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Characterization of prostanoid receptors mediating actions of the isoprostanes, 8-iso-PGE_2 and 8-iso-$PGF_{2\alpha}$, in some isolated smooth muscle preparations

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1 We investigated the contracting actions of the isoprostanes (isoPs), 8-iso-prostaglandin (PG) $F_{2\alpha}$ and 8-iso-PGE_2, in comparison to the effects of the thromboxane (TX) A_2-mimetic U 46619 and the traditional prostaglandin PGE_2 in the isolated rat aorta, isolated rat gastric fundus and the isolated guinea-pig ileum.

2 U 46619 and 8-iso-$PGF_{2\alpha}$ caused contractions in the rat aorta and rat gastric fundus in a concentration-dependent manner, whereas these agonists showed no effects in the guinea-pig ileum. However, 8-iso-PGE_2 and PGE_2 caused contractions in all isolated organs used.

3 The prostanoid TP-receptor antagonist SQ 29,548 (10 nM) significantly antagonized vasoconstrictions induced by the agonists used in the rat aorta. SQ 29,548 at a final concentration of 3 μM, but not at lower concentrations, significantly inhibited contractions induced by U 46619, 8-iso-$PGF_{2\alpha}$ and 8-iso-PGE_2 in the rat fundus. Responses to PGE_2 were unchanged. The prostanoid EP_1-receptor antagonist SC 51089 (3 μM) significantly inhibited contractions induced by 8-iso-PGE_2 and PGE_2 in the rat fundus and in the guinea-pig ileum. SC 51089 had no effect on responses to any of the agonists tested.

4 Our results show that 8-iso-PGE_2, in contrast to 8-iso-$PGF_{2\alpha}$, can also cause contractions by activation of the EP_1-receptors in the rat gastric fundus and the guinea-pig ileum. The findings of the present study do not support the existence of a unique isoP-receptor in the tissues used.
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Keywords: 8-iso-PGE_2; 8-iso-$PGF_{2\alpha}$; TP-receptor antagonist SQ 29,548; EP_1-receptor antagonist SC 51089; rat aorta, rat gastric fundus; guinea-pig ileum

Abbreviations: isoPs, isoprostanes; mN, millinewton; PG, prostaglandin; TX, thromboxane

Introduction

It has been found that prostaglandin (PG)-like compounds, which are termed isoprostanes (isoPs), are produced by free radical catalyzed peroxidation of arachidonic acid independent of the cyclo-oxygenase enzyme in human and animals (Morrow *et al*., 1990a,b; 1994a; 1998a,b). Compared with $PGF_{2\alpha}$, which is a weak vasoconstrictor, and PGE_2, which is mainly a vasodilator (Nicosia & Patrono, 1989), the isoPs, 8-iso-$PGF_{2\alpha}$ and 8-iso-PGE_2, are powerful vasoconstrictors *in vivo* and *in vitro* (Morrow *et al*., 1994b; Kromer & Tippins, 1996; Möbert *et al*., 1997; Sametz *et al*., 1999). These isoPs might exert their vasoconstrictor effects by activation of the TP-receptor (Takahashi *et al*., 1992) or by a unique isoP-receptor in vascular smooth muscles distinct from but structurally similar to the TP-receptor (Fukunaga *et al*., 1993; 1997).

The aim of this study was to investigate whether the isoPs, 8-iso-$PGF_{2\alpha}$ and 8-iso-PGE_2, are also able to cause effects in the rat gastric fundus and/or the guinea-pig ileum, which contain the prostanoid EP_1-receptor (Bennet *et al*., 1980; Coleman *et al*., 1985). To this end, we have compared the effects of both isoPs with that of the TXA_2-mimetic U 46619 and the traditional prostaglandin PGE_2 in the isolated rat aortic strip, isolated rat fundic strip and the isolated guinea-pig ileum. Furthermore, we investigated the influences of the selective TP-receptor antagonists, SQ 29,548 and ICI 192,605, and the selective EP_1-receptor antagonist SC 51089 (Hallinan *et al*., 1993; 1996; Malmberg *et al*., 1994), to clarify whether the effects of 8-iso-$PGF_{2\alpha}$ and 8-iso-PGE_2 are mediated by a unique isoP-receptor and/or also by other prostanoid receptors.

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Methods

Tissue preparation

Female Sprague-Dawley rats weighing 200–220 g and female guinea-pigs weighing 300–400 g (Forschungsinstitut für Versuchstierzucht und -haltung, Himberg, Austria), which had been fasting overnight, were stunned and exsanguinated.

Isolated smooth muscle preparations (rat aorta, rat gastric fundus and guinea-pig ileum) were suspended under a resting tension of 10 millinewton (mN) in 5 ml organ baths (Tyrode solution, 37°C, gassed with 95% O_2 and 5% CO_2). Changes in tension expressed in mN were measured isometrically by a Hugo Sachs Electronics (HSE) K30 isometric transducer connected to a bridge amplifier (HSE) and recorded on a Rikadenki multi pen recorder.

Rat aorta The thoracic aorta was quickly excised, cleaned of adhering connective tissue and cut helically to produce a strip.

Rat gastric fundus The stomach was quickly excised and the fundus was cut zigzag parallel to the longitudinal muscle fibres into a 2 mm wide strip.

Guinea-pig ileum The terminal ileum was excised, cut into 2 cm portions after discarding the 8–10 cm portion nearest the ileo-caecal junction and gently flushed intraluminally.

Experimental design

After an equilibration period of 60 min, concentration-response curves for 8-iso-$PGF_{2\alpha}$, 8-iso-PGE_2, U 46619 and PGE_2 were constructed cumulatively. At each concentration, the force was allowed to reach a plateau before the next higher concentration was added. Only one agonist was tested on each organ sample. To check whether the sensitivity of the organs to the actions of the agonists changed during the time course of the experiments, concentration-response curves were repeated three times. The contractions induced by these agonists in the organs used remained nearly unchanged with a maximum variation of $\pm 5\%$. The third concentration-response curve served as a control. Thereafter, the TP-receptor antagonist SQ 29,548 at final bath concentrations of 10, 30, 100, 300 nM and 3 μM or the EP_1-receptor antagonist SC 51089 at 0.3, 1 and 3 μM were applied to the isolated organs. For comparison to SQ 29,548 the influence of the selective TP-receptor antagonist ICI 192,605 at a concentration of 10 nM in the rat aorta and 3 μM in the rat gastric fundus was also investigated. Only one concentration of antagonists was examined in each experiment. Five min later (without washing) the concentration-response curves to the agonists were repeated. The effect of the EP_1-receptor antagonist AH 6809 (final bath concentration 3 μM) was investigated in the guinea-pig ileum for comparison with the effect of SC 51089. The inhibitory effect of AH 6809 (data not shown) did not differ from that of SC 51089. SC 51089 was used in all further experiments, because of its higher solubility.

In preliminary experiments, organ samples were exposed to antagonists for 5, 10 and 20 min before addition of agonists. At 5 or 10 min pre-incubation time no differences in the inhibitory effects were seen and after 20 min the effect of the antagonists decreased slightly. Therefore, a pre-incubation time of 5 min was chosen for the subsequent experiments.

Since the smooth muscle cells of the rat gastric fundus express both TP-receptors and EP_1-receptors (Bennet *et al.*, 1980), we investigated the influence of the TP-receptor antagonist SQ 29,548 given together with the EP_1-receptor antagonist SC 51089, each at a final bath concentration of 3 μM, on contractions induced by U 46619, 8-iso-PGE_2, 8-iso-$PGF_{2\alpha}$ and PGE_2.

Materials

8-iso-$PGF_{2\alpha}$, 8-iso-PGE_2, 9,11-dideoxy-9,11-methanoepoxy prostaglandin $F_{2\alpha}$ (U 46619), PGE_2 and SQ 29,548 were purchased from Cayman Chemical (Ann Arbor, MI, U.S.A.), SC 51089 and ICI 192,605 from BIOMOL Feinchemikalien GmbH (Hamburg, Germany). IsoPs, PGE_2, U 46619, SQ 29,548 and ICI 192,605 were dissolved in ethanol (stock solution) and SC 51089 in DMSO (25%). Further dilutions were made with 0.9% saline freshly before experiments.

The composition of Tyrode solution was (in mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.15, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, Glucose 5.6.

Data analysis

The data were plotted as the means $\pm$ s.e.mean of six experiments. Tension measured as gram (g) was converted to millinewton (mN). The negative logarithm of the concentration of agonist that caused 50% of the maximum contraction (pEC_{50}) was calculated for each concentration-response curve, using linear regression analysis after logit-log transformation for linearization of the curves (Griesbacher *et al.*, 1998; Tallarida & Murray, 1981). Potency of the four agonists used were estimated by comparison of the $pEC_{50} \pm$ s.e.mean and the slopes of the concentration-response curves. Slope values, which were calculated with GraphPAD InStat (GraphPAD, Software, Version 3.0, San Diego, CA, U.S.A.), are given with 95% confidence interval derived from linear regression analysis. For estimation of the antagonist affinity of SQ 29,548 and SC 51089 the concentration-response curves obtained during the influence of these antagonists were tested for deviation from parallelism (Geigy, 1982) and the horizontal distances in log units were used to construct a Schild plot (Arunlakshana & Schild, 1959). The pA_2 and Schild slope values as an estimate for the antagonist's affinity in the tissues used are given with 95% confidence interval derived from linear regression analysis.

Statistical analysis

Values are expressed as means $\pm$ s.e.mean. Statistical significance was calculated by a two way analysis of variance (ANOVA) followed by Dunnett multiple comparison test. $P<0.05$ was taken as significant and illustrated in the appropriate figures by an asterisk.

Results

Agonist potency

In the isolated rat aorta, the isoPs, 8-iso-PGE_2 and 8-iso-$PGF_{2\alpha}$ (Figures 1 and 2), the TXA_2-mimetic U 46619 (Figure 3) and PGE_2 (Figure 4) induced vasoconstrictions in a concentration-dependent manner. The following rank order of potency was determined by comparison of the pEC_{50} values (Table 1): U 46619 > 8-iso-PGE_2 > 8-iso-$PGF_{2\alpha}$ > PGE_2. The slopes of the concentration-response curves obtained for U 46619, 8-iso-PGE_2 and 8-iso-$PGF_{2\alpha}$ were nearly identical (Table 1). U 46619 showed a higher maximum response (E_{max}) than the isoPs at the maximum concentrations which were available (Figures 1, 2 and 3).

The vehicle (ethanol, final bath concentration 0.02% v v^{-1}) had no effect. Mean values of contractions (mN) were obtained from six experiments of each agonist used.

In the isolated rat gastric fundus, the agonists used induced contractions concentration-dependently (Figures 5 and 6). The following rank order of potency was determined by comparison of the pEC_{50} values (Table 1): PGE_2 > 8-iso-PGE_2 > U 46619 > 8-iso-$PGF_{2\alpha}$. 8-iso-PGE_2 showed a higher E_{max} than PGE_2. The slopes of the concentration-response curves obtained for U 46619 and 8-iso-$PGF_{2\alpha}$ were nearly identical, whereas that for 8-iso-PGE_2 was higher and that for PGE_2 lower (Table 1).

The vehicle (ethanol, final bath concentration 0.02% v v^{-1}) was without effect. Mean values of contractions (mN) were obtained from six experiments of each agonist used.

In the isolated guinea-pig ileum, PGE_2 and 8-iso-PGE_2 caused a concentration-dependent contraction, whereas U 46619 and 8-iso-$PGF_{2\alpha}$ had no effect (Figure 7). PGE_2 was

Table 1 pEC_{50} values and the slopes of the concentration-response curves obtained for U 46619, 8-iso-PGE_2, 8-iso-$PGF_{2\alpha}$ and PGE_2 in the rat aorta, rat fundus and guinea-pig ileum

	U 46619	*8-iso-PGE_2*	*8-iso-$PGF_{2\alpha}$*	*PGE_2*
Rat aorta				
pEC_{50}	9.7±0.08	9.2±0.06	8.7±0.07	8.3±0.08
Slope	1.5 (0.7–2.3)	1.5 (1.2–1.7)	1.6 (1.0–2.2)	0.7 (0.4–1.0)
Rat fundus				
pEC_{50}	9.0±0.05	9.6±0.06	8.6±0.08	10.4±0.05
Slope	10.9 (8.1–13.7)	13.9 (12.6–15.2)	10.3 (7.5–13.2)	8.3 (6.6–10.0)
Guinea-pig ileum				
pEC_{50}	no effect	9.4±0.09	no effect	10.8±0.06
Slope		1.5 (1.3–1.9)		1.6 (0.8–2.2)

pEC_{50} values are means±s.e.mean. 95% confidence interval shown in parentheses. $n=6$.

more potent than 8-iso-PGE_2, but the slopes of the concentration-response curves were nearly identical (Table 1). The vehicle (ethanol, final bath concentration 0.2% v v^{-1}) had no effect. Mean values of contractions (mN) were obtained from six experiments of each agonist used.

Influence of TP-receptor and EP_1-receptor antagonists

SQ 29,548 and ICI 192,605 The concentrations of SQ 29,548, which showed a significant inhibition, were extremely tissue-dependent, from 3 nM in the rat aorta to 3 μM in the rat fundus and without effect in the guinea-pig ileum.

In the isolated rat aorta, SQ 29,548 at a final bath concentration of 3, 10 and 30 nM inhibited the vasoconstrictions induced by 8-iso-PGE_2 (Figure 1), 8-iso-$PGF_{2\alpha}$ (Figure 2) and U 46619 (Figure 3) significantly and concentration-dependently. The weak vasoconstrictor effect induced by PGE_2 were also inhibited by SQ 29,548 (10 nM) significantly (Figure 4). ICI 192,605 at a concentration of 10 nM caused nearly the same shift to the right as SQ 29,548 (data not shown). Contractions induced by U 46610, 8-iso-PGE_2, 8-iso-$PGF_{2\alpha}$ and PGE_2 seem to be antagonized competitively by SQ 29,548; slopes of the Schild plots are not significantly different from unity (Table 2). The pA_2 values showed that the agonists were antagonized by SQ 29,548 in the same order of magnitude (Table 2). The slope parameters of the concentration-response curves in the presence of the antagonist were not significantly different from the slope of the control curves obtained by the agonists alone.

In the isolated rat gastric fundus, SQ 29,548 up to a concentration of 100 nM had no influence on the contractions induced by the agonists (data not shown). SQ 29,548 at concentrations of 0.3, 1 and 3 μM inhibited the contraction induced by 8-iso-$PGF_{2\alpha}$ and U 46619 (Figure 5A,B). A concentration of 3 μM inhibited the effects of 8-iso-PGE_2 significantly (Figure 6C). SQ 29,548 at a concentration of 0.3 μM inhibited the contraction induced by 8-iso-PGE_2 slightly but not significantly and at 1 μM significantly (data not shown). ICI 192,605 at a concentration of 3 μM caused nearly the same shift to the right as SQ 29,548 (data not shown). SQ 29,548 (3 μM) had no influence on the effects of PGE_2 (Figure 6C). It can be inferred that contractions induced by 8-iso-PGE_2 are antagonized by SQ 29,548 competitively, because the slope of the Schild plot was not significantly different from unity (Table 2).

On the one hand SQ 29,548 at 0.3 μM showed no inhibition of 8-iso-$PGF_{2\alpha}$ and U 46619 responses (Figure 5) and on the other hand at 3 μM the inhibition was so potent that EC_{50} values could not be calculated. Consequently, pA_2 values could not be estimated. The slope parameters of the concentrations-response curves in the presence of the antagonist were not

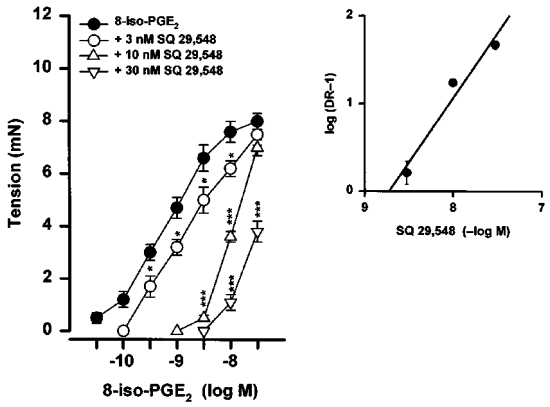

Figure 1 Contractions of the isolated rat aorta. Concentration-response curves to 8-iso-PGE_2 before and during SQ 29,548 at final bath concentrations of 3, 10 and 30 nM. The Schild plot of the antagonist data is shown in the right graph. Each symbol is the mean value of six experiments. Vertical bars represent s.e.mean. Significance of difference from controls: $*P<0.05$, $***P<0.001$.

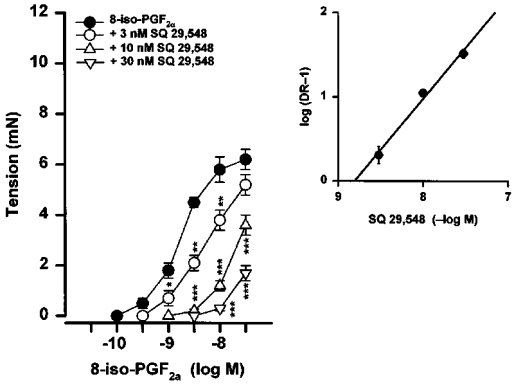

Figure 2 Contractions of the isolated rat aorta. Concentration-response curves to 8-iso-$PGF_{2\alpha}$ before and during SQ 29,548 at final bath concentrations of 3, 10 and 30 nM. The Schild plot of the antagonist data is shown in the right graph. Each symbol is the mean value of six experiments. Vertical bars represent s.e.mean. Significance of difference from controls: $*P<0.05$, $**P<0.01$, $***P<0.001$.

significantly different from the slope of the control curves obtained by 8-iso-PGE_2 alone.

In the guinea-pig ileum, SQ 29,548 at a concentration of 3 μM did not influence the contractions induced by 8-iso-PGE_2 (Figure 7). The vehicle used for SQ 29,548 (ethanol, final bath concentration 0.03% v v^{-1}) was without effect on contractions induced by the agonists used in all three isolated smooth

muscle preparations. By 1.5 h after application of SQ 29,548, the responsiveness to agonists of all organs used returned to values indistinguishable from controls after wash out.

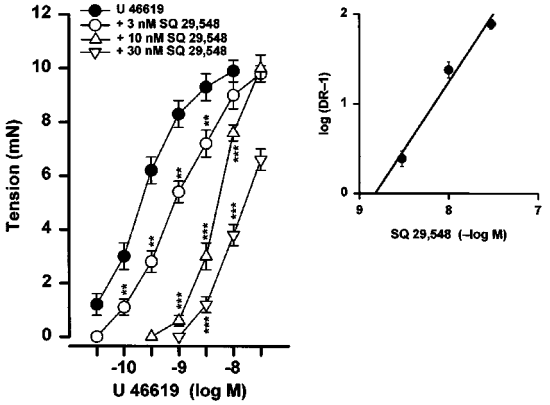

Figure 3 Contractions of the isolated rat aorta. Concentration-response curves to U 46619 before and during SQ 29,548 at final bath concentrations of 3, 10 and 30 nM. The Schild plot of the antagonist data is shown in the right graph. Each symbol is the mean value of six experiments. Vertical bars represent s.e.mean. Significance of difference from controls: $^{**}P<0.01$, $^{***}P<0.001$.

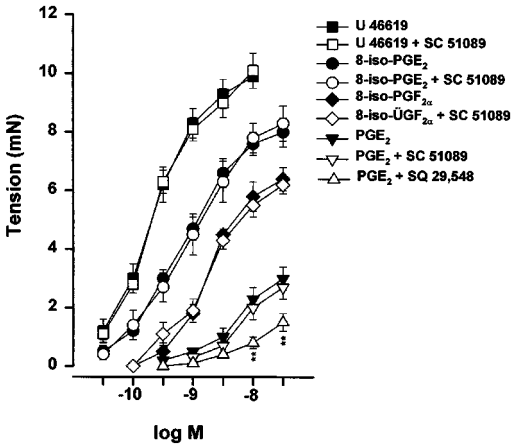

Figure 4 Contractions of the isolated rat aorta. Concentration-response curves to U 46619, 8-iso-PGE_2, 8-iso-$PGF_{2\alpha}$ before and during 3 μM SC 51089 and PGE_2 before and during 3 μM SC 51089 or 10 nM SQ 29,548 in the isolated rat aorta. Each symbol is the mean value of six experiments. Vertical bars represent s.e.mean. Significance of difference from controls: $^{**}P<0.01$.

SC 51089

In the rat aorta, SC 51089 at a final bath concentration of 3 μM caused no effect on vasoconstrictions induced by U 46619, 8-iso-PGE_2, 8-iso-$PGF_{2\alpha}$ and PGE_2 (Figure 4).

In the rat gastric fundus, SC 51089 at a concentration of 3 μM caused a slight but significant inhibition of contractions induced by 8-iso-PGE_2 and PGE_2 (Figure 6A). A concentration of 1 μM showed a slight but not significant inhibition (data not shown). SC 51089 (3 μM) had no influence on contractions induced by U 46619 and 8-iso-$PGF_{2\alpha}$ (Figure 6B). The inhibition of PGE_2 by SC 51089 at a concentration of 3 μM seems not to be competitive (Figure 6A).

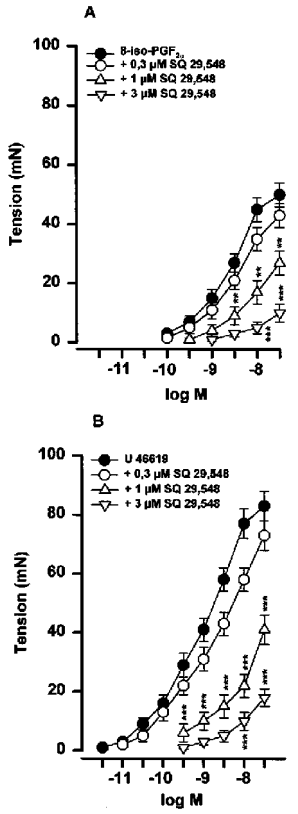

Figure 5 Contractions of the isolated rat gastric fundus. Concentration-response curves to (A) 8-iso-$PGF_{2\alpha}$ and to (B) U 46619 before and during SQ 29,548 at final bath concentrations of 0.3, 1 and 3 μM. Each symbol is the mean value of six experiments. Vertical bars represent s.e.mean. Significance of difference from controls: $^{**}P<0.01$, $^{***}P<0.001$.

Table 2 Affinity of SQ 29,548 and SC 51089 on contractions induced by U 46619, 8-iso-PGE_2, 8-iso-$PGF_{2\alpha}$ and PGE_2 in the isolated rat aorta, rat fundus and guinea-pig ileum

	SQ 29,548 *rat aorta*	*SQ 29,548* *rat aorta*	*SC 51089* *guinea-pig ileum*
U 46619			
pA_2	8.8 (8.5– 9.3)	>7.0	no effect of U 46619
Slope	1.5 (1.0–1.9)		
8-iso-PGE_2			
pA_2	8.7 (8.3–9.3)	6.5 (6.1–7.2)	6.7 (6.3–7.4)
Slope	1.5 (1.0–2.0)	1.4 (0.8–2.0)	1.2 (0.7–1.6)
8-iso-$PGF_{2\alpha}$			
pA_2	8.8 (8.5–9.3)	>7.0	no effect of 8-iso-$PGF_{2\alpha}$
Slope	1.2 (0.9–1.5)		
PGE_2			
pA_2	8.2 (7.7–9.2)	no effect	6.7 (6.2–7.9)
Slope	1.7 (0.7–2.7)		1.1 (0.5–1.7)

Values are shown as means. 95% confidence interval shown in parentheses. $n=6$.

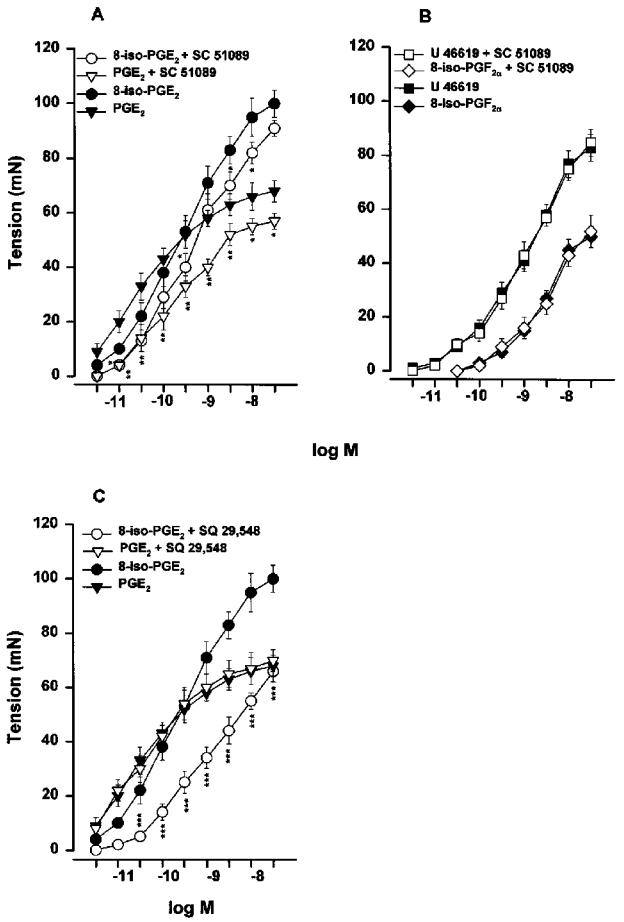

Figure 6 Contractions of the isolated rat gastric fundus. Concentration-response curves to (A) 8-iso-PGE_2 and PGE_2, (B) U 46619 and 8-iso-$PGF_{2\alpha}$ before and during 3 μM SC 51089 and to (C) 8-iso-PGE_2 and PGE_2 before and during 3 μM SQ 29,548. Each symbol is the mean value of six experiments. Vertical bars represent s.e.mean. Significance of difference from controls: $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

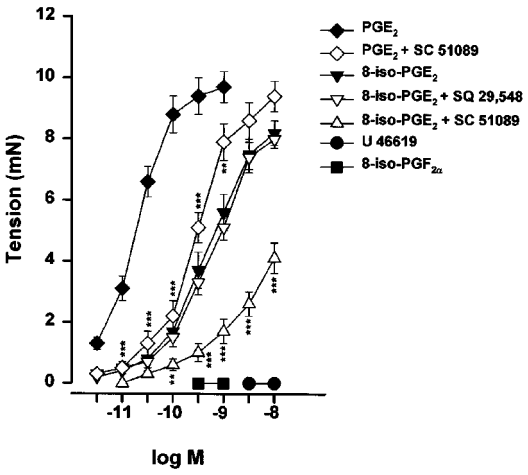

Figure 7 Contractions of the isolated guinea-pig ileum. Concentration-response curves to 8-iso-PGE_2 before and during 3 μM SQ 29,548 or during 3 μM SC 51089, and to PGE_2 before and during 3 μM SC 51089. U 46619 and 8-iso-$PGF_{2\alpha}$ showed no effects. Each symbol is the mean value of six experiments. Vertical bars represent s.e.mean. Significance of difference from controls: $^{**}P<0.01$, $^{***}P<0.001$.

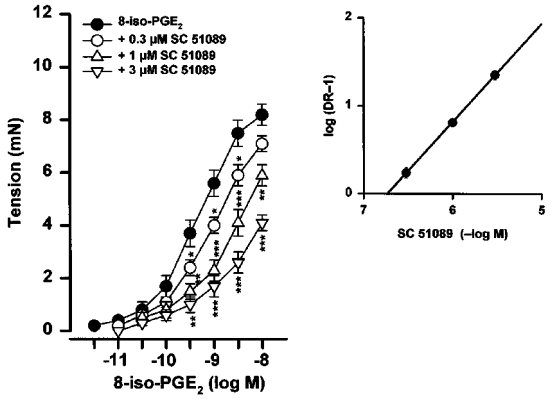

Figure 8 Contractions of the isolated guinea-pig ileum. Concentration-response curves to 8-iso-PGE_2 before and during SC 51089 at final bath concentrations of 0.3, 1 and 3 μM. The Schild plot of the antagonist data is shown in the right graph. Each symbol is the mean value of six experiments. Vertical bars represent s.e.mean. Significance of difference from controls: $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

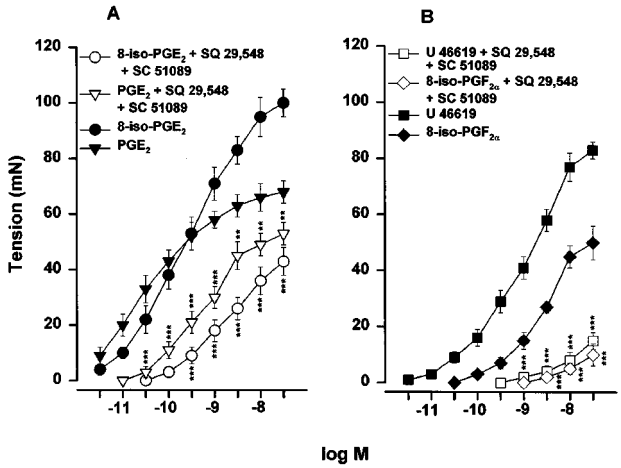

Figure 9 Contractions of the isolated rat gastric fundus. Concentration-response curves to (A) 8-iso-PGE_2 and PGE_2 before and during 3 μM SQ 29,548 together with 3 μM SC 51089 and to (B) U 46619 and 8-iso-$PGF_{2\alpha}$ before and during 3 μM SQ 29,548 together with 3 μM SC 51089. Each symbol is the mean value of six experiments. Vertical bars represent s.e.mean. Significance of difference from controls: $^{**}P<0.01$, $^{***}P<0.001$.

In the guinea-pig ileum, SC 51089 at a concentration of 0.3, 1 and 3 μM reduced the effects of 8-iso-PGE_2 significantly (Figure 8). SC 51089 at a concentration of 0.3 and 1 μM (data not shown) and 3 μM (Figure 7) inhibited contractions induced by PGE_2 significantly. In contrast to the rat fundus, the contractions induced by 8-iso-PGE_2 and PGE_2 were antagonized competitively by SC 51089; slopes of the Schild plots are not significantly different from unity (Table 2). The pA_2 value (6.7) determined for SC 51089 against PGE_2 was identical to that described in the product data sheet ($pA_2 = 6.5$). The slope parameters of the concentrations-response curves in the presence of the antagonist were not significantly different from the slope of the control curves obtained by the agonists alone. The vehicle used for SC 51089 (DMSO, final bath concentration 0.0075% v v^{-1}) was without effect on contractions induced by the agonists used in all

three isolated smooth muscle preparations. By 1.5 h after application of SC 51089, the responsiveness to agonists of all organs used returned to values indistinguishable from controls after wash out.

SQ 29,548 together with SC 51089 in the gastric fundus

SQ 29,548 (3 μM) given together with SC 51089 (3 μM) showed increased inhibitions of contractions induced by 8-iso-PGE_2 and PGE_2 compared with the inhibitory effects induced by the antagonists given alone (compare Figure 9A with 6A,C). The concentration-response curve of 8-iso-PGE_2 was shifted 1.4 log units to the right by SQ 29,548 (3 μM) given alone and 1.9 log units given together with SC 51089 (3 μM). The concentration-response curve of PGE_2 was shifted 1.0 log units to the right by SC 51089 (3 μM) given alone and 1.6 log units given together with SQ 29,548 (3 μM).

In contrast, SC 51089 (3 μM) did not amplify the inhibitory effect of SQ 29,548 at a concentration of 1 μM (data not shown) or of 3 μM (compare Figure 9B with 5A,B) on contractions induced by U 46619 and 8-iso-$PGF_{2\alpha}$.

Discussion

Initially, it was assumed that isoPs exert their vasoconstrictor effects by activation of the TP-receptor (Takahashi *et al*., 1992). However, it was shown that 8-iso-$PGF_{2\alpha}$ and 8-iso-PGE_2 are only weak agonists of platelet aggregation and acted more potently as antagonists of TP-receptor agonist-induced platelet aggregation (Morrow *et al*., 1992; Yin *et al*., 1994; Longmire *et al*., 1994). Receptor-binding studies led to the hypothesis that the isoPs caused their effects by activation of a unique isoP-receptor in vascular smooth muscle cells distinct from but structurally similar to TP-receptor (Fukunaga *et al*., 1993; 1997). However, all these investigations were performed either in platelets or in vascular smooth muscle preparations, in which TP-receptors are predominant (Eglen & Whiting, 1988). It could be shown that vasoconstrictions induced by PGE_2 and $PGF_{2\alpha}$ in vascular systems are also mediated by TP-receptors (Jones *et al*., 1982; Baxter *et al*., 1995; Amin *et al*., 1996). Therefore, the authors assume that the TP-receptor is the only prostanoid receptor in vascular smooth muscles, which is responsible for contractions induced by eicosanoids. The guinea-pig ileum has been suggested as a tissue which contains a preponderance of EP_1-receptors and has a lack of TP- and DP-receptors (Coleman *et al*., 1981; 1985; Coleman & Kennedy, 1985; Eglen & Whiting, 1988). It was also described that the rat gastric fundus contains a multiplicity of prostanoid receptor types, including TP-receptors as well as EP_1-receptors (Bennet *et al*., 1980). In the present study, the pEC_{50} values estimated for the agonists in the tissues used are comparable with that described in various studies (Zhang *et al*., 1996; Eglen & Whiting, 1988; Splawinski *et al*., 1973; Elmhurst *et al*., 1997).

Our results show different effectiveness of the agonists in each tissue used, which might be due to the different pattern of prostanoid receptors mentioned above. The results obtained with the TP-receptor antagonist, SQ 29,548 and ICI 192,605, and the EP_1-receptor antagonist SC 51089 confirmed these findings.

Although the potency of the isoPs in the rat aorta is weaker and also the E_{max} obtained by maximum available concentrations is lower than that of U 46619, the similarity in the pA_2 values determined for SQ 29,548 against U 46619 and the isoPs used in the rat aorta suggest that these agonists act *via* the same type of TP-receptor. The available concentrations of agonists were not always high enough to reach maximum responses at the high antagonist concentrations. This uncertainty should be taken into consideration for the interpretation of the pA_2 values. Nevertheless, it seems to be justified to compare the estimated pA_2 values with those described in the literature. The pA_2 value of 8.8 (8.5–9.3) against U 46619 lies between that described by Zhang *et al*. (1996) and Eglen & Whiting (1988) for SQ 29,548 against U 46619 (9.2 and 8.4, respectively). The pA_2 value of 8.8 (8.5–9.2) against 8-iso-$PGF_{2\alpha}$ is also in the range of that estimated by Zhang *et al*. (1996) which was 9.2 (9.0–9.4). Furthermore, these antagonistic effects of SQ 29,548 were competitive, because the slopes of the Schild plots were not significantly different from unity.

In the rat gastric fundus, the range of the antagonists concentrations, which showed inhibitory effects, was small (1 and 3 μM of SQ 29,548 on U 46619 and 8-iso-$PGF_{2\alpha}$ and 3 μM on 8-iso-PGE_2 contractions; 3 μM of SC 51089 on 8-iso-PGE_2 contractions). Therefore, we investigated the effects of SC 51089 given together with SQ 29,548. The results show that the EP_1-receptor antagonist SC 51089 enhanced the inhibitory effect of TP-receptor antagonist SQ 29,548 on contractions induced by 8-iso-PGE_2 but not that induced by U 46619 and 8-iso-$PGF_{2\alpha}$. This would imply the activation of both the TP-receptor and the EP_1-receptor by 8-iso-PGE_2 in contrast to the contractions of U 46619 and 8-iso-$PGF_{2\alpha}$ which seem to be mediated by activation of the TP-receptor alone.

The most interesting result of our study was that 8-iso-PGE_2 was able to induce contractions in the guinea-pig ileum and the rat fundus, which strongly suggests that it is able to activate the EP_1-receptor. This is supported by the observation that besides PGE_2, 8-iso-PGE_2 was the only one of the agonists used which caused a contraction in the guinea-pig ileum. These results confirm the findings of Elmhurst *et al*. (1997) that the effects of 8-iso-PGE_2 are mediated by activation of the EP-receptor as well as the TP-receptor in the canine colon. The pEC_{50} values for PGE_2 and 8-iso-PGE_2 in the colonic epithelium estimated by these authors were nearly identical with that obtained in the guinea-pig ileum in the present study. Additional confirmation was provided by the inhibitory effect of SC 51089 on contractions induced by 8-iso-PGE_2 in the guinea-pig ileum, in that identical pA_2 values were determined for SC 51089 against PGE_2 and 8-iso-PGE_2. The lack of 8-iso-$PGF_{2\alpha}$ induced effects in the guinea-pig ileum seems to exclude the existence of a unique isoP-receptor in this smooth muscle preparation. The similarity of a possible unique isoP-receptor with the TP-receptor, as it has been postulated by Fukunaga *et al*. (1993; 1997), makes a differentiation difficult as long as a selective isoP-receptor antagonist is not available. Therefore, it must be taken into consideration that the contracting effects of isoPs in the rat aorta and gastric fundus, which were inhibited by the two selective TP-receptor antagonists SQ 29,548 and ICI 192,605, are also more likely mediated by activation of the TP-receptor than a unique isoP-receptor.

Morrow & Roberts (1997) proposed that the stereochemistry of the side chains (cis-orientation) might be responsible for the vasoconstrictor actions of the isoPs. Recently, we found that the number of double bonds, in combination with the cis-orientation of the side chains, plays an important role in the vasoconstrictor effects of isoPs, because 8-iso-$PGF_{3\alpha}$ showed no and 8-iso-PGE_1 much weaker effects than 8-iso-PGE_2 and 8-iso-$PGF_{2\alpha}$ (Sametz *et al*., 1999). These structures seem to be important for the affinity of the isoPs to the TP-receptor.

In contrast, the results of the present study suggest that the ring structure of 8-iso-PGE_2 might be of priority for the affinity to the EP_1-receptor. Our data obtained in the rat fundus showed also that contractions induced by 8-iso-PGE_2, in contrast to PGE_2 contractions, were inhibited much less potently by the EP_1-receptor antagonist than by the TP-receptor antagonist. Furthermore, SC 51089 amplified the inhibition of 8-iso-PGE_2 induced by SQ 29,548 and on the other hand SQ 29,548 the inhibition of PGE_2 induced by SC 51089. For that reason, it seems that 8-iso-PGE_2 acts primarily *via* the TP-receptor and PGE_2 primarily *via* the EP_1-receptor, if both receptors are available.

In conclusion, our results show that 8-iso-PGE_2, in contrast to 8-iso-$PGF_{2\alpha}$, can also cause contractions by activation of the EP_1-receptors in the isolated rat gastric fundus and the isolated guinea-pig ileum. The findings of the present study do not imply the evidence for the existence of a unique isoP-receptor in the tissues used.

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