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Characterization of EP receptor subtypes responsible for prostaglandin E_2-induced pain responses by use of EP_1 and EP_3 receptor knockout mice

[1]Toshiaki Minami, [1]Hiroyuki Nakano, [2]Takuya Kobayashi, [3]Yukihiko Sugimoto, [4]Fumitaka Ushikubi, [3]Atsushi Ichikawa, [2]Shuh Narumiya & *,[5]Seiji Ito

[1]Department of Anesthesiology, Osaka Medical College, Takatsuki 569-8686, Japan; [2]Department of Pharmacology, Faculty of Medicine, Kyoto University, Kyoto 606-8315, Japan; [3]Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8315, Japan; [4]Department of Pharmacology, Asahikawa Medical College, Asahikawa 078-8510, Japan and [5]Department of Medical Chemistry, Kansai Medical University, Moriguchi 570-8506, Japan

1 Prostaglandin E_2 (PGE_2) is known to be the principal pro-inflammatory prostanoid and play an important role in nociception. To identify PGE receptor (EP) subtypes that mediate pain responses to noxious and innocuous stimuli, we studied them by use of EP_1 and EP_3 knockout ($EP_1^{-/-}$ and $EP_3^{-/-}$) mice.

2 PGE_2 could induce mechanical allodynia in $EP_1^{+/+}$, $EP_3^{+/+}$ and $EP_3^{-/-}$ mice, but not in $EP_1^{-/-}$ mice. *N*-methyl-D-aspartate (NMDA), the substrate of nitric oxide (NO) synthase L-arginine, or the NO donor sodium nitroprusside administered intrathecal (i.t.) could induce allodynia in $EP_3^{-/-}$ and $EP_1^{-/-}$ mice. Activation of EP_1 receptors appears to be upstream, rather than downstream, of NMDA receptor activation and NO production in the PGE_2-induced allodynia.

3 Although PGE_2 produced thermal hyperalgesia over a wide range of dosages from 50 pg to 0.5 μg kg^{-1} in $EP_3^{+/+}$ mice, it showed a monophasic hyperalgesic action at 5 ng kg^{-1} or higher doses in $EP_3^{-/-}$ mice. The selective EP_3 agonist, ONO-AE-248, induced hyperalgesia at 500 pg kg^{-1} in $EP_3^{+/+}$ mice, but not in $EP_3^{-/-}$ mice.

4 Saline-injected $EP_1^{-/-}$ mice showed hyperalgesia, which was reversed by i.t. PGE_2 in a dose-dependent manner.

5 There was no significant difference in the formalin-induced behaviours between $EP_1^{-/-}$ or $EP_3^{-/-}$ mice and the cognate wild-type mice.

6 These results demonstrate that spinal EP_1 receptors are involved in the PGE_2-induced allodynia and that spinal EP_3 receptors are involved in the hyperalgesia induced by low doses of PGE_2. However, the formalin-induced pain cannot be ascribed to a single EP receptor subtype EP_1 or EP_3. *British Journal of Pharmacology* (2001) **133,** 438–444

Keywords: PGE_2; allodynia; hyperalgesia; formalin test; EP_1; EP_3; knockout mice

Abbreviations: i.t., intrathecal; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; PGE_2, prostaglandin E_2; SNP, sodium nitroprusside

Introduction

Prostanoids are the products of the cyclo-oxygenase pathway of arachidonic acid metabolism and act as local mediators in various tissues under physiological and pathophysiological conditions (Narumiya *et al*., 1999). Since Vane (1971) first reported that aspirin-like drugs prevented the development of inflammation by blocking the synthesis of prostanoids, it has been widely accepted that prostanoids are involved in pain, fever, oedema, and various aspects of inflammation. Among them, prostaglandin E_2 (PGE_2) is considered to be the principal pro-inflammatory prostanoid and play an important role in nociceptive processing in the spinal cord as well as in the periphery (Yaksh *et al*., 1999). PGE_2 is released from the spinal cord *in vivo* upon various noxious stimuli and inflammatory insults. The intrathecal (i.t.) administration of PGE_2 into conscious mice induced hyperalgesia to noxious stimuli (Taiwo & Levine, 1988; Uda *et al*., 1990; Minami *et al*., 1994a) and allodynia to tactile innocuous stimuli (Minami *et al*., 1994a, c). Conversely, i.t. delivery of cyclo-oxygenase inhibitors blocked pain responses induced by subcutaneous formalin, and i.t. substance P and *N*-methyl-D-aspartate (NMDA) (Malmberg & Yaksh, 1992a, b).

The diversity of PGE_2 actions is believed to be the result of four PGE receptor subtypes EP_1–EP_4 coupled to different signal transduction pathways (Narumiya *et al*., 1999). The EP_1 receptor is coupled to intracellular Ca^{2+} mobilization. Whereas EP_2 and EP_4 receptors are coupled to stimulation of adenylate cyclase *via* Gs, EP_3 receptor is coupled to inhibition of adenylate cyclase *via* Gi. We previously showed that PGE_2 induced thermal hyperalgesia over a wide range of dosages between 50 pg–500 ng kg^{-1} with two apparent peaks of 0.5 ng kg^{-1} and 500 ng kg^{-1}. While the EP_3 agonist MB28767 showed a monophasic hyperalgesic action at as low

*Author for correspondence at: Department of Medical Chemistry, Kansai Medical University, 10–15 Fumizono, Moriguchi 570-8506, Japan; E-mail: ito@takii.kmu.ac.jp

as 50 pg kg^{-1}, the EP_2 agonist butaprost induced hyperalgesia at doses higher than 50 ng kg^{-1}, suggesting that the PGE_2-induced hyperalgesia is mediated by the EP_3 receptor at lower doses and by the EP_2 receptor at higher doses (Minami *et al*., 1994a). We also suggested that PGE_2-induced allodynia is mediated by the EP_1 receptor by use of EP receptor agonists and that the bifunctional EP_1 antagonist/EP_3 agonist ONO-NT-012 is a highly potent, simple competitive antagonist for the PGE_2-induced allodynia (Minami *et al*., 1994a; 1995b). On the other hand, Malmberg *et al*. (1994) suggested that i.t. EP_3 agonists produced thermal hyperalgesia and allodynia and that i.t. EP_1 antagonist significantly attenuated the second phase of formalin-induced pain. Previous studies demonstrated that PGE_2 plays versatile roles in pain transmission through different EP receptor subtypes in the spinal cord. However, studies in this area have been limited by the lack of potent and specific EP receptor antagonists so far.

To elucidate the pathophysiological significance of EP receptor subtypes, the gene targeting technique has been employed (Narumiya *et al*., 1999). In the present study, we examined the roles of EP_1 and EP_3 subtypes in the PGE_2-induced pain responses by use of the respective knockout mice ($EP_1^{-/-}$ and $EP_3^{-/-}$). Furthermore we examined the role of EP_1 and EP_3 subtypes in the formalin test.

Methods

Chemicals

PGE_2 and ONO-AE-248 (Zacharowski *et al*., 1999) were generous gifts from Ono Central Research Institute (Osaka, Japan). NMDA, L-arginine and sodium nitroprusside (SNP) were purchased from Sigma (St. Louis, MO, U.S.A.). All chemicals were dissolved in sterile saline on the day of the experiments and kept on ice until used. All drugs, including saline, were coded to assure blind testing.

Animals

$EP_1^{-/-}$ or $EP_3^{-/-}$ mice were obtained by the gene targeting technique (Ushikubi *et al*., 1998). The animals were housed under conditions of a 12-h light–dark cycle, a constant temperature of $22 \pm 2^{\circ}C$ and $60 \pm 10\%$ humidity.

Studies on allodynia and hyperalgesia

Studies on allodynia and hyperalgesia were carried out as described previously (Minami *et al*., 1999). 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 and drugs in vehicle were injected slowly into the subarachnoid space of conscious mice by a slight modification of the method of Hylden & Wilcox (1980).

For allodynia, the mice were divided into various groups ($n = 5-6$/group). Drug-treated groups were injected with 5 μl of vehicle containing various doses of test agents. Control mice were given physiological saline (5 μl). After the i.t. injection, each mouse was placed in an individual $13 \times 8.5 \times 13$ cm Plexiglas enclosure with wood chips on the floor and observed. Allodynia was assessed once every 5 min over a 5-min period by light stroking of the flank of the mice with a paintbrush. The allodynic 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, or strong efforts to escape. The maximum possible score for allodynia of six mice was $2 \times 6 = 12$ in any 5 min period and was taken as 100%.

For hyperalgesia, mice were placed on a hot plate maintained at 52.5°C, and the elapsed time until the mice showed the first avoidance responses (licking the feet, jumping or rapidly stamping the paws) was recorded. The response time of the mice to the hot plate was measured 30 min after i.t. injection, the points of the maximal hyperalgesic effect obtained with PGE_2 (Uda *et al*., 1990).

Formalin test

Formalin test was carried out as described previously (Nakano *et al*., 2000), essentially according to the procedure reported by Hunskaar & Hole (1987). The mice were divided into various groups ($n = 8-10$/group). Using a minimum of restraint, 20 μl of 2% formalin in 0.9% NaCl was injected subcutaneously into the right dorsal hind paw of the mouse using a microsyringe with a 26-gauge needle. After the formalin injection, each mouse was placed to the observation chamber. The amount of time spent licking and biting the injected paw was measured with a hand-held stop-watch for 5 min from 0 to 30 min. Two distinct periods of high licking activity can be identified, an early phase lasting the first 5 min and a late phase lasting from 15 to 30 min after the injection of formalin.

The animals were used only for one measurement in each experiment.

This study was conducted with the approval of the local ethics committee and in accordance with the guidelines of the Ethics Committee of the International Association for the Study of Pain (Zimmermann, 1983).

Statistics

Data for hyperalgesia were analysed by parametric ANOVA and statistical significance ($P < 0.05$) was further examined by Duncan's test. Data for allodynia and formalin test were analysed by non-parametric ANOVA and statistical significance ($P < 0.05$) was further examined by Williams' test and Dunnett's test, respectively, for multiple comparison.

Results

Effect of i.t. PGE_2 on allodynia in $EP_1^{-/-}$ and $EP_3^{-/-}$ mice

To assign a PGE receptor subtype to PGE_2-induced mechanical allodynia, we examined whether PGE_2 could induce allodynia in $EP_1^{-/-}$ and $EP_3^{-/-}$ mice. While i.t. administration of saline did not evoke any response to tactile stimuli applied to the flank in wild-type mice, i.t. PGE_2 (500 ng kg^{-1}) resulted in prominent agitation responses, such as vocalization, biting, and escape from the probe. As shown in Figure 1, i.t. PGE_2 (500 ng kg^{-1}) induced allodynia over

the 50-min experimental period in $EP_1^{+/+}$ and $EP_3^{+/+}$ mice. PGE_2 could induce mechanical allodynia in $EP_3^{-/-}$ mice, but not in $EP_1^{-/-}$ mice. These results clearly demonstrate that the EP_1 receptor is involved in the PGE_2-induced allodynia.

Effect of i.t. NMDA, L-arginine, and SNP on allodynia in $EP_1^{-/-}$ and $EP_3^{-/-}$ mice

We previously showed that PGE_2-induced allodynia is mediated by activation of NMDA glutamate receptor and following nitric oxide (NO) production in the spinal cord (Minami *et al*., 1994b; 1995a; 1999; Eguchi *et al*., 1999). Intrathecal administration of NMDA (500 ng kg^{-1}), the substrate of NO synthase L-arginine (250 μg kg^{-1}), or the NO donor SNP (500 ng kg^{-1}) induced mechanical allodynia in both $EP_1^{-/-}$ and $EP_3^{-/-}$ mice. When the scores of allodynia obtained for the overall 50 min were cumulated and expressed as a per cent of the maximum possible score, the allodynic score of any one agent was beyond 70% (Table 1). These values obtained in $EP_1^{-/-}$ and $EP_3^{-/-}$ mice were almost the same as those in wild-type mice.

Effect of i.t. PGE_2 on hyperalgesia in $EP_1^{-/-}$ and $EP_3^{-/-}$ mice

To characterize a PGE receptor subtype(s) involved in PGE_2-induced hyperalgesia, we examined whether PGE_2 could induce hyperalgesia in $EP_1^{-/-}$ and $EP_3^{-/-}$ mice. As shown in Figure 2a,b, there was no difference in the latency periods (17.2 ± 1.2 s and 17.8 ± 0.9 s) (mean$\pm$s.e.mean, $n=10$) between $EP_3^{+/+}$ and $EP_3^{-/-}$ mice 30 min after i.t. saline injection. While i.t. injection of PGE_2 to $EP_3^{+/+}$ mice produced a hyperalgesic action over a wide range of dosages from 50 pg to 500 ng kg^{-1} (Figure 2a), i.t. injection of PGE_2 to $EP_3^{-/-}$ mice produced a monophasic hyperalgesic action at a narrower range of dosages (5 ng–5 μg kg^{-1}) (Figure 2b). PGE_2 at a dose of 500 ng kg^{-1} reduced the latency periods to 10.1 ± 1.1 s and 11.5 ± 0.6 s in $EP_3^{+/+}$ and $EP_3^{-/-}$ mice, respectively. To clarify the difference in dose dependency of PGE_2 for hyperalgesia between $EP_3^{+/+}$ and $EP_3^{-/-}$ mice, we examined the induction of hyperalgesia by the selective EP_3 agonist ONO-AE-248. ONO-AE-248 reduced the latency period to 10.6 ± 0.6 s at 500 pg kg^{-1}, comparable to the potency of PGE_2, and 13.4 ± 1.1 s at 500 ng kg^{-1} in $EP_3^{+/+}$ mice (Figure 2c). On the other hand, ONO-AE-248 had no effect at 500 pg kg^{-1} and 500 ng kg^{-1} in $EP_3^{-/-}$ mice (Figure 2d). Loss of the functional EP_3 receptor in $EP_3^{-/-}$ mice was verified by the inability of the EP_3 agonist to induce hyperalgesia. Consistent with our pharmacological studies (Minami *et al*., 1994a), these results demonstrate that the PGE_2-induced hyperalgesia is mediated by the EP_3 subtype at lower doses of PGE_2.

Similar to $EP_3^{+/+}$ mice, PGE_2 reduced the latency period in $EP_1^{+/+}$ mice over a wide range of dosages (500 pg kg^{-1}–5 μg kg^{-1}) with a maximum effect at 50 ng kg^{-1} (8.5 ± 0.8 s, mean$\pm$s.e.mean, $n=10$), as compared with that (15.1 ± 1.2 s) of the saline-injected control (Figure 3a). Unexpectedly, untreated $EP_1^{-/-}$ mice and saline-injected $EP_1^{-/-}$ mice were in a hyperalgesic state (8.7 ± 0.4 s and 6.5 ± 0.7 s, respectively). The latency period was dose-dependently prolonged by i.t. injection of PGE_2 in $EP_1^{-/-}$ mice (Figure 3b).

Formalin test in $EP_1^{-/-}$ and $EP_3^{-/-}$ mice

The formalin test is an established model of pain associated with injury and inflammation and is a sensitive method to

Table 1 Allodynia induced by various agents in $EP_1^{-/-}$ and $EP_3^{-/-}$ mice

	Allodynia	
Agent	*$EP_1^{-/-}$* (%)	*$EP_3^{-/-}$* (%)
PGE_2	2.5 ± 2.5**	74.2 ± 9.2
NMDA	73.3 ± 7.7	80.0 ± 7.8
L-Arginine	76.7 ± 6.0	77 ± 9.4
SNP	80.0 ± 9.3	72 ± 11.7

PGE_2 (500 ng kg^{-1}), NMDA (500 ng kg^{-1}), L-arginine (250 μg kg^{-1}) or sodium nitroprusside (SNP) (500 ng kg^{-1}) was injected into the subarachnoid space of $EP_1^{-/-}$ and $EP_3^{-/-}$ mice. Data (mean$\pm$s.e.mean, $n=5$–6) are expressed as a per cent of the maximum possible cumulative score over the 50-min experiment period. **$P<0.01$ compared to $EP_1^{+/+}$ mice.

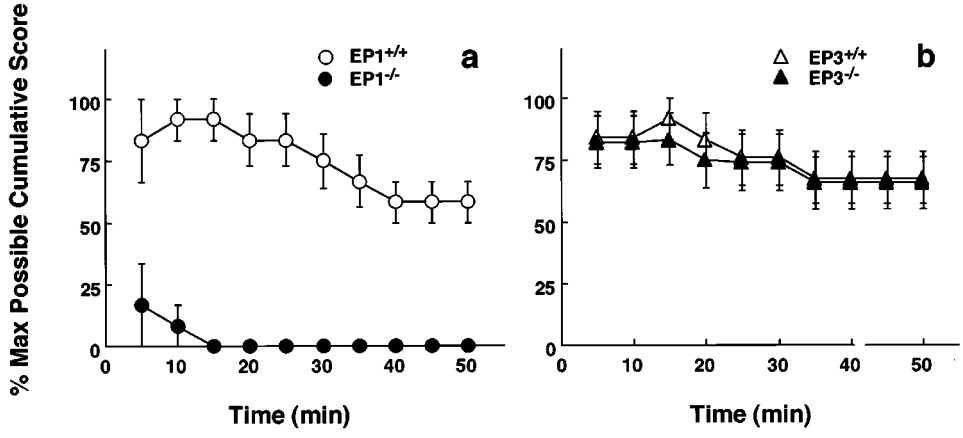

Figure 1 Time courses of allodynia induced by PGE_2 in wild-type and receptor-deficient mice. PGE_2 (500 ng kg^{-1}) was injected i.t. in $EP_1^{+/+}$ and $EP_1^{-/-}$ (a), and $EP_3^{+/+}$ and $EP_3^{-/-}$ (b) mice. Assessment of allodynia was made as described under 'Methods'. The value (mean$\pm$s.e.mean) represents the per cent of the maximum possible cumulative score of 5–6 mice evaluated every 5 min.

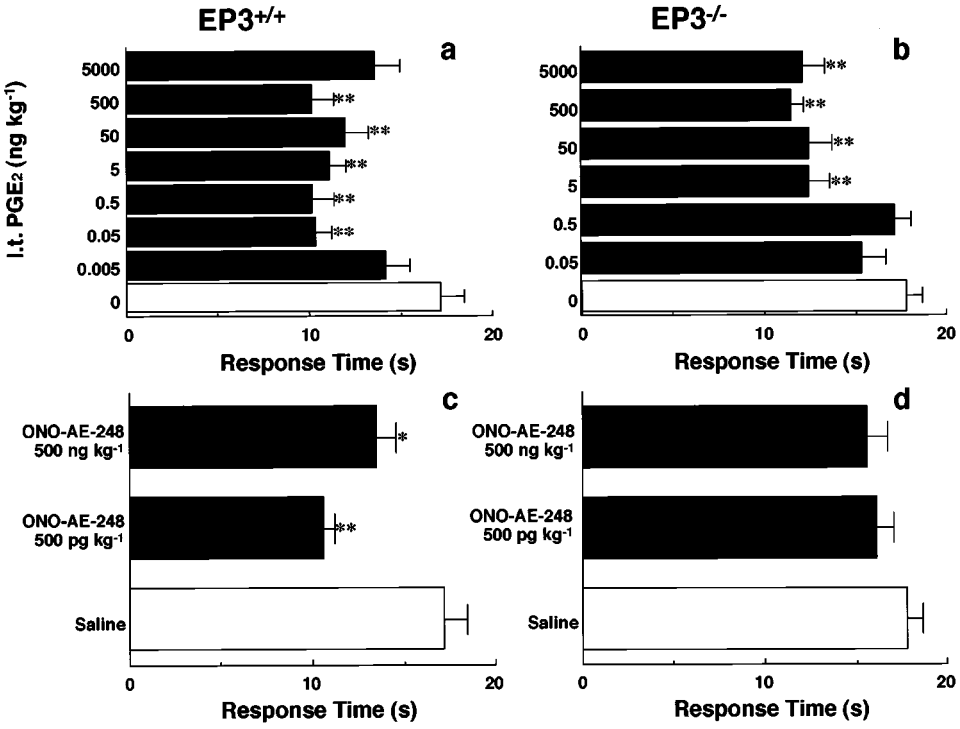

Figure 2 Hyperalgesia evoked by PGE_2 in $EP_3{}^{+/+}$ (a,c) and $EP_3{}^{-/-}$ (b,d) mice. An indicated dose of PGE_2 or ONO-AE-248 (an EP_3 agonist), or saline was injected into the subarachnoid space of conscious mice. Hyperalgesia was assessed 30 min after i.t. injection of agent. Each column represents the mean ± s.e.mean ($n = 8-10$). $^*P < 0.05$, $^{**}P < 0.01$, compared with the saline-injected control group.

evaluate various classes of analgesic drugs (Hunskaar & Hole, 1987). Formalin injected subcutaneously into the paw produces two phases of responding, an early phase (0–5 min), attributed to a direct effect of the formalin on nociceptors and a late phase (15–30 min), related to the subsequent development of inflammation and central sensitization. The formalin injection induced biphasic licking and biting responses of the injected paw, with an early phase determined for the first 5 min (41.7 ± 8.1 s and 44.1 ± 4.5 s) and a late phase determined for 15–30 min (171.7 ± 29.8 s and 196.2 ± 28.1 s) in $EP_1{}^{+/+}$ and $EP_3{}^{+/+}$ mice, respectively (Figure 4). The early-phase behaviours (30.2 ± 8.0 s and 46.0 ± 5.1 s) in $EP_1{}^{-/-}$ and $EP_3{}^{-/-}$ mice were not different from those in wild-type mice in the formalin test. In the late phase, the amount of time spent in licking the injected paw was partly reduced, but not significantly decreased in $EP_1{}^{-/-}$ mice (105.5 ± 20.8 s) and in $EP_3{}^{-/-}$ mice (127.1 ± 12.5 s). In contrast to the PGE_2-induced allodynia and hyperalgesia, these results demonstrate that licking and biting responses induced by formalin injection are not ascribed to a single EP_1 or EP_3 subtype.

Discussion

We previously demonstrated that i.t. PGE_2 induced both allodynia and hyperalgesia. On the basis of rankings of specificities of EP agonists for EP receptor subtypes, we suggested that the PGE_2-induced allodynia is mediated by the EP_1 receptor in the mouse spinal cord and that the PGE_2-induced hyperalgesia is mediated by the EP_3 receptor at lower doses and by the EP_2 receptor at higher doses (Minami *et al*., 1994a). Consistent with our earlier observations, while PGE_2 induced allodynia in $EP_3{}^{-/-}$ mice, it could not induce allodynia in $EP_1{}^{-/-}$ mice (Figure 1). This confirmed that the EP_1 receptor is involved in the PGE_2-induced allodynia. PGE_2 stimulates the release of glutamate and NO from the spinal cord in a Ca^{2+}-dependent manner (Nishihara *et al*., 1995; Sakai *et al*., 1998). The PGE_2-induced allodynia was blocked by NMDA and non-NMDA receptor antagonists and inhibitors for NO synthase and guanylate cyclase (Minami *et al*., 1994b; 1995a). We recently showed that the PGE_2-induced allodynia was not observed in mice deficient in ε1 subunit of NMDA receptors (Minami *et al*., 1999). Therefore, we postulated that i.t. PGE_2 induces allodynia through activation of the NMDA receptor and following NO production *via* the EP_1 receptor. Here we demonstrated that activations of the glutamate-NO system, i.e., NMDA, L-arginine, and SNP, induced allodynia in $EP_1{}^{-/-}$ and $EP_3{}^{-/-}$ mice (Table 1) to the same extent as in wild-type mice, suggesting that a site(s) of PGE_2 involving the induction of allodynia lies upstream, rather than downstream, of the glutamate-NO system.

Intrathecal injection of PGE_2 produced a monophasic hyperalgesia in $EP_3{}^{-/-}$ mice at higher doses than in $EP_3{}^{+/+}$ mice (Figure 2a,b). The selective EP_3 agonist ONO-AE-248 produced a hyperalgesic effect at 500 pg and 500 ng kg^{-1} in $EP_3{}^{+/+}$ mice, but not in $EP_3{}^{-/-}$ mice (Figure 2c,d). ONO-AE-248 at 500 pg kg^{-1} seemed to be more effective in producing hyperalgesia in $EP_3{}^{+/+}$ mice than at 500 ng kg^{-1}. These results confirm that the PGE_2-induced hyperalgesia is mediated by the EP_3 subtype at lower doses. Whether

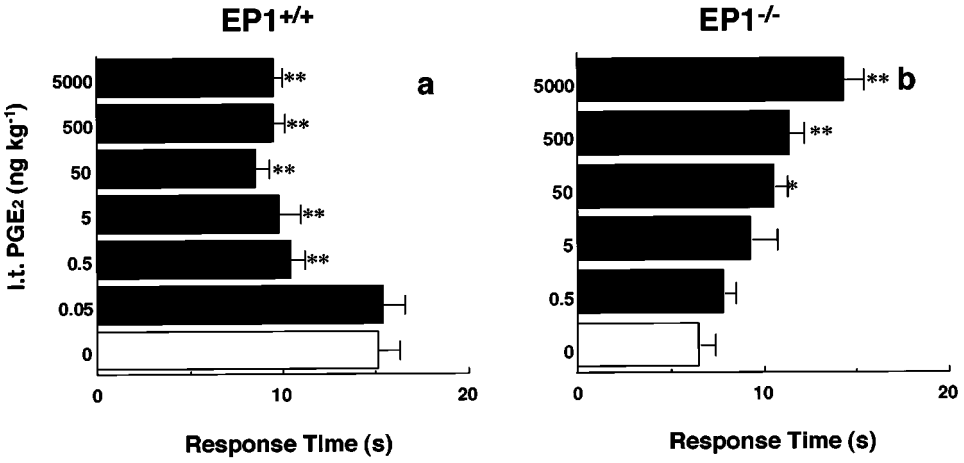

Figure 3 Hyperalgesia evoked by PGE_2 in $EP_1{}^{+/+}$ (a) and $EP_1{}^{-/-}$ (b) mice. An indicated dose of PGE_2 or saline was injected into the subarachnoid space of conscious mice. Hyperalgesia was assessed 30 min after i.t. injection of PGE_2. Each column represents the mean ± s.e.mean ($n = 8-10$). $^*P < 0.05$, $^{**}P < 0.01$, compared with the saline-injected control group.

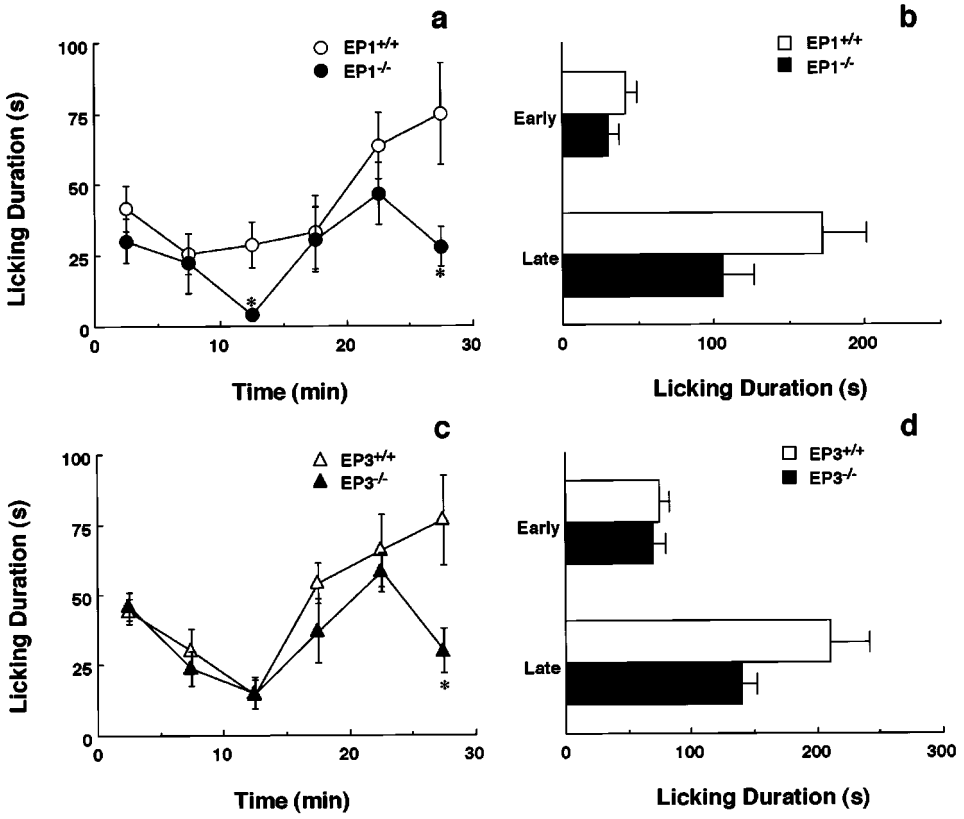

Figure 4 Formalin test in $EP_1{}^{+/+}$ and $EP_1{}^{-/-}$ (a,b), and $EP_3{}^{+/+}$ and $EP_3{}^{-/-}$ (c,d) mice. Assessment of the formalin test was made as described under 'Methods'. Each point represents the mean ± s.e.mean ($n = 9-10$). $^*P < 0.05$, compared with the wild-type mice.

hyperalgesia induced by high doses of PGE_2 is mediated by EP_2 receptors will be clarified by $EP_2{}^{-/-}$ mice. In this context, Kumazawa *et al.* (1996) previously showed by use of EP agonists that low concentrations of PGE_2 augmented the response of polymodal receptors by bradykinin through EP_3 receptors and that high concentrations of PGE_2 augmented heat responses through EP_2 receptors in the periphery. Unexpectedly, $EP_1{}^{-/-}$ mice showed a hyperalgesic action in the hot plate test and the hyperalgesia was alleviated by PGE_2 in a dose-dependent manner (Figure 3). Because i.t. PGE_2 produced hyperalgesia at as low as 500 pg kg^{-1} (Figures 2a and 3a), the observed hyperalgesic response in $EP_1{}^{-/-}$ mice may be due to unopposed activation of EP_3 receptor at the basal level of PGE_2 in the spinal cord. While

the exact mechanism of hyperalgesia in $EP_1^{-/-}$ mice remains to be clarified, the reversal of a hyperalgesic state in $EP_1^{-/-}$ mice by PGE_2 may be mediated by EP_4. The present study suggests that endogenous PGE_2 may play an inhibitory role in the appearance of hyperalgesia *via* the EP_1 receptor under physiological conditions and disruption of EP_1 receptors apparently unmasks hyperalgesic EP receptor subtypes.

Formalin injection produces a characteristic biphasic flinching, shaking or licking behaviour of the injected paw. Spinal involvement of PGE_2 in the formalin test has been proposed based on several observations: (i) i.t. injection of cyclo-oxygenase inhibitors produces a dose-dependent anti-nociceptive effect on the late phase, but limited effect on the early phase of the formalin test in the rats (Malmberg & Yaksh, 1992b); (ii) subcutaneously injected formalin increased PGE_2 release from the spinal cord (Malmberg & Yaksh, 1995); and (iii) i.t. injection of the EP_1 antagonists SC-51089 and SC-51234A produced significant suppression of the second phase, without any effect on the early phase, in the formalin test (Malmberg *et al*., 1994). Consistent with previous reports, there is no difference in the early response between wild-type mice and knockout mice. Although there was a considerable decrease in flinching behaviour in $EP_1^{-/-}$ and $EP_3^{-/-}$ mice 30 min after formalin injection, this difference was not significant when compared with the whole (15–30 min) late-phase behaviour (Figure 4). The difference in the extent of contribution of EP_1 in the formalin test may be due to the difference in testing paradigm and animal species. Because the suppressive effect of EP_1 antagonists on formalin-induced pain was weak as compared with those of cyclooxygenase inhibitors (Malmberg & Yaksh, 1992b; Malmberg *et al*., 1994), the formalin-induced behaviours are likely to be a complex interplay among EP receptor subtypes and other modifying factors at both peripheral and spinal levels.

The use of mice deficient in PG receptors has provided direct assignment to inflammatory responses including pain. In mice lacking the PGI receptor (IP $^{-/-}$), the acetic acid-induced writhing response or carrageenan-induced paw oedema was reduced to the same level as that observed when indomethacin was administered to wild-type mice (Murata *et al*., 1997). Because the IP $^{-/-}$ mice did not show any alteration in thermal nociceptive responses examined by hot plate and tail flick tests, they suggested that PGI_2 is a mediator of inflammatory swelling and pain in the periphery, but not involved in nociceptive transmission at the spinal level. In the present study, we extended our previous studies by use of $EP_1^{-/-}$ and $EP_3^{-/-}$ mice and present evidence that PGE_2 induces allodynia through the EP_1 receptor and hyperalgesia through the EP_3 receptor at lower doses. Continued investigations into the specific roles of the receptors that mediate the actions of PGs with other PG receptor knockout mice will further promote our understanding of PG actions in nociception and may potentially lead to the development of more specific and less toxic therapies for pain.

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