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Effects of 5-HT receptor antagonists on morphine-induced tolerance in mice

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Abstract

The effect of 5-HT receptor antagonists on tolerance to morphine antinociception was studied in mice. Slow release morphine suspension was injected subcutaneously (s.c.) in order to produce tolerance. When different doses of morphine (3, 6 and 9 mg/kg) were administered on the 4th day after injection of slow-release morphine suspension, tolerance to the test doses of morphine was observed. The tolerance obtained was decreased by pretreatment with the non-selective 5-HT receptor antagonist methysergide (1 and 2 mg/kg) or the 5-HT_2 receptor antagonist ritanserin (1 and 2 mg/kg). When the 5-HT receptor antagonists were used on the 2nd and 3rd day after injection of slow-release morphine suspension or on the 4th day (60 min before last dose of morphine), a maximum reduction in morphine tolerance was observed on the 3rd day. Pretreatment of animals with metergoline (1 and 2 mg/kg) or mianserin (1 and 2 mg/kg) also decreased the tolerance to morphine. It may be concluded that at least a 5-HT_2 receptor mechanism is involved in tolerance to morphine antinociception.

Keywords: Morphine tolerance; Methysergide; Metergoline; Ritanserin; Mianserin; Antinociception

1. Introduction

A number of studies have demonstrated that neurotransmitters such as serotonin and norepinephrine contribute to the full expression of opiate analgesia. The serotonergic system seems to be involved in morphine withdrawal jumping (Samanin et al., 1980) and quasi-morphine withdrawal (Neal and Sparber, 1990). Recent studies have shown that spinal serotonergic systems also contribute to spinal opiate-induced analgesia (Crisp et al., 1991). A multiplicity of binding sites for 5-HT exist in the central nervous system (CNS) of mammals, including humans. Currently, four major types are recognized, 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 (Conn and Sanders-Bush, 1987a; Dumuis et al., 1989; Glennon, 1987; Richardson and Engel, 1986; Tricklebank, 1987). Distinct 5-HT receptor subtypes have been subdivided into other 5-HT sites (Millan et al., 1991). It has been suggested that the analgesia of narcotic drugs can not be separated from their ability to produce tolerance and dependence. However, there is also evidence indicating the possible dissociation of morphine's analgesic effect from its tolerance-inducing effect (Cox et al., 1988; Miksic et al., 1980). Antinociception and the development of tolerance to the antinociceptive action of some opioid agonists have been suggested to be mediated by a 5-HT mechanism, probably by different modes with interaction of the 5-HT_1 and 5-HT_2 receptors (Ho and Takemori, 1989). In the present work, the effect of 5-HT receptor antagonists on the development of morphine tolerance has been evaluated.

2. Materials and methods

2.1. Animals

Male albino mice weighing 20–25 g were used in these experiments. They were kept 10 per cage (45 × 30 × 15 cm) at an environmental temperature of 22–24°C on a 12-h light-dark cycle. The animals had free access to food and water, except during the time of experiments. Each animal was used once only and was killed immediately after the experiments.

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2.2. Development of tolerance to morphine

The mice were treated subcutaneously (s.c.) with a slow-release morphine suspension (morphine sulphate, 300 mg/kg). To assess the degree of tolerance, the antinociceptive response to different doses of morphine (3, 6 and 9 mg/kg s.c.) was measured on the 4th and 5th day after injection of the slow-release morphine suspension. Maximum tolerance was obtained on the 4th day after injection of the slow-release morphine suspension.

2.3. Analgesia testing

On the 4th day after administration of the slow-release morphine suspension, antinociception was measured 15, 30, 45 and 60 min after injection of the test doses of morphine. Antinociception was determined in the tail-flick test, wherein noxious stimulation is effected by a beam of high intensity light focused on the animal's tail with a tail-flick apparatus (model 812, Hugo Sachs Electronik, Germany). The mean response latency prior to drug administration was 2–3 s and a 10-s cut off was imposed to avoid excessive tissue damage.

2.4. Drugs

The drugs used were: morphine sulphate (Mac Farlan Smith, England), methysergide (Sandoz, Switzerland), metergoline (Sandoz, Switzerland), ritanserin and mianserin (Research Biochemical, USA). Slow-release morphine suspension was prepared by suspending morphine sulphate, at a concentration of 30 mg/ml, in a mixture of 0.75 ml Arlacel (Sigma), 4.25 ml paraffin oil and 5 ml saline (Collier et al., 1972). The drugs were dissolved in physiological saline except ritanserin, which was dissolved in tartaric acid (1.0 M). The drugs were prepared immediately before injection and were administered in a volume of 10 ml/kg.

2.5. Statistical analysis

Analysis of variance (ANOVA) followed by Newman-Keuls' tests were used to evaluate the significance of the results obtained.

3. Results

3.1. Induction of tolerance to morphine

Mice were injected subcutaneously (s.c.) with slow-release morphine suspension and tolerance was tested on the 4th day (72 h later) after drug injection. Animals which had become tolerant showed only a small

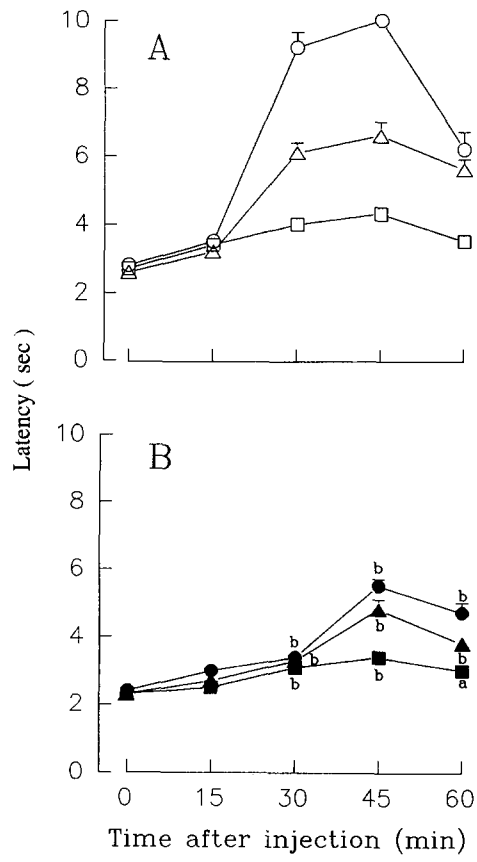

Fig. 1. Comparison of antinociception in non-tolerant and tolerant mice to morphine. Mice were injected (s.c.) with 9 (○), 6 (△) and 3 (□) mg/kg morphine in animals which did not receive slow-release morphine suspension (Graph A) or 9 (●), 6 (▲) and 3 (■) mg/kg in those which received the slow-release morphine suspension (Graph B). Slow-release morphine suspension was administered 72 h before injection of test doses of morphine. The antinociception was recorded 15, 30, 45 and 60 min after morphine administration. Each point represents the mean ± S.E.M. of nine mice tested every 15 min. $^{a}P < 0.05$, $^{b}P < 0.01$ different from saline-treated control animals.

antinociceptive effect 30 [$F(5,48) = 101.4$, $P < 0.01$], 45 [$F(5,48) = 188.5$, $P < 0.01$] and 60 [$F(5,48) = 20.4$, $P < 0.01$] min after different doses of morphine (3, 6 and 9 mg/kg) (Fig. 1).

3.2. Effects of methysergide and ritanserin on the tolerance produced by slow-release morphine suspension

The mice were injected with slow-release morphine suspension (s.c.) and antinociception induced by morphine was tested on the 4th day (72 h) after the suspension administration. When the animals were pretreated with methysergide (2 mg/kg, i.p.) 1, 24 and 48 h before the test dose of morphine (9 mg/kg), there was a significant increase in tail-flick latencies, 30 [$F(3,32) = 12.8$, $P < 0.01$] and 45 [$F(3,32) = 7.9$, $P < 0.01$] min after morphine injection (Fig. 2A). Pretreatment of the animals with ritanserin (2 mg/kg, i.p.) 1,

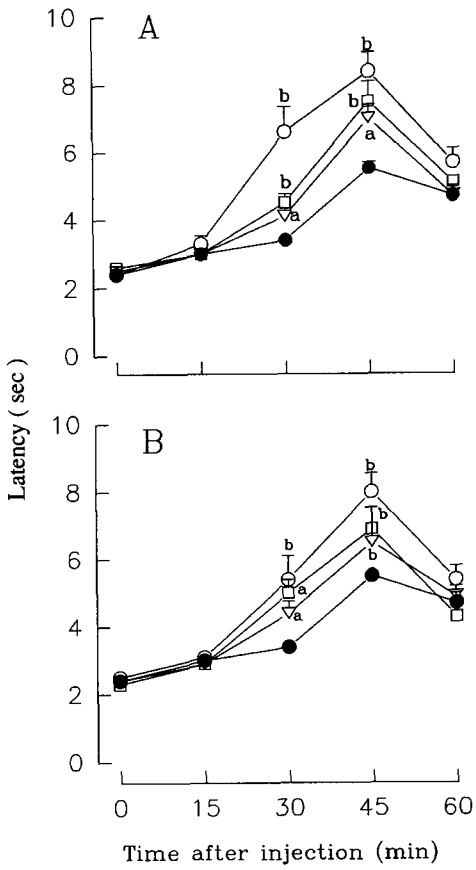

Fig. 2. Effects of 5-HT receptor antagonists on tolerance induced by slow-release morphine suspension. Animals were treated (i.p.) with saline (●; 10 ml/kg), methysergide (2 mg/kg; Graph A) or ritanserin (2 mg/kg; Graph B) on the 2nd day (□; 24 h), 3rd day (○; 48 h) or 4th day (▽; 71 h) after administration of the slow-release morphine suspension and the antinociceptive effect was tested with morphine (9 mg/kg) on the 4th day (72 h) after the suspension administration. Other details as Fig. 1. $^{a}P < 0.05$, $^{b}P < 0.01$ different from saline-treated control animals.

24 and 48 h before the test dose of morphine (9 mg/kg) also reduced tolerance to morphine, 30 [$F(3,32) = 4.4$, $P < 0.05$] and 45 [$F(3,32) = 4.1$, $P < 0.05$] min after morphine injection (Fig. 2B).

3.3. Effects of 5-HT receptor antagonists on morphine-induced tolerance

In a group of animals, 4 days (72 h) after treatment with the slow-release morphine suspension, the response to the test dose of morphine (9 mg/kg, s.c.) was decreased. Pretreatment of the animals with methysergide (1 and 2 mg/kg i.p.), or metergoline (1 and 2 mg/kg i.p.), ritanserin (1 and 2 mg/kg i.p.), mianserin (1 and 2 mg/kg i.p.) 24 h prior to the test dose of morphine (9 mg/kg), increased antinociceptive response to morphine, 30 [$F(8,72) = 3.1$, $P < 0.01$] and 45 [$F(8,72) = 4.7$, $P < 0.01$] min after morphine administration as compared with saline pretreatment (10 mg/kg, i.p., 24 h) (Table 1). The higher dose of methysergide (2 mg/kg) and ritanserin (2 mg/kg) increased the response to the low test dose of morphine (6 mg/kg) 30 [$F(2,24) = 8.5$, $P < 0.01$] and 45 [$F(2,24) = 5.2$, $P < 0.05$] min after the drug injection (Table 2).

Table 1
Antinociceptive response to a high dose of morphine (9 mg/kg) by tolerant animals in the presence or absence of 5-HT receptor antagonists

Pretreatment (mg/kg)	Time after morphine injection (min)			
	15	30	45	60
Saline 10 ml/kg	3.0±0.2	3.4±0.1	5.4±0.2	4.7±0.3
Methysergide 1	3.1±0.1	5.2±0.3 ^{b}	6.9±0.1 ^{b}	5.1±0.2
Methysergide 2	3.3±0.2	6.6±0.7 ^{b}	8.4±0.5 ^{b}	5.7±0.4
Metergoline 1	3.1±0.1	4.9±0.4 ^{b}	6.8±0.5 ^{b}	4.9±0.4
Metergoline 2	3.2±0.2	5.4±0.9 ^{a}	8.1±0.5	5.4±0.2
Mianserin 1	3.7±0.1	4.4±0.2 ^{b}	6.4±0.2 ^{b}	4.9±0.1
Mianserin 2	3.3±0.2	4.8±0.4 ^{a}	7.8±0.6 ^{b}	5.1±0.5
Ritanserin 1	3.6±0.1	4.6±0.2 ^{b}	6.8±0.2 ^{b}	4.8±0.2
Ritanserin 2	3.1±0.1	5.4±0.7 ^{b}	8.0±0.5 ^{b}	5.4±0.4

Animals were treated (i.p.) with different doses of 5-HT receptor antagonists on the 3rd day (48 h) after slow-release suspension administration. Antinociception was tested with morphine (9 mg/kg) on the 4th day (72 h) after injection of the slow-release morphine suspension. Antinociception was recorded 15, 30, 45 and 60 min after administration of the test dose of morphine. Each value represents the mean±S.E.M. for nine mice tested every 15 min. $^{a}P < 0.05$, $^{b}P < 0.01$ different from saline control group.

3.4. Effects of 5-HT receptor antagonists on antinociception

When the animals were treated with different doses of methysergide (1 and 2 mg/kg), metergoline (1 and 2

Table 2
Antinociceptive effects of a low dose of morphine (6 mg/kg) in tolerant mice in the presence or absence of 5-HT receptor antagonists

Pretreatment (mg/kg)	Time after morphine injection (min)			
	15	30	45	60
Saline 10 ml/kg	2.7±0.0	3.3±0.1	4.8±0.3	3.8±0.2
Methysergide 2	3.0±0.1	4.0±0.2 ^{b}	5.6±0.2 ^{b}	4.0±0.1
Ritanserin 2	2.8±0.1	3.7±0.1 ^{a}	5.4±0.1 ^{b}	3.9±0.1

Animals were injected (i.p.) with 5-HT receptor antagonists on the 3rd day (48 h) after slow-release suspension administration. Antinociception was tested with morphine (6 mg/kg) on the 4th day (72 h) after administration of the slow-release morphine suspension. The response was recorded 15, 30, 45 and 60 min after test dose of morphine. Each value represents the mean±S.E.M. of nine mice tested every 15 min. $^{a}P < 0.05$, $^{b}P < 0.01$ different from saline-treated control animals.

mg/kg) or mianserin (1 and 2 mg/kg) alone, neither of the drugs induced antinociception (data not shown).

4. Discussion

In the present work administration of slow-release morphine suspension induced tolerance to morphine. Thus, the antinociceptive response of test doses of morphine was decreased. The effect obtained is similar to that found by others (Van Der Laan and Hillen, 1986). The non-selective 5-HT receptor antagonists methysergide and metergoline decreased the development or expression of tolerance to morphine antinociception. This may indicate that 5-HT mechanisms may be involved in the induction of tolerance to the drug.

One major function long attributed to 5-HT is the modulation of nociception. In the periphery, 5-HT increases nociceptive transmission by sensitizing nociceptors localized on primary afferent fibres (Besson and Chaouch, 1987; Richardson and Engel, 1986). In contrast, central networks of 5-HT have traditionally been thought to play an opposite role; that is, to be involved in antinociception (Crisp et al., 1991; Millan et al., 1991). Consistent with such a possibility, serotonergic pathways from midbrain raphe nuclei project to the periaqueductal gray, thalamus and other cerebral structures involved in antinociceptive mechanisms, whereas there is a major descending serotonergic pathway from medullary raphe nuclei to the dorsal horn of the spinal cord, the site of primary processing of afferent nociceptive information (Besson and Chaouch, 1987; Bowker et al., 1982). Although it may be suggested that the antinociception of opioid drugs can not be separated from their ability to produce tolerance and dependence, other evidence indicates the possible dissociation of their analgesic effect from their tolerance-inducing effect (Cox et al., 1968; Miksic et al., 1980; Kaneto et al., 1985). The present findings show that the 5-HT_2 receptor antagonists mianserin (Neal and Sparber, 1986) and ritanserin (Leysen et al., 1981) reduced the development of tolerance to morphine's antinociceptive actions. This may further emphasize the involvement of 5-HT_2 in morphine tolerance. Previous results shown by other workers (Neal and Sparber, 1986) demonstrated that the acute administration of the 5-HT_2 receptor antagonist mianserin attenuated naloxone-precipitated withdrawal after a single dose of morphine in morphine-pelleted rats. Kleven and Sparber (1989) have also shown that acute mianserin treatment attenuated the behavioural suppression during a quasi-morphine withdrawal syndrome. Therefore, the similarity between naloxone-induced withdrawal and tolerance to morphine seems likely. This is in agreement with others who predicted that 5-HT_2 antagonists could block or attenuate the expression of both withdrawal and tolerance (Sparber et al., 1978). However, the involvement of the 5-HT_2 receptors seems more likely. Since at least two to three subtypes of 5-HT_2 receptors have been distinguished (Millan et al., 1991), the precise 5-HT_2 receptor subtypes involved need to be clarified.

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