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Short communication

Contribution of spinal μ_1-opioid receptors to morphine-induced antinociception

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Abstract

To determine the role of μ-opioid receptor subtypes, μ_1 and μ_2, in antinociception induced by intrathecal (i.t.) or intracerebroventricular (i.c.v.) injection of morphine, we assessed the effect of naloxonazine, a selective antagonist at μ_1-opioid receptors. The antinociceptive effects of morphine were measured using four different nociceptive tests. The selective μ_1 antagonist, naloxonazine (35 mg/kg, s.c.), 24 h before testing antagonized the antinociceptive effect of morphine on responses to chemical and thermal stimuli to a greater extent than that on responses to mechanical stimuli, as judged from ED_{50} values. The present results suggest that the antinociceptive activity of both i.t. and i.c.v. morphine on responses to chemical and thermal stimuli may be mediated through the μ_1-opioid receptor subtype (naloxonazine-sensitive sites). These findings may be interpreted as indicative of the existence of μ_1-receptor subtypes capable of mediating antinociception not only in supraspinal sites but also in spinal sites.

Keywords: Morphine; Naloxonazine; Antinociception; μ-Opioid receptor subtype

1. Introduction

There is evidence from a large variety of biochemical and pharmacological experimental approaches for the existence of μ-opioid receptor subtypes, μ_1 and μ_2 (Wolozin and Pasternak, 1981). The development of an irreversible and long-acting antagonist, naloxonazine (Ling et al., 1986), has made it possible to investigate the functions of μ_1-opioid receptors. Especially concerning antinociception, naloxonazine antagonizes morphine-induced antinociception supraspinally but not spinally (Heyman et al., 1988; Paul et al., 1989), and the antinociception induced by i.t. administration of morphine is well attenuated by β-funaltrexamine (Pick et al., 1991) which selectively and irreversibly blocks μ-opioid receptors in binding assays, without distinguishing between the μ_1- and μ_2-opioid receptor subtypes. It is, therefore, speculated that μ_1-opioid receptors mediate antinociception supraspinally, whereas μ_2-opioid receptors are responsible for spinal antinociception. In the above studies, the method utilized for evaluating the antinociceptive effects of morphine was the tail-flick test, which depends on tail withdrawal as the radiant noxious heat endpoint, and the response to thermal stimulation involves a spinal reflex. The tail-flick does not require the integrative action of higher brain centers (Irwin et al., 1951). It is not surprising, therefore, that the neural mechanisms that underlie morphine-induced antinociception in the tail-flick test are markedly different from those involved in the tail-pressure, formalin and hot-plate tests employed in the present study. We have, therefore, added the latter three nociceptive tests to assess antinociception. These nociceptive stimuli can induce nociceptive behavioural responses such as licking and biting that are thought to be mediated supraspinally.

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In the present study, the role of the μ-opioid receptor subtypes, μ_1 and μ_2, in the antinociceptive effects of morphine against the responses to mechanical, chemical and thermal noxious stimuli was examined using the irreversible μ_1-opioid receptor antagonist, naloxonazine. In addition, the sensitivity to i.c.v. or i.t. morphine alone was compared to the sensitivity to morphine in conjunction with naloxonazine.

2. Materials and methods

2.1. Animals

Male ddY mice weighing 22–25 g were used for all experiments. The animals were kept in a temperature-controlled room with a standard 12-h light–dark cycle (24 ± 0.5, 12-h dark/light cycle with lights on at 0900 h). Food and water were continuously available. Mice were tested only once. The experiments were performed with the approval of the Committee of Animal Experiments in Tohoku College of Pharmacy.

2.2. Injection procedure

Intracerebroventricular (i.c.v.) injections were made directly into the lateral ventricle according to the slightly modified method of Haley and MaCormick (1957). The method for intrathecal (i.t.) injections was adapted from that of Hylden and Wilcox (1980). For the injections into the subarachnoid space, a 29-gauge needle, matched to a 50-μl Hamilton microsyringe, was directed into an intervertebral space at the level of the 5th and 6th lumbar vertebrae. All i.c.v. and i.t. injections were made in a volume of 5 μl in unanaesthetized mice.

2.3. Drugs

Drugs administered i.c.v. or i.t. were: morphine hydrochloride (Sankyo, Tokyo) and naloxonazine (Research Biochemical International, MA, USA). Morphine was dissolved in sterile Ringer's solution. Naloxonazine was dissolved in dilute acetic acid (0.01%) and injected s.c. (35 mg/kg) in a volume of 0.1 ml/10 g body weight 24 h prior to testing.

2.4. Assessment of nociceptive threshold

Four assays of nociception were used: the tail-pressure test, the formalin test, the hot-plate test and the tail-flick test.

The method for the mouse tail-pressure test was previously described in detail (Sakurada et al., 1986). The base of the tail was pressed at a rate of 10 mm Hg/s and the level of pressure in mm Hg that evoked biting or licking behaviour was noted. Only mice responding behaviourally to mechanical nociceptive stimuli (40–50 mm Hg) were selected. A value of 100 mm Hg was taken as the cut-off pressure.

In the formalin test, mice were placed in transparent cages (22.0 cm × 15.0 cm × 12.5 cm high) which also served as observation chambers and were allowed to adapt to their environment for 1 h before testing. After this period, 20 μl of formalin (2.0% in saline) was injected subcutaneously (s.c.) in the plantar surface of the hind-paw using a microsyringe with 26-gauge needle. Recording of the response started immediately after formalin injection and continued for 10 min. Only biting or licking of the formalin-injected hindpaw was defined as a nociceptive response and the total time of the response was measured with a hand-held stop-watch during the observation period.

In the hot-plate test, the reaction time of the response was determined in a Plexiglass frame (5.0 cm × 7.0 cm × 4.0 cm high) on the steel plate maintained at a temperature 53 ± 0.5°C. 'Hind-paw' licking or biting was taken as a nociceptive response to heat. A cut-off time of 60 s was applied in the hot-plate test. Only mice responding behaviourally to thermal nociceptive stimuli from 10 to 20 s were selected.

The tail-flick test was adapted from the classical design of D'Amour and Smith (1941). Nociceptive thresholds were determined by using an automated tail-flick unit (Ugo Basile, Italy). Tail-flick latencies were recorded as the time from the start of the heat stimulus to withdrawal of the tail. The intensity of the light beam was adjusted so that baseline readings were generally between 2.0 and 3.0 s. The light beam was focused on the same spot, about 1.0 cm from the tip of the tail, for all animals. A maximum latency of 10 s was imposed to minimize tissue damage. The antinociceptive effect was calculated as % of MPE: % of MPE = $[(T2 - T1)/(10 - T1)] \times 100$ where T1 and T2 are pre-drug and post-drug responsive latency (in s), respectively.

2.5. Data analysis and statistics

Statistical significance of the data was estimated with a mixed two-factor analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. A level of probability of 0.05 or less was accepted as significant. The ED_{50} values and their 95% confidence limits (95% CL) for the antinociceptive effect of morphine were computed according to the method of Litchfield and Wilcoxon (1949) using Programs 11 and 47 of the Pharmacological calculations system of Tallarida and Murray (1987).

3. Results

3.1. Effects of naloxonazine on morphine-induced antinociception in the tail-pressure test

The antinociceptive activity of morphine was estimated 10 min after i.c.v. and 15 min after i.t. injection, times

Table 1
ED_{50} values for morphine antinociception to mechanical, chemical and thermal noxious stimuli in vehicle-treated control mice and in mice pretreated with naloxonazine (NXZ)

	Route	Morphine ED_{50} (nmol)		(B)/(A) ratio
		Vehicle (A)	Naloxonazine (B)	
Tail-pressure test	i.c.v.	1.40 (0.81–2.43)	1.80 (1.20–2.89)	1.29
	i.t.	0.80 (0.50–1.29)	1.00 (0.62–1.62)	1.25
Formalin test	i.c.v.	0.11 (0.06–0.19)	0.88[a] (0.52–1.48)	8.00
	i.t.	1.10 (0.72–1.69)	9.20[a] (5.21–16.25)	8.36
Hot-plate test	i.c.v.	1.15 (0.78–1.71)	6.40[a] (4.69–8.73)	5.57
	i.t.	0.80 (0.25–2.55)	10.50[a] (5.0–21.0)	13.13
Tail-flick test	i.c.v.	2.60 (1.72–3.94)	8.40[a] (5.30–13.31)	3.23
	i.t.	0.33 (0.15–1.68)	0.37 (0.20–1.97)	1.12

Results are expressed as ED_{50} and 95% confidence limits are given in parentheses.
[a] $P < 0.01$ when compared with vehicle-pretreated group.

previously established to be the time of peak antinociceptive effect of morphine. Morphine had an extremely potent and dose-dependent antinociceptive activity at both i.c.v. and i.t. injection sites, with ED_{50} of 1.40 and 0.80 nmol, respectively (Table 1). Naloxonazine did not change statistically the dose–response curves for i.t. morphine alone (Fig. 1). In naloxonazine-pretreated groups, the ED_{50} values were 1.80 and 1.00 nmol for i.c.v. and i.t. morphine, respectively (Table 1).

3.2. Effects of naloxonazine on morphine-induced antinociception in the formalin test

The injection of 2.0% formalin into the plantar surface of a hind-paw produced a nociceptive behavioural response consisting of biting or licking of the injected paw for about 80 s immediately after the injection. Licking or biting behaviour in the early and transient phase (the first phase), which is recognized as a result of the direct stimulation of the nerve endings by the stimulus, was more vigorous than that in the late and long-lasting phase (the second phase) which is due to subsequent inflammation (Dubuisson and Dennis, 1977). Since morphine inhibited the nociceptive response equally in both phases as did other centrally acting analgesics (unpublished), only the results from the first phase were used to investigate the involvement of opioids in the present study. Morphine was injected i.c.v. 5 min or i.t. 15 min prior to the start of the observation period in the first phase of formalin-induced nociception. Morphine, thus administered, inhibited the formalin-induced nociceptive response, with ED_{50} of 0.11 and 1.10 nmol, respectively (Table 1). Pretreatment with naloxonazine markedly attenuated the antinociceptive actions of supraspinal and spinal morphine (Table 1). Naloxonazine produced an approximately 8-fold shift to the right of the dose–response curve for i.t. morphine (Fig. 1). The i.t. injection of morphine at a high dose (38.4 nmol) in mice pretreated with naloxonazine elicited reciprocal scratching toward the flanks, biting or licking of the hind-paw, which peaked at 0–5 min and disappeared 15 min after i.t. morphine. Thus, the behavioural response to i.t. high-dose morphine did not interfere with formalin-evoked paw-licking, since formalin was injected 15 min after i.t. morphine. These behavioural changes induced by morphine were reported upon elsewhere (Sakurada et al., 1996).

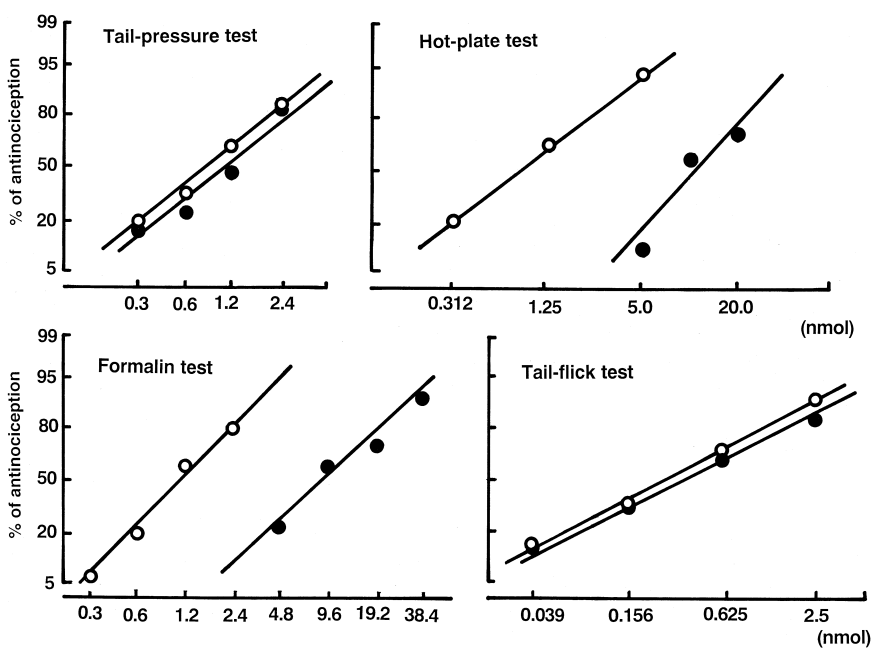

Fig. 1. Sensitivity of effect of intrathecally injected morphine to naloxonazine in four different nociceptive tests. Morphine in various doses was given alone (open symbols) and in combination with 35 mg/kg naloxonazine (filled symbols). A minimun of three doses with 10 animals per dose was used to obtain dose–response curves.

3.3. Effects of naloxonazine on morphine-induced antinociception in the hot-plate test

The antinociceptive activity of morphine was assessed at peak effect, which was 10 min after i.c.v. injection and 15 min after i.t. injection. The i.c.v. or i.t. administration of morphine dose dependently suppressed thermal nociception, with ED_{50} of 1.15 and 0.80 nmol, respectively (Table 1). The antinociception induced by i.c.v. and i.t. morphine was antagonized by pretreatment with naloxonazine. Naloxonazine shifted the dose–response curve 13-fold to the right (Fig. 1). Morphine (20 nmol), injected i.t. into mice pretreated with naloxonazine, evoked scratching, biting or licking during the first 5 min.

3.4. Effects of naloxonazine on morphine-induced antinociception in the tail-flick test

Testing, 10 min after i.c.v. or i.t. injection of morphine, showed a dose-dependent inhibition of the tail-flick response to thermal stimulation. The ED_{50} values for i.c.v. and i.t. administration of morphine were 2.60 and 0.33 nmol, respectively (Table 1). Pretreatment with naloxonazine resulted in a significant shift of the dose–response curve for i.c.v. morphine approximately 3-fold to the right (data not shown) without altering its sensitivity toward i.t. morphine (Fig. 1). ED_{50} values for i.c.v. and i.t. morphine in conjunction with naloxonazine were 8.40 and 0.37 nmol, respectively.

4. Discussion

The main finding of the present study was that the antinociceptive effect of both i.c.v. and i.t. morphine in the formalin and hot-plate tests was significantly antagonized by pretreatment with naloxonazine, an irreversible μ_1-opioid receptor antagonist, whereas naloxonazine failed to antagonize i.c.v. and i.t. morphine-induced antinociception in the tail-pressure test.

Binding studies indicate that naloxonazine, injected s.c., produces a long-lasting, selective blockade of μ_1-binding sites (Ling et al., 1986). With respect to [D-Ala^2, Me-Phe^4, Gly(ol)^5]enkephalin (DAMGO)-induced antinociception in the tail-flick test, i.c.v. DAMGO is highly sensitive to naloxonazine, whereas i.t. DAMGO is insensitive to naloxonazine and sensitive to β-funaltrexamine, which strongly binds μ_1- and μ_2-opioid receptors (Pick et al., 1991). In this latter series of experiments, the investigators suggested that μ_1-opioid receptors are important for supraspinal, but not spinal antinociception. However, it should be noted that their data were obtained in studies using a spinal reflex, the 'tail-flick' response, which is thought to measure changes in both the spinal reflex and the centrally modulated system involved in the spinal reflex. The present study also showed that, in the tail-flick test, naloxonazine antagonized the antinociceptive effect of i.c.v. injected morphine without affecting i.t. morphine-induced antinociception.

The results obtained in the formalin and hot-plate tests are different from those of the tail-flick test; in the first two tests naloxonazine attenuated markedly both supraspinal and spinal morphine antinociception. These findings implicate μ_1-receptors in the mediation of spinal morphine antinociception as well as in the supraspinal action of morphine. This implication is supported by the autoradiographic demonstration that μ_1- and μ_2-opioid receptor subtypes are localized in spinal and supraspinal structures involved in the modulation of nociception (Moskowitz and Goodman, 1985). It is important to note that the ED_{50} for i.t. morphine was 10-fold greater than the ED_{50} for i.c.v. morphine in the formalin test. These results are consistent with previously reported data that i.c.v. morphine is 8.5 times more potent than i.t. morphine as assayed by the behavioural response to i.t. injected substance P (Takahashi et al., 1987). Then, one can speculate that the antinociceptive effect of peripherally injected morphine may be due predominantly to an action at the supraspinal level in the formalin test as well as in the substance P-induced behaviour assay. In the tail-pressure test, neither supraspinal nor spinal morphine antinociception was sensitive to naloxonazine. The results obtained in different nociceptive tests indicate that μ_1-opioid receptors are primarily involved in morphine-induced antinociception in tests employing the formalin and hot-plate tests, whereas in the tail-pressure test μ_2-receptors may predominate. Thus, it is of extreme importance to obtain more information on the pharmacology of naloxonazine, using not only the tail-flick reflex but also other nociceptive behavioural responses which are thought to originate from higher centers in the brain.

In conclusion, pretreatment with naloxonazine markedly antagonized the antinociceptive effects of morphine on the hind-paw licking or biting response evoked by formalin and thermal stimuli, whereas morphine-induced antinociception was not inhibited by naloxonazine as assayed with mechanical stimuli. These findings suggest the presence of μ_1-receptors sensitive to naloxonazine in spinal sites as well as in supraspinal sites as assayed with the formalin and hot-plate tests.

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