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Suppression of naloxone-precipitated withdrawal jumps in morphine-dependent mice by stimulation of prostaglandin EP_3 receptor

Takayuki Nakagawa, Masabumi Minami, *Seishi Katsumata, Yuka Ienaga & [1] Masamichi Satoh

Departments of Molecular Pharmacology and *Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, 606-01, Japan

1 We have shown that intracisternal (i.c.) administration of interleukin-1β (IL-1β) attenuates naloxone-precipitated withdrawal jumps in morphine-dependent mice, and the effect was partly mediated by the corticotropin-releasing factor. To elucidate further other possible mechanisms involved in the inhibitory effect of IL-1β on morphine withdrawal jumping behaviour, in this study, we examined the involvement of the prostaglandin-synthesis pathway, because prostaglandins have been shown to mediate the several central effects of IL-1. Furthermore, we examined the effects of subtype-selective prostaglandin receptor agonists on morphine withdrawal jumping behaviour.

2 Mice were rendered morphine-dependent by subcutaneous implantation of a pellet containing 11.5 ± 0.3 mg morphine hydrochloride for 48 h. Morphine withdrawal syndromes were precipitated by intraperitoneal (i.p.) injection of naloxone (10 mg kg^{-1}). The degree of physical dependence on morphine was estimated by counting the number of jumps, one of the typical withdrawal signs in mice, for 40 min.

3 The inhibitory effect of IL-1β (1 ng/mouse) administered intracisternally 30 min before naloxone (10 mg kg^{-1}, i.p.) was significantly blocked by pretreatment with sodium salicylate (a cyclo-oxygenase inhibitor, 10 ng or 30 ng/mouse) administered intracisternally 15 min before IL-1β, while i.c. administration of sodium salicylate alone (3 ng, 10 ng or 30 ng/mouse) followed by i.c. administration of vehicle instead of IL-1β did not significantly change the number of jumps precipitated by naloxone.

4 Intracisternal administration of M&B28,767 (an EP_3-receptor agonist, 1 fg-30 ng/mouse) and sulprostone (an EP_1/EP_3-receptor agonist, 10 fg-100 ng/mouse) 30 min before naloxone (10 mg $kg,^{-1}$ i.p.) attenuated withdrawal jumps with a U-shaped dose-response, reaching a peak at 10 pg/mouse and 100 pg/mouse, respectively. On the other hand, i.c. administration of iloprost (an EP_1/IP-receptor agonist, 10 fg-100 ng/mouse), butaprost (an EP_2-receptor agonist, 10 fg-100 ng/mouse) or prostaglandin $F_{2\alpha}$ (a FP-receptor agonist, 10 fg-100 ng/mouse) 30 min before naloxone (10 mg kg^{-1}, i.p.) did not significantly change the number of jumps precipitated by naloxone.

5 These results indicate that the prostaglandin-synthesis pathway is, at least in part, involved in the inhibitory effect of IL-1β on naloxone-precipitated withdrawal jumps in morphine-dependent mice, and that the prostaglandin synthesized in the brain suppresses the morphine withdrawal jumping behaviour via the EP_3-receptor, but not via the EP_1-, EP_2-, IP- or FP-receptor.

Keywords: Morphine withdrawal; interleukin-1β; prostaglandin; EP_3-receptor; sodium salicylate

Introduction

Because of its potent analgesic effect, morphine is widely used in clinical management of pain, including chronic cancer pain and post-operative pain. However, its liability to develop physical and psychic dependence restricts the range of application and causes several social problems relevant to its non-clinical use. Although the mechanism involved in morphine dependence has been vigorously investigated, it still remains unclear. On the other hand, many studies on detoxification of narcotic addicts have been carried out, and several drugs including methadone and buprenorphine (opioids), as well as clonidine and dizocilpine (non-opioids), have been used to detoxify opioid addicts (Bhargava, 1994). Furthermore, several studies have shown that cytokines such as interferon-α (Dafny, 1983) and tumour necrosis factor-α (Okutomi *et al.*, 1992), and immunomodulating agents such as muramyl dipeptide (Dougherty *et al.*, 1987) and lipopolysaccharide (Okutomi *et al.*, 1992) can attenuate morphine withdrawal syndromes in animal models. In addition, we have recently found that intracisternal administration of interleukin-1β (IL-1β) attenuates naloxone-precipitated jumps in morphine-dependent mice, and the effect was partly mediated by corticotropin-releasing factor (CRF) (Katsumata *et al.*, 1995). To elucidate further other possible mechanisms responsible for the inhibitory effect of IL-1β on morphine withdrawal jumping behaviour, in this study we examined the involvement of the prostaglandin-synthesis pathway, because prostaglandins have been shown to mediate the several central effects of IL-1, including activation of the hypothalamo-pituitary axis (Katsuura *et al.*, 1988), induction of fever (Fontana *et al.*, 1984) and hyperalgesia (Oka *et al.*, 1993). Furthermore, we examined which subtype(s) of prostaglandin receptors is involved in the regulation of the morphine withdrawal syndrome by use of several subtype-selective prostaglandin agonists, that is, sulprostone (EP_1/EP_3-selective), iloprost (EP_1/IP-selective), butaprost (EP_2-selective), M&B28,767 (EP_3-selective) and prostaglandin $F_{2\alpha}$ (FP-selective) (Coleman *et al.*, 1994).

Methods

Animals

Male ddY mice weighing 20-24 g were used. They were kept at a constant ambient temperature of 23 ± 1°C under a 12 h light/dark cycle with free access to food and water.

[1] Author for correspondence.

Development of morphine dependence

Mice were rendered morphine-dependent as previously described (Katsumata *et al.*, 1995). First, the mice used in this study were selected by the tail-pinch assay (Takagi *et al.*, 1966). The base of the tail including the anal mucosa was pinched by an artery clip with 500 g pressure. We used the mice which bit the clip within 2 s. Then, the mouse was rendered morphine-dependent by s.c. implantation of a morphine pellet at the back of the neck for 48 h. Pellet implantation was carried out between 13h00min and 15h00min according to the method of Hui & Roberts (1975). Thirty minutes after implantation of the morphine pellet, none of the mice bit the clip within 6 s in the tail-pinch assay. Forty-eight hours after implantation of the morphine pellet, the nociceptive threshold was measured by tail-pinch assay again in the mouse still implanted with the pellet which still contained sufficient morphine hydrochloride to cause analgesia in a naive mouse. The mice which bit the clip within 2 s were used for further studies. Since the development of physical dependence on morphine is reportedly well correlated with the development of tolerance (Way, 1993), we checked the development of tolerance to morphine by the tail-pinch assay and selected the tolerant animals. Then each mouse was placed into a plexiglass cylinder 25 cm in diameter and 30 cm in height for 30 min in order to acclimatize it to the experimental environment. The mice which showed jumping behaviour during this procedure were discarded.

Effect of sodium salicylate on inhibition of morphine withdrawal jumping behaviour by IL-1β

After the 30 min habituation period, the implanted pellet was removed without anaesthesia (Hui & Roberts, 1975). Soon after removal of the pellet, sodium salicylate (3, 10 or 30 ng/5 μl/mouse) or vehicle (5 μl of 0.1% BSA in PBS) was administered intracisternally according to the methods of Ueda *et al.* (1979) and then the mice were returned to the plexiglass cylinder. Fifteen minutes after pretreatment with sodium salicylate, IL-1β (1 ng/5 μl/mouse) or vehicle (5 μl of 0.1% BSA in PBS) was administered intracisternally. Thirty minutes after the i.c. administration of IL-1β or vehicle, naloxone (10 mg kg^{-1}) was administered intraperitoneally. Immediately after naloxone was administered, the mice were returned to the plexiglass cylinder and the number of jumps was counted for 40 min. These experimental procedures were performed between 13h00min and 17h00min.

Effects of prostaglandin receptor agonists on morphine withdrawal jumping behaviour

After the 30 min habituation period, the implanted pellet was removed. Soon after removal of the pellet, sulprostone (10 fg–100 ng/5 μl/mouse), iloprost (10 fg–100 ng/5 μl/mouse), butaprost (10 fg–100 ng/5 μl/mouse), M&B28,767 (1 fg–30 ng/5 μl/mouse), prostaglandin $F_{2\alpha}$ (10 fg–100 ng/5 μl/mouse) or vehicle (5 μl/mouse) was administered intracisternally to the morphine-dependent mice. Thirty minutes after the i.c. administration of the drug, naloxone (10 mg kg^{-1}) was administered intraperitoneally. Then the mice were immediately returned to a plexiglass cylinder and the number of jumps was counted for 40 min. These experimental procedures were performed between 13h00min and 17h00min.

Materials

Molecular sieve 4A 1/8 was purchased from Nacalai Tesque (Kyoto, Japan). Morphine hydrochloride was from Takeda Chemical Industries, Ltd. (Osaka, Japan). The morphine pellets containing 11.5±0.3 mg of morphine hydrochloride each were prepared according to the method of Hui & Roberts (1975) except for the use of morphine hydrochloride instead of morphine sulphate. Naloxone hydrochloride and sodium salicylate were from Sigma Chemical Co. (St. Louis, U.S.A.). Human recombinant IL-1β was a gift from Otsuka Pharmaceutical Co. (Tokushima, Japan). M&B28,767 and butaprost were gifts from Rhone-Poulenc Rorer Ltd (Dagenham, U.K.) and Bayer Ltd (Slough, U.K.), respectively. Sulprostone and iloprost were gifts from Schering AG. (Berlin, Germany). Prostaglandin $F_{2\alpha}$ was purchased from Funakoshi Ltd (Tokyo, Japan). Sulprostone, butaprost, M&B28,767 and prostaglandin $F_{2\alpha}$ were dissolved in ethanol and stored at −20°C. For intracisternal (i.c.) injection of these drugs, ethanol was removed by evaporation under nitrogen gas and the drugs were dissolved in phosphate buffered saline (PBS). Iloprost was supplied in aqueous solution and was diluted with PBS to a given concentration. IL-1β and sodium salicylate were dissolved in PBS containing 0.1% bovine serum albumin (BSA). Naloxone hydrochloride was dissolved in saline.

Statistical analysis

Data are presented as means±s.e.mean of the total number of jumps during 40 min. The data were analyzed by the Mann-Whitney U-test. Differences with $P<0.05$ were considered significant.

Results

Effect of sodium salicylate on inhibition of morphine withdrawal jumps by IL-1β

In the mice administered vehicle intracisternally (0.1% BSA in PBS) twice at 45 min and 30 min before the i.p. injection of naloxone (10 mg kg^{-1}), the mean number of jumps was 54.9±11.1. As we previously reported (Katsumata *et al.*, 1995), i.c. administration of IL-1β (1 ng/mouse) 30 min before naloxone significantly decreased the number of jumps (13.0±4.2, $P<0.01$). I.c. administration of sodium salicylate (3, 10 or 30 ng/mouse) at 15 min before IL-1β (1 ng/mouse) attenuated the inhibitory effect of IL-1β in a dose-dependent manner, that is, the mean number of jumps was 29.3±9.3, 37.6±2.9 or 52.1±9.2, respectively. A significant attenuation was observed in mice pretreated with sodium salicylate at doses of 10 and 30 ng/mouse ($P<0.01$). In the case of i.c. administration with sodium salicylate (3, 10 or 30 ng/mouse)

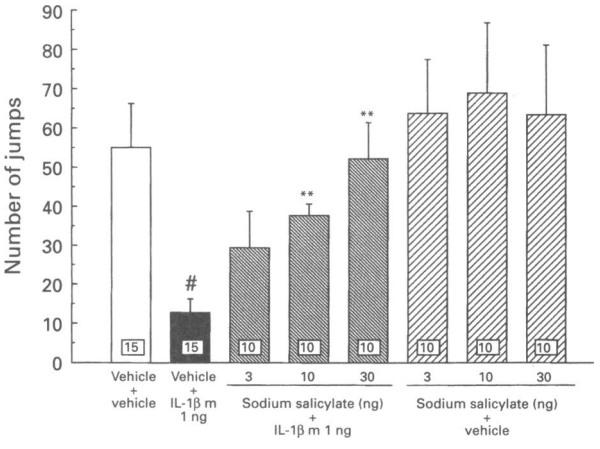

Figure 1 Effect of pretreatment with sodium salicylate on inhibition of naloxone-precipitated jumping behaviour by IL-1β (1 ng) in morphine-dependent mice. Intracisternal pretreatment with sodium salicylate or vehicle was carried out 15 min before intracisternal administration of IL-1β (1 ng) or vehicle. Each column represents mean number of jumps±s.e.mean during 40 min. #$P<0.05$ compared with the group administered vehicle intracisternally 15 min after pretreatment with vehicle. **$P<0.01$ compared with the group administered IL-1β (1 ng) intracisternally, 15 min after pretreatment with vehicle.

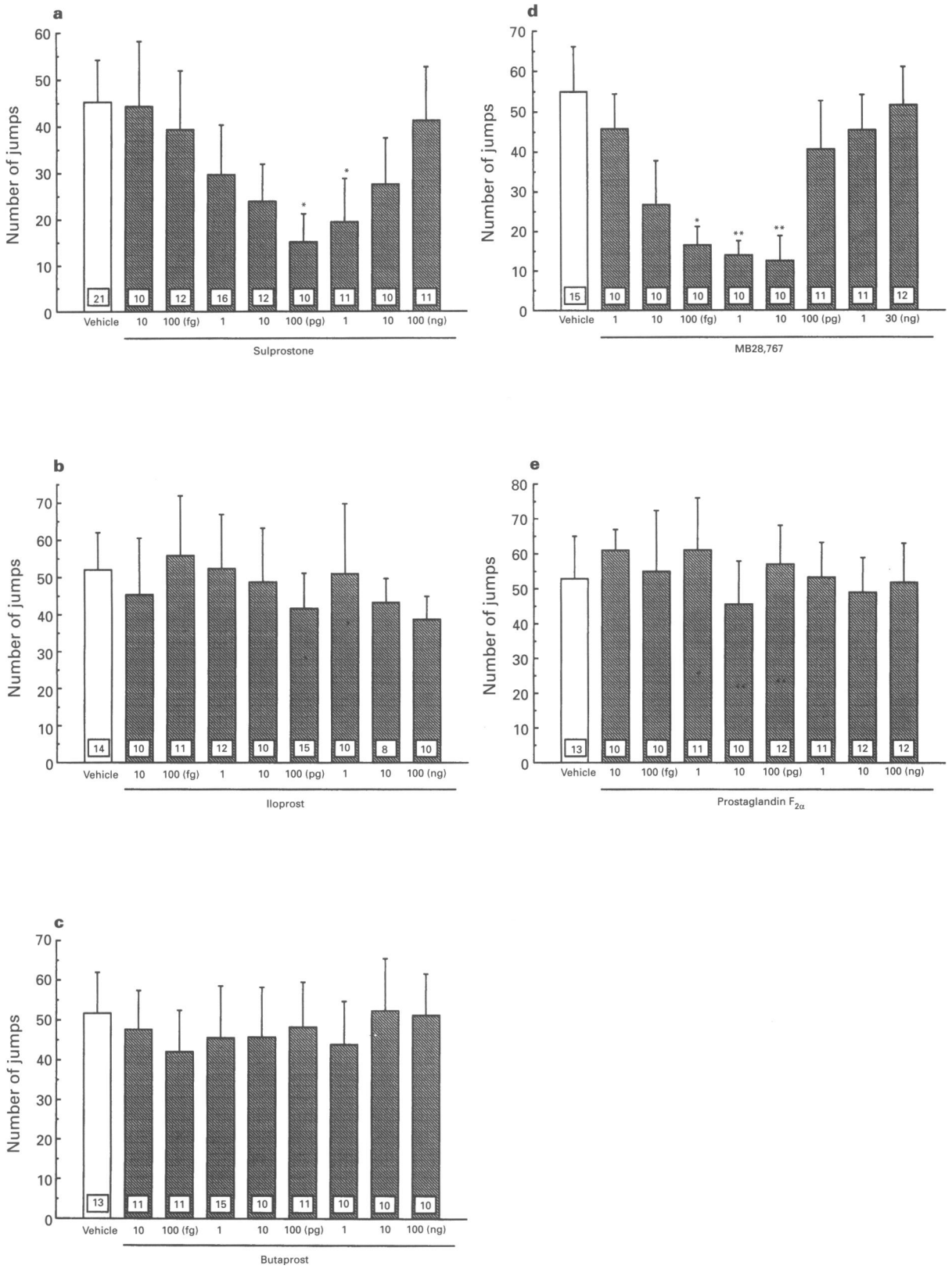

Figure 2 Effects of prostaglandin receptor agonists on naloxone-precipitated jumping behaviour in morphine-dependent mice; (a) sulprostone; (b) iloprost; (c) butaprost; (d) M&B28,767; (e) prostaglandin $F_{2\alpha}$. Each agonist in various doses or vehicle was administered intracisternally 30 min before naloxone (10 mg kg^{-1}, i.p.). Each column represents the mean number of jumps ± s.e.mean during 40 min. $^{*}P<0.05$, $^{**}P<0.01$ compared with the group administered vehicle intracisternally 30 min before naloxone (10 mg kg^{-1}, i.p.).

at 15 min before i.c. injection of vehicle, the mean number of jumps was 63.6 ± 13.7, 68.8 ± 17.9 or 63.3 ± 17.7, respectively. The number of jumps tended to increase, but no significant change was observed compared with the group administered vehicle intracisternally twice (Figure 1).

Effects of prostaglandin receptor agonists on morphine withdrawal jumps

As shown in Figure 2a, i.c. administration of sulprostone (an EP_1/EP_3 receptor agonist, $EP_3 > EP_1$ in agonist potency, Negishi *et al.*, 1994) 30 min before naloxone (10 mg kg^{-1}, i.p.), reduced morphine withdrawal jumps with a U-shaped dose-response curve. Significant effects were observed at the doses of 100 pg/mouse and 1 ng/mouse (15.1 ± 6.1 and 19.5 ± 9.4 jumps, respectively, $P < 0.05$), compared with the group administered vehicle intracisternally (45.3 ± 8.9).

Similarly, i.c. administration of M&B28,767, an EP_3-receptor agonist, 30 min before naloxone (10 mg kg^{-1}, i.p.), attenuated morphine withdrawal jumps with a U-shaped dose-response curve (Figure 2d). Significant effects were observed at doses of 100 fg/mouse (16.5 ± 4.7 jumps, $P < 0.05$) and 1 and 10 pg/mouse (13.9 ± 3.7 and 12.5 ± 6.3 jumps respectively, $P < 0.01$) compared with the group administered vehicle intracisternally (52.0 ± 11.4).

On the other hand, i.c. administration of iloprost (an EP_1/IP-receptor agonist, 10 fg–100 ng/mouse), butaprost (an EP_2-receptor agonist, 10 fg–100 ng/mouse) or prostaglandin $F_{2\alpha}$ (a FP-receptor agonist, 10 fg–100 ng/mouse) did not change significantly the number of jumps precipitated by naloxone (10 mg kg^{-1}, i.p.) (Figure 2b, c, e).

Discussion

In this study, we have shown that the inhibitory effect of IL-1β on naloxone-precipitated jumping behaviour in morphine-dependent mice was antagonized by pretreatment with sodium salicylate, a cyclo-oxygenase inhibitor, while sodium salicylate itself did not significantly affect morphine withdrawal jumps. As this finding suggests that the inhibitory effects of IL-1β might be mediated by prostaglandins synthesized in the brain, we further examined the effects of prostaglandin receptor agonists on morphine withdrawal jumps. We found that both M&B28,767 (an EP_3-agonist) and sulprostone (an EP_1/EP_3-agonist) attenuated morphine withdrawal jumps with a U-shaped dose response curve, but iloprost (an EP_1/IP-agonist), butaprost (an EP_2-agonist) or prostaglandin $F_{2\alpha}$ (a FP-agonist) were without effect. These results suggest that naloxone-precipitated jumps are suppressed by activation of the EP_3-receptor, but not by that of the EP_1-, EP_2, IP- or FP-receptor, in the brain. M&B28,767 produced an inhibitory effect on morphine withdrawal jumps at lower doses than sulprostone. This result is consistent with the previous finding that M&B28,767 inhibits forskolin-induced cyclic AMP production in the CHO cells expressing EP_3 receptor with much higher potency than sulprostone, while both agonists bind to the cell membrane with nearly equal binding potencies (Negishi *et al.*, 1994). Although it is likely that the inhibitory effect of IL-1β on morphine withdrawal jumping behaviour is mediated through the activation of the EP_3-receptor by the prostaglandin E_2 the synthesis of which is facilitated by IL-1β, unavailability of a selective antagonist for the EP_3-receptor (Coleman *et al.*, 1994) makes it impossible to confirm such a speculation.

Sugimoto *et al.* (1994) have shown the widespread distribution of EP_3-receptor mRNA in the mouse brain and its localization on neuronal cells by an *in situ* hybridization technique. On the other hand, we and other groups reported on the distribution of the mRNA for the μ-opioid receptor, which is considered to play an important role in the development of morphine-dependence and expression of withdrawal symptoms, in the rat brain (Minami *et al.*, 1994; Mansour *et al.*, 1994; Delfs *et al.*, 1994). Comparing the distribution of the EP_3-receptor mRNA with that of the μ-opioid receptor mRNA, the expression of both mRNAs are observed in many brain regions, including the amygdala, globus pallidus, medial thalamus, periaqueductal gray and locus coeruleus which have been shown to be the brain regions involved in morphine withdrawal syndromes. Maldonado & Koob (1993) reported that electrolytic lesioning of the locus coeruleus decreased the withdrawal syndrome in morphine-dependent rats. Injection of naloxone into the amygdala (Calvino *et al.*, 1979; Tremblay & Charton, 1981), medial thalamus (Wei *et al.*, 1972; 1973; Tremblay & Charton, 1981) or globus pallidus. (Tremblay & Charton, 1981) precipitated withdrawal in morphine-dependent rats. Furthermore, microinjection of methylnaloxonium into the amygdala (Stinus *et al.*, 1990), locus coeruleus and periaqueductal gray matter (Maldonado *et al.*, 1992) produced a place aversion in morphine-dependent rats. In these brain regions, these two receptors might co-exist on the same neurones and the interaction between these receptors might be involved in the effect of EP_3 receptor agonist in suppressing morphine withdrawal jumps.

It is well known that acute administration of opioids inhibits adenylate cyclase in many brain regions (Childers, 1991) and decreases cyclic AMP-dependent protein phosphorylation (Guitart & Nestler, 1989). On the other hand, chronic administration of opioids like morphine leads to increased levels of adenylate cyclase and activity of cyclic AMP-dependent protein kinase (Guitart & Nestler, 1989; 1990; Terwilliger *et al.*, 1991). Furthermore, a recent report has shown that expression of type VIII adenylate cyclase mRNA is increased in the locus coeruleus, amygdala and thalamus after chronic treatment with morphine in mice, and that this increment in the mRNA level is well correlated with the intensity of naloxone-precipitated jumping behaviour (Matsuoka *et al.*, 1994). The activated adenylate cyclase system probably causes an explosive production of cyclic AMP when the persistent inhibition of adenylate cyclase is abolished by administration of opioid antagonists like naloxone. Indeed, an increase in the cyclic AMP level was observed when NG108-15 cells which had been pretreated with chronic morphine, were treated with naloxone (Sharma *et al.*, 1975). It has been proposed that such an explosive increment in the intracellular concentration of cyclic AMP may be involved in the expression of the withdrawal syndrome (Nestler *et al.*, 1993). This idea is supported by the findings that the agents which increase cyclic AMP levels, such as the phosphodiesterase inhibitor, 3-isobutyl-l-methylxanthine and dibutyryl cyclic AMP, enhance morphine withdrawal jumps and that the agents having the reciprocal effect, such as a phosphodiesterase stimulator, imidazole, attenuate the withdrawal behaviour (Collier & Francis, 1975).

Stimulation of the EP_3 receptor, as well as μ-opioid receptor, has been shown to inhibit adenylate cyclase (Sugimoto *et al.*, 1992). Although three isoforms of mouse EP_3 receptor are produced through alternative splicing, all of these isoforms named as $EP_{3\alpha}$, $EP_{3\beta}$ and $EP_{3\gamma}$ are shown to couple to inhibitory G proteins and M&B28,767 inhibits forskolin-induced cyclic AMP accumulation in the order of $EP_{3\alpha} > EP_{3\beta} > EP_{3\gamma}$ (Sugimoto *et al.*, 1993; Irie *et al.*, 1993). These findings suggest that EP_3 agonists like M&B28,767 and sulprostone suppress morphine withdrawal jumping behaviour through the inhibition of enhanced production of cyclic AMP. Irie *et al.* (1993) have reported the dual coupling of $EP_{3\gamma}$ isoform to adenylate cyclase. M&B28,767 inhibits forskolin-induced cyclic AMP accumulation but enhances the basal activity of adenylate cyclase in the cells expressing the $EP_{3\gamma}$ isoform. Furthermore, they revealed that both basal and forskolin-induced cyclic AMP accumulations were enhanced by the stimulation of the $EP_{3\gamma}$ isoform in the cells treated with pertussis toxin. Such a dual coupling of $EP_{3\gamma}$ receptor to both stimulatory and inhibitory G proteins might result in the U-shaped dose-response curve obtained with EP_3 receptor agonists.

Ca^{2+} channels are thought to be the other possible signal transduction molecules involved in the inhibitory effect of EP_3 receptor agonists on morphine withdrawal jumps. Calcium channel blockers have been shown to suppress naloxone-precipitated morphine withdrawal syndromes (Ramkumar & El-Fakahany, 1988). Furthermore, clonidine, which suppresses some of the morphine withdrawal symptoms in rats and hu-

man subjects, inhibits not only adenylate cyclase but also Ca^{2+} channels via α_2-adrenoceptors (Lipscombe *et al.*, 1989). Prostaglandin E_2 is also shown to suppress Ca^{2+} channels in rat sympathetic neurones, while it is unclear which subtype of EP receptor is involved in such a suppression (Ikeda, 1992).

In the present study, we have shown that stimulation of the EP_3 receptor suppressed morphine withdrawal jumping behaviour. This finding suggests the possibility that EP_3 receptor agonists can be used to relieve patients with opiate withdrawal syndrome. Moreover, it provides possible clues for elucidation of the mechanisms of opiate dependence and the opiate withdrawal syndrome.

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