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Characterization of thromboxane A_2/prostaglandin endoperoxide receptors in aorta

Rongan Zhang, Martin L. Ogletree, Suzanne Moreland *

Department of Pharmacology, Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, NJ 08543, USA

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Abstract

Thromboxane A_2/prostaglandin endoperoxide receptor antagonists were studied in rat and guinea-pig aortas contracted with U-46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$) or 8-epi-prostaglandin $F_{2\alpha}$. In rat aorta, the antagonists competitively inhibited contractions evoked by either agonist with a rank order of potency as follows: BMS-180291 ([1s-(*exo,exo*)]-2-[[3-[4-[(pentylamino)carbonyl]-2-oxazolyl]-7-oxabicyclo[2.2.1]hept-2-yl]methyl]-benzenepropanoic acid) $\geq$ SQ 29,548 ([1s-[1α,2β-(5z),3β,4α)]-7-[3-[[2-[(phenylamino)carbonyl]hydrozino]methyl]-7-oxobicyclo-[2.2.1]hept-2-yl]-5-heptanoic acid) > daltroban (4-[2-(4-chlorobenzenesulfonylamino) ethyl]-benzene acetic acid) $\geq$ SQ 30,741 ([1s-[1β,2α(5z),3α,4β]]-7-[3-[[[[(oxa)amino]acetyl] amino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl-5-heptanoic acid) = AA-2414 (2,4,5-trimethyl-3,6-dioxo-ζ-phenyl-1,4-cyclohexadien-1-heptanoic acid). In guinea-pig aorta, the antagonists competitively antagonized contractions elicited by either agonist with the following rank order of potency: SQ 29,548 = AA-2414 $\geq$ SQ 30,741 > daltroban. Antagonism by BMS-180291 in guinea-pig aorta was not strictly competitive. These findings indicate that thromboxane A_2/prostaglandin endoperoxide receptors in rat aortas are different from those in guinea pigs. Because the actions of both agonists were equivalently antagonized by each of the antagonists in both rat and guinea-pig aortas, the results do not support the hypothesis that U-46619 and 8-epi-prostaglandin $F_{2\alpha}$ elicit contractions via different receptor subtypes in the aorta.

Keywords: Thromboxane; Smooth muscle, vascular; U-46619; 8-epi-prostaglandin $F_{2\alpha}$; BMS-180291; SQ 29,548; Daltroban; BM-13505; SQ 30,741; AA-2414

1. Introduction

Thromboxane A_2 and prostaglandin endoperoxides are vasoactive, lipid-derived mediators that are formed primarily via cyclooxygenase in response to activation of numerous cell types by a variety of stimuli. They induce platelet aggregation and smooth muscle contraction via the activation of specific thromboxane A_2/prostaglandin endoperoxide receptors. Isoprostanes, such as 8-epi-prostaglandin $F_{2\alpha}$, are thromboxane A_2/prostaglandin endoperoxide receptor agonists in smooth muscle, but they are produced independent of cyclooxygenase by a mechanism involving free radical attack on phospholipids (Morrow et al., 1990; Morrow et al., 1992). As such, their synthesis is not inhibited by aspirin-like drugs, in contrast to cyclooxygenase-derived thromboxane A_2 and prostaglandin endoperoxides. Pharmacological studies of thromboxane A_2/prostaglandin endoperoxide receptors using vascular smooth muscles and platelets from different species have raised questions regarding the relevance of interspecies differences and/or receptor subtypes (Coleman et al., 1981; Furci et al., 1991; Mais et al., 1985; Schror, 1993; Swayne et al., 1988). Ogletree and Allen (1992) concluded that rat and guinea-pig thromboxane A_2/prostaglandin endoperoxide receptors in smooth muscle are pharmacologically different. More recently, evidence has emerged suggesting the existence in rat aortic smooth muscle cells of isoprostane receptors that are different from thromboxane A_2/prostaglandin endoperoxide receptors (Fukunaga et al., 1993). The present study was designed to compare the responses of aortic rings from rats and guinea pigs using the agonists, U-46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$; Bundy, 1975) and 8-epi-prostaglandin $F_{2\alpha}$ (Morrow et al., 1990), and five structurally different thromboxane A_2/prostaglandin endoperoxide receptor antagonists, AA-2414 (2,4,5-trimethyl-3,6-dioxo-ζ-phenyl-1,4-cyclohexadien-1-heptanoic acid;

* Corresponding author. Tel.: (1-609) 252-5396; Fax: (1-609) 252-6607.

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Ashida et al., 1989), daltroban (4-[2-(4-chlorobenzenesulfonylamino)ethyl]-benzene acetic acid; BM-13505; Stegmeier et al., 1983), BMS-180291 ([1s-(exo,exo)]-2-[[3-[4-[(pentylamino)carbonyl]-2-oxazolyl]-7-oxabicyclo[2.2.1]-hept-2-yl]methyl]-benzenepropanoic acid; Ogletree et al., 1993), SQ 29,548 ([1s-[1α,2β-(5z),3β,4α)]-7-[3-[[2-[(phenylamino)carbonyl]hydrozino]methyl]-7-oxobicyclo-[2.2.1]hept-2-yl]-5-heptanoic acid; Ogletree et al., 1985), and SQ 30,741 ([1s-[1β,2α(5z),3α,4β]]-7-[3-[[[[(oxa)-amino]acetyl]amino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl-5-heptanoic acid; Ogletree et al., 1986). The rank orders of potency of the five antagonists were compared in search of evidence for distinct isoprostane receptors or thromboxane A_2/prostaglandin endoperoxide receptor subtypes in the aortas of the two species.

2. Materials and methods

2.1. Tissue preparation

Male Sprague-Dawley rats weighing 225–250 g and male Hartley guinea pigs weighing 450–500 g were killed by inhalation of CO_2. The thoracic aortas were quickly removed and placed in a petri dish containing warm physiological salt solution. Adherent connective tissue was carefully removed and rings of 5 mm width were cut. The endothelium was left intact. Each ring was mounted in a 10 ml water-jacketed tissue bath for isometric force recording using two self-closure stainless steel hooks; one was connected to a micrometer for adjusting tissue length and the other to a Grass FT03 force transducer for force measurement. A multi-channel polygraph (Grass Model 7D) was used to monitor mechanical responses of the tissues. All tissue baths were filled with physiological salt solution warmed to 37°C and aerated with 5% CO_2 in O_2 to maintain a pH of 7.4. The physiological salt solution was of the following composition, in mM: 118.4 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.9 $CaCl_2$, 25.0 $NaHCO_3$ and 10.1 D-glucose.

2.1.1. Test compounds

Stock solutions of 10 mM of AA-2414, daltroban, BMS-180291, SQ 29,548 and SQ 30,741 were prepared daily in 100% dimethyl sulfoxide. Subsequent concentrations of the compounds were diluted in water or dimethyl sulfoxide. U-46619 and 8-epi-prostaglandin $f_{2\alpha}$ were prepared in 95% ethanol as 10 mM stocks and diluted in water. The highest concentration of dimethyl sulfoxide (0.03%) was also present in the vehicle control tissues (without antagonist).

2.2. Experiment protocol

Aortic rings were gradually stretched to 1 (guinea pig) or 2 (rat) g preload over an equilibration period of 60 min. The rings were then contracted with 50 mM KCl to test for viability. The tissues were washed 3 times with fresh physiological salt solution while force returned to baseline. Rings were equilibrated for another 30 min during which time the preload was maintained by adjusting the tissue length.

For some rings, cumulative concentration–response curves to half-log concentration increments of U-46619 were obtained starting at 1 nM. At each concentration, the force was allowed to plateau before the addition of the next concentration. Once the rings reached maximal force, they were washed 4–5 times with warm physiological salt solution until they relaxed to baseline. The process took approximately 90–120 min. The rings were then divided into 4 or 5 groups of 4 rings each. A single concentration of antagonist was added to each group and allowed a 20 min incubation period before repeating the U-46619 concentration–response curve.

Attempts were made to study the effects of the thromboxane A_2/prostaglandin endoperoxide receptor antagonists on contractions elicited by 8-epi-prostaglandin $F_{2\alpha}$ using a protocol identical to that described for U-46619. In preliminary experiments in rat aortas, however, during the second 8-epi-prostaglandin $F_{2\alpha}$ concentration–response determination, numerous spontaneous phasic contractions were superimposed on the more slowly developing tonic contractions. Thus, for rings contracted with 8-epi-prostaglandin $F_{2\alpha}$, a single concentration of antagonist or vehicle was incubated with a group of 4 rings for 20 min before concentration–response relationships for 8-epi-prostaglandin $F_{2\alpha}$ were obtained. The modification of the protocol was used for 8-epi-prostaglandin $F_{2\alpha}$ contractions of both rat and guinea-pig aortas.

2.3. Data analysis

The data were plotted as the mean ± S.E.M. of at least 4 rings from different animals. Force was expressed as g. The EC_{50}, the concentration of agonist that caused 50% of the maximum contraction, was calculated from the equation for the line constructed by connecting the two adjacent points containing 50% of the maximum contraction in the concentration–response curve. The dissociation constant K_B was calculated from the following equation: $K_B = [\text{antagonist}]/[(EC'_{50}/EC_{50}) - 1]$ Here the EC'_{50} and EC_{50} were calculated from concentration–response curves in the presence of the antagonist or vehicle, respectively. The EC_{50} and K_B values were reported as geometric means. Schild analysis was used to yield pA_2 values and slopes of the Schild plots. The 95% confidence intervals of the EC_{50}, K_B, and pA_2 values, as well as the slope, were calculated as described by Tallarida and Jacob (1979). Rank orders of potency of the five thromboxane A_2/prostaglandin endoperoxide receptor antagonists in the aortas of each species contracted with either U-46619 or 8-epi-prostaglandin $F_{2\alpha}$ were determined by comparison of the pA_2 values and 95% confidence intervals.

3. Results

U-46619 (1–300 nM) caused concentration-dependent contractions of rat aortic rings; the EC_{50} value was 8.0 nM (95% CI = 7.5–8.4 nM, $n = 76$) and the maximum response was $6.5 \pm 0.09\,g$. The isoprostane, 8-epi-prostaglandin $F_{2\alpha}$ (0.1–30 μM), also caused concentration-dependent contractions in rat aortic rings with an EC_{50} value of 1.2 μM (95% CI = 1.0–1.5 μM, $n = 20$) and maximal force of $6.2 \pm 0.21\,g$. The efficacy of 8-epi-prostaglandin $F_{2\alpha}$ was comparable to that of U-46619, but the potency of 8-epi-prostaglandin $F_{2\alpha}$ was 150-fold less than U-46619. Pretreatment of the rat aortic rings with each of five thromboxane A_2/prostaglandin endoperoxide receptor antagonists caused parallel rightward shifts in the concentration–response curves for both U-46619 and 8-epi-prostaglandin $F_{2\alpha}$. The antagonism was competitive and reversible; slopes of the Schild plots were not significantly different from unity (Table 1). The rank order of potency for the five antagonists was as follows: BMS-180291 ≥ SQ 29,548 > daltroban ≥ SQ 30,741 = AA-2414.

U-46619 (1–300 nM) contracted the guinea-pig aortic rings in a potent and concentration-dependent manner with an EC_{50} value of 10 nM (95% CI = 9.9–11 nM, $n = 79$) and maximal force of $7.8 \pm 0.20\,g$. 8-epi-prostaglandin $F_{2\alpha}$ also caused concentration-dependent contractions of guinea-pig aortas; the EC_{50} value was 2.0 μM (95% CI = 1.7–2.4 μM, $n = 20$) and the maximal force was $6.0 \pm 0.22\,g$. Thus, 8-epi-prostaglandin $F_{2\alpha}$ was somewhat less efficacious and 200-fold less potent than U-46619 in the guinea-pig aorta. Among the five thromboxane A_2/prostaglandin endoperoxide receptor antagonists, AA-2414, daltroban, SQ 29,548, and SQ 30,741 caused parallel rightward shifts in both the U-46619 and 8-epi-prostaglandin $F_{2\alpha}$ concentration–response curves (Fig. 1). The rank order of potency was: SQ 29,548 = AA-2414 ≥ SQ 30,741 > daltroban. Table 1 summarizes the values for pA_2, K_B, and slope from the Schild analysis for the compounds.

The antagonism of contractions induced by U-46619 in guinea-pig aorta by BMS-180291 was different from the other four antagonists. BMS-180291 caused antagonism of U-46619-evoked contraction in guinea-pig aorta that was not strictly competitive, i.e., slope > unity; thus, the potency of the compound was difficult to quantitate. As shown in Fig. 1, at 0.1 nM and 0.3 nM, BMS-180291

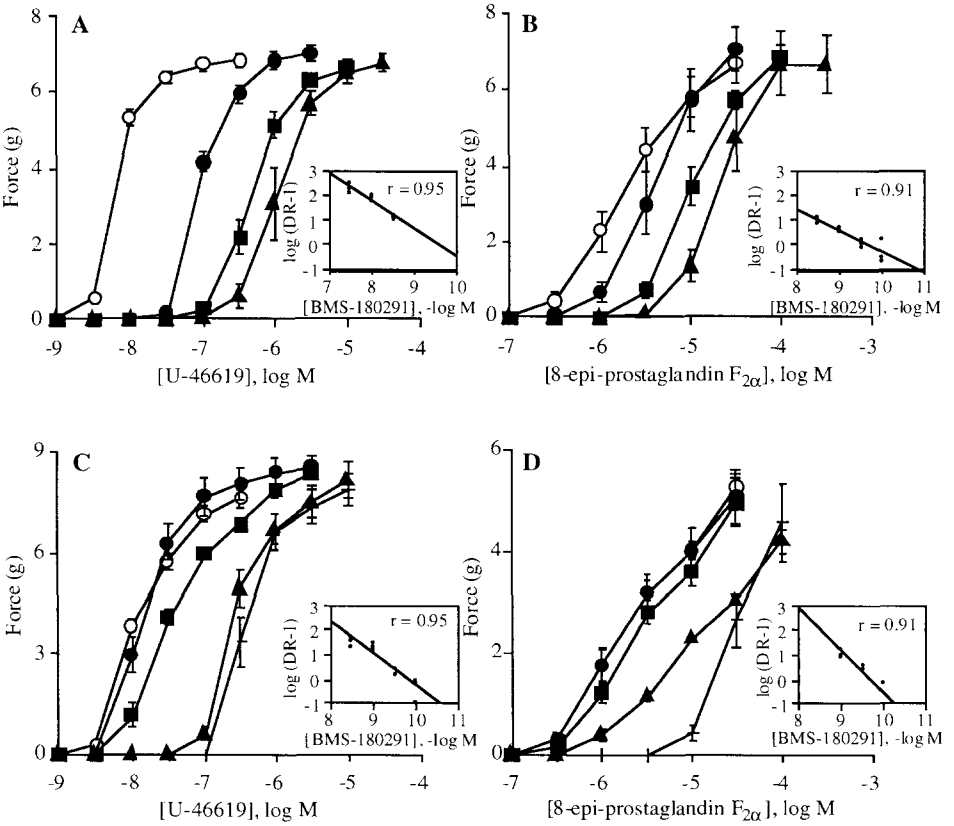

Fig. 1. Antagonism by BMS-180291 of cumulative concentration–response curves in rat and guinea-pig aorta. Curves were obtained in the presence of vehicle (open circles, $n = 16$–20) and 0.1 (filled circles, $n = 4$), 0.3 (filled squares, $n = 4$), 1 (filled triangles, $n = 4$), and 3 nM (crosses, $n = 4$) BMS-180291. Schild plots of the data are shown in the inset; DR represents the dose ratio of the EC_{50} values. (A) Concentration–response curves for U-46619 in rat aorta. (B) Concentration–response curves for 8-epi-prostaglandin $F_{2\alpha}$ in rat aorta. (C) Concentration–response curves for U-46619 in guinea-pig aorta. (D) Concentration–response curves for 8-epi- prostaglandin $F_{2\alpha}$ in guinea-pig aorta.

Table 1
Effects of AA-2414, daltroban, BMS-180291, SQ 29,548, and SQ 30,741 on contractions in rat and guinea-pig aortic rings elicited by U-46619 and 8-epi-prostaglandin $F_{2\alpha}$

	AA-2414	Daltroban	BMS-180291	SQ 29,548	SQ 30,741
Rat					
U-46619					
pA_2	7.8 (7.8–7.9)	8.2 (8.0–8.5)	9.5 (9.2–9.9)	9.2 (8.9–9.4)	8.2 (7.9–8.5)
Slope	0.96 (0.90–1.02)	1.08 (0.87–1.28)	1.12 (0.87–1.37)	0.97 (0.83–1.11)	0.87 (0.66–1.07)
K_B	16 (15–17)	4.9 (3.8–6.3)	0.19 (0.15–0.24)	0.78 (0.66–0.93)	8.6 (7.0–10)
8-epi-prostaglandin $F_{2\alpha}$					
pA_2	7.9 (7.7–8.1)	8.3 (8.1–8.4)	9.7 (9.5–9.9)	9.2 (9.0–9.4)	7.9 (7.7–8.0)
Slope	0.89(0.78–1.00)	0.89 (0.62–1.15)	0.85 (0.63–1.08)	0.86 (0.72–1.00)	0.86 (0.68–1.04)
K_B	17 (15–20)	5.8 (4.6–7.5)	0.23 (0.17–0.31)	0.82 (0.67–1.0)	17 (13–22)
Guinea pig					
U-46619					
pA_2	8.4 (8.3–8.5)	7.3 (7.1–7.4)	9.8 (9.7–10)	8.5 (8.4–8.6)	8.3 (8.1–8.5)
Slope	1.00 (0.91–1.08)	1.04 (0.89–1.20)	1.32 (1.06–1.58)	1.11 (0.98–1.24)	0.93 (0.82–1.04)
K_B	3.9 (3.5–4.4)	52 (43–64)	—	2.9 (2.4–3.4)	7.0 (6.1–8.2)
8-epi-prostaglandin $F_{2\alpha}$					
pA_2	8.5 (8.4–8.7)	7.3 (7.2–7.5)	9.7 (9.5–9.8)	8.9 (8.5–9.4)	8.1 (7.9–8.3)
Slope	0.87 (0.63–1.12)	0.98 (0.80–1.15)	1.75 (1.18–2.31)	0.73 (0.47–0.99)	0.95 (0.78–1.13)
K_B	3.9 (3.1–4.8)	48 (39–59)	—	2.4 (1.7–3.5)	8.8 (7.1–11)

Data are shown as mean, 95% confidence interval shown in parentheses. K_B values are in nM.

displayed little inhibitory effect on U-46619-induced contractions in the guinea-pig aortic rings. Increasing the concentration of BMS-180291 to 1 nM caused a maximal > 10-fold shift in the concentration–response curve. A higher concentration of BMS-180291 (3 nM) produced essentially no additional shift in the concentration–response relationship. One possible explanation for this finding is that the tissues were not exposed to BMS-180291 long enough to reach equilibrium before the U-46619 concentration–response curves were begun. The experiment was repeated using a 90 min, rather than 20 min, equilibration time, but this modification did not alter the result. Additionally, neither 10 nor 100 nM SQ 29,548 (K_B = 2.9 nM) was able to further shift the concentration–response curves elicited by U-46619 in the presence of 3 nM BMS-180291. As with U-46619, BMS-180291 antagonized, but not in a classic competitive manner, the 8-epi-prostaglandin $F_{2\alpha}$-induced contractions in guinea-pig aorta (Fig. 1). Values of pA_2, K_B, and slope from the Schild analysis are summarized in Table 1.

4. Discussion

The present study compared activities of five structurally different thromboxane A_2/prostaglandin endoperoxide receptor antagonists on U-46619- and 8-epi-prostaglandin $F_{2\alpha}$-induced constrictor responses in aortic rings from rat and guinea pig. U-46619, a stable analog of prostaglandin H_2 and a thromboxane A_2/prostaglandin endoperoxide receptor agonist, caused concentration-dependent contractions in the aortas from both species with equal potency. 8-epi-prostaglandin $F_{2\alpha}$, an F_2-isoprostane, also contracted aortas of both species in a concentration-dependent fashion, but with significantly less potency than U-46619. The difference in potency between U-46619 and 8-epi-prostaglandin $F_{2\alpha}$ in rat and guinea-pig aortas, 150- and 200-fold respectively, is approximately 10-fold greater than that reported previously (Ogletree, 1992) for these agonists in bovine coronary arteries (EC_{50}: 4 nM U-46619 and 58 nM 8-epi-prostaglandin $F_{2\alpha}$, agonistic potency ratio: 15-fold). U-46619 and 8-epi-prostaglandin $F_{2\alpha}$ produced equivalent maximal force in rat aortas, indicating comparable levels of efficacy. In guinea-pig aortas U-46619 produced significantly greater maximal force than 8-epi-prostaglandin $F_{2\alpha}$, suggesting that 8-epi-prostaglandin $F_{2\alpha}$ may not be a full agonist in this tissue. Substantial differences in agonist potency ratio for U-46619 and 8-epi-prostaglandin $F_{2\alpha}$ were found when comparing contractile responses in vascular tissue from different species. Although the more pronounced differences were observed in separate studies, it may be argued that the different agonist potency ratios reflect differences in thromboxane A_2/prostaglandin endoperoxide receptor molecular structure in vascular smooth muscle from bovine coronary arteries, rat aortas and guinea-pig aortas. It is also possible that intact endothelium on aortas in the present study and the lack of intact endothelium on spirals of bovine coronary artery in the prior study could have contributed to differences in ratios of net contractile agonist potency. In this regard, it is reasonable to expect that activation of endothelium might be affected by thromboxane

A_2/prostaglandin endoperoxide receptor agonists differently than contraction of smooth muscle or activation of platelets, because there is also evidence for thromboxane A_2/prostaglandin endoperoxide receptor subtypes in these different cell types (Furci et al., 1991; Mais et al., 1985; Raychowdhury et al., 1994).

In rat aortas, AA-2414, daltroban, BMS-180291, SQ 29,548, and SQ 30,741 were competitive antagonists of U-46619- and 8-epi-prostaglandin $F_{2\alpha}$-induced contractions with the same rank orders of potency: BMS-180291 $\geq$ SQ 29,548 > daltroban $\geq$ SQ 30,741 = AA-2414. In guinea-pig aortas, AA-2414, daltroban, SQ 29,548, and SQ 30,741 competitively antagonized U-46619- and 8-epi-prostaglandin $F_{2\alpha}$-induced contractions with a different rank order of potency: SQ 29,548 = AA-2414 $\geq$ SQ 30,741 > daltroban. Slopes of the Schild plots for antagonism of U-46619- and 8-epi-prostaglandin $F_{2\alpha}$-elicited guinea-pig aortic contractions by BMS-180291 were significantly different from unity, indicating that the compound does not behave as a strictly competitive antagonist in the guinea pig; this is different from its competitive antagonism in the rat. It is, nevertheless, obvious on inspection that BMS-180291 was the most potent antagonist tested against U-46619 and 8-epi-prostaglandin $F_{2\alpha}$ in guinea pig and rat aortas. Therefore, the ranking of antagonist potencies in rat and guinea-pig aortas differed primarily in the potency of AA-2414 relative to daltroban against both U-46619 and 8-epi-prostaglandin $F_{2\alpha}$. In the rat AA-2414 and SQ 30,741 tied for the least active, but in the guinea pig daltroban was considerably less active than any of the other antagonists tested. These findings confirm that the thromboxane A_2/prostaglandin endoperoxide receptors in rat aortas are different from those in guinea-pig aortas (Ogletree and Allen, 1992), which is consistent with significant interspecies differences in thromboxane A_2/prostaglandin endoperoxide receptor pharmacology.

The unusual nature of BMS-180291 antagonism was also reported by Ogletree et al. (1993) in guinea-pig tracheal rings which contain pharmacologically identical thromboxane A_2/prostaglandin endoperoxide receptors to those in the guinea-pig aorta (Ogletree and Allen, 1992). Responses to higher concentrations of both U-46619 and 8-epi-prostaglandin $F_{2\alpha}$ in guinea-pig aortas involved receptors that were not antagonized by BMS-180291. This suggests involvement of either more than one thromboxane A_2/prostaglandin endoperoxide receptor subtype or activation by the agonists of contraction-linked receptors distinct from thromboxane A_2/prostaglandin endoperoxide receptors. In anecdotal experiments, the U-46619-induced contraction that was resistant to 3 nM BMS-180291 was not blocked by addition of 100 nM SQ 29,548. This finding argues for activation by U-46619 of both a thromboxane A_2/prostaglandin endoperoxide receptor and one or more contraction-linked, non-thromboxane A_2/prostaglandin endoperoxide receptors in guinea-pig smooth muscles. The reason for the different nature of antagonism by BMS-180291 in guinea-pig tissues is not clear. As suggested by Ogletree et al. (1993), it may be related to the apparently very slow dissociation of BMS-180291 from its receptors in guinea-pig smooth muscle.

The potencies determined in the present study of the thromboxane A_2/prostaglandin endoperoxide antagonists daltroban, SQ 29,548, and SQ 30,741, as well as the agonist U-46619, in rat and guinea-pig aortas are comparable to those previously reported by Ogletree and Allen (1992). The similarity of the results highlights the reproducibility of Schild analysis for the ranking of thromboxane A_2/prostaglandin endoperoxide receptor antagonists. It should be noted that U-46619 is known to possess weak activity as an agonist at F_2-isoprostane receptors in the dog iris, which is an activity not shared by 8-epi-prostaglandin $F_{2\alpha}$ (Ogletree, 1992). Furthermore, high concentrations of U-46619 may activate adenylate cyclase, but it is not clear whether 8-epi-prostaglandin $F_{2\alpha}$ produces similar effects.

The lesser potency of 8-epi-prostaglandin $F_{2\alpha}$ in the rat aorta seems contrary to previous findings by Morrow et al. (1990) and Takahashi et al. (1992) that 8-epi-prostaglandin $F_{2\alpha}$ is an extremely potent renal vasoconstrictor in rats. Renal arteries were not included in the present study; thus we cannot directly address this issue. More recently, Fukunaga et al. (1993) found that the K_i for inhibition of [^{3}H]SQ 29,548 binding to rat aortic smooth muscle cells by 8-epi-prostaglandin $F_{2\alpha}$ was 16-fold higher than that by U-46619. However, they also presented evidence suggesting the existence of distinct, high affinity F_2-isoprostane receptors in cultured rat aortic smooth muscle cells. Employing inositol 1,4,5-triphosphate production and [^{3}H]thymidine incorporation as measures of functional responses in cultured cells, these investigators found significant responses to 8-epi-prostaglandin $F_{2\alpha}$ at concentrations as low as 1 nM and 100 nM, respectively. U-46619 at 100 nM produced only a small effect on inositol 1,4,5-triphosphate and no effect on DNA synthesis, suggesting that the measured effects of 8-epi-prostaglandin $F_{2\alpha}$ were possibly independent of thromboxane A_2/prostaglandin endoperoxide receptors. Because the proliferative and secretory phenotype of cultured rat aortic smooth muscle cells is different from the contractile smooth muscle cells in fresh aortic rings, many additional explanations for the findings in cultured cells should be explored.

We found no evidence for a distinct, contraction-linked F_2-isoprostane receptor that could be pharmacologically differentiated from the thromboxane A_2/prostaglandin endoperoxide receptor. In the present study, effects of five structurally different antagonists were evaluated in U-46619- and 8-epi-prostaglandin $F_{2\alpha}$-contracted aortas from rats and guinea pigs. In rat aortas, each thromboxane A_2/prostaglandin endoperoxide antagonist yielded almost identical pA_2 values for antagonizing U-46619 and 8-epi-prostaglandin $F_{2\alpha}$-elicited contractions, indicating essentially identical thromboxane A_2/prostaglandin endoperoxide receptors were involved in mediating vasoconstrictor

responses of the agonists. The same conclusion can be reached in guinea-pig aortas treated with these thromboxane A_2/prostaglandin endoperoxide receptor antagonists. Because the actions of U-46619 and 8-epi-prostaglandin $F_{2\alpha}$ were equally antagonized by each of the five antagonists in rat and guinea-pig aortas, the results from this study do not support the hypothesis that U-46619 and 8-epi-prostaglandin $F_{2\alpha}$ contract aortic smooth muscle via different thromboxane A_2/prostaglandin endoperoxide receptor subtypes.

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