British Journal of Pharmacology (2000) 131, 745–755

www.nature.com/bjp

Characterization of prostanoid receptors mediating contraction of the gastric fundus and ileum: studies using mice deficient in prostanoid receptors

[1,2]Yuji Okada, [1]Akiyoshi Hara, [1]Hong Ma, [1]Chun-Yang Xiao, [1]Osamu Takahata, [2]Yutaka Kohgo, [3]Shuh Narumiya & *,[1]Fumitaka Ushikubi

[1]Department of Pharmacology, Asahikawa Medical College, Midorigaoka-Higashi 2-1-1-1, Asahikawa 078-8510, Japan; [2]Third Department of Internal Medicine, Asahikawa Medical College, Midorigaoka-Higashi 2-1-1-1, Asahikawa 078-8510, Japan and [3]Department of Pharmacology, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8315, Japan

1 Receptors mediating prostanoid-induced contractions of longitudinal sections of gastric fundus and ileum were characterized by using tissues obtained from mice deficient in each type and subtype of prostanoid receptors.

2 The fundus and ileum from mice deficient in either EP_3 ($EP_3^{-/-}$ mice), EP_1 ($EP_1^{-/-}$ mice) and FP ($FP^{-/-}$ mice) all showed decreased contraction to PGE_2 compared to the tissues from wild-type mice, whereas contraction of the fundus slightly increased in $EP_4^{-/-}$ mice.

3 17-phenyl-PGE_2 also showed decreased contraction of the fundus from $EP_3^{-/-}$, $EP_1^{-/-}$ and $FP^{-/-}$ mice. Sulprostone showed decreased contraction of the fundus from $EP_3^{-/-}$ and $FP^{-/-}$ mice, and decreased contraction of the ileum to this compound was seen in tissues from $EP_3^{-/-}$, $EP_1^{-/-}$ and $FP^{-/-}$ mice. In $DP^{-/-}$ mice, sulprostone showed increased contraction.

4 DI-004 and AE-248 caused the small but concentration-dependent contraction of both tissues, and these contractions were abolished in tissues obtained from $EP_1^{-/-}$ and $EP_3^{-/-}$ mice, respectively, but not affected in other mice.

5 Contractions of both fundus and ileum to $PGF_2\alpha$ was absent at lower concentrations (10^{-9} to 10^{-7} M), and suppressed at higher concentrations (10^{-6} to 10^{-5} M) of the agonist in the $FP^{-/-}$ mice. Suppression of the contractions at the higher $PGF_2\alpha$ concentrations was also seen in the fundus from $EP_3^{-/-}$, $EP_1^{-/-}$ and $TP^{-/-}$ mice and in the ileum from $EP_3^{-/-}$ and $TP^{-/-}$ mice.

6 Contraction of the fundus to PGD_2 was significantly enhanced in $DP^{-/-}$ mice, and contractions of the fundus and ileum to this PG decreased in $FP^{-/-}$ and $EP_3^{-/-}$ mice.

7 Contractions of both tissues to I-BOP was absent at 10^{-9} to 10^{-7} M and much suppressed at higher concentrations in $TP^{-/-}$ mice. Slight suppression to this agonist was also observed in the tissues from $EP_3^{-/-}$ mice.

8 PGI_2 induced small relaxation of both tissues from wild-type mice. These relaxation reactions were much potentiated in $EP_3^{-/-}$ mice. On the other hand, significant contraction to PGI_2 was observed in both tissues obtained from $IP^{-/-}$ mice.

9 These results show that contractions of the fundus and ileum induced by each prostanoid agonist are mediated by actions of this agonist on multiple types of prostanoid receptors and in some cases modified by its action on relaxant receptors.

British Journal of Pharmacology (2000) **131,** 745–755

Keywords: Prostanoids; prostaglandins; thromboxane; prostacyclin, prostanoid analogues; prostanoid receptor; knockout mouse; gastric fundus; ileum; smooth muscle

Abbreviations: I-BOP, 1S-[1α,2β(5Z),3α(1E,3S),4α]-7-[3-(hydroxy-4-(p-iodophenoxy)-1-butenyl)-7-oxabicyclo-[2.2.1]hept-2-yl]-5′-heptenoic acid; ICI-81008, (±) ω-tetranor-16-m-trifluoromethylphenoxy $PGF_2\alpha$; U-46619, (15S)-hydroxy-11α, 9α(epoxymethano)prosta-5Z,13E-dienoic acids

Introduction

Smooth muscle preparations, including those from the gastrointestinal tract, have been used for analysis of actions of prostanoids and their analogues, and have been used for pharmacological identification and characterization of the prostanoid receptors by comparing the potencies of various ligands. These studies have identified five types of prostanoid receptors and named them the DP, EP, FP, IP and TP as receptors for PGD_2, PGE_2, $PGF_2\alpha$, PGI_2 and TXA_2 (Coleman *et al*., 1990), based on the fact that naturally occurring prostanoids show highest affinity to the respective receptor. Moreover, there are four subtypes of the EP; the EP_1, EP_2, EP_3 and EP_4, to which various PGE_2 analogues show specific rank orders of affinities (Coleman *et al*., 1994; Ushikubi *et al*., 1995). These receptors can be functionally grouped into three categories: the relaxant receptors, consisting of the IP, DP, EP_2 and EP_4, mediate smooth muscle relaxation; the contractile receptors, consisting of the TP, FP and EP_1, mediate its contraction; and the EP_3 which is an inhibitory receptor inhibiting smooth muscle relaxation (Narumiya *et al*., 1999).

PGE_2 contracts longitudinal smooth muscles of the gastric fundus, ileum and colon and relaxes circular smooth muscles of these organs (Coleman *et al*., 1994). However, it has not

*Author for correspondence; E-mail: ushikubi@asahikawa.med.ac.jp

been fully elucidated which types and subtypes of the receptors mediate these actions of PGE_2. $PGF_2\alpha$ was reported to contract rat stomach fundus strip (Dong *et al*., 1986) and longitudinal smooth muscles of human stomach, ileum and colon (Bennett *et al*., 1981). While TXA_2 and its analogues were reported to be a potent constrictor of human stomach, ileum and colon (Bennett *et al*., 1981) and of the rat gastric fundus (Bennett & Sanger, 1982), it failed to contract guinea-pig ileum (Coleman *et al*., 1981), suggesting some species difference. Although the DP belongs to a group of the relaxant receptors, contractile actions of PGD_2 were reported in rat fundus (Horton & Jones, 1974) and colon (Diener & Gabato, 1994), and in gerbil colon and rat stomach (Hamberg *et al*., 1975). These results suggest that contractions of smooth muscles induced by PGD_2 are mediated by the contractile receptor(s) other than the DP. Nevertheless, these contractile receptors have not been well characterized in the gastrointestinal smooth muscles. PGI_2 relaxed longitudinal muscle strips of human stomach, ileum and colon (Bennett *et al*., 1981), whereas contractile effect of PGI_2 on the gastric fundus and gerbil colon were reported (Crane *et al*., 1978, Bennett *et al*., 1980).

The contractile effects of the prostanoids and their analogues on the gastrointestinal tract are mediated mainly by direct actions on smooth muscles, while some effects are likely to be mediated by indirect actions *via* the enteric nervous system. For example, it has been reported that the IP and some EPs participate in the enhanced neurotransmission in the guinea-pig ileum (Poll *et al*., 1988; Fukunaga *et al*., 1993), and that the IP mediates depolarization of NANC neurones of the rat colon (Qian & Jones, 1995; Rudd *et al*., 2000). In accordance with these results, mRNAs of the EP_3 were expressed in neurons of murine gastric myenteric ganglia (Morimoto *et al*., 1997), and the EP_3 and IP were detected in rat nodose ganglia (Ek *et al*., 1998; Matsumura *et al*., 1995).

Some longitudinal smooth muscle preparations have been traditionally considered to contain predominantly one subtype of the EPs. For example, guinea-pig and dog fundus were considered to contain the EP_1 (Coleman *et al*., 1985), and chick ileum has been used as a tissue containing mainly the EP_3 (Coleman *et al*., 1987). Although these assumptions were derived from studies using PGE_2 analogues considered to be specific to each subtype of EPs, these ligands have been recently revealed to bind to various types of the prostanoid receptors including several subtypes of the EPs (Kiriyama *et al*. 1997). Moreover, there are almost no agonists and antagonists strictly specific for each of EPs (Kiriyama *et al*. 1997; Abramovitz *et al*., 2000). Furthermore, agonists are inherently difficult to be used as pharmacological tools because their perceived selectivity is so dependent on the coupling of the receptors upon which they act. It therefore has been very difficult to evaluate the contribution of each prostanoid receptors to the contractile effects of the prostanoids based on pharmacological analysis alone. According to the recent molecular biological studies using *in situ* hybridization and Northern blot techniques, almost all types and subtypes of the prostanoid receptors are expressed in all levels of the gastrointestinal tract (Morimoto *et al*., 1997; Ding *et al*., 1997; Wright *et al*., 1999). These observations, along with lack of ligands specific to each prostanoid receptor, further complicate interpretation of the effects of the prostanoids on gastrointestinal smooth muscles.

In the present study, we use longitudinal smooth muscles of gastric fundus and ileum derived from mice lacking each of the prostanoid receptors, and identify the prostanoid receptors mediating the contractile effects of the prostanoids and their analogues. We also estimate the extent of contribution of each receptor to the contractions, and clarify the relaxant actions mediated by some receptors, which modify the contractions.

Methods

Animals

Generation and maintenance of $IP^{-/-}$ (Murata *et al*., 1987), $FP^{-/-}$ (Sugimoto *et al*., 1987), $EP_1{}^{-/-}$ and $EP_3{}^{-/-}$ (Ushikubi *et al*., 1998), $EP_4{}^{-/-}$ (Segi *et al*., 1998), $EP_2{}^{-/-}$ (Hizaki *et al*., 1999) and $DP^{-/-}$ mice (Matsuoka *et al*., 2000) has been reported. Generation of $TP^{-/-}$ mice will be reported elsewhere. These mice and wild-type control mice except $EP_4{}^{-/-}$ mice have been back-crossed to C57BL/6 mice more than six times. The $EP_4{}^{-/-}$ mice have mixed genetic background of 129sv/ola and C57BL/6 mice, because all the $EP_4{}^{-/-}$ mice born from back-crossed parents died within 2 days after birth from patent ductus arteriosus (Nguyen *et al*., 1997; Segi *et al*., 1998). For the experiments using $EP_4{}^{-/-}$ mice, wild-type mice having similar mixed genetic background with $EP_4{}^{-/-}$ mice were used as a control. All studies were performed using 8–12-week-old male and female mice. The animals had free access to water and food until the day of experiments.

Measurement of tension

Mice were killed by cervical dislocation. The entire stomach was excised, and gastric fundus was separated from the body in Tyrode solution (composition in mM): NaCl 136.8, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 2.5, NaH_2PO_4 0.4, $NaHCO_3$ 11.9 and glucose 5.5, pH 7.3, and mounted longitudinally in the bath filled with Tyrode solution. The terminal portion of the ileum was excised and cut into segments of 1 cm long. Preparations were longitudinally mounted in 10 ml organ baths containing Tyrode solution for recording of tension with isometric transducers connected to a polygraph recorder (Star Medical PAS-401, Osaka, Japan). The bathing solution was aerated with 95% O_2 and 5% CO_2, and was maintained at 31°C.

After the equilibration period under 0.5 g of tension, the preparations were contracted with ACh (100 μM) four times at intervals of 20 min for stabilization, and then contraction with PGE_2 (1 μM) was tested to ascertain the reactivity of each tissue to PGE_2. To examine contractile actions, prostanoids and their analogues were added cumulatively except in the experiments examining the contraction of the ileum induced by PGI_2 and carbacyclin, where the compounds were added at a concentration of 1 μM. The degree of the contractile responses were presented as a percentage of that induced by ACh (100 μM), which was added just before challenge with PGE_2.

In examining the relaxant actions of PGD_2, PGI_2 and carbacyclin in the fundus, these compounds were added at a concentration of 1 μM after the preparations were pre-contracted by $PGF_2\alpha$ to a degree of 70–80% of that induced by ACh (100 μM). The relaxant actions of these compounds in the ileum were examined on the preparations mechanically stretched by a tension of 1.0 g, because $PGF_2\alpha$-induced responses of this tissue declined too rapidly and could not be discriminated from the relaxation induced by these compounds.

To evaluate only the direct actions of the prostanoids and their analogues on smooth muscle, we minimized the neuronal effects by adding atropine (0.8 μM) and phenoxybenzamine (0.7 μM) to the bathing solution. Indomethacin (2.8 μM) was also included to inhibit the endogenous production of prostanoids.

Compounds

PGD_2, PGE_2, sulprostone, 17-phenyl-PGE_2, $PGF_2\alpha$, PGI_2, carbacyclin and I-BOP were purchased from Cayman Chemical Co. (Ann Arbor, MI, U.S.A.). DI-004 and AE-248 (Suzawa *et al*., 2000) were kindly donated by Ono Pharmaceutical (Osaka, Japan). Stock ethanol solutions of these compounds were stored at $-20^{\circ}C$ and diluted with phosphate-buffered saline for use. The concentrations of the stock solutions were generally 10 mM except for I-BOP, which was at a concentration of 1 mM.

Data analysis

All data are expressed as mean ± s.e.mean. Statistical comparison were made with a two-way repeated measures analysis of variance (ANOVA) followed by Dunnett's test for multiple comparison (Dunnett, 1955). Differences were considered significant if $P<0.05$. In the text, *n* refers to the number of animals used. ED_{50} values were calculated by non-linear regression analysis using Prism II, a computer program (GraphPad Software, San Diego, U.S.A.).

Results

Contractions induced by acetylcholine and the prostanoid ligands in wild-type mice

In the fundus and ileum of wild-type mice, acetylcholine (100 μM) induced the tensions of 3.8 ± 0.2 and 0.89 ± 0.03 g, respectively ($n=15$). These values were not significantly different from those in mice lacking one of the prostanoid receptors (data not shown). All the naturally occurring prostanoids and their analogues used in this study contracted both the fundus and ileum with various potencies and maximum effects as shown in Table 1. The rank order of potencies was I-BOP > 17-phenyl-PGE_2 = sulprostone = $PGF_2\alpha$ > PGE_2 > PGI_2 = PGD_2 in the fundus. In the ileum, it was I-BOP > $PGF_2\alpha$ > 17-phenyl-PGE_2 > sulprostone > PGD_2 = PGE_2. The rank order of maximum tensions was $PGF_2\alpha$ = I-BOP = 17-phenyl-PGE_2 > sulprostone > PGE_2 > PGD_2 = PGI_2 in the fundus. In the ileum, it was sulprostone > PGE_2 = 17-phenyl-PGE_2 > $PGF_2\alpha$ = I-BOP > PGD_2. At a concentration of 3 μM, AE-248, carbacyclin and DI-004 contracted the fundus by 45, 35 and 17%, respectively. In the ileum, DI-004- and AE-248-induced contractions were 10 and 28%, respectively. At a concentration of 1 μM, PGI_2 and carbacyclin contracted the ileum by 16 and 26%, respectively.

Effects of PGE_2

Concentration-dependent contractile responses were elicited by PGE_2 in the fundus and ileum of wild-type mice (Figure 1). In the fundus, the response reached a plateau at 10 μM, and the ED_{50} value and maximum tension were 489 ± 57 nM and 97 ± 4%, respectively (Figure 1a; Table 1). In the ileum, the response reached a plateau at 3 μM, and the ED_{50} value and maximum tension were 390 ± 32 nM and 90 ± 2%, respectively (Figure 1b; Table 1). In $EP_2^{-/-}$, $IP^{-/-}$, $TP^{-/-}$ and $DP^{-/-}$ mice, PGE_2-induced responses showed similar concentration-response curves with that in wild-type mice both in the fundus and ileum (Figure 1a,b). However, in $EP_1^{-/-}$, $EP_3^{-/-}$ and $FP^{-/-}$ mice, we found decreased response to PGE_2 (Figure 1a, b). In $EP_1^{-/-}$ mice, the maximum tensions decreased by 23 and 12% in the fundus and ileum, respectively. ED_{50} values were about three times higher in both preparations than those in wild-type mice. In $EP_3^{-/-}$ mice, the maximum tensions decreased by 59% in both the fundus and ileum. ED_{50} values were about 10 times higher in both preparations than those in wild-type mice. In $FP^{-/-}$ mice, the maximum tensions decreased by 38 and 59% in the fundus and ileum, respectively. However, ED_{50} values were not so different from those in wild-type preparations. These results show that PGE_2 contracts the fundus and ileum *via* the EP_1, EP_3 and FP. While the estimated rank order of potencies of the PGE_2 to these receptors was $EP_3 > EP_1 > FP$ in both the fundus and ileum, that of the maximum responses to PGE_2 *via* these receptors was $EP_3 > FP > EP_1$ and $EP_3 = FP > EP_1$ in the fundus and ileum, respectively.

We found increased response to PGE_2 in the fundus of $EP_4^{-/-}$ mice compared with that in the fundus of wild-type mice ($EP_4^{+/+}$ mice), which have the similar mixed genetic background with $EP_4^{-/-}$ mice and showed similar response to PGE_2 with that in wild-type mice back-crossed to C57BL/6 mice (Figure 1c). The maximum tension was 109 ± 3% in $EP_4^{-/-}$ mice, which was 12% higher than that in wild-type mice. There was no such difference in the ileum (Figure 1d). These results suggest that the EP_4 mediates some relaxant effects of PGE_2 in the fundus. However, we refrained from including the $EP_4^{-/-}$ mice in the following experiments, because of the mixed genetic background of these mice in addition to the small number of pups grown up to adults, and

Table 1 EC_{50} values and maximum tensions in contractions of the fundus and ileum induced by the prostanoids and their analogues in wild-type mice

	Fundus		*Ileum*	
Ligands	EC_{50} (nM)	*Maximum tension* (% of ACh contraction)	EC_{50} (nM)	*Maximum tension* (% of ACh contraction)
PGD_2	3030 ± 448	40 ± 3 (11)	319 ± 32	78 ± 3 (16)
PGE_2	489 ± 57	97 ± 4 (13)	390 ± 33	90 ± 2 (21)
$PGF_2\alpha$	181 ± 18	155 ± 5 (11)	27 ± 3	85 ± 3 (16)
PGI_2	1480 ± 160	38 ± 3 (8)	n.d.	16 ± 3 (8)
I-BOP	1.9 ± 0.7	154 ± 4 (6)	12 ± 3	85 ± 3 (5)
17-phenyl-PGE_2	131 ± 11	146 ± 4 (11)	41 ± 5	89 ± 2 (13)
Sulprostone	141 ± 15	126 ± 5 (8)	60 ± 10	95 ± 5 (12)
DI-004	n.d.	17 ± 1 (19)	n.d.	10 ± 1 (15)
AE-258	n.d.	45 ± 2 (13)	n.d.	28 ± 1 (14)
Carbacyclin	n.d.	35 ± 4 (6)	n.d.	26 ± 3 (6)

n.d. = ED_{50} values could not be determined, because the response did not reach a plateau. Maximum tensions induced by the ligands, to which the responses did not reach a plateau, represent the tensions induced at highest concentrations used. The numbers in parenthesis indicate *n*.

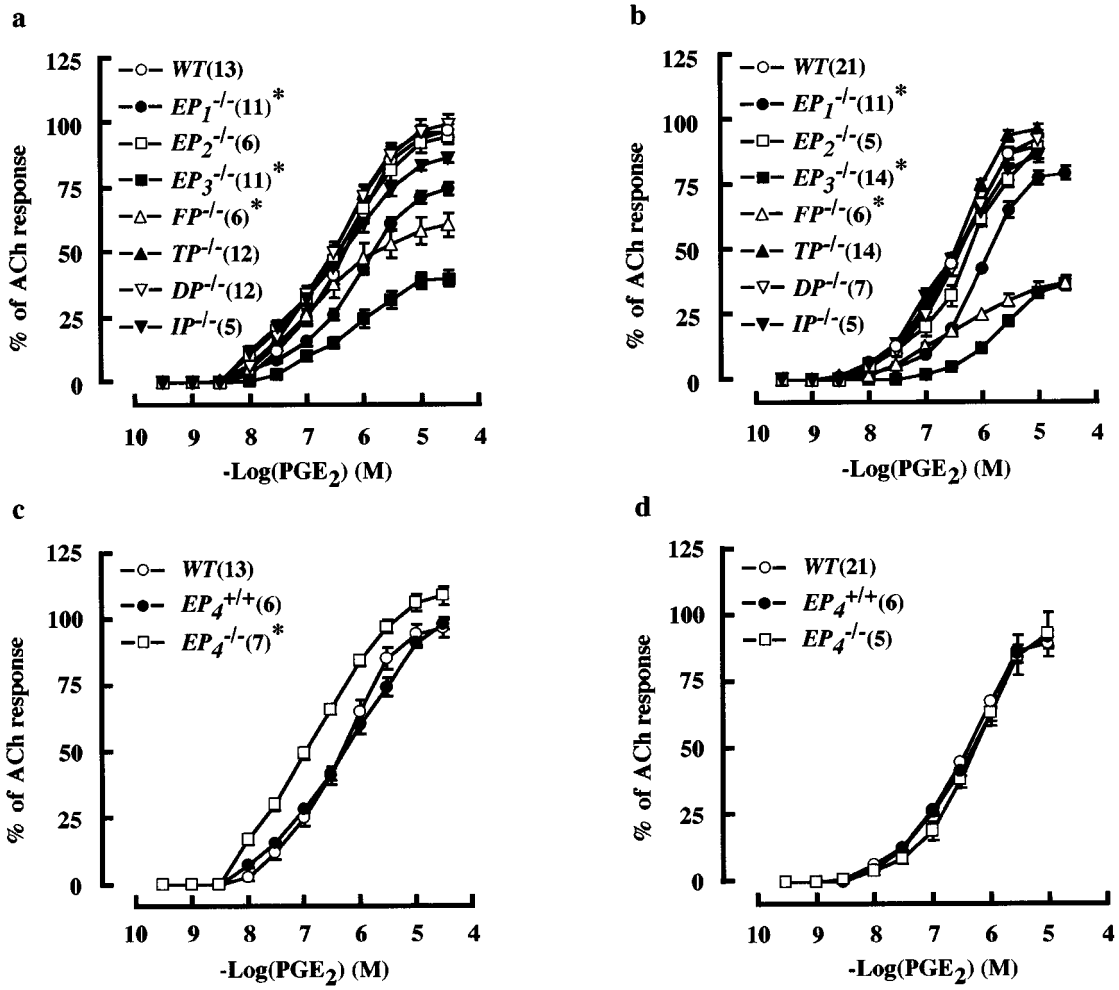

Figure 1 Contractions induced by PGE_2 in longitudinal sections of the fundus (a,c) and ileum (b,d). In (c) and (d), the data from wild-type mice (*WT*) in (a) and (b) are presented again for comparison. $EP_4^{+/+}$ mice represent wild-type control mice having mixed genetic background similar to $EP_4^{-/-}$ mice as described in Methods (c and d). Each point shows the mean value with s.e.mean shown by vertical bars. The numbers in parenthesis indicate *n*. $^*P<0.05$ vs *WT*.

because the extent of relaxation mediated by the EP_4 is small. We also excluded $EP_2^{-/-}$ mice, because the effect mediated by the EP_2 was negligible if present (Figure 1a,b).

Effects of DI-004 and AE-248

In wild-type mice, concentration-dependent contractions were elicited by DI-004, and tensions at 3 μM of concentrations were 17 ± 1 and $10\pm1\%$ in the fundus and ileum, respectively (Figure 2a,b). AE-248 also induced contractions with tensions of 45 ± 2 and $28\pm1\%$, respectively (Figure 2c,d). While their potencies were not so high, the responses to DI-004 and AE-248 were completely abolished in $EP_1^{-/-}$ and $EP_3^{-/-}$ mice, respectively. These results indicate the high specificities of DI-004 and AE-248 to the EP_1 and EP_3, respectively. In accordance with this result, the samples prepared from other knockout mice showed similar concentration-response curves with those from wild-type mice (Figure 2).

Effects of 17-phenyl-PGE_2 and sulprostone

In the fundus and ileum from wild-type mice, concentration-dependent contractions induced by 17-phenyl-PGE_2 reached a plateau at 3 and 1 μM with ED_{50} values of 131 ± 11 and 41 ± 5 nM, respectively. Maximum tensions developed in the fundus and ileum were 146 ± 4 and $89\pm2\%$, respectively (Figure 3a,b; Table 1). These values were significantly higher than those induced by EP_1-specific agonist DI-004, suggesting the action of 17-phenyl-PGE_2 on other receptor(s) in addition to the EP_1. In fact, we found decreased responses to the ligand in $EP_1^{-/-}$, $EP_3^{-/-}$ and $FP^{-/-}$ mice in both organs (Figure 3a, b). In the fundus, maximum tensions and ED_{50} values were almost the same among $EP_1^{-/-}$, $EP_3^{-/-}$ and $FP^{-/-}$ mice, which suggest that all of these three receptors contribute equally to the contractions induced by 17-phenyl-PGE_2 (Figure 3a). In the ileum, different concentration-response curves were obtained among $EP_1^{-/-}$, $EP_3^{-/-}$ and $FP^{-/-}$ mice (Figure 3b). In $EP_1^{-/-}$ mice, only slight decrease in the maximum tension was observed. In $FP^{-/-}$ mice, 59% decrease of the maximum tension and decrease in ED_{50} value were observed. In $EP_3^{-/-}$ mice, 58% decrease of the maximum tension was accompanied by a right-ward shift of the concentration-response curve. These results show that the rank order of potencies of 17-phenyl-PGE_2 to these receptors is $EP_3>EP_1>FP$ and that of tensions induced by this ligand is $EP_3=FP>EP_1$.

In the fundus and ileum of wild-type mice, concentration-dependent contractions induced by sulprostone did not reach a plateau, suggesting that this ligand acts on some receptors with low affinities. Tensions induced by sulprostone at 10 μM of concentration were 126 ± 5 and $95\pm5\%$, respectively (Figure 3c,d). These values were significantly higher than those

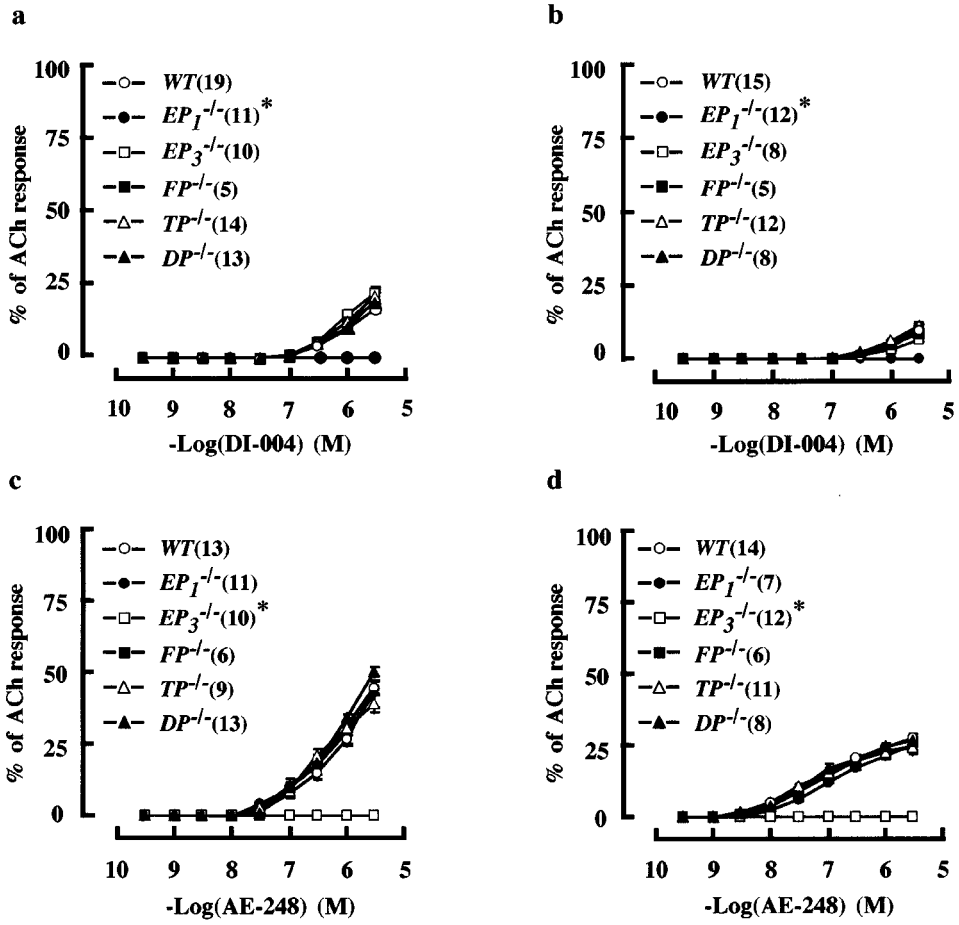

Figure 2 Contractions induced by DI-004 (a,b) and AE-248 (c,d) in longitudinal sections of the fundus (a,c) and ileum (b,d). Each point shows the mean value with s.e.mean shown by vertical bars. The numbers in parenthesis indicate *n*. *$P<0.05$ vs *WT*.

induced by EP_3-specific agonist AE-248, suggesting further that sulprostone may act on other receptor(s) in addition to the EP_3. In the fundus, the response was decreased by 21% in the $FP^{-/-}$ mice and 42% in the $EP_3{}^{-/-}$ mice, which was accompanied with right-ward shift of the concentration-response curve. In $DP^{-/-}$ mice, the response was significantly increased up to 166 ± 6%, suggesting that sulprostone acts on the DP, which mediates relaxation of the fundus as shown later. These results show that sulprostone induced the contraction of the fundus *via* the EP_3 and FP, and that this contraction was apparently reduced by the relaxant effect *via* the DP. In the ileum, the response to sulprostone was reduced in $EP_1{}^{-/-}$, $EP_3{}^{-/-}$ and $FP^{-/-}$ mice. While the tensions induced by sulprostone at 1×10^{-5} M of concentration were comparable, the potency of the ligand was about three ranks of order lower in the $EP_3{}^{-/-}$ mice than in the $FP^{-/-}$ mice. The response was slightly decreased in the $EP_1{}^{-/-}$ mice especially at middle concentrations of the ligand used. These results indicate that sulprostone induces the contraction of the ileum mainly *via* the EP_3 at low concentrations, and *via* the EP_1 and FP in addition to EP_3 at middle and high concentrations, respectively.

Effects of $PGF_2\alpha$

In the fundus and ileum of wild-type mice, $PGF_2\alpha$ elicited contractions concentration-dependently with EC_{50} values of 181 ± 18 and 27 ± 3 nM, respectively. Maximum tensions induced by $PGF_2\alpha$ were 155 ± 5 and 85 ± 3%, respectively (Figure 4). In the fundus of the $EP_1{}^{-/-}$, $EP_3{}^{-/-}$ and $TP^{-/-}$ mice, maximum tensions decreased by 21, 33 and 29%, respectively. However, EC_{50} values were similar to that in wild-type mice (Figure 4a). The maximum tension in the fundus of $FP^{-/-}$ mice decreased by 29%, and EC_{50} value increased by about one rank of order compared with those in wild-type mice (Figure 4a). These results shows that the rank order of affinities of $PGF_2\alpha$ was $FP>EP_1=EP_3=TP$, and that the tension induced by $PGF_2\alpha$ depended equally on these receptors at high concentrations. In the ileum of the $EP_3{}^{-/-}$, $FP^{-/-}$ and $TP^{-/-}$ mice, maximum tensions decreased by 50, 50 and 16%, respectively. While EC_{50} value in the $EP_3{}^{-/-}$ and $TP^{-/-}$ mice was similar to that in wild-type mice, it was one rank of order higher in $FP^{-/-}$ mice (Figure 4b). These results show that the rank order of potencies of $PGF_2\alpha$ was $FP>EP_3=TP$, and that of maximum tensions was $FP=EP_3>>TP$.

Constrictions induced by PGD_2

The maximum tension induced by PGD_2 in wild-type mice was higher in the ileum than in the fundus, and was 78 ± 3 and 40 ± 3%, respectively. However, the maximum tension induced by PGD_2 reached up to 122 ± 3% in $DP^{-/-}$ mice (Figure 5a), indicating that PGD_2 has a potency to induce strong relaxation of the fundus *via* the DP. In fact, PGD_2 showed a strong relaxant action on the fundus precontracted by $PGF_2\alpha$, and which disappeared in $DP^{-/-}$ mice (Figure 5c). Because of this relaxant action of PGD_2, PGD_2-induced contractions of the fundus *via* the receptors other than the DP were apparently weakened. However, this relaxant effect of PGD_2 was not

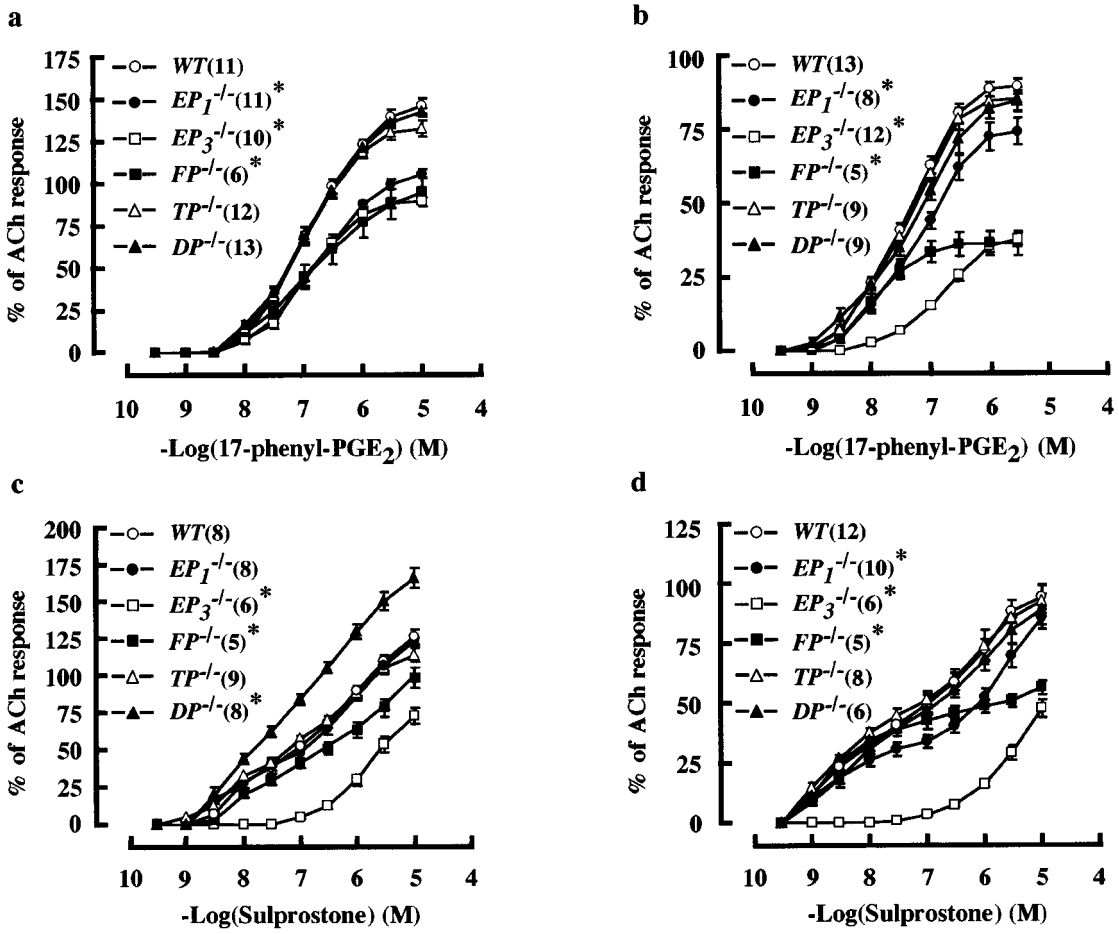

Figure 3 Contractions induced by 17-phenyl-PGE_2 (a,b) and sulprostone (c,d) in longitudinal sections of the fundus (a,c) and ileum (b,d). Each point shows the mean value with s.e.mean shown by vertical bars. The numbers in parenthesis indicate *n*. $^*P<0.05$ vs *WT*.

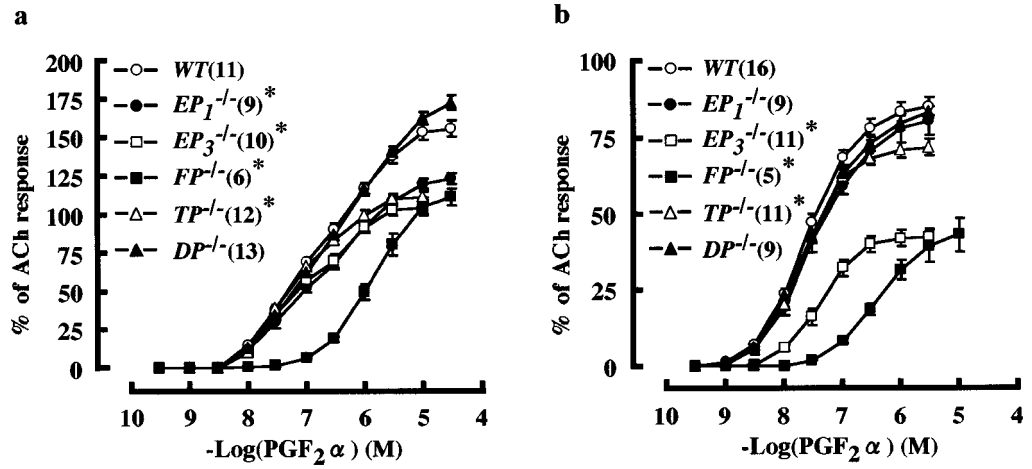

Figure 4 Contractions induced by $PGF_2\alpha$ in longitudinal sections of the fundus (a) and ileum (b). Each point shows the mean value with s.e.mean shown by vertical bars. The numbers in parenthesis indicate *n*. $^*P<0.05$ vs *WT*.

found in the ileum (Figure 5b). In the fundus of $EP_3^{-/-}$ and $FP^{-/-}$ mice, maximum tensions decreased by 64 and 54%, respectively. In the ileum of $EP_3^{-/-}$ and $FP^{-/-}$ mice, maximum tensions decreased by 60 and 62%, respectively. EC_{50} values in both the fundus and ileum of $FP^{-/-}$ mice were about one rank order higher than those in wild-type mice (Figure 5a,b). These results show that the potency of PGD_2 to the FP is higher than that to the EP_3, and that the tensions induced by PGD_2 depend equally on these receptors at high concentrations.

Contractions induced by I-BOP

Contractile responses were elicited by I-BOP concentration-dependently in the fundus and ileum of wild-type mice. Both in the fundus and ileum of $EP_3^{-/-}$ mice, maximum

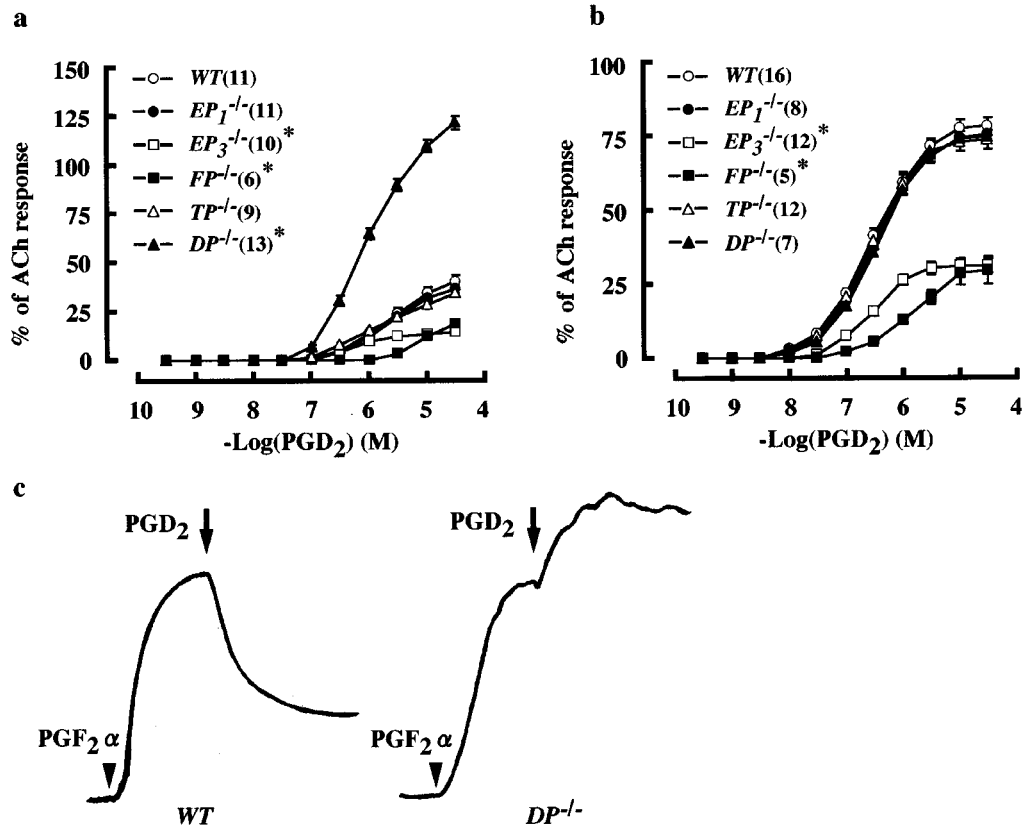

Figure 5 Contractions induced by PGD_2 in longitudinal sections of the fundus (a) and ileum (b), and relaxations induced by PGD_2 (c). (a,b) Each point shows the mean value with s.e.mean shown by vertical bars. The numbers in parenthesis indicate *n*. $^*P<0.05$ vs *WT*. (c) Arrowheads and arrows indicate the addition of $PGF_2\alpha$ (0.3 μM) and PGD_2 (1 μM), respectively.

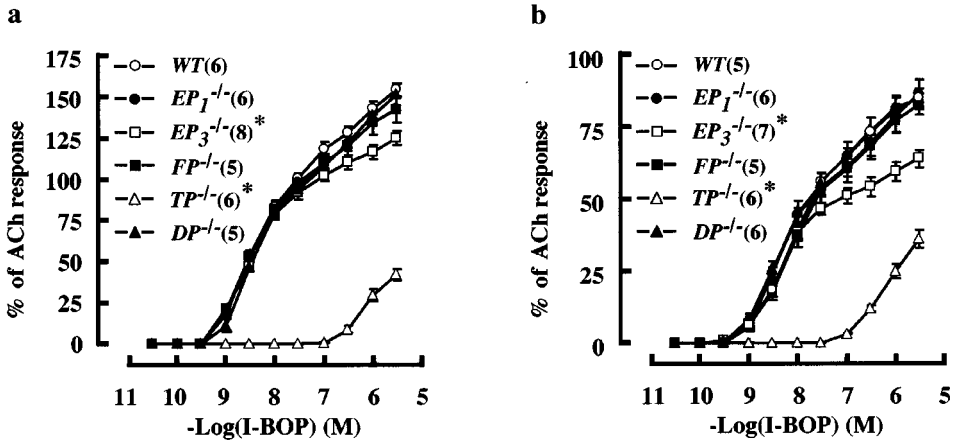

Figure 6 Contractions induced by I-BOP in longitudinal sections of the fundus (a) and ileum (b). Each point shows the mean value with s.e.mean shown by vertical bars. The numbers in parenthesis indicate *n*. $^*P<0.05$ vs *WT*.

tensions decreased by 19 and 25%, respectively. In $TP^{-/-}$ mice, these decreases were 73 and 58%, respectively, and the concentration-response curves shifted to the right (Figure 6). In $EP_1^{-/-}$, $FP^{-/-}$, and $DP^{-/-}$ mice, similar concentration-response curves to that in wild-type mice were obtained (Figure 6). These results show that I-BOP acts mainly on the TP, and that it cross-acts on the EP_3 at high concentrations.

Contractions induced by PGI_2 and carbacyclin

PGI_2 and its derivative, carbacyclin, showed relaxant effects on the fundus and ileum (Figure 7). In the fundus of wild-type mice precontracted by $PGF_2\alpha$, PGI_2 and carbacyclin induced slight contraction. However, their relaxant actions were apparent in mice lacking the EP_3 (Figure 7a,c), *via* which these ligands mainly show contractile effects as shown in Figure 8. Moreover, contractile actions of PGI_2 increased in $IP^{-/-}$ mice, indicating the participation of the IP. Similar relaxant actions were also found in the ileum to which mechanical tension of 1.0 g was applied (Figure 7b,d). Because of these relaxant actions of PGI_2 and carbacyclin *via* the IP, their contractile effects were markedly augmented in $IP^{-/-}$ mice compared with those in wild-type mice (Figure 8a,b,d). However, this augmentation was not seen in the fundus of $IP^{-/-}$ mice contracted by carbacyclin (Figure 8c),

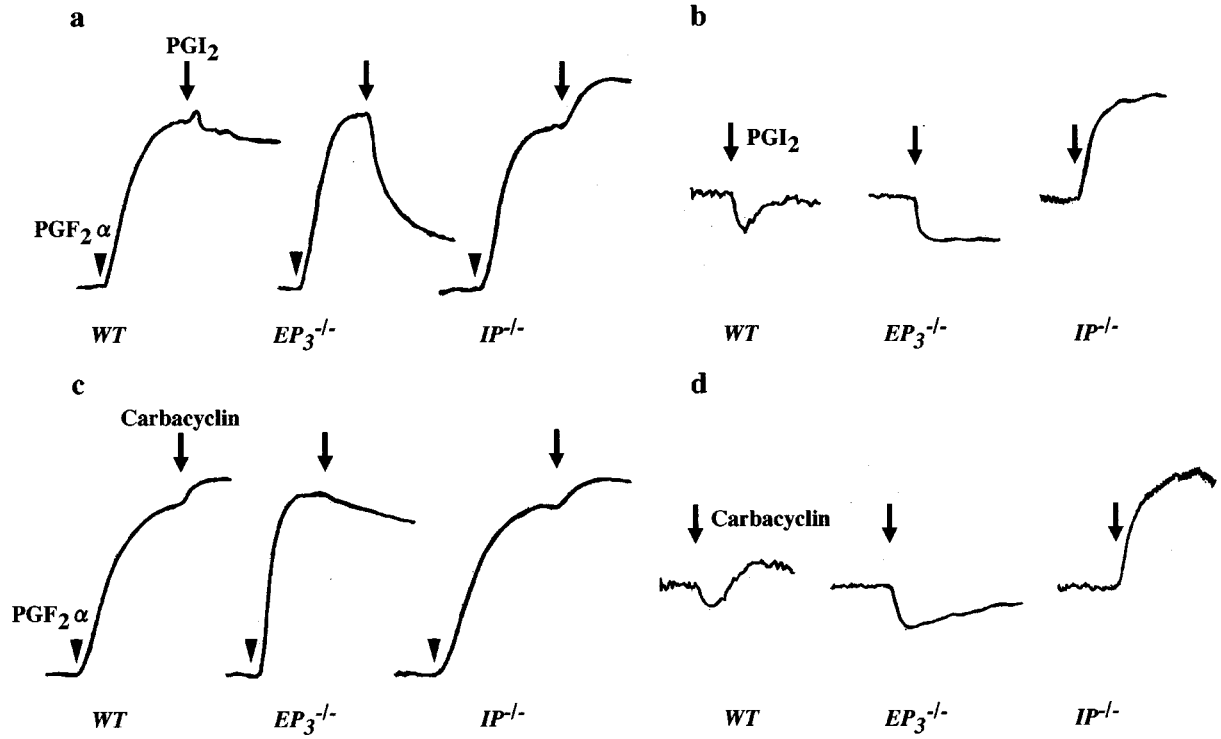

Figure 7 Relaxant actions of PGI_2 (a,b) and carbacyclin (c,d) in longitudinal sections of the fundus (a,c) and ileum (b,d). (a,b) Arrowheads and arrows indicate the addition of $PGF_2\alpha$ (0.3 μM) and PGI_2 (1 μM), respectively. (c,d) Arrowheads and arrows indicate the addition of $PGF_2\alpha$ (0.3 μM) and carbacyclin (1 μM), respectively.

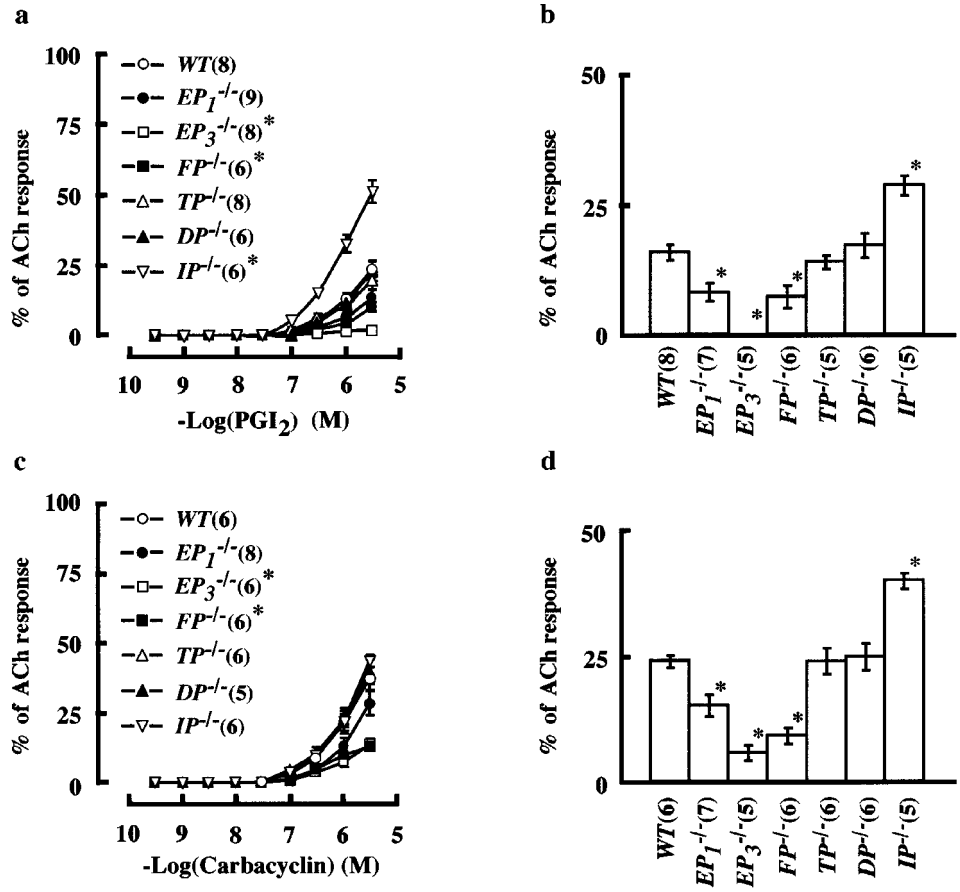

Figure 8 Contractions induced by PGI_2 (a,b) and carbacyclin (c,d) in longitudinal sections of the fundus (a,c) and ileum (b,d). (a,c) Each point shows the mean value with s.e.mean shown by vertical bars. (b,d) Each column shows the mean value with s.e.mean shown by vertical bars. The numbers in parenthesis indicate *n*. $^*P<0.05$ vs *WT*.

Table 2 Actions of the prostanoids and their analogues on the prostanoid receptors

Receptors	*PGE_2*	*17-phenyl-PGE_2*	*Sulprostone*	*DI-004 (EP_1)*	*Ligands AE248 (EP_3)*	*$PGF_2\alpha$*	*PGD_2*	*I-BOP (TP)*	*PGI_2*
EP_1	<	<	<	<	–	<*	–	–	<
EP_2	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–
EP_3	<	<	<	–	<	<	<	<	<
EP_4	>*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–
FP	<	<	<	–	–	<	<	–	<
TP	–	–	–	–	–	<	–	<	–
DP	–	–	>*	–	–	–	>>*	–	–
IP	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	>

*Actions detected only in the fundus. n.d. = not determined. <, contraction; > relaxation; –, no action.

reflecting that the relaxant action of carbacyclin in the fundus was weak (Figure 7c).

Concentration-response curves of contractions induced by PGI_2 and carbacyclin started from concentration of 1×10^{-7} M, and did not reach a plateau at concentrations of up to 3×10^{-6} M in the fundus of wild-type mice, suggesting that these ligands act on the receptor(s) other than the IP with low potencies (Figure 8a,c). PGI_2-induced contractions of the fundus were almost completely depressed in $EP_3^{-/-}$ mice and decreased by 57 and 44% at a concentration of 3 μM in $FP^{-/-}$ and $EP_1^{-/-}$ mice, respectively (Figure 8a). Carbacyclin-induced contractions of the fundus decreased by 64, 65 and 24% at a concentration of 3 μM in $EP_3^{-/-}$, $FP^{-/-}$ and $EP_1^{-/-}$ mice, respectively (Figure 8c). Similar results were obtained in the ileum, when the contractions induced by these ligands at a concentration of 1 μM were examined. Thus, PGI_2-induced contractions of the ileum disappeared almost completely in $EP_3^{-/-}$ mice and decreased by 54 and 24% in $FP^{-/-}$ and $EP_1^{-/-}$ mice, respectively (Figure 8b). Carbacyclin-induced contractions of the ileum decreased by 76, 62 and 37% in $EP_3^{-/-}$, $FP^{-/-}$ and $EP_1^{-/-}$ mice, respectively (Figure 8d). These results indicate that PGI_2 and carbacyclin contract the fundus and ileum *via* the EP_3, FP and EP_1, and that they have potency to relax these tissues *via* their own receptor, the IP.

Discussion

In this study, we examined the direct contractile actions of prostanoids and their analogues on longitudinal smooth muscles of the murine gastric fundus and ileum to define which types and/or subtypes of the prostanoid receptors participate in their actions. The rank order of potencies of I-BOP and naturally occurring prostanoids was comparable to those reported in longitudinal muscles of human stomach, ileum and colon; U-46619 > PGE_2 > $PGF_2\alpha$ > PGD_2, except the order between PGE_2 and $PGF_2\alpha$. In the fundus and ileum, the EC_{50} values of PGE_2 were 489 and 390 nM, respectively (Table 1). These values were one or two ranks of order higher than reported K_d values of PGE_2 to each of the EPs (Kiriyama *et al*., 1997). This may be derived from the large contribution of the contractions mediated by the FP, which could shift log concentration-curves of PGE_2 to the right because of low potency of PGE_2 to the FP.

PGE_2-induced contractions were mediated by the EP_3, FP and EP_1. While contractile actions of PGE_2 have been reported to be mediated by the EP_1 and/or EP_3, this study clearly showed the participation of the FP in these actions of PGE_2 especially at high concentrations. While novel agonists, DI-004 and AE-248, showed strict specificity to the respective receptors, their potencies and efficacies were not so high. 17-phenyl-PGE_2, which has been considered as an agonist with relative specificity to the EP_1, showed affinities to the EP_3 and FP in addition to the EP_1. Moreover, the contractile actions of this ligand in the ileum were mainly mediated by the EP_3 at low concentrations and by the FP at high concentrations, suggesting little involvement of the EP_1. Sulprostone, which has been considered as an agonist with relative specificity to the EP_3, contracted the fundus and ileum mainly *via* the EP_3. However, it also acts on the FP in the fundus and on the EP_1 and FP in the ileum. Moreover, the contribution of the FP was comparable to that of the EP_3 at 10 μM of concentration in the ileum. It is notable that sulprostone shows the relaxant action *via* the DP in the fundus, which was obscured by the contractile actions *via* the EP_3 and FP. These results show that long-used subtype specific ligands, such as 17-phenyl-PGE_2 and sulprostone, are actually not so specific, and that novel agonists with strict specificities, such as DI-004 and AE-248, should be used to characterize each subtype of the EPs. However, specific antagonists are inherently more useful as pharmacological tools because selectivity of agonists has been sometimes misleading due to their activation on multiple receptors.

$PGF_2\alpha$ is used for the treatment of paralytic ileus occurring after the abdominal operation to facilitate the movement of gastrointestinal tract. The FP-mediated smooth muscle contraction was found in rat gastric fundus, which are potently contracted by ICI-81008, an agonist for the FP (Dong *et al*., 1986). However, it was unknown which types and subtypes of the prostanoid receptors participate in the $PGF_2\alpha$-induced contractions. This study clearly showed that $PGF_2\alpha$ acts on the EP_1, EP_3 and TP in addition to the FP, which contributes to the high efficacies of $PGF_2\alpha$ in both the fundus and ileum.

The DP couples to Gs and raise intracellular cyclic AMP concentration (Hirata *et al*., 1994), which leads to smooth muscle relaxation. However, PGD_2 sometimes contracts smooth muscles *via* the receptor(s) other than the DP. For example, contractile effects of PGD_2 on human airway and rat colon smooth muscle were reported to be mediated by the TP (Coleman & Sheldrick, 1989; Diener & Gabato, 1994). In this study, we found the contribution of the FP and EP_3 instead of the TP in PGD_2-induced contractions of both the fundus and ileum. On the other hand, PGD_2 showed high potential as a relaxant *via* the DP in the fundus. This relaxant action of PGD_2 on longitudinal smooth muscle of the alimentary tract was demonstrated for the first time in this study, whereas the action was reported in the circular smooth muscle of the rabbit stomach (Whittle *et al*., 1979).

While contractile actions of TXA_2 on airway and vascular smooth muscles are well known, those on smooth muscle of gastrointestinal tract have not been fully characterized. We found strong contractile actions of I-BOP, a TXA_2 analogue,

on both the fundus and ileum, which were mediated mainly *via* the TP and slightly *via* the EP_3. Moreover, I-BOP showed highest potency among the ligands used in this study, suggesting the physiological or pathophysiological roles of TXA_2 played *via* the direct actions on gastrointestinal smooth muscles, which has been largely attributed to its action on vasculature and blood platelets.

There have been few reports on the relaxant actions of PGI_2 on longitudinal smooth muscle of gastrointestinal tract. However, rat colon does appear to contain inhibitory IP receptor, where PGI_2 inhibits spontaneous myogenic activity (Dong *et al*., 1986). We found the relaxant actions of PGI_2 and carbacyclin in both the fundus and ileum, which however were masked by their contractile actions *via* the EP_1, EP_3 and FP. These results suggest that PGI_2 and its analogues have potency to work as relaxants on various gastrointestinal smooth muscle, and that this potency has been long overlooked due to the actions of these ligands on the contractile receptors.

While contractions induced by the prostanoids and their analogues were essentially similar between in the fundus and ileum, the FP and EP occupancies seem to be more efficatious in the ileum than in the fundus. Moreover, apparent differences between the fundus and ileum were noted in relaxant actions mediated by the DP and EP_4. Sulprostone and PGD_2 relaxed only the smooth muscle of the fundus, suggesting the difference in the expression level of the DP between smooth muscles of these organs. PGE_2 also showed a relaxant action *via* the EP_4 only in the fundus. While physiological significance of these differences is not clear at present, specific DP ligands, if present, could efficiently relax the fundus and might be used as therapeutic and diagnostic tools.

Binding affinities of various prostanoids and their analogues to the cloned murine and human prostanoid receptors expressed in cultured cells have been reported (Kiriyama *et al*., 1997; Abramovitz *et al*., 2000). While these studies showed that various prostanoids and their analogues bind to a broad array of receptors, they did not examine their functional potencies. In this study, we can estimate these potencies in smooth muscle contractions, and found that these potencies are generally in good agreement with the binding affinities of the prostanoid ligands to the prostanoid receptors. For example, PGE_2 bound to the FP with K_i value of 100 nM in addition to four subtypes of EPs, and PGD_2 bound to the FP and EP_3 with K_i values of 47 and 280 nM, respectively, in addition to the DP (Kiriyama *et al*., 1997). These are comparable to the potencies estimated from contractions induced by the PGE_2 and PGD_2 (Figures 1 and 5). These results suggest that ligands, which were shown to bind to a variety of receptors, indeed act on these receptors and work as agonists. However, the FP did not mediate I-BOP-induced contraction, in spite of its binding to this receptor with the same affinity as that to the EP_3 (Kiriyama *et al*., 1997) (Figure 6). This may suggest that some ligands including I-BOP, which was shown to bind to some prostanoid receptors, may work as an antagonist for these receptors.

In conclusion, we have used mice lacking each of the prostanoid receptors and performed a systemic analysis of the contractions of the fundus and ileum induced by the prostanoid and their analogues. This study has revealed that a broad array of receptors mediate the actions of each ligand, some of which were not previously known. This information can be utilized in analysing prostanoid actions not only in smooth muscle preparations but also in other biological systems, and may contribute to the understanding of the prostanoid actions under various physiological and pathological conditions.

We thank K. Nakaya and T. Yokoyama for help in breeding and maintenance of mice, and K. Inaba for secretarial assistance. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan and by the Research Grant for Cardiovascular Diseases (11C-1) from the Ministry of Health and Welfare. This work was also supported by grants from the Ono Medical Research Foundation, the Takeda Science Foundation, the Smoking Research Foundation and the Suhara Memorial Foundation.

References

ABRAMOVITZ, M., ADAM, M., BOIE, Y., CARRIERE, M., DENIS, D., GODBOUT, C., LAMONTAGNE, S., ROCHETTE, C., SAWYER, N., TREMBLAY, N.M., BELLEY, M., GALLANT, M., DUFRESNE, C., GAREAU, Y., RUEL, R., JUTEAU, H., LABELLE, M., OUIMET, N. & METTERS, K.M. (2000). The utilization of recombinant prostanoid receptors to determine the affinities and selectivities of prostaglandins and related analogs. *Biochim. Biophys. Acta,* **1483,** 285–293.

BENNETT, A. & SANGER, G.J. (1982). Pinane thromboxane A_2 analogues are non-selective prostanoid antagonists in rat and human stomach muscle. *Br. J. Pharmacol.,* **77,** 591–596.

BENNETT, A., HENSBY, C.N., SANGER, G.J. & STAMFORD, I.F. (1981). Metabolites of arachidonic acid formed by human gastrointestinal tissues and their actions on the muscle layers. *Br. J. Pharmacol.,* **74,** 435–444.

BENNETT, A., JAROSIK, C., SANGER, G.J. & WILSON, D.E. (1980). Antagonism of prostanoid-induced contractions of rat gastric fundus muscle by SC-19220, sodium meclofenamate, indomethacin or trimethoquinol. *Br. J. Pharmacol.,* **71,** 169–175.

COLEMAN, R.A. & SHELDRICK, L.G. (1989). Prostanoid-induced contraction of human bronchial smooth muscle is mediated by TP-receptors. *Br. J. Pharmacol.,* **96,** 688–692.

COLEMAN, R.A., GRIX, S.P., HEAD, S.A., LOUTTIT, J.B., MALLETT, A. & SHELDRICK, R.L.G. (1994). A novel inhibitory receptor in piglet saphenous vein. *Prostaglandins,* **47,** 151–168.

COLEMAN, R.A., HUMPHREY, P.P., KENNEDY, I., LEVY, G.P. & LUMLEY, P. (1981). Comparison of the actions of U-46619, a prostaglandin H_2-analogue, with those of prostaglandin H_2 and thromboxane A_2 on some isolated smooth muscle preparations. *Br. J. Pharmacol.,* **73,** 773–778.

COLEMAN, R.A., KENNEDY, I., HUMPHREY, P.P.A., BUNCE, K. & LUMLEY, P. (1990). Prostanoids and their receptors. In *Comprehensive Medicinal Chemistry Vol. 3*: Membranes & Receptors. ed. Emmett, J.C. pp. 643–714. Oxford: Pergamon Press.

COLEMAN, R.A., KENNEDY, I. & SHELDRICK, R.L.G. (1985). AH6809, a prostanoid EP_1 receptor blocking drug. *Br. J. Pharmacol.,* **85,** 273P.

COLEMAN, R.A., KENNEDY, I., SHELDRICK, R.L.G. & TOLOWINSKA, I.Y. (1987). Further evidence for the existence of three subtypes of PGE_2-sensitive (EP-) receptors. *Br. J. Pharmacol.,* **91,** 407P.

CRANE, B.H., MAISH, T.L., MADDOX, Y.T., COREY, E.J., SZEKELY, I. & RAMWELL, P.W. (1978). Effects of prostaglandin I_2 and analogs on platelet aggregation and smooth muscle contraction. *J. Pharmacol. Exp. Ther.,* **206,** 132–138.

DIENER, M. & GABATO, D. (1994). Thromboxane-like actions of prostaglandin D_2 on the contractility of the rat colon in vitro. *Acta Physiol. Scand.,* **150,** 95–101.

DING, M., KINOSHITA, Y., KISHI, K., NAKATA, H., HASSAN, S., KAWANAMI, C., SUGIMOTO, Y., KATSUYAMA, M., NEGISHI, M., NARUMIYA, S., ICHIKAWA, A. & CHIBA, T. (1997). Distribution of prostaglandin E receptors in the rat gastrointestinal tract. *Prostaglandins,* **53,** 199–216.

DONG, Y.J., JONES, R.L. & WILSON, N.H. (1986). Prostaglandin E receptor subtypes in smooth muscle: agonist activities of stable prostacyclin analogues. *Br. J. Pharmacol.,* **87,** 97–107.

DUNNETT, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *Am. J. Stat. As.,* **50,** 1096–1121.

EK, M., KUROSAWA, M., LUNDEBERG, T. & ERICSSON, A. (1998). Activation of vagal afferents after intravenous injection of interleukin-1β: role of endogenous prostaglandins. *J. Neurosci.,* **18,** 9471–9479.

FUKUNAGA, Y., MINE, Y., YOSHINAGA, S., TAKEUCHI, T., HARA, F. & YAGASAKI, O. (1993). Role of prostacyclin in acetylcholine release from myenteric plexus of guinea-pig ileum. *Eur. J. Pharmacol.,* **233,** 237–242.

HAMBERG, M., HEDQVIST, P., STRANDBERG, K., SVENSSON, J. & SAMUELSSON, B. (1975). Prostaglandin endoperoxides IV. Effects on smooth muscle. *Life Sci.,* **16,** 451–462.

HIRATA, M., KAKIZUKA, A., AIZAWA, M., USHIKUBI, F. & NARUMIYA, S. (1994). Molecular characterization of a mouse prostaglandin D receptor and functional expression of the cloned gene. *Proc. Natl. Acad. Sci. U.S.A.,* **91,** 11192–11196.

HIZAKI, H., SEGI, E., SUGIMOTO, Y., HIROSE, M., SAJI, T., USHIKUBI, F., MATSUOKA, T., NODA, Y., TANAKA, T., YOSHIDA, N., NARUMIYA, S. & ICHIKAWA, A. (1999). Abortive expansion of the cumulus and impaired fertility in mice lacking the prostaglandin E receptor subtype EP_2. *Proc. Natl. Acad. Sci. U.S.A.,* **96,** 10501–10506.

HORTON, E.W. & JONES, R.L. (1974). Biological activity of prostaglandin D_2 on smooth muscle. *Br. J. Pharmacol.,* **52,** 110P–111P.

KIRIYAMA, M., USHIKUBI, F., KOBAYASHI, T. & NARUMIYA, S. (1997). Ligand binding specificities of the eight types and subtypes of the mouse prostanoid receptors expressed in Chinese hamster ovary cells. *Br. J. Pharmacol.,* **122,** 217–224.

MATSUMURA, K., WATANABE, Y., ONOE, H. & WATANABE, Y. (1995). Prostacyclin receptor in the brain and central terminals of the primary sensory neurons: an autoradiographic study using a stable prostacyclin analogue [^{3}H]-iloprost. *Neuroscience,* **65,** 493–503.

MATSUOKA, T., HIRATA, M., TANAKA, H., TAKAHASHI, Y., MURATA, T., KABASHIMA, K., SUGIMOTO, Y., KOBAYASHI, T., USHIKUBI, F., AZE, Y., YOSHIDA, N., HONDA, Y., NAGAI, H. & NARUMIYA, S. (2000). Prostaglandin D_2 as a mediator of allergic asthma. *Science,* **287,** 2013–2017.

MORIMOTO, K., SUGIMOTO, Y., KATSUYAMA, M., OIDA, H., TSUBOI, K., KISHI, K., KINOSHITA, Y., NEGISHI, M., CHIBA, T., NARUMIYA, S. & ICHIKAWA, A. (1997). Cellular localization of mRNAs for prostaglandin E receptor subtypes in mouse gastrointestinal tract. *Am. J. Physiol.,* **272,** G681–G687.

MURATA, T., USHIKUBI, F., MATSUOKA, T., HIRATA, M., YAMAZAKI, A., SUGIMOTO, Y., ICHIKAWA, A., AZE, Y., TANAKA, T., YOSHIDA, N., UENO, A., OH-ISHI, S. & NARUMIYA, S. (1997). Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. *Nature,* **388,** 678–682.

NARUMIYA, S., SUGIMOTO, Y. & USHIKUBI, F. (1999). Prostanoid receptors: structures, properties and functions. *Physiol. Rev.,* **79,** 1193–1226.

NGUYEN, M., CAMENISCH, T., SNOUWAERT, J.N., HICKS, E., COFFMAN, T.M., ANDERSON, P.A., MALOUF, N.N. & KOLLER, B.H. (1997). The prostaglandin receptor EP_4 triggers remodelling of the cardiovascular system at birth. *Nature,* **390,** 78–81.

POLL, C., GRIX, S. & COLEMAN, R.A. (1988). Further evidence for the existence of neuronal prostanoid receptors which enhance neurotransmission in the guinea-pig ileum. *Br. J. Pharmacol.,* **88,** 806P.

QIAN, Y.M. & JONES, R.L. (1995). Inhibition of rat colon contractility by prostacyclin (IP-) receptor agonists: involvement of NANC neurotransmission. *Br. J. Pharmacol.,* **115,** 163–171.

RUDD, J.A., QIAN, Y., TSUI, K.K. & JONES, R.L. (2000). Non-prostanoid prostacyclin mimetics as neuronal stimulants in the rat: comparison of vagus nerve and NANC innervation of the colon. *Br. J. Pharmacol.,* **129,** 782–790.

SEGI, E., SUGIMOTO, Y., YAMASAKI, A., AZE, Y., OIDA, H., NISHIMURA, T., MURATA, T., MATSUOKA, T., USHIKUBI, F., FUKUMOTO, M., TANAKA, T., YOSHIDA, N., NARUMIYA, S. & ICHIKAWA, A. (1998). Patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice. *Biochem. Biophys. Res. Commun.,* **246,** 7–12.

SUGIMOTO, Y., YAMASAKI, A., SEGI, E., TSUBOI, K., AZE, Y., NISHIMURA, T., OIDA, H., YOSHIDA, N., TANAKA, T., KATSUYAMA, M., HASUMOTO, K., MURATA, T., HIRATA, M., USHIKUBI, F., NEGISHI, M., ICHIKAWA, A. & NARUMIYA, S. (1997). Failure of parturition in mice lacking the prostaglandin F receptor. *Science,* **277,** 681–683.

SUZAWA, T., MIYAURA, C., INADA, M., MARUYAMA, T., SUGIMOTO, Y., USHIKUBI, F., ICHIKAWA, A., NARUMIYA, S. & SUDA, T. (2000). The role of prostaglandin E receptor subtypes (EP1, EP2, EP3, and EP4) in bone resorption: an analysis using specific agonists for the respective EPs. *Endocrinology,* **141,** 1554–1559.

USHIKUBI, F., HIRATA, M. & NARUMIYA, S. (1995). Molecular biology of prostanoid receptors: An overview. *J. Lipid Mediat.,* **12,** 343–359.

USHIKUBI, F., SEGI, E., SUGIMOTO, Y., MURATA, T., MATSUOKA, T., KOBAYASHI, T., HIZAKI, H., TUBOI, K., KATSUYAMA, M., ICHIKAWA, A., TANAKA, T., YOSHIDA, N. & NARUMIYA, S. (1998). Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP_3. *Nature,* **395,** 281–284.

WHITTLE, B.J., MUGRIDGE, K.G. & MONCADA, S. (1979). Use of the rabbit transverse stomach-strip to identify and assay prostacyclin, PGA_2, PGD_2 and other prostaglandins. *Eur. J. Pharmacol.,* **53,** 167–172.

WRIGHT, D.H., NANTEL, F., METTERS, K.M. & FORD-HUTCHINSON, A.W. (1999). A novel biological role for prostaglandin D_2 is suggested by distribution studies of the rat DP prostanoid receptor. *Eur. J. Pharmacol.,* **377,** 101–115.

(Received May 22, 2000
Revised July 3, 2000
Accepted July 26, 2000)