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Short communication

Pretreatment with pertussis toxin blocks morphine- but not β-endorphin-induced antinociception in the mouse

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Abstract

We have previously demonstrated that the antinociception induced by morphine and β-endorphin given intracerebroventricularly (i.c.v.) is mediated by the stimulation of respective μ- and ε-opioid receptors. The effects of i.c.v. pretreatment with pertussis toxin on the antinociception induced by morphine and β-endorphin given i.c.v. were studied in male ICR mice. Antinociception was assessed by the tail-flick and hot-plate tests. Pretreatment with pertussis toxin (0.5 μg) given i.c.v. 96 h earlier blocks the antinociception induced by i.c.v. administered morphine in both tail-flick and hot-plate tests. The same pretreatment did not affect the antinociception induced by i.c.v. administered β-endorphin. Our results indicate that morphine-, but not β-endorphin-induced antinociception is mediated by pertussis toxin sensitive G-proteins.

Keywords: Antinociception; Morphine; β-Endorphin; Pertussis toxin; G-protein

1. Introduction

Pretreatment of mice or rats intracerebroventricularly (i.c.v.) with pertussis toxin has been demonstrated to attenuate antinociception and other effects induced by morphine and other μ-opioid receptor agonists (Chung et al., 1994; Parolaro et al., 1990a, b; Suzuki et al., 1991; Parenti et al., 1986; Shah et al., 1994; Funada et al., 1993). The results of these in vivo studies indicate that pharmacological effects induced by μ-opioid receptor agonists are mediated by pertussis toxin sensitive G-proteins.

We have previously demonstrated that the antinociception induced by morphine and β-endorphin given supraspinally is mediated by the stimulation of different types of opioid receptors (Tseng, 1995). The antinociception induced by supraspinally administered morphine is mediated by μ-opioid receptors while the antinociception induced by supraspinal β-endorphin is mediated by ε-opioid receptors. The question is then raised whether the ε-receptor mediated antinociception induced by i.c.v. administered β-endorphin is mediated by pertussis toxin sensitive G-proteins. Chung et al. (1994) recently reported that, unlike the antinociception induced by morphine which is blocked by the pretreatment with pertussis toxin, the antinociception induced by β-endorphin was not blocked by the pretreatment with pertussis toxin. The finding is extremely important because it indicates that the β-endorphin-induced response is not mediated by the pertussis toxin sensitive G-protein. The present experiments were then designed using different experimental protocols to study the effects of pretreatment with pertussis toxin on antinociception induced by morphine and β-endorphin in the mouse.

2. Materials and methods

2.1. Animals

Male ICR mice weighing 25–30 g (Sasco, Omaha, NE, USA) were used for the studies. Animals were housed five per cage in a room maintained at 22 ± 0.5°C with an alternating 12 h light-dark cycle. Food and water were available ad libitum. Animals were used only once in all experiments.

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2.2. Assessment of antinociception

Antinociception was determined by the tail-flick (D'Amour and Smith, 1941) and the hot-plate (Eddy and Leimbach, 1953) tests. For measurement of the latency of the tail-flick response, mice were gently held with one hand with the tail positioned in the apparatus (Model TF6, EMDIE Instrument Co., Maidens, VA, USA) for radiant heat stimulation. The tail-flick response was elicited by applying radiant heat to the dorsal surface of the tail. The intensity of the heat stimulus in the tail-flick test was adjusted so that the animal flicked its tail within 3–5 s. For the hot-plate test, mice were placed individually on the hot-plate (55°C) and the time from the placement of the mouse on the hot-plate to the onset of licking of the hind paw (reaction time) was measured. The dimension of the hot-plate apparatus was 30 × 30 × 30 cm (Model 39 Hot Plate, iitc Life Science, Woodland Hills, CA, USA). Control latencies for the hot-plate test were approximately 9 s. The inhibition of the tail-flick and hot-plate responses was expressed as 'percentage of the maximum possible effect (% MPE)' which was calculated as $[(T1 - T0)/(T2 - T0)] \times 100$, where $T0$ and $T1$ were the tail-flick and hot-plate latencies before and after the injection of opioid agonist and $T2$ was the cut-off time, which was set at 10 and 30 s for the tail-flick and hot-plate tests, respectively.

2.3. Experimental protocol

I.c.v. injection was made according to the procedure described by Haley and McCormick (1957) with an injection volume of 4 μl. Mice were pretreated i.c.v.

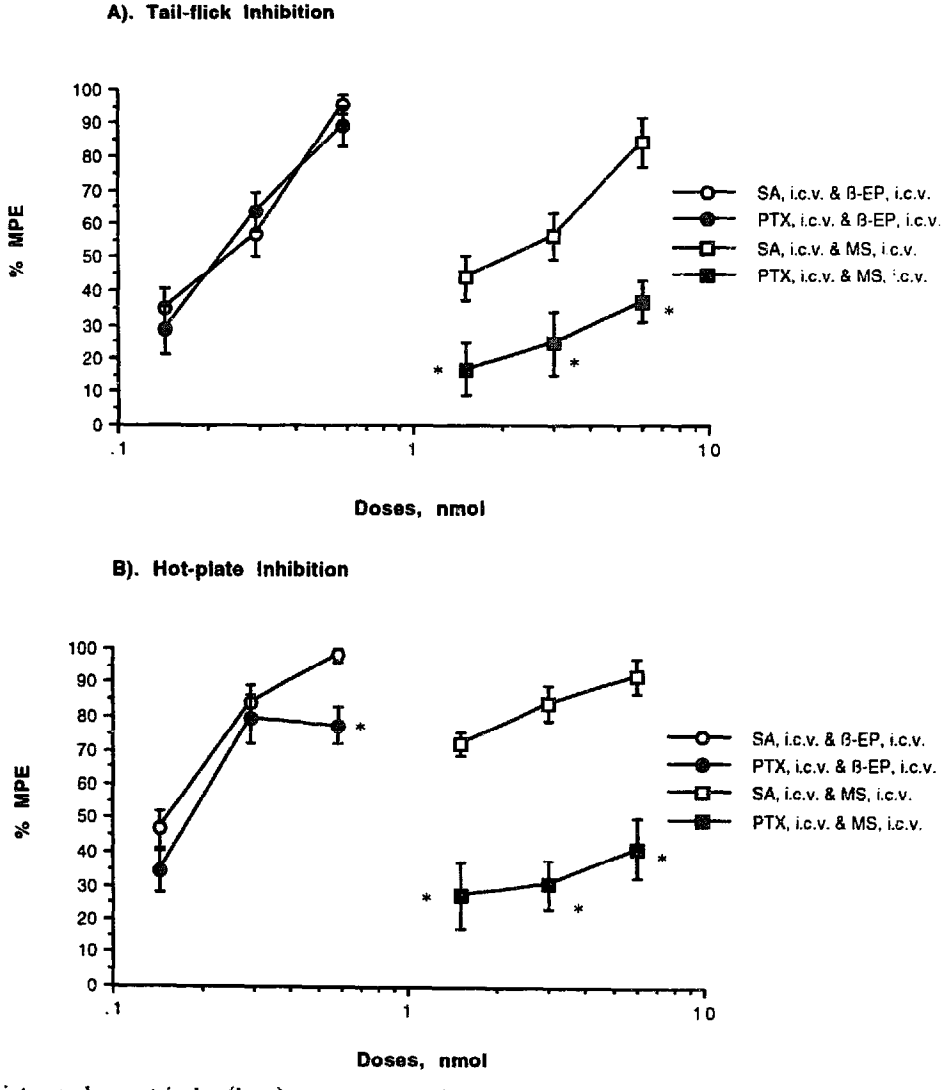

Fig. 1. The effects of intracerebroventricular (i.c.v.) pretreatment with pertussis toxin on the inhibition of the tail-flick (A) and hot-plate (B) responses induced by i.c.v. administered morphine or β-endorphin. Groups of mice were pretreated i.c.v. with pertussis toxin (1.0 μg) 96 h earlier and were injected i.c.v. with various doses of morphine or β-endorphin. The tail-flick and hot-plate response were measured 30 and 15 min, respectively, after the injection of β-endorphin and at 20 and 10 min, respectively, after the injection of morphine. % MPE: percentage of maximum possible effect; SA, saline; PTX, pertussis toxin; MS, morphine sulfate; β-EP, β-endorphin. The vertical bars indicate the standard error of the mean. * $P < 0.05$, significantly different from mice given saline pretreatment.

with pertussis toxin (0.5 μg) 96 h before the i.c.v. injection of various doses of β-endorphin or morphine. The tail-flick and hot-plate responses were measured at 30 and 15 min, respectively, after the injection for β-endorphin and at 20 and 10 min, respectively, after the injection for morphine. These times were determined in previous studies in which the effects reached a maximum after injection.

2.4. Statistics

Statistical analysis was made by Student *t*-test or analysis of variance followed by Newman-Keul's test (comparison between multiple groups). Difference was considered significant at $P \leq 0.05$.

2.5. Drugs

Drugs used were pertussis toxin (Research Biochem., Natick, MA, USA), morphine sulfate (Mallinckrodt Chemical, St. Louis, MO, USA) and β-endorphin (Peninsula Laboratory, Belmont, CA, USA).

3. Results

Morphine at doses from 1.5 to 6.0 nmol or β-endorphin from 0.14 to 0.58 nmol given i.c.v. produced a dose-dependent inhibition of the tail-flick or hot-plate response in mice pretreated i.c.v with saline 96 h earlier I.c.v. pretreatment with pertussis toxin 96 h earlier markedly attenuated the inhibition of both tail-flick and hot-plate responses induced by i.c.v. administered morphine (Fig. 1A). On the other hand, the same pretreatment with pertussis toxin did not have any effect on the inhibition of both tail-flick and hot-plate responses induced by β-endorphin, except that the hot-plate inhibition induced by a high dose of β-endorphin (0.58 nmol) was slightly attenuated (Fig. 1B). Pretreatment with pertussis toxin did not have any effect on the baseline tail-flick and hot-plate latencies measured before i.c.v. injection of morphine or β-endorphin. (The mean tail-flick latency ± S.E.M. for groups of mice injected with saline 96 h earlier was 3.5 ± 0.1 s and that with pertussis toxin was 3.2 ± 0.3 s; the mean hot-plate latency ± S.E.M. for groups of mice injected with saline was 9.4 ± 0.4 s and that with pertussis toxin was 9.9 ± 0.6 s.)

4. Discussion

In the present study, we found that pretreatment with pertussis toxin attenuated the antinociception induced by i.c.v. administered morphine. The results of our studies are consistent with previous findings by others (Chung et al., 1994; Parolaro et al., 1990a, b; Parenti et al., 1986; Shah et al., 1994). There is ample evidence to support the notion that μ-opioid receptors transduce their signals via G-proteins (for review, see Law, 1995). The effect of pertussis toxin in inhibiting the morphine-induced antinociception could involve ADP-ribosylation of α-subunit of the G-protein catalyzed by pertussis toxin.

It has been previously demonstrated that the antinociception induced by morphine and β-endorphin given supraspinally is mediated by the stimulation of μ- and ε-opioid receptors, respectively (Tseng, 1995). This contention is supported by the findings that morphine-induced antinociception is blocked by selective opioid receptor antagonists, β-funaltrexamine, CTOP but not by a selective ε-opioid receptor antagonist, β-endorphin-(1–27) while β-endorphin-induced antinociception is blocked by β-endorphin-(1–27), but not by β-funaltrexamine or CTOP (Suh and Tseng, 1988, 1990; Suh et al., 1988). The present study was then designed to address the question that the ε-opioid receptor mediated antinociception induced by i.c.v. β-endorphin is also mediated by pertussis toxin-sensitive G-proteins.

We found that, in contrast to the results with morphine-induced response, i.c.v. pretreatment with pertussis toxin did not affect the inhibition of the tail-flick and hot-plate responses to i.c.v. administered β-endorphin. The results suggest that, unlike μ-opioid receptors which are coupled to pertussis toxin-sensitive G proteins, ε-opioid receptors are not coupled to pertussis toxin-sensitive G proteins for the production of antinociception and provide another piece of evidence that antinociception induced by morphine and β-endorphin is mediated by the stimulation of different types of opioid receptors.

However, we also found that the inhibition of the hot-plate response induced by a high dose but not low doses of β-endorphin was slightly attenuated by the pretreatment with pertussis toxin. Unlike the tail-flick response, which is a spinally mediated reflex and the inhibition of the hot-plate response induced by i.c.v. administered β-endorphin is caused by the activation of a descending pain control system, the hot-plate response is mainly a supraspinally organized task. Since β-endorphin is a nonspecific ε-opioid receptor agonist, it is possible that the hot-plate inhibition induced by a high dose of β-endorphin is mediated in part by the stimulation of opioid receptors other than ε-opioid receptors, such as μ- or κ-opioid receptors.

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