opiate withdrawal. Treatments are the same as in Fig. 1. Naloxone 10 mg/kg was injected i.p. 3 h after the last administration of morphine or NaCl solutions. Withdrawal was expressed as the number of jumps in 15 min after naloxone injection. $n = 15–20$ mice per group. * $P < 0.01$ vs. number of jumps in the group pretreated with 150 mM NaCl before chronic morphine treatment.

4. Discussion

Our experiments revealed that DSP-4 pretreatment in mice could prevent opiate withdrawal manifestations. The locus coeruleus has been implicated in behavioural and neurovegetative manifestations of opiate withdrawal (Maldonaldo et al., 1992; Rasmussen et al., 1990). In fact, locus coeruleus electrolytic lesions have been shown to reduce withdrawal manifestations in rats (Maldonaldo and Koob, 1993). Thus, our results showed that lesions of locus coeruleus projections but not of the locus coeruleus itself were able to prevent opiate withdrawal manifestations. In fact, DSP-4 did not alter the structural appearence of the locus coeruleus perikarya but induced neurodegenerative changes of its projections (Fritschy and Grzanna, 1989), as revealed by the decrease in norepinephrine content in these brain regions.

A striking parallel between the time course of the behavioural signs and the hyperactivity of locus coeruleus neurones was observed during opiate withdrawal (Rasmussen et al., 1990; Aghajanian, 1978). It has been shown that, in the rat, DSP-4 treatment could decrease locus coeruleus firing rate between 10 and 50 days after administration (Olpe et al., 1983). These data could partly explain our results since opiate withdrawal symptoms have been

correlated with an increase in the locus coeruleus firing rate (Aghajanian, 1978).

Funada et al. (1994) have shown that DSP-4 administration to morphine-tolerant mice 24 h before naloxone challenge prevented withdrawal manifestations. Our model is quite different since morphine treatment was given to mice pretreated with DSP-4 7 days before. Under these experimental conditions, the neurodegenerative effects of DSP-4 are complete (Fritschy et al., 1990) and opiate tolerance was induced in animals in which locus coeruleus projections had been lesioned.

In the rat, lesions of the dorsal catecholamine bundle induced by 6-hydroxydopamine (Britton et al., 1984) did not prevent opiate withdrawal manifestations. This suggests that other locus coeruleus projections that are susceptible to the neurodegenerative effect of DSP-4 (central tegmental tract, cerebellar and spinal cord projections and dorsal periventricular system projections) could be implicated in opiate withdrawal.

In the rat, DSP-4 administration had no effect on behavioural manifestations of opiate withdrawal (Chieng and Christie, 1995). The animal species and the different DSP-4 dosage could account for the discrepancy between these results and ours. Moreover, in these experiments, tolerance was not evaluated before withdrawal testing. Furthermore, withdrawal manifestations in rat are sometimes difficult to interpret. In contrast, opiate withdrawal-induced repetitive jumping in mice is easy to quantify.

Our results show that degeneration of locus coeruleus projections susceptible to DSP-4 had no effect on opiate tolerance. The involvement of locus coeruleus in opiate tolerance has been advocated on electrophysiological (Aghajanian, 1978; Kogan et al., 1992) and biochemical (Rasmussen et al., 1990) grounds. Especially, during chronic morphine treatment, locus coeruleus cells escape from the inhibitory effect of morphine on their firing rate (Aghajanian, 1978). The development of opiate tolerance implies the involvement of supraspinal noradrenergic centres since intracerebroventricularly but not intrathecally injected α-adrenoceptor antagonists are able to prevent morphine tolerance (Kaneto and Inoue, 1990). The locus coeruleus is the largest supraspinal noradrenergic nucleus and projects massively to spinal and supraspinal neuronal centres. Therefore, all these experimental facts suggest its involvement in opiate tolerance. Our results clearly show that other noradrenergic centres are probably involved in the development of opiate tolerance.

In conclusion, these features suggest that locus coeruleus projections are a major noradrenergic component in the expression of opiate withdrawal but not in the development of opiate tolerance in mice.

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