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Influence of prazosin and clonidine on morphine analgesia, tolerance and withdrawal in mice

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Abstract

Rapid development of tolerance and dependence limits the usefulness of morphine in long-term treatment. We examined the effects of clonidine (α_2-adrenoceptor agonist) and prazosin (α_1-adrenoceptor antagonist) on morphine analgesia, tolerance and withdrawal. Morphine tolerance was induced using a 3-day cumulative twice-daily dosing regimen with s.c. doses up to 120 mg/kg. Tolerance was assessed on day 4, as loss of the antinociceptive effect of a test dose of morphine (5 mg/kg). After 10 h, morphine withdrawal was precipitated with naloxone (1 mg/kg). Prazosin had no analgesic effect alone but dose-dependently potentiated morphine analgesia in morphine-naive mice. Another α_1-adrenoceptor antagonist, corynanthine, had similar effects. Prazosin also increased the analgesic potency of the morphine test dose in morphine-tolerant mice. Naloxone-precipitated vertical jumping was not affected, but weight loss was reduced by prazosin. Acutely administered clonidine potentiated morphine analgesia and alleviated opioid withdrawal signs, as expected. We conclude that in addition to the already established involvement of α_2-adrenoceptors in opioid actions, also α_1-adrenoceptors have significant modulatory role in opioid analgesia and withdrawal.

Keywords: Morphine withdrawal; Morphine tolerance; Analgesia; Prazosin; Clonidine

1. Introduction

Pain modulation is a dynamic process, which involves many interactions among complex ascending and descending neuronal systems. Opioidergic and noradrenergic pathways have very important roles in nociception and analgesia (Childers, 1991; Furst, 1999; Summers and McMartin, 1993). Activation of opioid and α_2-adrenergic receptors inhibits the transmission of pain sensation at spinal and supraspinal levels. While α_2-adrenoceptors inhibit nociception and have analgesic synergy with opioids, there is evidence that α_1-adrenoceptors may facilitate nociception and oppose opioid analgesia. Microinjection of the α_1-adrenoceptor antagonist, prazosin, into the nucleus raphe magnus, a brain nucleus involved in nociceptive processing, produces antinociception similar to the α_2-adrenoceptor agonist clonidine (Sagen and Proudfit, 1985). It has also been reported that prazosin augments clonidine analgesia in amphibians (Brenner et al., 1994). Morphine microinjection into the ventrolateral periaqueductal gray inhibits nociceptive tail responses, an effect mediated by acetylcholine, 5-HT and α_2-adrenergic receptors in the spinal cord. In some experiments, however, microinjection of morphine into the periaqueductal gray has also facilitated nociceptive responses; this facilitation of hot plate responses was mediated by α_1-adrenoceptors in the spinal cord (Holden et al., 1999).

Rapid development of tolerance and dependence limits the usefulness of morphine and other potent opioids in long-term treatment. Morphine tolerance and dependence are complex phenomena, involving also the brain noradrenergic system (Harrison et al., 1998; Milanes and Laorden, 2000). Tolerance also develops to the analgesic activity of clonidine. The analgesic and sedative effects of α_2-adrenoceptor agonists are attenuated in chronic administration, and there is cross-tolerance between morphine and α_2-adrenoceptor agonists (Roerig, 1995; Ware and Paul, 2000). Clonidine has been used clinically for treatment of opioid withdrawal. There is very limited information on the possible involvement of α_1-adrenoceptors in opioid tolerance, dependence and withdrawal.

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Selective α_1-adrenoceptor antagonists may thus potentiate morphine analgesia and have potential to counteract some of the undesired effects of morphine. Prazosin is a relatively selective α_1-adrenoceptor antagonist. High doses of prazosin also block α_2-adrenoceptors but with less potency at α_{2A}-adrenoceptors, compared to α_{2B}- and α_{2C}-adrenoceptors. In this study, the effects of acute administration and chronic pretreatment with different doses of prazosin on morphine analgesia, tolerance and withdrawal were examined to clarify the importance of α_1-adrenergic receptor activation in modulation of opioid effects. The involvement of α_2-adrenoceptors in these processes was also investigated with use of the classical α_2-adrenoceptor agonist, clonidine, similarly employed as single and repeated doses.

2. Materials and methods

2.1. Animals

Male C57 Bl/6 J mice weighing 22–35 g were used. Animals were allowed free access to food and tap water, and were kept under artificial light for 12 h each day and in a room with controlled temperature (21 °C) and humidity (50 ± 10%). All experiments were approved by the local committee for animal welfare and were in accordance with the European Communities Council Directive of 24 November 1986 (86/906/EEC). Each mouse was tested only once. The group *n* was 9 to 10.

2.2. Drugs

Morphine HCl (Sigma), prazosin HCl (Tocris), cirazoline HCl (Tocris), corynanthine HCl (Tocris), clonidine HCl (Sigma) and naloxone HCl (RBI) were used. Prazosin and corynanthine were dissolved in polyethylene glycol and other drugs were dissolved in physiological saline. Appropriate vehicle controls were used. All drugs were administered in a volume of 10 ml/kg s.c. or i.p. as applicable.

2.3. Antinociceptive assay

The tail-flick method of D'Amour and Smith (1941) was used with a cutoff time of 10 s using a commercial tail-flick analgesiameter (Ugo Basile, Comercio, Italy). The basal tail-flick scores were first measured followed by a second tail-flick measurement 30 min after i.p. administration of the test drugs (prazosin 0.01, 0.1, 0.25, 0.5, 1 or 2 mg/kg, or clonidine 5 mg/kg, or cirazoline 0.3 mg/kg, or corynanthine 0.5 mg/kg, or saline). Then, a 5 mg/kg test dose of morphine was administered to all mice, and 30 min later, tail-flick scores were measured for the third time. Tail-flick scores were converted to %MPE (Maximal Possible Effect): %MPE=(measured tail-flick score—average tail-flick score of saline-treated control group)/(cutoff time—average tail-flick score of saline-treated control group) × 100%.

2.4. Induction of morphine tolerance and dependence

A 3-day cumulative dosing regimen was used for the induction of morphine tolerance and dependence. The treatment schedule consisted of twice daily s.c. doses of morphine given at 30 mg/kg (a.m.) and 45 mg/kg (p.m.) on day 1; 60 and 90 mg/kg on day 2; and 120 mg/kg twice on day 3. Animals were assessed for both tolerance and dependence on the 4th day, as described by Way et al. (1969).

2.5. Effects of prazosin and clonidine on expression of morphine tolerance and dependence

On the 4th day, 12 h after the second 120 mg dose of morphine, an acute dose of test drug (clonidine or prazosin) or saline was given i.p. 30 min before the morphine test dose (5 mg/kg) for assessment of tolerance. Tolerance was assessed based on loss of the antinociceptive effects of morphine, using the tail-flick test. Different doses of clonidine (0.5, 1, 2 and 5 mg/kg) and prazosin (0.01, 0.1, 0.25, 0.5 and 1 mg/kg) were tested. On the 4th day, after the assessment of tolerance, a final dose of morphine (120 mg/kg) was given. Ten hours later, different doses of prazosin, clonidine or saline were given. Thirty minutes after the drug administration, naloxone (1 mg/kg s.c.) was given to precipitate morphine withdrawal. Morphine withdrawal was assessed as the occurrence of withdrawal signs following naloxone. The mice were observed in groups of five for 15 min in a transparent cylinder (16 cm height, 12 cm diameter) for the occurrence of withdrawal jumping. They were then kept in well-ventilated cages without access to food and water for another 2 h 45 min. The weight loss rate was calculated for each animal.

2.6. Repeated pretreatment with test drugs (prazosin and clonidine)

Clonidine, prazosin or saline were injected i.p. twice a day, immediately preceding the morphine injections during the induction period. Tolerance and withdrawal were assessed with the same procedures as described previously except that test drugs were not given preceding the test dose of morphine (5 mg/kg) or before naloxone.

2.7. Statistical analysis

Analgesia was measured as tail-flick latencies and converted to %MPE. Naloxone-precipitated morphine withdrawal was evaluated based on the number of jumps and percentage loss of body weight. The effects of each drug were tested using one-way analysis of variance (ANOVA) followed by Scheffé's post hoc test using a computer program (SPSS 8.0 for Windows, SPSS, Chicago, IL). Different drugs were compared using independent sample *t*-tests. All data are presented as mean ± S.E.M. The level of significance was set at $P < 0.05$.

3. Results

3.1. Acute prazosin potentiated morphine analgesia and opposed morphine tolerance

Prazosin given alone had no analgesic effect, neither in drug-naive (Fig. 1A) nor in morphine-treated mice (result not shown). Single doses of prazosin administered 30 min before a test dose (5 mg/kg) of morphine increased the analgesic efficacy of the morphine test dose, as evidenced by increased reaction latencies in the tail-flick test (up to 84 ± 5% and 90 ± 4% of MPE after 0.25 and 0.5 mg/kg, compared to 38 ± 1% of MPE in the control group receiving only morphine) (Fig. 1A). Another α_1-adrenoceptor antagonist, corynanthine, also potentiated morphine analgesia, and had no analgesic effect when given alone. Cirazoline (0.3 mg/kg), an α_1-adrenoceptor agonist, did not affect morphine analgesia (Fig. 1B).

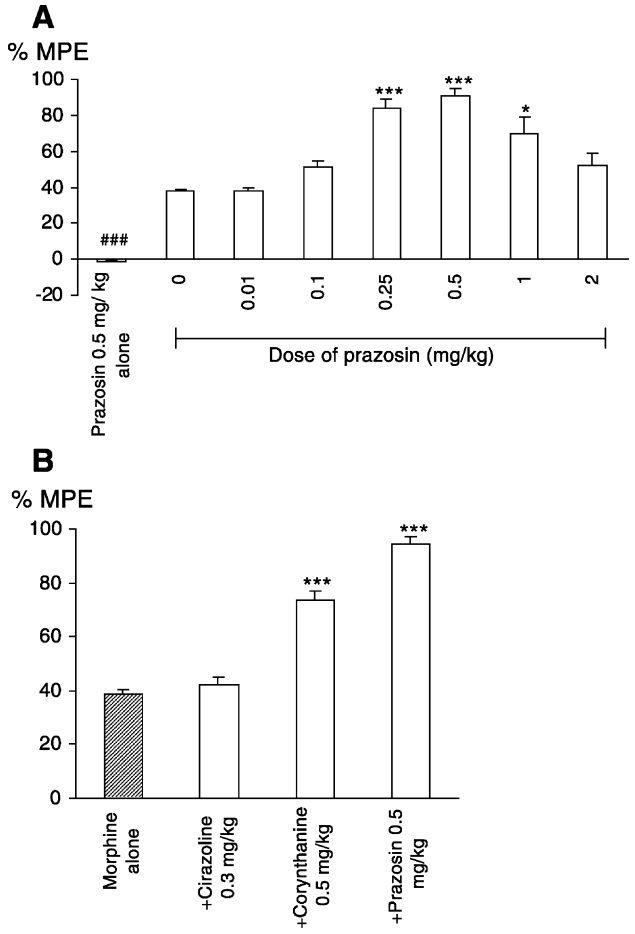

Fig. 1. (A) Acute prazosin potentiated morphine (5 mg/kg) analgesia in mice, but prazosin alone had no analgesic effect. Tail-flick scores were converted to percentage of Maximal Possible Effect (%MPE). Data are means ± S.E.M., *$P<0.05$ and ***$P<0.001$; ANOVA with Scheffé's post hoc test. ###, $P<0.001$; t-test compared to morphine alone. (B) Acute prazosin and corynanthine potentiated morphine (5 mg/kg) analgesia in mice, but cirazoline had no influence. ***$P<0.01$; ANOVA with Scheffé's post hoc test.

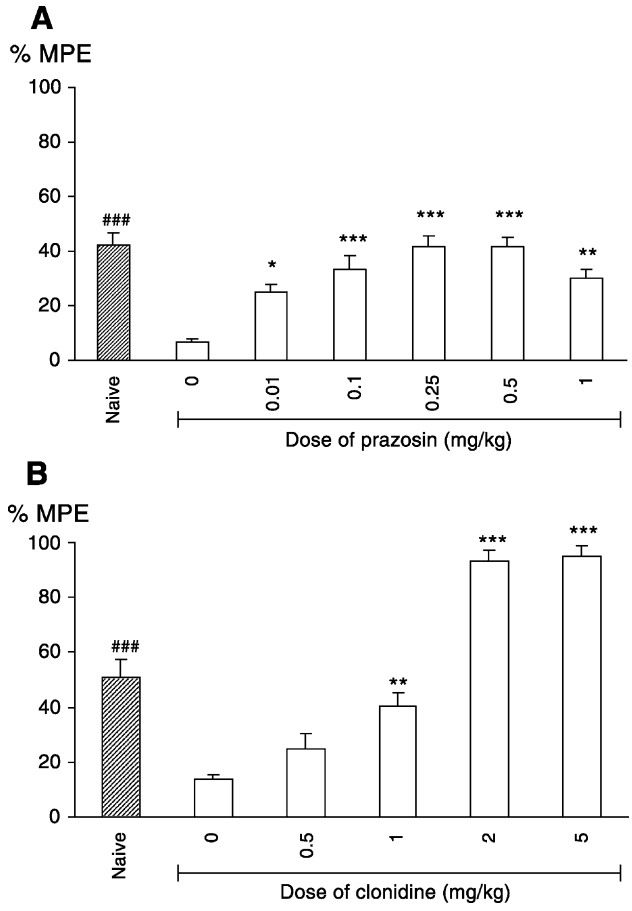

Fig. 2. Acute effects of prazosin (A) and clonidine (B) on analgesia scores after a test dose of morphine (5 mg/kg) in morphine-tolerant mice (open bars). Naive mice (hatched bar) received saline injections instead of morphine in the induction period. Asterisks denote significant differences compared to control groups (morphine tolerant, no prazosin or no clonidine, Scheffé's test). ###, $P<0.001$, t-test compared to morphine-treated control groups.

Tolerance to the analgesic effect of the test dose of morphine was evident in the mice pretreated with morphine for 3 days (e.g., 7 ± 1% of MPE vs. 42 ± 5% of MPE in drug-naive mice, Fig. 2A). Morphine tolerance was dose-dependently opposed by prazosin. Prazosin 0.25 and 0.5 mg/kg given 30 min before the test dose of morphine restored the analgesic efficacy of the test dose of morphine to the same level of analgesia as observed in drug-naive mice (42 ± 3 and 41 ± 4%), but not to the level observed in drug-naive mice receiving both prazosin and 5 mg/kg morphine (see Fig. 1). Larger (1 mg/kg) and smaller (0.01 and 0.1 mg/kg) doses of prazosin also decreased the expression of morphine tolerance statistically significantly (Fig. 2A).

3.2. Clonidine alone had potent analgesic effects

Clonidine is known to be a potent analgesic in mice and to potentiate morphine analgesia (Fairbanks and Wilcox, 1999). Dose–response experiments and interaction studies

with morphine were therefore not repeated. We observed that a single dose of clonidine (5 mg/kg i.p.), given to drug-naive mice, was sufficient to induce an analgesic response amounting to 93 ± 3% of MPE. When given to morphine-treated mice preceding the 5 mg/kg test dose of morphine, clonidine appeared to retain its analgesic potency (40 ± 5%, 93 ± 4% and 95 ± 4% of MPE for 1, 2 and 5 mg/kg of clonidine) (Fig. 2B).

3.3. Prazosin modified naloxone-precipitated morphine withdrawal

Single doses of prazosin given 30 min before naloxone (1 mg/kg) to morphine-dependent mice had no effects on the number of vertical jumps observed in the first 15 min after naloxone administration (not shown). Prazosin, however, dose-dependently and very effectively prevented the weight loss observed in morphine-dependent mice after administration of naloxone (Fig. 3A).

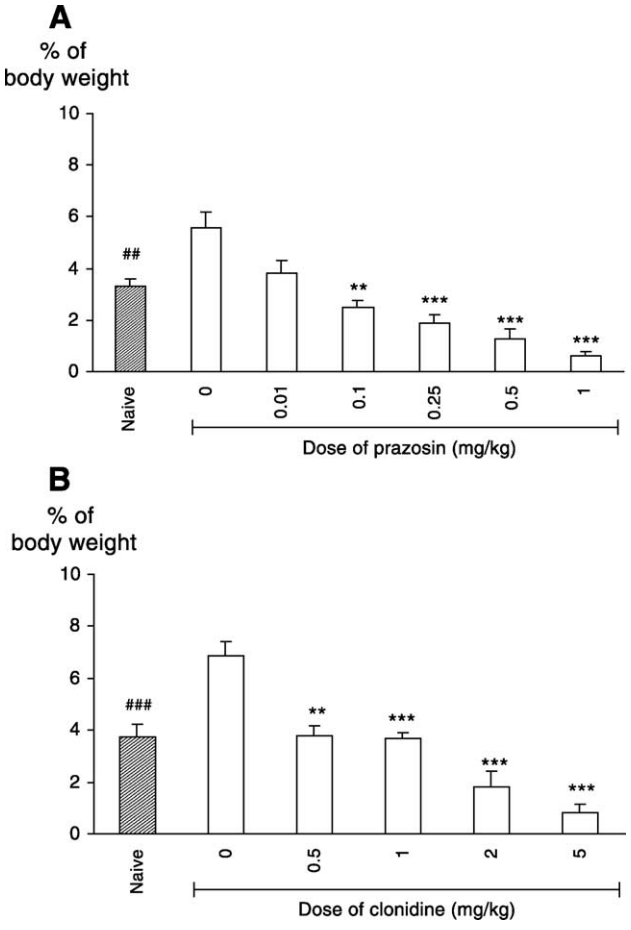

Fig. 3. Acute effects of prazosin (A) and clonidine (B) on weight loss (percent of body weight in 3 h) after naloxone administration to morphine-dependent mice. Asterisks denote significant differences compared to control group; Scheffé's test. ###, $P<0.001$, ##, $P<0.01$; *t*-test compared to morphine-treated control group.

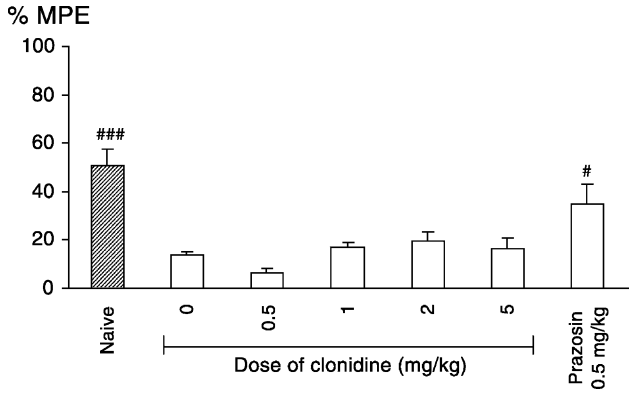

Fig. 4. Effects of repeated pretreatment with clonidine (0.5–5 mg/kg) or prazosin (0.5 mg/kg) on analgesia scores after a test dose of morphine (5 mg/kg) in morphine-tolerant mice. ###, $P<0.001$, #, $P<0.05$; *t*-test compared to morphine-treated control group.

3.4. Clonidine attenuated naloxone-precipitated morphine withdrawal

Single doses of clonidine decreased the number of naloxone-induced vertical jumps (after 5 mg/kg, 38 ± 7 jumps in 15 min compared to 63 ± 3 in the control group). Naloxone-induced weight loss was also clearly prevented by acute clonidine administration (Fig. 3B).

3.5. Repeated prazosin alleviated morphine tolerance but clonidine did not

Repeated prazosin (0.5 mg/kg) pretreatment enhanced the analgesic efficacy of the test dose of morphine in morphine-tolerant mice (from 14 ± 2% to 35 ± 9% of MPE). Repeated clonidine pretreatment had no significant analgesic effect in morphine-tolerant mice (Fig. 4).

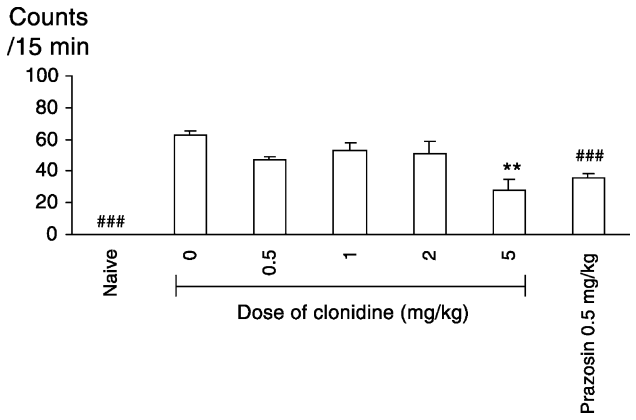

Fig. 5. Effects of repeated pretreatment with clonidine or prazosin on naloxone-induced vertical jumping (jumps/15 min) in morphine-dependent mice. ###, $P<0.001$; *t*-test compared to morphine-treated control group. **$P<0.01$; ANOVA with Scheffé's post hoc test.

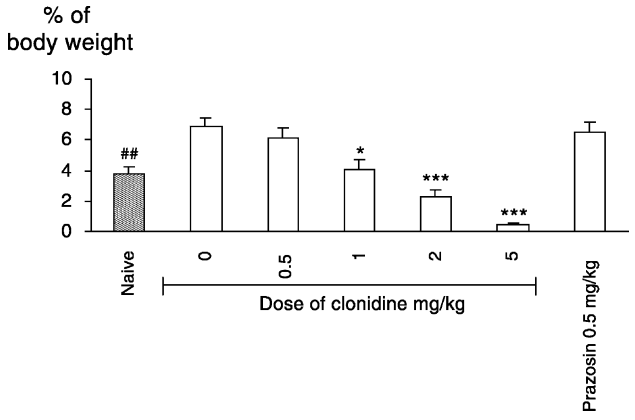

Fig. 6. Effects of repeated pretreatment with clonidine or prazosin on weight loss (percent of body weight in 3 h) after naloxone administration to morphine-dependent mice. Asterisks denote significant differences compared to morphine-treated control group (morphine tolerant, no clonidine, Scheffé's test). ##, $P<0.01$; *t*-test compared to morphine-treated control group.

3.6. Repeated prazosin and clonidine reduced naloxone-induced vertical jumping, but only clonidine opposed the weight loss

Small repeated doses of clonidine failed to influence the vertical jumping induced by naloxone-precipitated withdrawal in morphine-dependent mice. Repeated administration of 5 mg/kg of clonidine, before each dose of morphine during the induction treatment, reduced naloxone-precipitated jumping by approximately 55% (Fig. 5). Repeated pretreatment with prazosin (0.5 mg/kg) also reduced naloxone-induced jumping to some extent (by 43%, Fig. 5). The higher doses (1, 2 and 5 mg/kg) of repeated clonidine pretreatment also decreased the weight loss rate after naloxone-induced morphine withdrawal, but repeated prazosin (0.5 mg/kg) had no effect on weight loss (Fig. 6).

4. Discussion

The α_2-adrenoceptor agonist employed in the present experiments, clonidine, is known to have analgesic efficacy and to potentiate morphine analgesia. In this study, clonidine also appeared to attenuate the expression of morphine tolerance and withdrawal upon acute administration. The interpretation of these results is, however, complicated by the analgesic and sedative properties of α_2-adrenoceptor agonists (Buerkle and Yaksh, 1998). Chronic clonidine pretreatment did not prevent the emergence of tolerance to morphine. The possible development of cross-tolerance between clonidine and morphine (Roerig, 1995; Ware and Paul, 2000) was not specifically investigated in this study.

Acute administration of prazosin and corynanthine potentiated morphine analgesia, although they had no analgesic effects when given alone. The centrally active α_1-adrenoceptor agonist, cirazoline, did not influence morphine analgesia. Prazosin also appeared to attenuate the expression of morphine tolerance. Large doses of prazosin were clearly less efficacious than moderate doses of prazosin in the range of 0.25–0.5 mg/kg. We presume that high doses of prazosin can also block α_2-adrenergic receptors, which participate in opioid antinociception. It has previously been reported that prazosin at 2 mg/kg had no analgesic effect in mice and did not influence morphine analgesia (Dambisya et al., 1991a). However, it has also been reported that systemic administration of prazosin and another α_1-adrenoceptor antagonist, terazosin, displayed analgesic activity in the writhing test in mice (Sierralta et al., 1995).

Repeated prazosin pretreatment appeared to inhibit the development of morphine tolerance; in these experiments, prazosin was administered before each dose of morphine during tolerance induction, but not before the test dose of morphine. Consequently, it is likely that the observed effect-potentiation of the analgesic efficacy of the test dose of morphine in morphine-tolerant mice-reflects a modulatory effect of prazosin on the mechanisms involved in the development of morphine tolerance, in contrast to the analgesic potentiation seen after its acute administration.

Acute clonidine and also prazosin administration attenuated the weight loss of the mice after naloxone-precipitated opioid withdrawal in a dose-dependent manner, but in the case of clonidine, this may have been caused by the sedative and anti-secretory effects of the drug. Prazosin is not known to have similar pharmacological effects. Acute clonidine administration also significantly decreased naloxone-induced vertical jumping, but prazosin did not. Repeated pretreatment with clonidine did not decrease vertical jumping, and was not effective in preventing weight loss. Chronic prazosin pretreatment, in contrast, decreased vertical jumping, but did not decrease the weight loss rate of morphine-withdrawn mice.

Pain sensation is a very complex phenomenon, and many neuronal systems and transmitters are involved in nociception. Pain sensation can be divided into four components: transduction, transmission, modulation and perception. Opioidergic and noradrenergic systems have important but complex roles in modulation of nociception. Opioid-induced analgesia is due to actions at several sites in the central nervous system, both spinal and supraspinal. Morphine and other μ-opioid receptor agonists selectively inhibit various nociceptive reflexes, but other sensory modalities remain unaffected. Three mechanisms appear to be involved in opioid analgesia. Opioid receptors on the terminals of primary afferent nerves meditate inhibition of substance P release in the dorsal horn of the spinal cord. Morphine also antagonizes the effects of substance P by exerting postsynaptic inhibitory actions on interneurons and on the output neurons of the spinothalamic tract that convey nociceptive information to higher centers in the brain. Profound analgesia can also be produced by supra-

spinal mechanisms, most notably by effects mediated through the periaqueductal gray matter, the nucleus raphe magnus and the locus coeruleus. Opioid receptor activation in these centers results in enhanced activity of descending aminergic bulbospinal pathways that inhibit the processing of nociceptive information in the spinal cord (Reisine and Pasternak, 1996).

Noradrenaline has an important role in the modulation of pain transmission especially at the level of the spinal cord (Proudfit and Monsen, 1999). Intrathecal or direct spinal injection of noradrenaline produces antinociception and decreased dorsal horn neuronal activity. Noradrenaline has, however, bidirectional modulatory effects on nociception. Activation of α_{1A}- but not $\alpha_{1B/D}$-adrenoceptors mediates potentiation of spinal nociceptive reflexes (Hedo and Lopez-Garcia, 2001). Noradrenaline enhances motoneuronal responses to stimulation of nearby ventral interneurons via activation of α_{1A}-adrenoceptors (Wada et al., 1997). The principal adrenoceptor subtype mediating noradrenaline antinociception has recently been identified as the α_{2A}-adrenoceptor based on studies in gene-targeted mice (Stone et al., 1997). In addition, α_{2C}-adrenoceptors may also contribute to spinal analgesia (Fairbanks et al., 2002).

Increased central noradrenergic activity potentiates morphine analgesia (Bohn et al., 2000b), but acute morphine administration inhibits noradrenergic neurons in the locus coeruleus and reduces noradrenaline release in its projection areas (Rossetti et al., 1993). Some centrally acting sympathomimetic agents, such as ephedrine, which act primarily through enhancing the release of stored catecholamines, potentiate morphine analgesia, although they do not have analgesic effects when given alone (Dambisya et al., 1991b; Tekol et al., 1994).

There are four major systems of descending noradrenergic neurons that have been demonstrated to modulate nociception. Stimulation of the first three causes antinociception. Stimulation of the fourth system produces hyperalgesia. The first system consists of bulbospinal noradrenaline neurons located in the A6 cell group (nucleus locus coeruleus) and the A7 region of the dorsolateral medulla. The second is the A1 catecholamine nucleus projecting to the nucleus raphe magnus, and activating descending inhibitory neuronal pathways. The third system originates in the rostral part of the A5 cell group and may include the A7 nucleus, and projects to the dorsal horn of the spinal cord. The fourth system of noradrenaline neurons originates in the caudal A5 nucleus and projects to and tonically inhibits spinally projecting 5-HT neurons located in the nucleus raphe magnus in the ventromedial medulla. Blockade of this action by α_1-adrenoceptor antagonist produces analgesia (Proudfit, 1988).

The microinjection of morphine into the A7 region has dual effects; it produces both hyperalgesia, i.e., facilitation of nociception, meditated by α_1-adrenoceptors in the spinal cord, and antinociception that is mediated by spinal α_2-adrenoceptors (Holden et al., 1999). Morphine appears to indirectly activate two different populations of spinally projecting noradrenergic neurons in the A7 cell group. Morphine disinhibits A7 neurons by inhibiting local GABA interneurons that tonically inhibit A7 neurons (Bajic et al., 2001). Inhibition of nociception is also produced by morphine activating enkephalin and/or SP neurons that activate spinally projecting A7 neurons to produce α_2-adrenergic antinociception (Proudfit and Monsen, 1999).

The locus coeruleus (A6) is a brain nucleus important for modulation of nociception (Zhang et al., 1998), opioid tolerance and dependence. It is rich in μ-type opioid receptors, α_{2A}- and α_1-adrenergic receptors. It is a central site for the regulation of the brain noradrenergic system, and also participates in the regulation of peripheral sympathetic nervous activity. The sympathetic system mediates many symptoms of the opioid withdrawal syndrome (Devoto et al., 2002). The potent noradrenergic neurotoxic compound, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4), blocked vertical jumping induced by naloxone-precipitated morphine withdrawal, but DSP-4 did not influence the development of morphine tolerance (Dossin et al., 1996). The mechanisms of morphine tolerance and dependence appear to be different (Bohn et al., 2000a). Cerebral α_{2A}- and α_1-adrenergic receptors may both play important roles in the development of opioid tolerance (Kihara and Kaneto, 1986), dependence (Christie et al., 1987) and withdrawal (Maldonado, 1997).

The α_2-adrenoceptor agonist clonidine is in clinical use in the treatment of opioid withdrawal symptoms. The molecular mechanisms by which α_2-adrenoceptor agonists alleviate opioid withdrawal are not entirely clear, but clonidine acts in the brain, inhibiting adrenergic outflow from the brainstem, and inhibition of sympathetic outflow may inhibit some of the withdrawal signs (Kamibayashi and Maze, 2000). In the current experiments, we studied the effects of both acute and chronic administration of the α_1-adrenoceptor antagonist prazosin, and the α_2-adrenoceptor agonist clonidine on morphine tolerance and withdrawal in mice. As expected, neither treatment was capable of total prevention or suppression of the symptoms of naloxone-precipitated morphine withdrawal, but both agents exerted some modulatory effects on mouse behaviour.

Chronic morphine treatment might down-regulate not only opioid receptors but also α_2-adrenoceptors and related signal transduction mechanisms (Ingram et al., 1998), thus favouring the appearance of α_1-adrenoceptor-mediated responses (Tanganelli et al., 1989). Our results showed that chronic clonidine administration did not prevent the development of morphine tolerance, but chronic prazosin partly alleviated some signs of morphine dependence, indicating mediation by α_1-adrenoceptors.

Our results indicate that in addition to acutely administered α_2-adrenoceptor agonists, α_1-adrenoceptor antagonists might also be useful in potentiation of opioid analgesia and in alleviation of opioid tolerance and some opioid with-

drawal signs. This is based on the involvement of α_1-adrenoceptors in the brain and spinal cord in facilitation of the transmission of pain, and of spinal α_2-adrenoceptors in inhibition of nociceptive transmission. Thus, it might be beneficial to combine an α_1-adrenoceptor antagonist with morphine to treat chronic pain, instead of or in addition to α_2-adrenoceptor agonists. It is an advantage that prazosin can also be used for long-term treatment because cross-tolerance should not develop. Subtype-selective centrally acting α_1-adrenoceptor antagonists may have therapeutic advantages over prazosin for this purpose, as the hemodynamic effects of prazosin are an obvious clinical limitation for its use in the treatment of pain.

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