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Chronic neurosteroid treatment prevents the development of morphine tolerance and attenuates abstinence behavior in mice

Doodipala S. Reddy, Shrinivas K. Kulkarni *

Department of Pharmacology, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160 014, India

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Abstract

The effect of neurosteroids on the development of morphine tolerance and dependence was examined in mice. Development of tolerance to the antinociceptive effect of morphine sulfate (10 mg/kg, twice daily for 9 days) was measured in the tail-flick test and dependence was assessed from naloxone (2 mg/kg)-precipitated withdrawal jumps on day 10 of testing. Concomitant chronic administration of neurosteroids, allopregnanolone (0.5 mg/kg), pregnenolone sulfate (2 and 5 mg/kg) or dehydroepiandrosterone sulfate (2 and 5 mg/kg), followed by morphine (10 mg/kg) prevented the development of tolerance to the antinociceptive effect of morphine and suppressed the naloxone-precipitated withdrawal jumps. In contrast, dehydroepiandrosterone acetate (5 mg/kg) failed to modulate the morphine tolerance and dependence. The inhibitory effect was also seen upon concomitant administration of a neurosteroid precursor, progesterone (1–10 mg/kg), and a mitochondrial diazepam binding inhibitor receptor agonist, 4′-chlordiazepam (0.25–1 mg/kg), while an adrenocorticosteroid, hydrocortisone (1 and 10 mg/kg), failed to do so. However, acute treatment with these neurosteroids was not associated with any decrease in withdrawal jumping behavior in morphine-dependent mice. Neurosteroids themselves, at doses employed in the study, did not exert any effects on antinociception. These results support a role for neurosteroids in the development of tolerance to and dependence on morphine and suggest the potential utility of specific neuroactive steroids in its treatment.

Keywords: Neurosteroids; Morphine; Pregnenolone sulfate; Allopregnanolone; Progesterone; 4′-Chlordiazepam; Opiate tolerance; Opiate dependence

1. Introduction

Research over the past decade has characterized various neurosteroids synthesized within both the central and peripheral nervous systems (Paul and Purdy, 1992; Robel and Baulieu, 1994; Kulkarni and Reddy, 1995) and elucidated their multiple effects on various neurotransmitter systems (Majewska, 1992; Lambert et al., 1995; Schumacher et al., 1996). It is now well recognized that the neuroactive steroids, allopregnanolone, allotetrahydrodeoxycorticosterone and progesterone can potentiate, whereas pregnenolone sulfate and dehydroepiandrosterone sulfate inhibit $GABA_A$ receptor Cl^- channel responses (Mienville and Vicini, 1989; Majewska, 1992; Lan and Gee, 1994; Goodnough and Hawkinson, 1995; Gee et al., 1995). A pathway for neurosteroidogenesis has been delineated in brain (Robel and Baulieu, 1994) and the mitochondrial diazepam-binding inhibitor receptors have been found to regulate neurosteroid biosynthesis (Papadopoulos et al., 1992; Korneyev et al., 1993). The 3α-hydroxylated pregnane steroids have been shown to be potent anticonvulsants (Kokate et al., 1994; Frye, 1995), anxiolytics (Crawley et al., 1986; Bitran et al., 1991, 1995; Reddy and Kulkarni, 1997a,c), neurotrophics (Schumacher et al., 1996; Reddy and Kulkarni, 1997d) and antistress agents (Purdy et al., 1991; Barbaccia et al., 1996; Reddy and Kulkarni, 1996), while sulfated neurosteroids enhance memory performance in rodents (Floods et al., 1988, 1992; Mayo et al., 1993).

Development of tolerance to opiates is characterized by shortened duration and decreased intensity of the euphoric, analgesic and sedative effects. A withdrawal syndrome after abrupt discontinuation or after administration of opioid receptor antagonists occurs within hours of opiate discontinuation. Although the neurobiological basis for the development of tolerance and opiate dependence remains unclear, a wide variety of drugs have been shown to influence the opiate tolerance and withdrawal syndrome by

* Corresponding author. Fax: (91-172) 541-142.

influencing multiple neurotransmitter, ion channel and intracellular messenger pathways (Bhargava, 1994). Recent studies have demonstrated that concomitant neurosteroid treatment prevents the development of tolerance and facilitates recovery from the benzodiazepine withdrawal syndrome (Reddy and Kulkarni, 1997b). Further, it is hypothesized that neurosteroids may have a neuromodulatory role during the development of tolerance and may alleviate at least some of the signs of morphine abstinence (Kulkarni and Reddy, 1995). The purpose of the present study was to determine the effect of chronic neurosteroids on the development of morphine tolerance and naloxone-precipitated withdrawal syndrome.

2. Materials and methods

2.1. Animals

Male albino mice of the Swiss strain (20–25 g, Haryana Agricultural University, Hissar, India) were housed 5 per cage at room temperature and allowed to adapt to laboratory conditions for at least 2 days before the initiation of any experiment. The animals were housed under a standard light/dark cycle with free access to food and water, except during testing. The experiments were performed between 9.00 and 17.00 h at the ambient temperature. Mice were only given a drug or vehicle once.

2.2. Induction of tolerance and dependence

For the induction of tolerance to and dependence on morphine, the mice received morphine sulfate (10 mg/kg) twice daily at 9.30 and 17.00 h for 9 days as described previously (Verma and Kulkarni, 1995).

2.3. Measurement of morphine tolerance

Development of tolerance to the antinociceptive effect of morphine was assessed on days 1, 3 and 9 by the tail-flick test (D'Armour and Smith, 1941; modified by Kulkarni, 1980). Briefly, the tail-flick was evoked by a source of radiant heat, which was focused on the distal part of the tail. Each mouse was tested twice before drug

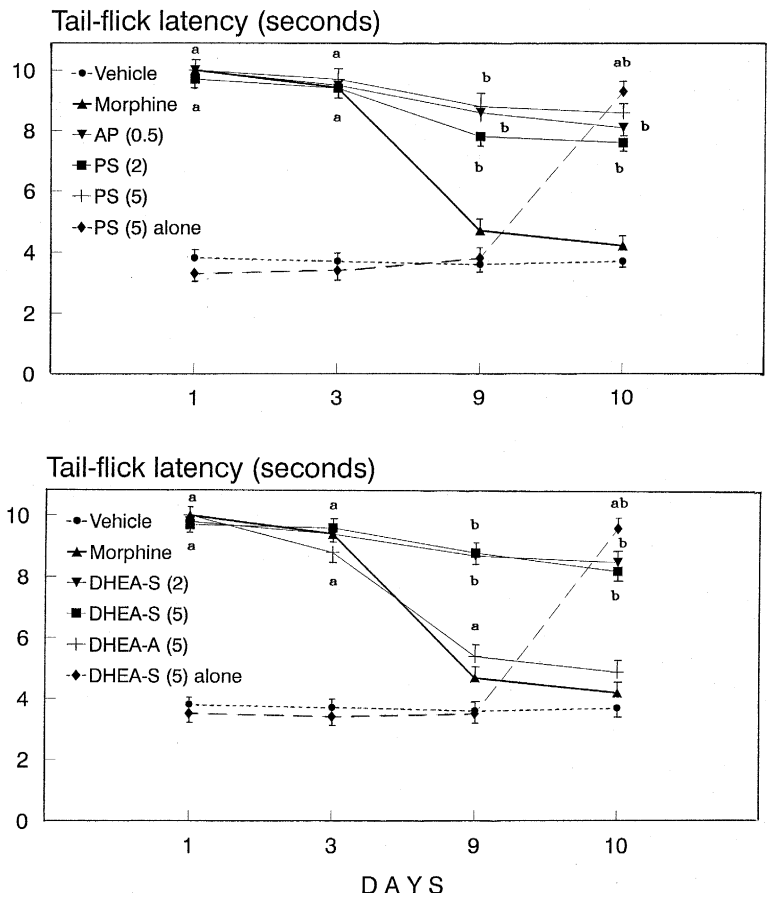

Fig. 1. Effect of chronic neurosteroids on the development of tolerance to the analgesic effect of morphine in mice. Neurosteroids, allopregnanolone (AP) (0.5 mg/kg, i.p.), pregnenolone sulfate (PS) (2 and 5 mg/kg, i.p.), dehydroepiandrosterone sulfate (DHEA-S) (2 and 5 mg/kg, i.p.) or dehydroepiandrosterone acetate (DHEA-A) (5 mg/kg, i.p.), was administered concomitantly with morphine or saline for days 1–9 and the schedule was reversed on day 10 of testing by injecting the respective vehicle followed by morphine. Each point represents the mean ± S.E.M. for 5–8 animals per group. [a] $P < 0.05$ compared to vehicle-treated group; [b] $P < 0.05$ when compared to morphine (10 mg/kg)-treated group (Duncan new multiple range test).

administration and the reaction times were averaged to obtain a baseline. The intensity of the heat stimulus was adjusted so that the mouse flicked its tail after 2–4 s. Only mice with a baseline reaction time from 2 to 4 s were used. A cut-off time of 10 s was imposed to prevent tissue damage. A minimum of three trials was recorded for each mouse 60 min after morphine injection.

2.4. Measurement of morphine withdrawal

Development of morphine dependence was assessed from naloxone-precipitated withdrawal jumps. Immediately after the tail-flick test on day 10, the mice were injected with naloxone (2 mg/kg) and were individually placed in a Plexiglass box (45 × 30 × 30 cm). The number of withdrawal jumps was recorded over a 20-min period.

2.5. Treatment schedule

In chronic studies, mice received neurosteroid and non-neurosteroids, either alone or in combination, twice daily for 9 days followed 30 min later by morphine (10 mg/kg) injection. Control experiments were performed on day 10 with the same group of animals to determine whether neurosteroids or non-neurosteroids affect the development of tolerance or whether they simply alter the behavioral expression of the antinociceptive response. On this day, the treatments were reversed so that the animals that had been treated with neurosteroids or non-neurosteroids followed by morphine on days 1–9 were challenged with vehicle followed by morphine, and animals that had been treated with vehicle followed by morphine on days 1–9 were challenged with the respective drugs followed by morphine. In addition, the animals that had received chronic treatment with neurosteroids or non-neurosteroids followed by vehicle for 9 days were challenged with the respective drug followed by morphine on day 10.

All drugs were injected intraperitoneally 30 min before morphine injection, except progesterone which was injected subcutaneously 60 min before morphine. Control groups received the respective vehicle. The neurosteroids and their dosages were chosen based on results of our previous studies (Reddy and Kulkarni, 1996, 1997a,b,c,d).

2.6. Drugs

Morphine sulfate (Government Analytical Laboratory, Chandigarh, India), allopregnanolone, pregnenolone sulfate, dehydroepiandrosterone sulfate (Sigma, St. Louis, MO, USA), progesterone (Unichem, Bombay, India), dehydroepiandrosterone acetate (Cipla, Bombay, India), hydrocortisone (Wyeth, Bombay, India), 4′-chlordiazepam (Research Biochemicals International, Natick, MA, USA) were used in the present study. All neurosteroids were dispersed in 0.1% Tween and diluted with saline, except progesterone and hydrocortisone which were dissolved in vegetable oil.

2.7. Statistical analysis

All results are expressed as means ± S.E.M. The data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test. Differences with $P < 0.05$ between experimental groups at each point were considered statistically significant.

3. Results

3.1. Effect of neurosteroids, allopregnanolone, pregnenolone sulfate, dehydroepiandrosterone sulfate and dehydroepiandrosterone acetate, on the development of tolerance to the antinociceptive effect of morphine

Mice receiving the chronic treatment with morphine (10 mg/kg) showed the maximal antinociceptive effect on days 1 and 3 of treatment (Fig. 1). However, the animals rapidly developed tolerance to the antinociceptive response as the reaction time reached the baseline latency by day 9 of testing. Chronic treatment with neurosteroids, allopregnanolone (0.5 mg/kg), pregnenolone sulfate (2 and 5 mg/kg) or dehydroepiandrosterone sulfate (2 and 5 mg/kg), followed by morphine (10 mg/kg) for 9 days showed significant antinociception on days 1, 3 and 9 of testing. Challenging each group with saline followed by

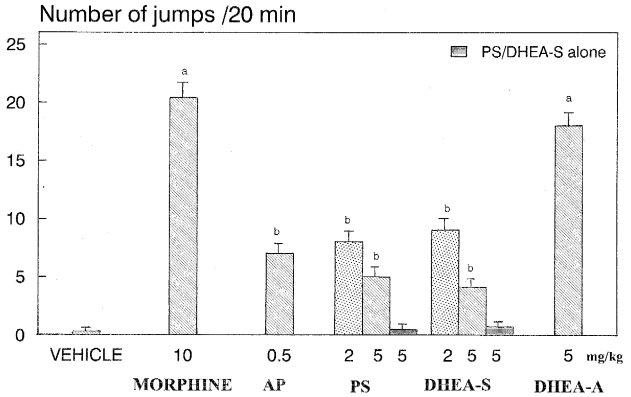

Fig. 2. Effect of chronic neurosteroids on naloxone-induced withdrawal jumps in morphine-dependent mice. Neurosteroids, allopregnanolone (AP) (0.5 mg/kg, i.p.), pregnenolone sulfate (PS) (2 and 5 mg/kg, i.p.), dehydroepiandrosterone sulfate (DHEA-S) (2 and 5 mg/kg, i.p.) or dehydroepiandrosterone acetate (DHEA-A) (5 mg/kg), was given concomitantly with morphine or saline for days 1–9 and the naloxone (2 mg/kg, i.p.)-induced withdrawal behavior was assessed on day 10 of testing. In the case of the PS or DHEAS only groups, the mice were treated chronically with vehicle followed by PS (5 mg/kg) or DHEAS (5 mg/kg) for 9 days and were challenged with naloxone (2 mg/kg) on day 10 of testing. Each bar represents the mean ± S.E.M. for 5–8 animals per group. [a] $P < 0.05$ compared to vehicle-treated group; [b] $P < 0.05$ when compared to morphine (10 mg/kg)-treated group (Duncan new multiple range test).

morphine on day 10 also evoked a significant antinociceptive response. In contrast, repeated administration of dehydroepiandrosterone acetate (5 mg/kg) and morphine (10 mg/kg) also evoked significant antinociception on days 1 and 3 as compared with the control group receiving vehicle alone. However, on day 9 of testing, the time of reaction to radiant heat reached the baseline. On day 10, the tail-flick latency was no greater than that of the control group receiving vehicle followed by morphine.

Mice chronically treated with vehicle followed by pregnenolone sulfate (5 mg/kg) or dehydroepiandrosterone sulfate (5 mg/kg) on days 1–9 did not show any significant antinociceptive effects, and also no antinociception by morphine when tested on day 10 (Fig. 1). Further, mice that had been treated with vehicle and morphine (10 mg/kg) on days 1–9, when challenged with allopregnanolone (0.5 mg/kg), pregnenolone sulfate (5 mg/kg) or dehydroepiandrosterone sulfate (5 mg/kg) followed by morphine on day 10 did not exhibit any antinociception as compared to the vehicle plus morphine (10 mg/kg)-treated control group (data not shown).

3.2. Effect of neurosteroids, allopregnanolone, pregnenolone sulfate, and dehydroepiandrosterone sulfate and dehydroepiandrosterone acetate, on naloxone-induced withdrawal jumping in morphine-dependent mice

Fig. 2 shows the number of jumps per mouse during the abstinence syndrome elicited on day 10 by single dose of naloxone (2 mg/kg) in mice receiving repeatedly vehicle followed by morphine (10 mg/kg) for 9 days. Chronic treatment with neurosteroids, allopregnanolone (0.5 mg/kg), pregnenolone sulfate (2 and 5 mg/kg) or dehydroepiandrosterone sulfate (2 and 5 mg/kg), for 9 days significantly decreased the jumping response induced by naloxone in morphine-dependent animals at all the doses used. However, repeated pretreatment with dehydroepiandrosterone acetate (5 mg/kg) for 9 days in morphine-treated mice, then challenge with saline and morphine on day 10 failed to reduce naloxone-precipitated jumping.

Control mice chronically treated with vehicle and injected with neurosteroids, pregnenolone sulfate (5 mg/kg)

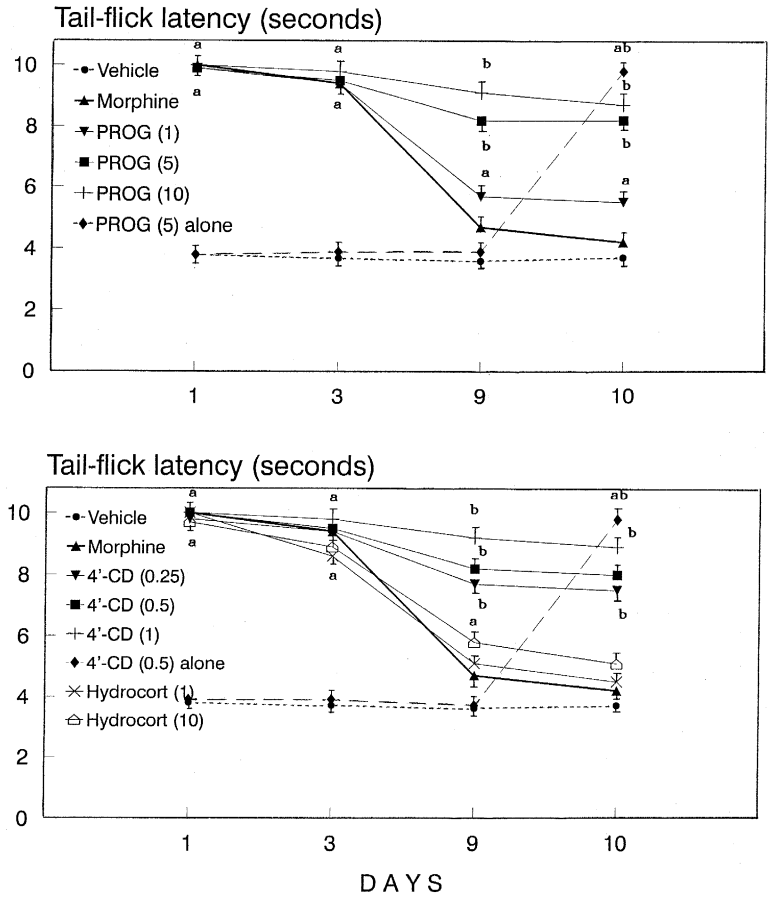

Fig. 3. Effects of different doses of progesterone and 4′-chlordiazepam and hydrocortisone on the development of tolerance to the analgesic effect of morphine in mice. Progesterone (PROG) (1–10 mg/kg, s.c.), 4′-chlordiazepam (4′-CD) (0.25–1 mg/kg, i.p.) or hydrocortisone (Hydrocort) (1 and 10 mg/kg, s.c.) was administered concomitantly with morphine or saline for days 1–9 and the schedule was reversed on day 10 of testing by injecting the respective vehicle followed by morphine. Each point represents the mean ± S.E.M. for 5–8 animals per group. [a] $P < 0.05$ compared to vehicle-treated group; [b] $P < 0.05$ when compared to morphine (10 mg/kg)-treated group (Duncan new multiple range test).

or dehydroepiandrosterone sulfate (5 mg/kg), for 9 days did not show any behavioral signs of opiate withdrawal after naloxone (2 mg/kg) (Fig. 2).

The acute effect of neurosteroids on the naloxone-induced jumps in mice receiving chronic saline followed by morphine on days 1–9 was also evaluated in order to delineate any non-specific effects of neurosteroids on behavioral withdrawal signs. Vehicle-treated mice, when challenged with naloxone (2 mg/kg), displayed 20.4 ± 2.08 jumps. Allopregnanolone (0.5 mg/kg) and pregnenolone sulfate (5 mg/kg), when administered 30 min prior to naloxone to morphine-dependent mice, produced 17.9 ± 3.24 and 18.7 ± 2.62 jumps, respectively, which did not differ from the vehicle control. Similarly, the jumps induced by dehydroepiandrosterone sulfate (5 mg/kg) (21.3 ± 3.46), progesterone (5 mg/kg) (20.6 ± 2.93) or 4′-chlordiazepam (0.5 mg/kg) (19.1 ± 2.34) in morphine-dependent mice were also not significantly different from those of the vehicle control.

3.3. Effect of progesterone, 4′-chlordiazepam and hydrocortisone on the development of tolerance to the antinociceptive effect of morphine

As shown in Fig. 3, mice receiving progesterone (1–10 mg/kg), a neuroactive steroid and precursor for neurosteroids, 4′-chlordiazepam (0.25–1 mg/kg), a mitochondrial diazepam binding inhibitor receptor agonist and neurosteroid inducer, followed by morphine (10 mg/kg) on days 1–9 exhibited significant dose-dependent antinociception throughout the testing period. However, the lower dose of progesterone (1 mg/kg) evoked a tolerance to the antinociceptive effect of morphine that was similar to that in the vehicle-treated group, while mice receiving the higher doses of progesterone (5 and 10 mg/kg) exhibited significant antinociception throughout the testing period. Chronic treatment with hydrocortisone (1 and 10 mg/kg), an adrenocorticosteroid, failed to modify the development of tolerance to the antinociceptive effect of morphine. Mice chronically treated with vehicle followed by progesterone (5 mg/kg) or 4′-chlordiazepam (0.5 mg/kg) on days 1–9 did not show any significant antinociceptive effects (Fig. 3). Further, mice that had been treated with vehicle and morphine (10 mg/kg) on days 1–9, when challenged with progesterone (5 mg/kg) or 4′-chlordiazepam (0.5 mg/kg) followed by morphine on day 10, did not exhibit any antinociception compared to the control mice treated with vehicle or morphine (10 mg/kg) (Fig. 3).

3.4. Effect of progesterone, 4′-chlordiazepam and hydrocortisone on naloxone-induced withdrawal jumping in morphine-dependent mice

As depicted in Fig. 4, repeated treatment with progesterone (5 and 10 mg/kg) or 4′-chlordiazepam (0.25–1 mg/kg) for 9 days, followed by saline and morphine on day 10 significantly decreased the jumping behavior induced by naloxone in morphine-dependent animals in a dose-related manner. Whereas mice receiving the lower dose of progesterone (1 mg/kg) showed a tendency to a suppressed naloxone-precipitated jumping behavior, the effect was not statistically significant. Similarly, chronic pretreatment with hydrocortisone (1 and 10 mg/kg) for 9 days also failed to alter the naloxone-induced jumps in morphine-dependent mice.

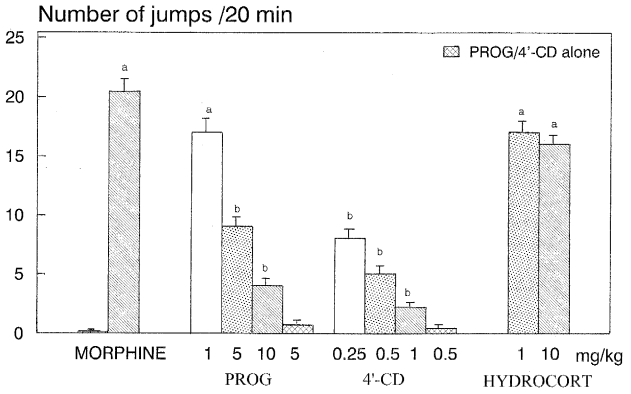

Fig. 4. Effects of different doses of progesterone, 4′-chlordiazepam and hydrocortisone on naloxone-precipitated withdrawal jumps in morphine-dependent mice. Progesterone (PROG) (1–10 mg/kg, s.c.), 4′-chlordiazepam (4′-CD) (0.25–1 mg/kg, i.p.) or hydrocortisone (Hydrocort) (1 and 10 mg/kg, s.c.) was administered concomitantly with morphine or saline for days 1–9 and the naloxone (2 mg/kg)-induced withdrawal jumping was assessed on day 10 of testing. In the case of PROG or 4′-CD alone groups, mice were treated chronically with vehicle followed by PROG (5 mg/kg) or 4′-CD (0.5 mg/kg) for 9 days and were challenged with naloxone (2 mg/kg) on day 10 of testing. Each bar represents the mean ± S.E.M. for 5–8 animals per group. [a] $P < 0.05$ compared to vehicle-treated group; [b] $P < 0.05$ when compared to morphine (10 mg/kg)-treated group (Duncan new multiple range test).

Control mice chronically treated with vehicle and injected with progesterone (5 mg/kg) or 4′-chlordiazepam (0.5 mg/kg) for 9 days did not show any behavioral signs of withdrawal after naloxone (2 mg/kg) (Fig. 4).

4. Discussion

In the present experiments chronic treatment with the neurosteroids, allopregnanolone, pregnenolone sulfate and progesterone, and the mitochondrial diazepam binding inhibitor receptor agonist, 4′-chlordiazepam, was remarkably effective to prevent the development of tolerance to the antinociceptive effect of morphine and to reduce the incidence of withdrawal jumping in morphine-dependent mice. Chronic treatment with non-neuroactive steroids, dehydroepiandrosterone acetate and hydrocortisone, did not modify the development of tolerance and the expression of morphine withdrawal. Moreover, repeated administration of neurosteroids alone for 9 days did not modify the tail-flick latencies in vehicle-treated animals.

The present study provided evidence for the first time that neurosteroids are effective to attenuate opiate tolerance to and dependence on opiates. Although the neurosteroid, 3α-androstanediol, has analgesic effects in animal models (Frye et al., 1996), only few studies have described a role for neurosteroids in the development of morphine tolerance. Chronic concurrent administration of estradiol and testosterone abolishes morphine tolerance (Ahmadiani and Hanafi, 1996). As with benzodiazepines (Tejwani et al., 1993), it is possible that the effects of neurosteroids on morphine tolerance and dependence involve the $GABA_A$ receptor Cl^- channel. Neurosteroids may also modulate the chronic morphine-induced increase in Ca^{2+} channel function (Diaz et al., 1995a,b) by inhibiting voltage-gated Ca^{2+} channel currents (Spence et al., 1991; Ffrench-Mullen et al., 1994). However, the findings in the present study do not allow this type of conclusion.

Chronic administration of dehydroepiandrosterone sulfate ester, but not the acetate, blocked morphine tolerance and dependence. The metabolism of dehydroepiandrosterone between sulfated and unsulfated forms occurs bi-directionally within the brain and is sensitive to the steroid sulfatase inhibitor, estrone-3-O-sulfamate (Li et al., 1995). Thus, it is possible to suggest that it is the sulfated form of dehydroepiandrosterone that is pharmacologically active. The difference in the activity of the sulfate and the acetate forms of dehydroepiandrosterone may be related to pharmacokinetic or pharmacodynamic properties. Although hydrocortisone is not a natural steroid in mice, the negative results with this steroid do not exclude a possible role of adrenocorticosteroids in the development of opioid tolerance (Piazza and Le Moal, 1996).

Recent evidence suggests that progesterone exerts diverse behavioral actions through its possible in vivo conversion to allopregnanolone (Bitran et al., 1993; Reddy and Kulkarni, 1996). Moreover, progesterone has an antiglucocorticoid action, and therefore can directly and indirectly modulate tolerance and dependence phenomena due to morphine. Since mitochondrial diazepam-binding inhibitor receptors regulate the biosynthesis of neurosteroids (Krueger and Papadopoulos, 1992), the 4′-chlordiazepam-induced attenuation of the development of tolerance to morphine and of the related abstinence behavior may involve such a mechanism acting on neurosteroid formation.

Neurosteroids attenuated the naloxone-induced withdrawal jumping following their repeated administration for 9 days, but were inactive after acute administration. The lack of suppressant effect following acute neurosteroid administration may be related to their ability to prevent the development of dependence rather than to suppression of the withdrawal manifestations. This possibility finds support from the results of experiments without pretreatment on day 10 in groups receiving chronic neurosteroids followed by morphine on days 1–9. Neurosteroids may also bind to the intracellular progestin receptor after metabolic conversion and start gene transcription (Rupprecht et al., 1993). This long-term regulatory effect of neurosteroids on the neurons may result in prevention of the development of tolerance to and dependence on opiates.

In summary, the present findings indicate that concomitant chronic administration of the neurosteroids, allopregnanolone, pregnenolone sulfate and dehydroepiandrosterone sulfate, attenuates the development of morphine tolerance and naloxone-precipitated abstinence behavior in mice. The neurosteroid precursor, progesterone, and the mitochondrial diazepam-binding inhibitor receptor agonist and neurosteroid inducer, 4′-chlordiazepam, may also prevent the development of tolerance to and dependence on morphine. These data suggest a potential for neurosteroids in the management of the opiate tolerance and withdrawal syndrome.

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