ELSEVIER

Biochemical Pharmacology

Biochemical Pharmacology 62 (2001) 157–160
Rapid communication

Major roles of prostanoid receptors IP and EP_3 in endotoxin-induced enhancement of pain perception

Akinori Ueno[a], Hideki Matsumoto[a], Hiroaki Naraba[a], Yuri Ikeda[a], Fumitaka Ushikubi[b], Toshiyuki Matsuoka[c], Shuh Narumiya[c], Yukihiko Sugimoto[d], Atsushi Ichikawa[d], Sachiko Oh-ishi[a,*]

[a]*Department of Pharmacology, School of Pharmaceutical Sciences, Kitasato University, 5–9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan*
[b]*Department of Pharmacology, Asahikawa Medical College, Asahikawa 078-8510, Japan*
[c]*Department of Pharmacology, Faculty of Medicine, Kyoto University, Kyoto 606-8501, Japan*
[d]*Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8501, Japan*

Accepted 9 March 2001

Abstract

To know the roles of prostaglandin I (IP) and prostaglandin E (EP) receptors in pain perception, we compared the acetic acid-induced writhing response in mice deficient in prostaglandin receptors, i.e. IP, EP_1, EP_2, EP_3, or EP_4, with or without lipopolysaccharide (LPS) pretreatment. Without LPS pretreatment, IP-receptor deficient mice showed a significantly smaller number of responses, as previously reported, whereas mice deficient in any of the EP-receptor subtypes showed a number of writhings similar to those of wild-type mice. When mice were pretreated with LPS for 24 hr to induce cyclooxygenase-2 expression, the wild-type as well as EP_1-, EP_2-, or EP_4-receptor-deficient mice showed a similar enhanced writhing response, whereas IP- and EP_3-receptor-deficient mice had a significantly less enhanced number of writhings. These results indicate that IP and EP_3 are the major prostaglandin receptors mediating the enhanced acetic acid-induced writhing response in mice pre-exposed to LPS, i.e. in endotoxin-enhanced inflammatory nociception.

Keywords: Writhing responses; Prostaglandin E receptor; IP receptor; EP_3 receptor; Lipopolysaccharide

1. Introduction

Prostaglandins are well-known bioactive lipids possessing various opposing biological activities and are characterized as modulators of not only physiological functions but also pathological or clinical symptoms in various organs of the body [1,2]. Therefore, extensive studies using agonists for characterizing prostaglandin receptors have been conducted to determine their specific biological action, and recently more precise studies involving cloning of the receptors of various subtypes have been done to clarify the structure of their functional domains [3,4]. In addition, the biological roles of these receptors have been elucidated by using these receptor-deficient mice [5,6].

The relationship between prostaglandin receptors and polymodal pain receptors has been explored, and in several studies it was determined that the E-series of prostaglandins cause hyperalgesia in humans and animals [7,8]. Berkenkopf and Weichman [9], however, reported that PGI_2 may be the main metabolite involved in the writhing response, based on the detection of 6-keto-$PGF_{1\alpha}$ in the exudate. By using IP-receptor knockout mice [10], we also determined previously that PGI_2 could be a major mediator for nociception in the mouse writhing reaction.

We have found that LPS-pretreated mice showed an enhanced number of writhings induced by acetic acid; this enhancement may be caused by the expression of inducible cyclooxygenase-2 which can increase the production of several prostanoids [11]. In this communication, we report which receptor subtype is responsible for the enhanced writhing response in LPS-pretreated mice.

* Corresponding author. Present address: Kitasato Institute, 5–9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan. Tel.: +81-3-3444-6161BR$_1$, Ext. 2120; fax: +81-3-5791-6120.

E-mail address: oh-ishi@kitasato.or.jp (S. Oh-ishi).

Abbreviations:, prostaglandin E receptor subtype 1; IP, prostaglandin I receptor; LPS, lipopolysaccharide; and WT, wild-type mice.

0006-2952/01/$ – see front matter
PII: S0006-2952(01)00654-2

2. Materials and methods

2.1. Agents

Carbacyclin (Biomol Research), PGE_2 (Cayman Chemical Co.), LPS (*Escherichia coli* 0111:B_4, Sigma), and indomethacin (Sigma) were purchased from the companies listed.

2.2. Animals

Male and female ICR mice were obtained from Japan SLC. Mice deficient in IP receptors [10] or EP_1, EP_2, and EP_3 receptors [12,13] were prepared as described previously, and back-crossed over at least six generations to C57BL/6 mice. The EP_4-receptor knockouts used in this study had a mixed genetic background of 129sv/ola and C57BL/6 mice, because all of the EP_4 knockouts born from the back-crossed parents died within 2 days after birth from patent ductus arteriosus [14]. Data for male and female mice were pooled, because there was no difference in writhing responses between the two sexes.

2.3. Induction of writhing reaction and administration of drugs

The writhing reaction was induced in mice by an intraperitoneal injection of 0.9% acetic acid solution at a dose of 5 mL/kg, as previously described [11]. Some animals received LPS (10 μg/0.1 mL of saline per mouse), given intraperitoneally 24 hr before the induction of writhing. A suspension of 1 mg indomethacin/mL in 1% sodium carboxymethylcellulose solution, 10 mg/kg, was injected subcutaneously 30 min before the induction of writhing.

2.4. Statistical analysis

Data are expressed as the means ± SEM of the indicated number of animals. Statistical analysis was conducted with Student's *t*-test or one-way ANOVA followed by Dunnett's *t*-test.

3. Results and discussion

As shown in Fig. 1, we confirmed that IP receptor-deficient mice without LPS pretreatment showed a significantly smaller number of writhing responses than wild-type mice without the pretreatment, as previously reported [10]. However, the writhing numbers of EP_1-, EP_2-, EP_3-, or EP_4-receptor-deficient mice without LPS pretreatment were not significantly different from those of wild-type mice.

When the writhing response was induced after the 24-hr pretreatment with LPS, the number of writhings of all mice increased, as shown in Fig. 1. These increases were determined to be significantly different from each vehicle control

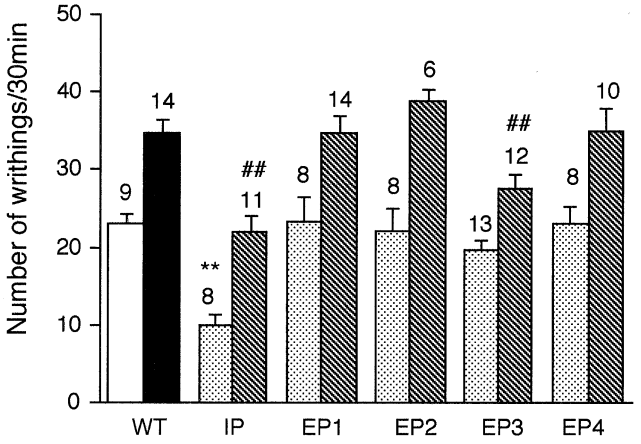

Fig. 1. Writhing response in wild-type (WT) mice and mice deficient in the prostaglandin receptors IP, EP_1, EP_2, EP_3, or EP_4 with or without LPS pretreatment. Mice were pretreated with vehicle (white and dotted columns) or 10 μg/mouse of *E. coli* LPS (black and hatched columns) intraperitoneally 24 hr before the injection of acetic acid. Each column expresses the mean number of writhing responses (with SEM) counted for 30 min; the number of mice in each group is shown at the top of each column. Symbols are omitted, but every writhing number of the LPS-pretreated group was significantly different from the corresponding control at least by $P < 0.05$. Key: (**) significantly different at $P < 0.01$, when compared with vehicle-pretreated wild-type mice (white column); and (##) significantly different at $P < 0.01$, from the LPS-pretreated wild-type mice (black column). ANOVA, followed by Dunnett's *t*-test, was used to determine statistical significance.

group, and these results are very similar to the findings in ICR mice in which cyclooxygenase-2 expression was detected after LPS treatment, as previously reported [11]. However, when we compared the increased writhing numbers caused by LPS pretreatment of each receptor-deficient mouse, IP- and EP_3-receptor-deficient mice showed significantly lower numbers than the other receptor subtype-deficient mice.

Treatment of the wild-type, EP_1- or EP_3-receptor-deficient mice with indomethacin, given at 10 mg/kg subcutaneously 30 min before the induction of LPS-enhanced writhing, suppressed the number of writhings for 30 min to 5.6 ± 0.8 (N = 5), 8.0 ± 2.1 (N = 5), or 7.0 ± 0.8 (N = 5), respectively. These three values were not statistically different from each other, and these indomethacin-suppressed levels in the wild-type as well as the EP_1- and EP_3-receptor-deficient mice were also almost the same as the indomethacin-treated writhing levels that had been obtained previously in wild-type and IP-receptor-deficient mice that had not received LPS treatment. Thus, all these results suggest that IP and EP_3 receptors respond to the enhanced writhing caused by LPS.

Analysis of the time courses of the writhing response is shown in Fig. 2. The writhing number in the first 10-min period was the largest, and it decreased with time, in the LPS-treated wild-type mice as well as in the LPS-treated EP_1-, EP_2-, or EP_4-receptor-deficient mice. However, the number in the first 10-min period was the same or smaller than that in the second 10-min period in IP- and EP_3-

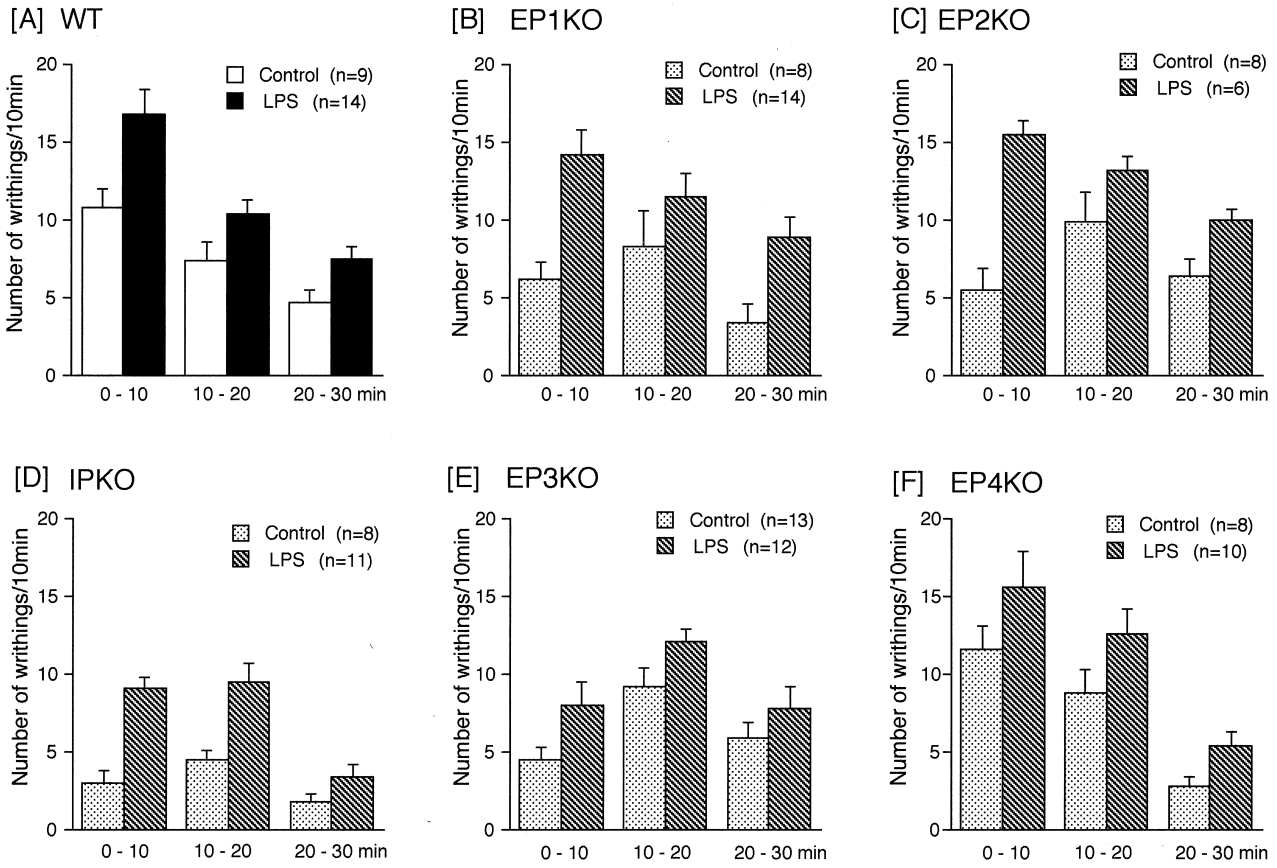

Fig. 2. Comparison of writhing numbers in wild-type and prostaglandin receptor-deficient mice. The number of writhing responses was counted for every 10-min period for 30 min for wild-type (A: WT) mice and mice deficient in prostaglandin receptors. EP_1-, EP_2-, IP-, EP_3- and EP_4-receptor knockouts are represented in (B) EP1KO, (C) EP2KO, (D) IPKO, (E) EP3KO, and (F) EP4KO, respectively. Vehicle controls (white and dotted columns); LPS-pretreated (black and hatched columns). Values are means ± SEM. The number of animals used (N) is shown in each panel.

receptor-deficient mice. This prominent difference of LPS-induced enhanced responses in IP- and EP_3-receptor-deficient mice in comparison with the other groups of mice indicated that the enhanced response by LPS pretreatment may be caused mainly by an increment in the first 10-min stimulation of IP and EP_3 receptors.

Figure 3 shows the effects of exogenous prostaglandins on the acetic acid-induced writhing reaction in mice. In this experiment, 1–3 ng of prostaglandins was administered, because in a previous study [11] 3 ng of 6-keto-$PGF_{1\alpha}$ and 1 ng of PGE_2 per mouse were detected in peritoneal exudates obtained 15 min after acetic acid injection. PGD_2, PGE_2, or the PGI_2 analog carbacyclin, injected simultaneously with acetic acid into indomethacin-treated mice, restored the writhing response; carbacyclin plus PGE_2 showed the largest effect. This result, together with the previous finding that 6-keto$PGF_{1\alpha}$ and PGE_2 were the main prostaglandins detected in the peritoneal exudates in mice injected with acetic acid [11], explain the smaller increase of the enhanced response of LPS-pretreated IP- or EP_3-receptor-deficient mice. Although the level of PGD_2 detected in the peritoneal exudate of LPS-treated mice was less than half of that of PGE_2 [11], involvement of PGD_2 could not be excluded, because PGD synthase-deficient mice have been reported to lack allodynia [15]. The level of $PGF_{2\alpha}$ detected in the exudate may be too low to induce pain (Fig. 3), but we do not know if PG receptors including FP are induced or up-regulated by LPS treatment. Therefore, a clear conclusion regarding the involvement of DP and FP in this model remains to be studied.

Using the acetic acid-induced writhing reaction in intact SPF mice [10], we previously reported that PGI_2 is a major prostaglandin involved in inflammatory pain. This fact was confirmed in the present study in mice that had not been pretreated with LPS. However, some researchers have reported that PGE_2 may be an important mediator in pain perception in various polymodal pain models [7,8]. In a previous study, mRNAs of receptors for prostaglandins, such as IP, EP_1, and EP_3, were demonstrated by *in situ* hybridization to be present in the dosal root ganglia, which is known as a pathway for polymodal pain perception [16]. Our present results clearly demonstrate a role for endogenous PGE_2 in pain perception via the EP_3 receptor in the case of LPS-pretreated mice, in addition to a role for PGI_2.

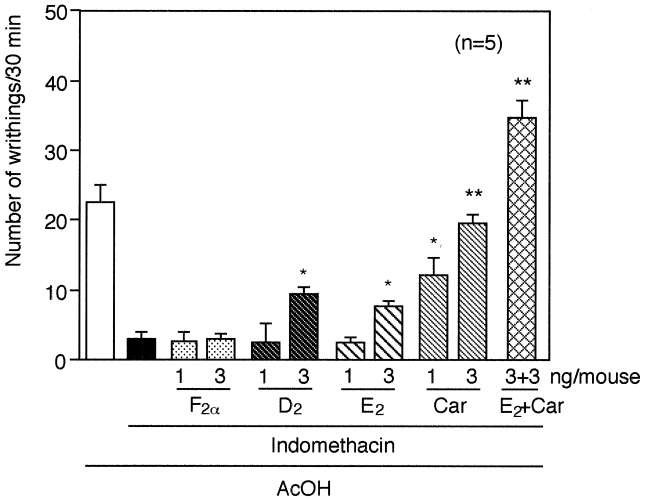

Fig. 3. Effects of exogenous prostaglandins on the number of writhings of indomethacin-treated mice. Normal mice were pretreated subcutaneously with indomethacin (10 mg/kg) 30 min before the experiment, and then writhing responses were induced by simultaneous injection of acetic acid and prostaglandins [$PGF_{2\alpha}$ ($F_{2\alpha}$), PGD_2 (D_2), PGE_2 (E_2), carbacyclin (Car)] or a mixture of PGE_2 and carbacyclin [E_2 + Car], at the indicated doses. Each column indicates the mean number of writhings (with SEM) for 30 min. Key (*) and (**) indicate a significant difference at $P < 0.05$ and $P < 0.01$, respectively, when compared with the indomethacin-treated control (black column).

The LPS-pretreated writhing model used in the present report may be a good animal or human model for the study of bacterial infections or invasion.

Taken together, we conclude that the nociception of the writhing response in non-LPS-treated mice could be mediated mainly by the IP receptor, as reported previously [10], and that the perception of enhanced pain in LPS-pretreated mice requires the mediation of both IP and EP_3 receptors. However, in addition to the studies with these receptor-deficient mice, future development of specific antagonists or agonists for each prostanoid receptor may help us to determine more precisely the characteristics of these receptors, such as specificity or cross-reactivities, which are presently not fully understood.

Acknowledgments

This work was supported, in part, by a Grant-in-aid (10672155) for scientific research from the Ministry of Education, Science, Sports, and Culture of Japan. The authors are grateful to Mr. K. Fujisaku for technical assistance.

References

[1] Samuelsson B, Goldyne M, Granstrom E, Hamberg M, Hammastrom S, Malmstein C. Prostaglandins and thromboxane. Annu Rev Biochem 1978;47:997–1029.
[2] McGiff JC. Prostaglandins, prostacyclin, and thromboxanes. Annu Rev Pharmacol Toxicol 1981;21:479–509.
[3] Narumiya S, Hirata N, Namba T, Hayashi Y, Ushikubi F, Sugimoto Y, Negishi M, Ichikawa A. Structure and function of prostanoid receptors. J Lipid Mediat 1993;6:155–61.
[4] Ushikubi F, Hirata M, Narumiya S. Molecular biology of prostanoid receptors; an overview. J Lipid Mediat Cell Signal 1995;12:343–59.
[5] Narumiya S, Sugimoto Y, Ushikubi F. Prostanoid receptors: structure, properties, and function. Physiol Rev 1999;79:1193–226.
[6] Bley KR, Hunter JC, Eglen RM, Smith JA. The role of IP prostanoid receptors in inflammatory pain. Trends Pharmacol Sci 1998;19:141–7.
[7] Kumazawa T, Mizumura K, Koda H, Fukusako H. EP receptor subtypes implicated in the PGE2-induced sensitization of polymodal receptors in response to bradykinin and heat. J Neurophysiol 1996;75:2361–8.
[8] Ferreira SH, Nakamura M, de Abreu Castro MS. The hyperalgesic effects of prostacyclin and prostaglandin E_2. Prostaglandins 1978;16:31–7.
[9] Berkenkopf J, Weichman BM. Production of prostacyclin in mice following intraperitoneal injection of acetic acid, phenylbenzoquinone and zymosan: its role in the writhing response. Prostaglandins 1988;36:693–709.
[10] Murata T, Ushikubi F, Matsuoka T, Hirata M, Yamasaki A, Sugimoto Y, Ichikawa A, Aze Y, Tanaka T, Yoshida N, Ueno A, Oh-ishi S, Narumiya S. Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. Nature 1997;388:678–82.
[11] Matsumoto H, Naraba H, Ueno A, Fujiyoshi T, Murakami M, Kudo I, Oh-ishi S. Induction of cyclooxygenase-2 causes an enhancement of writhing response in mice. Eur J Pharmacol 1998;352:47–52.
[12] Hizaki H, Segi E, Sugimoto Y, Hirose M, Saji T, Ushikubi F, Matsuoka T, Noda Y, Tanaka T, Yoshida N, Narumiya S, Ichikawa A. Abortive expansion of the cumulus and impaired fertility in mice lacking the prostaglandin E receptor subtype EP_2. Proc Natl Acad Sci USA 1999;96:10501–6.
[13] Sugimoto Y, Narumiya S, Ichikawa A. Distribution and function of prostanoid receptors: studies from knockout mice. Prog Lipid Res 2000;39:289–314.
[14] Segi E, Sugimoto Y, Yamasaki A, Aze Y, Oida H, Nishimura T, Murata T, Matsuoka T, Ushikubi F, Hirose M, Tanaka T, Yoshida N, Narumiya S, Ichikawa A. Patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice. Biochem Biophys Res Commun 1998;246:7–12.
[15] Eguchi N, Minami T, Shirafuji N, Kanaoka Y, Tanaka T, Nagata A, Yoshida N, Urade Y, Ito S, Hayaishi O. Lack of tactile pain (allodynia) in lipocalin-type prostaglandin D synthase-deficient mice. Proc Natl Acad Sci USA 1999;96:726–30.
[16] Oida H, Hamba T, Sugimoto Y, Ushikubi F, Ohishi H, Ichikawa A, Narumiya S. *In situ* hybridization studies of prostacyclin receptor mRNA expression in various mouse organs. Br J Pharmacol 1995;116:2828–37.