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Characterization of EP-receptor subtypes involved in allodynia and hyperalgesia induced by intrathecal administration of prostaglandin E_2 to mice

*†Toshiaki Minami, *†Isao Nishihara, *Rumiko Uda, [1]†‡Seiji Ito, *Masayoshi Hyodo & †Osamu Hayaishi

*Department of Anesthesiology, Osaka Medical College, Takatsuki 569; †Department of Cell Biology, Osaka Bioscience Institute, Suita 565 and ‡Department of Medical Chemistry, Kansai Medical University, Moriguchi 570, Japan

1 Intrathecal (i.t.) administration of prostaglandin E_2 (PGE_2) to conscious mice induced allodynia, a state of discomfort and pain evoked by innocuous tactile stimuli, and hyperalgesia as assessed by the hot plate test. We characterized prostaglandin E receptor subtypes (EP_{1-3}) involved in these sensory disorders by use of 7 synthetic prostanoid analogues.

2 Sulprostone ($EP_1 < EP_3$) induced allodynia over a wide range of dosages from 50 pg to 5 $\mu g\,kg^{-1}$. The maximal allodynic effect was observed at 5 min after i.t. injection, and the response gradually decreased over the experimental period of 50 min. This sulprostone-induced allodynia showed a time course similar to that induced by PGE_2.

3 17-Phenyl-ω-trinor PGE_2 ($EP_1 > EP_3$) and 16,16-dimethyl PGE_2 ($EP_1 = EP_2 = EP_3$) were as potent as PGE_2 in inducing allodynia, and more potent than sulprostone. Butaprost (EP_2), 11-deoxy PGE_1 ($EP_2 = EP_3$), MB 28767 (EP_3), and cicaprost (prostaglandin I_2 (IP-) receptor) induced allodynia, but with much lower scores. 13,14-Dihydro-15-keto PGE_2, a metabolite of PGE_2, did not induce allodynia.

4 16,16-Dimethyl PGE_2 as well as PGE_2 induced hyperalgesia over a wide range of dosages (16,16-dimethyl PGE_2: 5 pg–0.5 $\mu g\,kg^{-1}$ PGE_2: 50 pg–0.5 $\mu g\,kg^{-1}$) with two apparent peaks at 0.5 $ng\,kg^{-1}$ and 0.5 $\mu g\,kg^{-1}$. Sulprostone ($EP_1 < EP_3$) and 17-phenyl-ω-trinor PGE_2 ($EP_1 > EP_3$) showed a bell-shaped hyperalgesia at lower doses of 5 pg–5 $ng\,kg^{-1}$ and 50 pg–50 $ng\,kg^{-1}$, respectively. MB 28767 (EP_3) showed a monophasic hyperalgesic action over a wide range of dosages at 50 pg–5 $\mu g\,kg^{-1}$. Butaprost (EP_2) induced hyperalgesia at doses higher than 50 $ng\,kg^{-1}$.

5 These results demonstrate that PGE_2 may exert allodynia through the EP_1-receptor and hyperalgesia through EP_2- and EP_3-receptors in the mouse spinal cord.

Keywords: EP agonist; IP agonist; allodynia; hyperalgesia; spinal cord

Introduction

Prostaglandins are ubiquitously distributed in virtually all mammalian tissues and organs, and it has been well documented that they are involved in various aspects of inflammation including pain (Coleman *et al.*, 1989). Accumulating evidence indicates that prostaglandins are critical for the augmented processing of pain information at the spinal level. Intrathecal (i.t.) injection of acetylsalicylic acid, indomethacin, and other nonsteroidal anti-inflammatory drugs has been shown to produce analgesia (Yaksh, 1982; Malmberg & Yaksh, 1992a,b). We and others (Taiwo & Levine, 1986; 1988; Uda *et al.*, 1990) previously reported that i.t. injection of PGD_2 and PGE_2 induced hyperalgesic effects.

Quite recently, we demonstrated that i.t. administration of PGE_2 into mice also induces allodynia, a state of discomfort and pain evoked by innocuous tactile stimuli; the mice showed squeaking, biting, and scratching movements in response to low-threshold stimuli (Minami *et al.*, 1994a,b). Hyperalgesia is defined as an increased response to a stimulus that is normally painful, whereas the closely related term allodynia is defined as pain due to a stimulus that does not normally provoke pain (Merskey, 1986). Although hyperalgesia and allodynia are often associated consequences of damage to peripheral nerves or the central nervous system, it has recently been suggested that different receptor systems in the spinal cord may be involved in these two sensory disorders (Yaksh & Aimone, 1989).

PGE_2 produces a broad range of biological actions in diverse tissues through its binding to specific receptors on plasma membranes (Samuelsson *et al.*, 1978; Moncada *et al.*, 1985). The diversity of PGE_2 actions is due to PGE-receptor subtypes coupled to different signal transduction pathways. PGE receptors are pharmacologically divided into at least three subtypes, EP_1, EP_2 and EP_3, and they are considered to be coupled to Ca^{2+} mobilization and stimulation and inhibition of adenylate cyclase, respectively (Coleman *et al.*, 1987; 1989). It has been suggested that the EP_1-receptor is involved in contraction of gastrointestinal and tracheal smooth muscles (Coleman & Kennedy, 1985), and stimulation of neurotransmitter release (Ehrenpreis *et al.*, 1973). Important functions of PGE_2 mediated via EP_2-receptors include the negative regulation of the immune system (Monick *et al.*, 1987) and inflammation (Coleman *et al.*, 1989). It has also been suggested that the EP_2-receptor may be involved in relaxation in trachea (Gardiner, 1986) and ileum circular muscle (Lawrence *et al.*, 1992), and vasodilatation of various blood vessels (Coleman *et al.*, 1989). On the other hand, the EP_3-receptor is assumed to be involved in inhibition of gastric acid secretion (Chen *et al.*, 1988), modulation of neurotransmitter release in central and peripheral neurones (Hedqvist & von Euler, 1972), and inhibition of sodium and water reabsorption in kidney tubules (Sonnenburg & Smith, 1988; Nakao *et al.*, 1989). However, the EP-receptor subtypes in the spinal cord have not yet been characterized. Although there are few prostanoid analogues which show absolute selectivity for the individual EP_1-, EP_2- and EP_3-subtypes, many are now known as potent agonists for EP-receptors.

In the present study, we examined the effect of i.t. admini-

[1] Author for correspondence at Suita address.

stration of various agonists selective for EP-receptor subtypes on nociception and non-nociception in the spinal cord of conscious mice.

Methods

Intrathecal administration

Male ddY-mice weighing 22 ± 2 g were used in this study. The animals were housed under conditions of a 12-h light-dark cycle and a constant temperature of 22 ± 2°C and 60 ± 10% humidity. A 27-gauge stainless-steel needle (0.35 mm, o.d.) attached to a microsyringe was inserted between the L_5 and L_6 vertebrae by a slight modification of the method of Hylden & Wilcox (1980). Drugs in vehicle were injected slowly into the subarachnoid space of conscious mice at 22 ± 2°C. The volume of the i.t. injection was 5 μl. It was previously confirmed by use of Commassie brilliant blue and ^{3}H-labelled prostaglandins that the injected solution does not extend to the cervical segments (Uda *et al.*, 1990).

Studies on allodynia

Studies on allodynia were carried out essentially according to the method of Yaksh & Harty (1988). The mice were divided into various groups ($n = 6-8$/group). Control mice were given physiological saline (5 μl). Drug-treated groups were injected with 5 μl of vehicle containing various doses of test agents. After the i.t. injection, each mouse was placed in an individual 13 × 8.5 × 13 cm Plexiglas enclosure with wood chips on the floor and observed. Allodynia was assessed once every 5 min over a 50-min period by light stroking of the flank of the mice with a paintbrush. The allodynia response was ranked as follows: 0, no response; 1, mild squeaking with attempts to move away from the stroking probe; 2, vigorous squeaking evoked by the stroking probe, biting at the probe, and strong efforts to escape.

Hot plate test

Mice were placed on a hot plate maintained at 55°C, and the elapsed time until the mice showed the first avoidance responses (licking the feet, jumping, or rapidly stamping the paws) was recorded as described by Woolfe & MacDonald (1944). The response time of the mice to the hot plate was measured at 30 min after i.t. injection.

The animals were used for only one measurement in each experiment. This study was conducted in accordance with the guidelines of the Ethics Committee of the International Association for the Study of Pain (Zimmermann, 1983).

Drugs

13,14-Dihydro-15-keto PGE_2, 16,16-dimethyl PGE_2, and 17-phenyl-ω-trinor PGE_2 were purchased from Cayman Chemical (Ann Arbor, MI, U.S.A.). The following prostaglandin analogues were generous gifts: PGE_2 and 11-deoxy PGE_1 from Ono Central Research Institute (Osaka, Japan); butaprost from Dr P.J. Gardiner of Bayer, U.K.; sulprostone and cicaprost from Dr K.-H. Thierauch, Schering AG, Berlin; MB 28767 (15S-hydroxy-9-oxo-16-phenoxy-ω-tetranorprost-13E-enoic acid) from Rhône-Poulenc Rorer, U.K. Table 1 summarizes the selectivity profiles of the EP- and IP-receptor agonists employed here as reported by Lawrence *et al.* (1992).

Stock solutions of the prostanoids were stored in ethanol solution at − 20°C. For injection, an aliquot of the desired stock agonist solution was put into a borosilicate tube and the ethanol was removed by evaporation to dryness under nitrogen gas. Sterile saline was then added to dissolve the agonist. Each agonist was dissolved in sterile saline on the day of the experiment and kept on ice until used. All drugs, including saline, were coded to assure blind testing.

Statistics

The statistical analyses were carried out by analysis of variance (ANOVA). Statistical significance ($P < 0.05$) was further examined with Duncan's test for multiple comparison.

Results

Effect of i.t. EP agonists on allodynia

Recently we reported that PGE_2 induced allodynia and hyperalgesia over a wide range of dosages from 0.5 pg to 50 μg kg^{-1} (Minami *et al.*, 1994a). In order to specify the EP-receptor(s) involved in the PGE_2-induced allodynia, we examined the effect of various EP agonists and cicaprost, an IP agonist, on allodynia in conscious mice. The i.t. administration of sulprostone (0.5 μg kg^{-1}), an EP_1 and EP_3 agonist, resulted in prominent agitation responses such as vocalization, biting, and escape from the probe, to tactile stimuli applied to the flank. Brushing of the face or tactile stimulation of the forepaws did not give any response, indicating that allodynia appeared limited to the caudal dermatomes of the body. The i.t. administration of saline had no effect on allodynia. As shown in Figure 1, the sulprostone (0.5 μg kg^{-1})-induced allodynia showed its maximum expression at 5 min after i.t. injection and gradually decreased during the 50-min experimental period in a manner similar to that induced by PGE_2. When the score obtained for the overall 50 min was cumulated and expressed as a percentage of the maximum possible score, the score of sulprostone was comparable to that of PGE_2 at 0.5 μg kg^{-1} (Figure 2). Allodynic scores of butaprost, an EP_2 agonist, MB 28767, an EP_3 agonist, and cicaprost were much lower than that of PGE_2 at 0.5 μg kg^{-1}.

Figure 3 shows the dose-dependencies of allodynia evoked

Table 1 Specificity of prostaglandin E and prostaglandin I analogues at EP- and IP-receptors

Prostanoid	*EP_1*	*EP_2*	*EP_3*	*IP*
PGE analogue				
PGE_2	+++	+++	+++	
16,16-Dimethyl PGE_2	++++	++(+)	++++	
Sulprostone	++	0	++++	
17-Phenyl-ω-trinor PGE_2	+++	+	++	
Butaprost	(+)	++	0	
11-Deoxy PGE_1	+	++(+)	++	
MB 28767	(+)	(+)	+++	
PGI analogue				
Cicaprost	(+)	(+)	0	++++

Rankings of EP- and IP-receptors are from Lawrence *et al.* (1992). One + corresponds to a potency difference of approximately one order of magnitude.

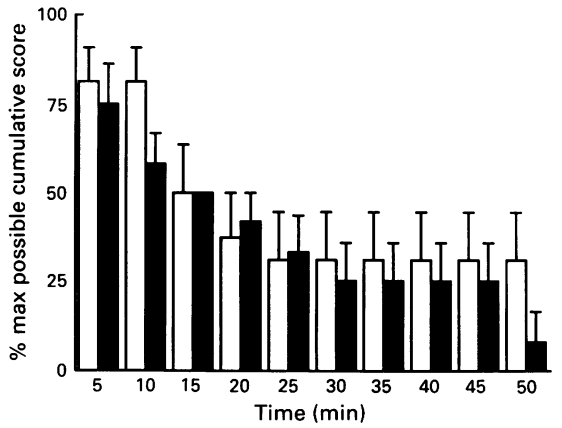

Figure 1 Time courses of allodynia induced by i.t. prostaglandin E_2 (PGE_2) and sulprostone. Mice were injected i.t. with 0.5 $\mu g\,kg^{-1}$ of PGE_2 (open columns) or sulprostone (solid columns). Assessment of allodynia was made as described under Methods. Each column (mean ± s.e.mean) represents the percentage of the maximum possible cumulative score of 6 mice evaluated every 5 min.

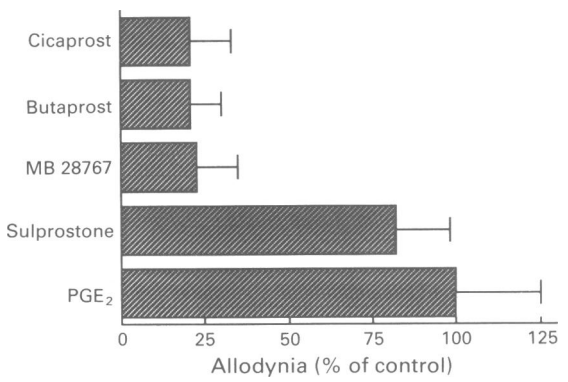

Figure 2 Effect of prostaglandin E_2 (PGE_2) and prostanoid agonists on allodynia in conscious mice. PGE_2 or prostanoid agonist (0.5 $\mu g\,kg^{-1}$) was injected into the subarachnoid space. Assessment of allodynia was made as described under Methods. When the score obtained for the overall 50-min experimental period was cumulated and expressed as a percentage of the maximum possible score, the allodynic score of PGE_2 was 43.8% and taken as 100%. Values shown are the mean ± s.e.mean of responses in 6 mice.

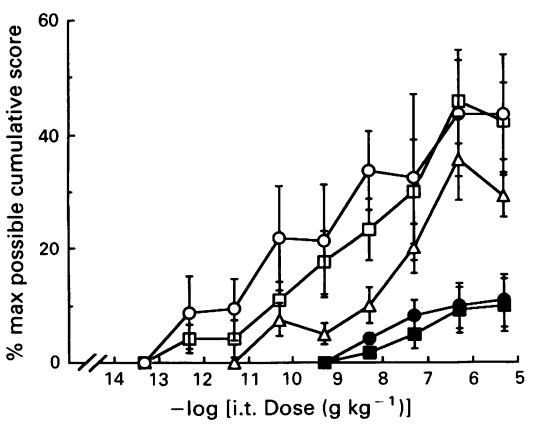

Figure 3 Dose-dependent effects of i.t. injection of prostaglandin E_2 (PGE_2) and various EP agonists on allodynia in conscious mice. Mice were injected with various doses of PGE_2 (○), 17-phenyl-ω-trinor PGE_2 (□), sulprostone (△), MB 28767 (●), or butaprost (■) into the subarachnoid space. Assessment of allodynia was made as described under Methods. Values shown are the mean ± s.e.mean of responses in 6 mice.

by EP agonists. The allodynia induced by sulprostone was observed at a dose as low as 50 $pg\,kg^{-1}$ and the maximal effect (35.8% of the maximum possible score) was observed at 5 $\mu g\,kg^{-1}$, with a half-maximal stimulation occurring at about 50 $\mu g\,kg^{-1}$. This agonist-induced allodynia showed similar time courses over a wide range of doses of 50 pg–5 $\mu g\,kg^{-1}$ (data not shown). Sulprostone-induced mice did not display clonic seizure and convulsion even at a dose as high as 5 $\mu g\,kg^{-1}$. 17-Phenyl-ω-trinor PGE_2 ($EP_1 > EP_3$) showed its maximum effect (45.8% of the maximum possible score) at 0.5 $\mu g\,kg^{-1}$, with a half-maximal stimulation occurring at about 5 $ng\,kg^{-1}$. Dose-dependency of 16,16-dimethyl PGE_2, a potent EP_1, EP_2 and EP_3 agonist, for allodynia showed a bell-shaped curve, and the maximum effect was observed at 50 $ng\,kg^{-1}$ (data not shown). Butaprost (Figure 3), MB 28767 (Figure 3), and 11-deoxy PGE_1 (data not shown) showed shallow log concentration-response curves with 10% of the maximum possible score at 5 $\mu g\,kg^{-1}$. 13,14-Dihydro-15-keto PGE_2, a metabolite of PGE_2, did not induce allodynia. Taken together, these results demonstrate that the EP_1-receptor may be involved in the PGE_2-induced allodynia in the spinal cord.

Effect of i.t.-administered EP agonists on hyperalgesia assessed by the hot plate test

As reported previously (Uda *et al.*, 1990), PGE_2-induced hyperalgesia was observed between 3 and 30 min after i.t. injection of PGE_2. In the present study, the response time of mice to the hot plate (55°C) was measured at 30 min after i.t. injection of various doses of EP agonists or vehicle so that the effect of EP agonist-induced allodynia would be minimized. There was no significant difference in the latency

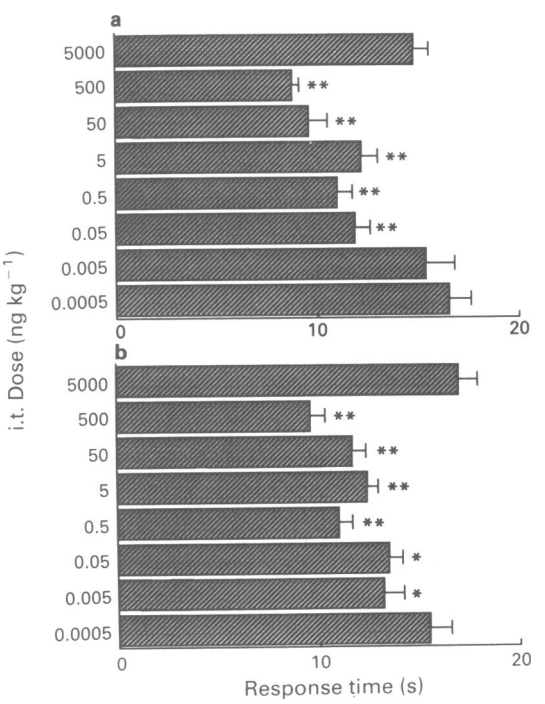

Figure 4 Dose-dependent effects of i.t. injection of prostaglandin E_2 (PGE_2) and 16,16-dimethyl PGE_2 on hyperalgesia assessed by the hot plate test. Mice were injected with various doses of PGE_2 (a) or 16,16-dimethyl PGE_2 (b) into the subarachnoid space. The time until the mice showed the first avoidance response to the hot plate test (55°C) was measured at 30 min after i.t. injection. Each column represents the mean ± s.e.mean of responses in ten mice. Statistical analyses were carried out by Duncan's test. $0.01 \leqslant {}^{*}P < 0.05$; $^{**}P < 0.01$, as compared with the saline-injected control group (16.2 ± 0.5 s).

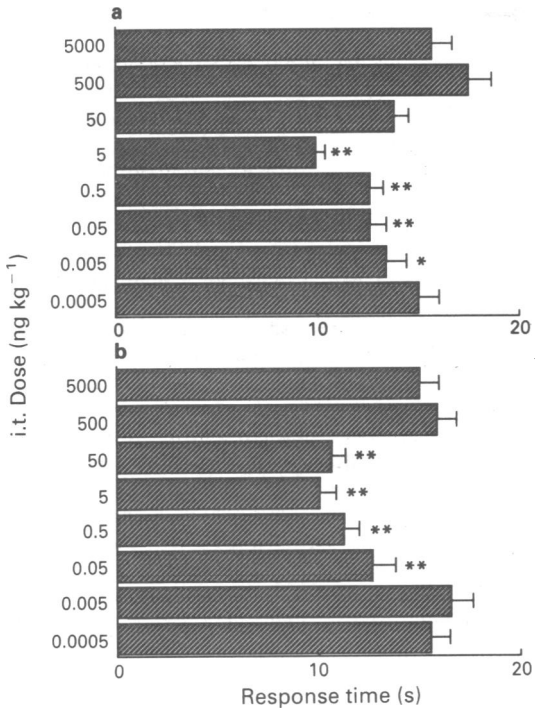

Figure 5 Dose-dependent effects of i.t. injection of sulprostone and 17-phenyl-ω-trinor PGE_2 on hyperalgesia assessed by the hot plate test. Mice were injected with various doses of sulprostone (a) or 17-phenyl-ω-trinor PGE_2 (b) into the subarachnoid space. Details as in the legend for Figure 4, $0.01 \leqslant {}^{*}P < 0.05$; ${}^{**}P < 0.01$, as compared with the saline-injected control group (16.2 ± 0.5 s).

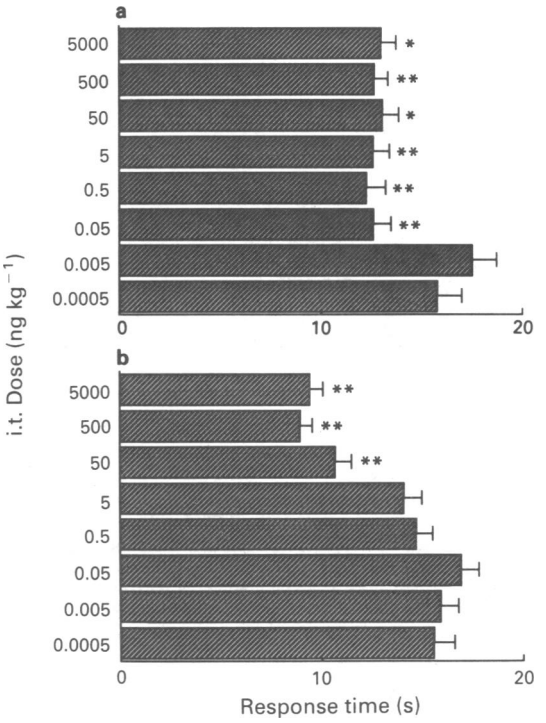

Figure 6 Dose-dependent effects of i.t. injection of MB 28767 and butaprost on hyperalgesia assessed by the hot plate test. Mice were injected with various doses of MB 28767 (a) or butaprost (b) into the subarachnoid space. Details as in the legend for Figure 4. $0.01 \leqslant {}^{*}P < 0.05$; ${}^{**}P < 0.01$, as compared with the saline-injected control group (16.2 ± 0.5 s).

period between the saline control (16.2 ± 0.5 s, mean ± s.e. mean) and the untreated control (15.4 ± 0.4 s).

As reported previously (Minami *et al.*, 1994a), PGE_2 (EP_1, EP_2, EP_3) produced its hyperalgesic action over a wide range of dosages from 50 pg to 0.5 μg kg^{-1}. Similarly, 16,16-dimethyl PGE_2 (EP_1, EP_2, EP_3) produced its hyperalgesic action at doses of 5 pg–0.5 μg kg^{-1} (Figure 4); this action showed two peaks, one at 0.5 ng kg^{-1} (11.0 ± 0.7 vs. 16.2 ± 0.5 s for the control) and one at 0.5 μg kg^{-1} (9.6 ± 0.7 s), and returned to the control level at 5 μg kg^{-1}, the highest concentration employed. On the other hand, sulprostone ($EP_1 < EP_3$) and 17-phenyl-ω-trinor PGE_2 ($EP_1 > EP_3$) produced bell-shaped dose dependency for hyperalgesic action at doses of 5 pg–5 ng kg^{-1} and 50 ng kg^{-1}, respectively (Figure 5). Neither sulprostone nor 17-phenyl-ω-trinor PGE_2 produced a significant change at higher doses examined. Unlike its effects on allodynia, MB 28767 (EP_3) produced its hyperalgesic action over a wide range of dosages from 50 pg to 5 μg kg^{-1}, and butaprost (EP_2) produced its hyperalgesic action at higher doses of 50 ng–5 μg kg^{-1} (Figure 6). 13,14-Dihydro-15-keto PGE_2 produced a weak hyperalgesic action at doses of 0.5 ng and 5 ng kg^{-1} (data not shown). Cicaprost (IP) produced no significant change. These results demonstrate that EP_2- and EP_3-receptors may be involved in the PGE_2-induced hyperalgesia in the spinal cord.

Discussion

We recently reported that the i.t. administration of PGE_2 to conscious mice induced allodynia and hyperalgesia (Uda *et al.*, 1990; Minami *et al.*, 1994a,b). The dose-dependency of PGE_2 for allodynia was apparently correlated with that for hyperalgesia. While the PGE_2-induced allodynia was dose-dependently relieved by the strychnine-sensitive glycine receptor agonist, taurine, the NMDA receptor antagonist ketamine, and a high dose of the α_2-adrenoceptor agonist, clonidine, the PGE_2-induced hyperalgesia assessed by the hot plate test was not suppressed by taurine or clonidine. To test the hypothesis that the mechanism of PGE_2-induced allodynia might be different from that of PGE_2-induced hyperalgesia (Minami *et al.*, 1994a), we used 7 synthetic prostaglandin analogues having different specificities for EP-receptor subtypes as summarized in Table 1.

The present study provides evidence that allodynia and hyperalgesia induced by PGE_2 are mediated by different EP-receptor subtypes. It is clear that the EP_1-receptor is involved in the PGE_2-induced allodynia for the following reasons: first, 17-phenyl-ω-trinor PGE_2 ($EP_1 > EP_3$) was more potent than sulprostone ($EP_1 < EP_3$) (Figure 3). Second, butaprost (EP_2), 11-deoxy PGE_1 ($EP_2 = EP_3 > EP_1$) and MB 28767 (EP_3) induced allodynia, but with much lower scores. On the other hand, the PGE_2-induced hyperalgesia was complicated, demonstrating that the hyperalgesia observed here may reflect the action of PGE_2 or EP agonists at multiple sites inherent in *in vivo* experiments. Both PGE_2 and 16,16-dimethyl PGE_2 induced a biphasic hyperalgesia over a wide range of dosages (PGE_2: 50 pg–0.5 μg kg^{-1}, 16,16-dimethyl PGE_2: 5 pg–0.5 μg kg^{-1}) with two apparent peaks at 0.5 ng and 0.5 μg kg^{-1} (Figure 4). Sulprostone ($EP_1 < EP_3$) and 17-phenyl-ω-trinor PGE_2 ($EP_1 > EP_3$) showed a bell-shaped dose-response for hyperalgesia at lower doses of 5 pg–5 ng kg^{-1} and 50 pg–50 ng kg^{-1}, respectively (Figure 5). MB 28767 (EP_3) showed a monophasic hyperalgesic action over a wide range of dosages of 50 pg–5 μg kg^{-1}. Butaprost (EP_2) induced hyperalgesia at doses higher than 50 ng kg^{-1} (Figure 6). These results suggest that PGE_2 may exert hyperalgesia through EP_2- and EP_3-receptors. Collectively, with the data obtained with butaprost, sulprostone, and MB 28767, the PGE_2-induced hyperalgesia is likely to be mediated by the EP_3-receptor at lower doses of PGE_2 and by the EP_2-receptor at higher doses. However, differences in receptor density or efficiency of receptor-effector coupling in the central nervous system could dramatically alter relative agonist potencies.

Therefore we cannot neglect the possibility that butaprost may exert hyperalgesia through the EP_3-receptor.

Prostaglandins have been studied as potential nociceptive transmitters, but as observed in the present experiments, these studies are complicated by the existence of multiple receptor subtypes and hampered by the absence of selective antagonists. Recently EP_1-, EP_2- and EP_3-receptors have been successfully cloned from mouse cDNA libraries (Sugimoto *et al.*, 1992; Honda *et al.*, 1993; Watabe *et al.*, 1993). There are some discrepancies in specificities of EP agonists and antagonists for EP-receptors between membranes from cDNA-transfected cells and tissues reported previously. For example, EP-receptors in isolated tissues that are susceptible to blockade by SC19220 and AH6809 have been designated as EP_1-receptors (Coleman *et al.*, 1985). However, because AH6809 showed only weak inhibition of the binding of [^{3}H]-PGE_2 to membranes prepared from EP_1-receptor cDNA-transfected CHO cells, it was suggested that there may be other forms of EP_1-receptor sensitive to AH6809 or that the action of AH6809 is species-specific and does not work on the mouse receptor (Watabe *et al.*, 1993). In this context, our previous observation that PGE_2-induced hyperalgesia was blocked by AH6809 (Uda *et al.*, 1990) is not inconsistent with the present results indicating that hyperalgesia induced by PGE_2 was mediated by EP_3- and/or EP_2-receptors (Figures 5 and 6). It was also reported that the lack of binding activity of butaprost toward the EP_2-receptor cloned from mouse mastocytoma P-815 cells suggests another form of EP_2-receptor subtype (Honda *et al.*, 1993). Furthermore, there are two isoforms of EP_3-receptor with different properties at the levels of signalling and desensitization in mice (Negishi *et al.*, 1993). It has also been shown that four isoforms exist of bovine EP_3-receptor that couple to different G proteins to activate different second messenger systems (Namba *et al.*, 1993). In addition to these findings, precise localization of EP-receptor subtypes in the spinal cord by *in situ* hybridization will be of help in understanding the diversity of cellular responses to PGE_2.

In summary, we have demonstrated here, on the basis of rankings of specificities of EP agonists for EP-receptor subtypes, that EP_1- and EP_3-receptors, and possibly EP_2-receptor, may exist in mouse spinal cord and be involved in pain transmission at the level of spinal cord. Spinal EP-receptor systems may exert pain transmission on high and low threshold afferents with mutual interactions of L-glutamate and other neuroactive substances (Taiwo & Levine, 1986; Uda *et al.*, 1990; Malmberg & Yaksh, 1992a,b; Minami *et al.*, 1994a,b). This is relevant because there is renewed interest and investigation into the mechanisms of hyperalgesia and associated allodynia (Willis, 1992). Because PGE_2 clearly plays important but complex roles in both acute and chronic pain via different pain processing pathways, the continued pharmacological approach along with the development of specific EP-receptor agonists and antagonists may provide us with an opportunity to learn more about the physiological and pathological roles of PGE_2 in pain.

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