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Individual variations of prostanoid agonist responses in rabbit aorta: evidence for the independent regulation of prostanoid receptor subtypes

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1 The frequency and selectivity of individual variations of prostanoid agonist responses in aortic strips from a population of male rabbits was studied. Three levels of responsiveness to the thromboxane mimetic U46619 occurred: responders (R), intermediate responders (IR), and non-responders (NR). R could be subdivided into R_1 and R_2 based on an enhanced potency of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in R_2. In the total population ($n = 92$), the phenotype frequency was: R, 69%; IR, 11%; and NR, 20%. In a subgroup of this population in which R_1 and R_2 phenotypes were determined ($n = 63$), the phenotype frequency was: R_1, 54%; R_2, 19%; IR, 6%; and NR, 21%.

2 The four rabbit aorta phenotypes, R_1, R_2, IR, and NR, were characterized with respect to the rank orders of prostanoid agonist potency, agonist intrinsic activities, and the effects of the thromboxane receptor antagonist SQ29548. The rank order of prostanoid agonist potency was U46619 > $PGF_{2\alpha}$ > PGE_2 in R_1 and R_2, and $PGF_{2\alpha}$ > PGE_2 > U46619 in IR and NR. For each prostanoid agonist, the intrinsic activity was highest in R ($R_1 \simeq R_2$), intermediate in IR, and lowest in NR. In R_1, SQ29548 inhibited all prostanoid agonist responses equally. The contractile effects of $PGF_{2\alpha}$ and PGE_2 were partially resistant to inhibition by SQ29548 in R_2. Prostanoid agonist responses were not inhibited by SQ29548 in IR.

3 The potency of histamine was equivalent in R_1, R_2, IR, and NR.

4 It is concluded that there are individual variations in the functional expression of thromboxane receptor sensitivity, i.e., prostanoid agonist responses inhibited by SQ29548. Also, there are individual variations in the functional expression of the sensitivity of a non-thromboxane receptor or receptors, i.e., prostanoid agonist responses not inhibited by SQ29548. It has been proposed by others that prostanoid receptors be termed P-receptors and that the prostanoid agonist to which they are most sensitive be indicated by a preceding letter, e.g., TP- for thromboxane receptor and FP- for $PGF_{2\alpha}$-selective receptor. Accordingly, we proposed a working hypothesis that suggests the four phenotypes could result from the independent regulation of the functional expression of TP- and FP-receptor subtypes with (a) R_2 containing both the TP- and FP-receptor subtypes in a fully functional state; (b) R_1 containing only the functional TP-receptor; (c) IR containing only the functional FP-receptor; and (d) NR containing only a low efficacy FP-receptor system.

5 The mechanisms underlying the observed individual variations are unknown but could include changes in receptor number or affinity, changes in receptor-effector coupling, changes in a second messenger system, or changes in tissue degradative or uptake processes. Further study is needed to differentiate between these possibilities.

Introduction

The rabbit aorta exhibits a relatively greater sensitivity to the contractile effects of the prostaglandin endoperoxides, prostaglandin G_2 (PGG_2) and PGH_2, and to thromboxane A_2 (TxA_2) than to other prostaglandins (Bunting *et al.*, 1976a; Gryglewski *et al.*, 1976; Coleman *et al.*, 1980; Vane, 1983). This tissue is also relatively resistant to the smooth muscle relaxant effects of PGE_2 and PGI_2 (Bunting *et al.*, 1976b; Forstermann *et al.*, 1984). These properties enabled Piper & Vane (1969) to characterize rabbit aorta contracting substance (RCS) as an unstable biological activity released during anaphylaxis in isolated lungs from sensitized guinea-pigs. The pharmacological characteristics of RCS and TxA_2 were very similar,

and both had short half-lives in solution (approximately 30 s), suggesting that RCS consisted primarily of TxA_2 and not the prostaglandin endoperoxides which have half-lives in solution of approximately 5 min (Hamberg *et al.*, 1974; 1975; Svensson *et al.*, 1975; Needleman *et al.*, 1976).

It is generally believed that the prostaglandin endoperoxides and TxA_2 produce vascular smooth muscle contraction by interacting with the same receptor, a putative TxA_2/PGH_2 receptor (Lefer, 1985; Burch *et al.*, 1985). The study of this receptor has been complicated by the chemical instability of the natural agonists. This problem has been overcome, however, by using chemically stable analogues. One such compound, U46619, is a stable analogue of PGH_2 (Bundy, 1975) that exhibits a pharmacological profile of activity that closely resembles that of TxA_2 (Coleman *et al.*, 1981). Inasmuch as TxA_2 has been identified as the active component of RCS, it was surprising to find in preliminary experiments that some rabbit aortic strips did not respond to the TxA_2-mimetic, U46619, even though they were responsive to other contractile agonists, e.g., noradrenaline and KCl (unpublished observations). Additional preliminary studies demonstrated that four distinct levels of prostanoid agonist responsiveness could be demonstrated, with the potency of U46619 identifying three groups (responsive, intermediate responsive, and non-responsive); and the U46619 responsive group was subdivided based on a difference in the potency of $PGF_{2\alpha}$. These observations prompted us to examine the frequency and selectivity of the variations of prostanoid agonist responses in aortic strips from a population of male rabbits. In addition, a pharmacological characterization of the prostanoid receptor(s) mediating contractile effects in the four groups of phenotypic responders was attempted by determining the rank order of prostanoid agonist potency (Coleman *et al.*, 1984) and by examining the inhibitory effects of the TxA_2/PGH_2 receptor antagonist SQ29548 (Ogletree *et al.*, 1985; Darius *et al.*, 1985).

Methods

Tissue isolation and preparation

Male, New Zealand White rabbits (2–3.5 kg, obtained from Hare-Marland, Hewitt, N.J.) were allowed to acclimatize to the animal room environment for at least two weeks before experimentation. Animals were stunned and exsanguinated, and the thoracic aorta from the aortic arch to the diaphragm removed and placed in oxygenated Krebs-Henseleit solution (room temperature) of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11. The aortae were cleaned of extraneous connective tissue, cut into spiral strips (Furchgott & Bhadrakom, 1953), and suspended in double-jacketed organ baths filled with 20 ml of Krebs-Henseleit solution (37°C), which was aerated with a mixture of 95% O_2 and 5% CO_2. The aortic strips were maintained under a resting tension of 4 g, and the Krebs-Henseleit solution was changed every 15 min for at least 2 h before the addition of drugs. Mechanical responses were recorded by Grass FT03 force-displacement transducers connected to a Grass model 7 polygraph. The analogue output of the polygraph was collected and converted to gram tension values using a DL 24 Data Logger (Buxco Electronics, Sharon, CT). The output of the Data Logger was stored and analysed on an IBM PC personal computer using commercially available software from Branch Technology (Dexter, MI). Concentration-response curves were constructed by the method of stepwise cumulative addition as described by van Rossum (1963). All aortic strips used in the present study were denuded of endothelium (Furchgott & Zawadski, 1980) in an attempt to rule out any influence of either the spontaneous or induced release of endothelial-dependent relaxant factor.

Variation of agonist potency in rabbit aortic strips

In this series of experiments, four aortic strips were obtained per animal and then randomized before suspending in organ baths. For each tissue, a single concentration-response curve to U46619, $PGF_{2\alpha}$, PGE_2, or histamine was determined. Agonist responses were expressed as either the % of the maximum agonist response (EC values) or as the % of the steady-state level of contraction induced by 80 mM KCl (EC_{KCl} values), which was added after the completion of each concentration-response curve. The randomization of aortic strips ensured that a given agonist would not be consistently tested against a strip from the same portion of the aorta. Thus, any change in maximal tissue contraction due to the portion of the aorta from which the strip was taken (Altura & Altura, 1970) would be randomized for all agonists.

Determination of dissociation constants

The potency of the TxA_2/PGH_2 receptor antagonist, SQ29548, against the prostanoid agonists was quantitated by determining dissociation constants (K_B) by the dose-ratio method described by Furchgott (1972) using the following equation:

$$K_B = [\text{Antagonist}]/\text{dose-ratio} - 1$$

where [Antagonist] is the molar concentration of antagonist, and the dose-ratio is the ratio of equieffective agonist concentrations obtained in the presence and absence of antagonist. Eight aortic strips were

obtained from one animal and randomly divided into four pairs. One tissue of each pair served as a control for each of the four agonists, while the remaining tissues were incubated with SQ29548 for 45 min before constructing agonist concentration-response curves on the four tissue pairs as previously described. Dose-ratios were determined between the control and treated tissue pairs at the level of the $EC_{50,KCl}$ value for U46619, $PGF_{2\alpha}$ and histamine, and the $EC_{30,KCl}$ value for PGE_2. The $EC_{30,KCl}$ was used for PGE_2 because $EC_{50,KCl}$ values for PGE_2 were not obtained in the presence of SQ29548. Higher concentrations of PGE_2 were not used in order to avoid adding excessive concentrations of ethanol (PGE_2 diluent) to the tissue bath. Dissociation constants were expressed as negative log values (pK_B).

Statistical analyses

The data are expressed as means ± s.e. Statistically significant differences between two means were determined by Student's *t* test for unpaired observations, whereas significant differences between three or more means were determined by one-way analysis of variance (Sokal & Rohlf, 1969). EC values and EC_{KCl} values were determined from regression lines of probit-transformed log concentration-response curves. The intrinsic activity for each agonist was measured as the fraction of the steady-state level of contraction induced by 80 mM KCl. Regression lines were calculated by the method of least squares.

Drugs and solutions

Histamine dihydrochloride and the Tris salt of $PGF_{2\alpha}$ were purchased from Sigma Chemical Company. U46619 ((5Z, 9α, 11α, 13E, 15S)-11, 9-(epoxymethano) prosta-5, 13-dien-1-oic acid) and PGE_2 were purchased from Upjohn Diagnostics. SQ29548 ((1S-(1α, 2β (5Z), 3β, 4α))-7-(3-((2-((phenylamino)-carbonyl) hydrozino)methyl)-7-oxabicyclo (2.2.1)-hept-2-yl)-5-heptenoic acid) was a gift from the Squibb Institute for Medical Research, Princeton, NJ. Stock solutions (10 mg ml^{-1}) of PGE_2, U46619, and SQ29548 were prepared in 95% ethanol. These stock solutions were stored at − 20°C, and fresh dilutions of these stock solutions were prepared just before each experiment. Ethanol stock solutions of SQ29548 were used within 2 weeks. Aliquots of the stock ethanol solution of PGE_2, U46619 and SQ29548 were first diluted in 1 mM Na_2CO_3 in order to avoid precipitation. All subsequent dilutions were made in water and the drug solutions were kept on ice for the duration of the experiment. The concentrations of ethanol in stock solutions did not induce contractions or relaxation (phenylephrine-contracted aorta) and had no effect on agonist responses in the rabbit aorta.

Results

Variation of U46619 responses in rabbit aortic strips

In the course of our initial experiments to characterize the smooth muscle pharmacology of U46619, some rabbit aortic strips that were contracted by noradrenaline, 5-hydroxytryptamine, histamine and KCl were not contracted by a supramaximal concentration of U46619 (Figure 1). Tissues that failed to respond to U46619 were termed non-responders (NR) and normally responsive tissues were designated responders (R). In R, indomethacin (3×10^{-6} M) did not affect the potency of U46619, suggesting that endogenously synthesized prostaglandins were not mediating contractions induced by U46619 (data not shown). It was thought that these variations in U46619 potency could represent differences in receptor sensitivity along the length of the aorta. However, when eight strips were serially cut per aorta and tested for the potency of U46619, the EC_{50} values were consistent, varying by less than a factor of two in strips from a given

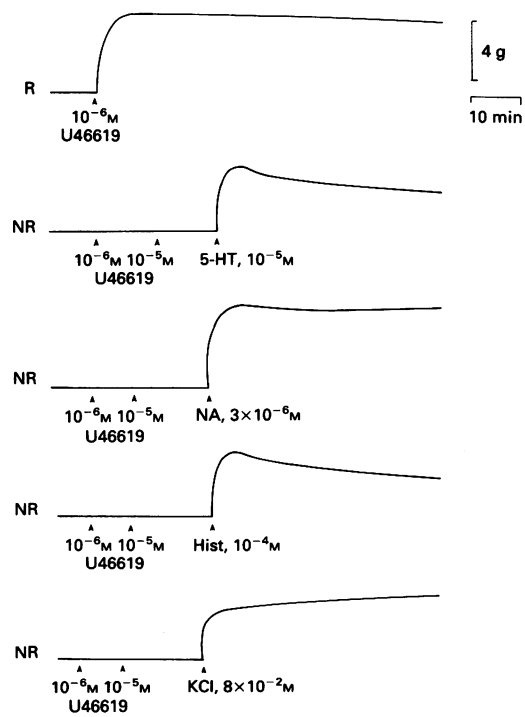

Figure 1 The effects of U46619 and other contractile agonists in U46619 responsive (R) and U46619 non-responsive (NR) rabbit aortic strips. 5-HT, 5-hydroxytryptamine; NA, noradrenaline; Hist, histamine. Molar concentrations of each agonist were added at the arrows.

responding aorta ($n = 4$); and U46619 failed to produce contractions in any strip from a given non-responding aorta ($n = 3$). Thus, the potency of U46619 in a single aortic strip could be used to indicate the responsiveness of the entire aorta to U46619. It was also considered possible that the non-responsiveness to U46619 might be due to an exaggerated smooth muscle relaxant response induced by the prostanoid agonists that would be sufficient to inhibit the contractile effects. However, neither PGE_2, which can induce relaxation of other smooth muscle preparations (Apperley *et al.*, 1979; Coleman & Kennedy, 1985; Erian & Turker, 1985; Dong *et al.*, 1986), nor U46619, which can increase tissue levels of cyclic AMP at high concentrations (Best *et al.*, 1979), produced a relaxation response in either U46619 responsive or non-responsive aortic strips which were contracted with 10^{-7} M phenylephrine (data not shown). It was also considered unlikely that endothelial-derived factors were involved because preliminary experiments demonstrated no acetylcholine-induced relaxation in strips contracted with 10^{-7} M phenylephrine.

Variations of agonist responses in aortic strips from a population of male rabbits

We examined the frequency of U46619 non-responsiveness in a population of male New Zealand White rabbits and also determined the effects of the prostanoid agonists, $PGF_{2\alpha}$ and PGE_2, in R and NR aortic strips. Histamine was tested as a control agonist, because it could contract both R and NR. Concentration-response curves to U46619, $PGF_{2\alpha}$, PGE_2, and histamine were constructed on aortic strips from 35 randomly selected rabbits. Aortae from 9 of the 35 rabbits were NR, whereas tissues from 24 animals were R (Figure 2 and Table 1). In addition, aortae from 2 rabbits in this group of 35 exhibited a previously unobserved intermediate level of sensitivity to U46619. These tissues were termed intermediate responders (IR; discussed subsequently). In aortic strips from R, U46619 was essentially a full agonist and produced contractions in low concentrations ($EC_{50} = 0.019\,\mu M$), whereas in NR, U46619 produced no contractions or at best, a trivial contraction ($< 2\%$ of KCl maximum contraction) at a concentration of $10\,\mu M$ (Figure 2a). Histamine, on the other hand, produced identical effects in both R and NR (Figure 2b). In R, the prostanoid agonists, $PGF_{2\alpha}$ and PGE_2, were less potent than U46619 and were not full agonists, based on the maximum KCl response (Figures 2c and d, respectively). The contractile effects of $PGF_{2\alpha}$ and PGE_2 were markedly diminished in NR, producing maximum responses of 7% and 10%, respectively (Figure 2c and d, respectively). Therefore, in contrast to U46619, which was essentially incapable of producing contractions in NR, $PGF_{2\alpha}$ and PGE_2 exhibited slight, yet significant, levels of contractions in these tissues.

The population study was continued in order to increase the sample size of IR aortae. In these studies, a U46619 concentration-response curve was performed on a single strip from each aorta to determine whether the phenotype was R, IR, or NR. The remaining aortic strips from R and NR aortae were used for studies unrelated to the present investigation. Four additional IR aortae were obtained by testing aortae from 29 rabbits, for a total of 6 IR aortae out of a population of 64 rabbits. Concentration-response curves to U46619, $PGF_{2\alpha}$, PGE_2 and histamine were determined in aortic strips from these six IR aortas. The results of these experiments are shown with the corresponding data from R aortae in Figure 3 and Table 1. The responses to histamine were identical in R and IR (Figure 3b). The intrinsic activities for each prostanoid agonist were lower in IR than R. When EC_{50} values were compared, the potency of $PGF_{2\alpha}$ was approximately 40 fold higher in IR ($EC_{50} = 0.016\,\mu M$) than in R ($EC_{50} = 0.72\,\mu M$), and the potency of U46619 was approximately 180 fold lower in IR ($EC_{50} = 3.4\,\mu M$) than in R ($EC_{50} = 0.019\,\mu M$). Therefore, when the differences in intrinsic activities are factored out by comparing EC_{50} values, it can be seen that the rank order of prostanoid agonist potency in R was U46619 > $PGF_{2\alpha}$ > PGE_2, and in IR was $PGF_{2\alpha}$ > PGE_2 > U46619. Because of the relatively low polygraph sensitivity setting in the initial series of experiments, it was not possible to quantitate EC_{50} values of the small responses induced by $PGF_{2\alpha}$ and PGE_2 in NR accurately. It appears from Figure 2 that the rank order of agonist potency in NR is $PGF_{2\alpha}$ > PGE_2 > U46619.

Since the 80 mM KCl response was used to normalize all agonist responses, this value should represent a reliable maximum response value in all phenotypes for comparisons between groups to be valid. As can be seen in Table 1, the KCl response values for each group of tissues in R, IR, and NR were similar. The 95% confidence limits for all values were overlapping. When KCl values in R, IR, and NR for each agonist group were evaluated by one-way analysis of variance, the 4.6 g value in the $PGF_{2\alpha}$ tissues of the IR phenotype was significantly different from the 5.6 g value in the $PGF_{2\alpha}$ tissues in the R phenotype ($0.05 > P > 0.01$). However, the extent of this variation (less than 20%) is consistent with normal variability of KCl response values of aortic strips (Altura & Altura, 1970). Thus when prostanoid agonist responses in R, IR, and NR were compared, the rank order of prostanoid agonist potency in R differed from the rank orders of agonist potency in IR and NR. For each prostanoid agonist, the rank order of intrinsic activity was R > IR > NR. In addition, the contractile responses of histamine were identical in

R, IR, and NR, and the absolute magnitudes of KCl-induced contractions were similar in the three groups (Table 1), suggesting that the variations of prostanoid agonists were not due to non-selective changes.

Due to practical considerations, the effects of PGD_2 were not systematically examined in all tissues. However, in a few preliminary experiments, we found that PGD_2 did not induce a maximum contraction in R relative to U46619 (66% of KCl response, $n = 8$ aortic strips from 2 rabbits) and was approximately 100 times less potent than U46619. In IR, PGD_2 produced a maximum response of approximately 45% of the KCl response and was approximately 30 times less potent than $PGF_{2\alpha}$ ($n = 4$ aortic strips from 1 rabbit). In NR, PGD_2 produced less than 10% of the KCl response at 10^{-5} M ($n = 8$ aortic strips from 2 rabbits). Although these results are preliminary, the potency and maximal responses of PGD_2 were very similar to those found for PGE_2 in the various phenotypes.

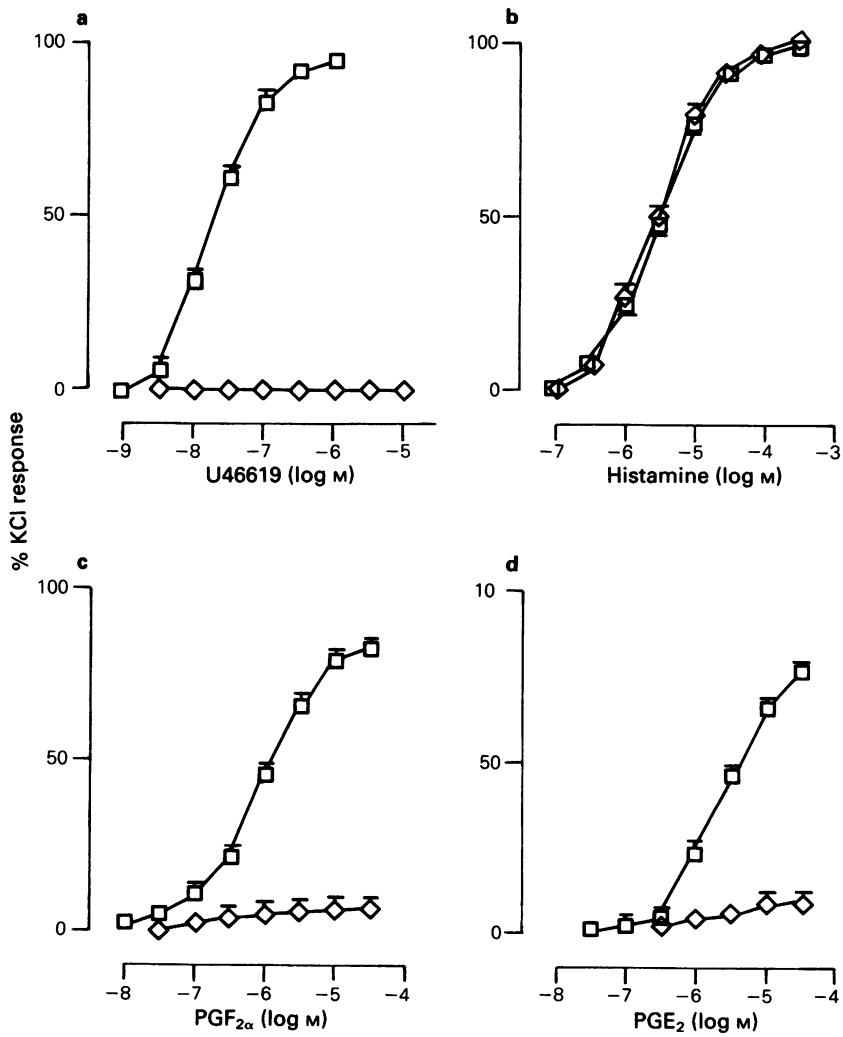

Figure 2 The effects (a) U46619, (b) histamine, (c) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and (d) PGE_2 in U46619 responsive (R; □) and U46619 non-responsive (NR; ◇) rabbit aortic strips. Results are expressed as the % of the 80 mM KCl response. $n = 24$ and 9 for R and NR, respectively. Vertical lines show s.e. when it is larger than the symbol representing the point.

The effects of SQ29548 on agonist responses in R and IR

The differences in the rank orders of agonist potency in R, as compared to IR and NR, suggest that different prostanoid receptor subtypes may be mediating the contractile effects in the various kinds of U46619-responsive tissues (Furchgott, 1972). Variations in the rank order of agonist potency may also be due to different efficacies (Furchgott, 1972). Therefore, variations in the rank order of agonist potency are not sufficient to demonstrate different receptor subtypes. In an attempt to rule out the problem of differences in efficacy, the apparent affinity of the selective TxA_2/PGH_2 receptor antagonist, SQ29548, was determined against agonist responses in R and IR. The affinity of a competitive antagonist should be the same for a given receptor regardless of the intrinsic efficacy of the agonist (Furchgott, 1972). The effects of SQ29548 against agonist responses in eight aortae designated R were tested; however, the results from one aorta were clearly different from the other seven. The results from the atypical aorta led to the eventual subdivision of R into R_1 and R_2 based on the potency of $PGF_{2\alpha}$ and the unusual inhibitory effects of SQ29548 (data from this aorta, designated R_2, are included in Figure 7; discus-

Table 1 Agonist responses in R, IR and NR rabbit aorta phenotypes

	R	*IR*	*NR*
U46619			
$EC_{50,KCl}$ (μM)	0.025 (0.020–0.030)	—	—
EC_{50} (μM)	0.019 (0.016–0.022)	3.4 (2.0–5.7)	—
Maximum response (% KCl)	95 (91–99)	39 (36–42)	0.2 (−0.3–0.7)
KCl response (g)	5.5 (5.1–5.8)	4.6 (3.3–5.9)	5.2 (4.6–5.8)
Histamine			
$EC_{50,KCl}$ (μM)	3.4 (2.5–4.6)	2.6 (2.0–3.3)	3.2 (2.1–4.8)
EC_{50} (μM)	3.1 (2.5–3.8)	2.5 (2.0–3.2)	2.8 (2.2–3.6)
Maximum response	99 (97–101)	100 (97–103)	99 (94–103)
KCl response (g)	5.5 (5.1–6.0)	5.6 (4.2–7.0)	5.3 (5.0–5.7)
$PGF_{2\alpha}$			
$EC_{50,KCl}$ (μM)	1.5 (1.0–2.2)	—	—
EC_{50} (μM)	0.72 (0.51–1.0)	0.016 (0.007–0.036)	—
Maximum response (% KCl)	83 (79–87)	46 (33–59)	7 (2.4–12)
KCl response (g)	5.6 (5.3–5.9)	4.6 (3.4–5.9)	5.2 (4.7–5.7)
PGE_2			
$EC_{50,KCl}$ (μM)	4.8 (3.7–6.3)	—	—
EC_{50} (μM)	2.0 (1.7–2.5)	0.63 (0.42–0.96)	—
Maximum response (% KCl)	77 (73–81)	50 (42–60)	10 (3–17)
KCl response	5.3 (5.0–5.6)	4.7 (3.7–5.6)	5.3 (4.7–5.8)

The data shown represent means and 95% confidence limits (numbers in parentheses) of EC_{50} values, $EC_{50,KCl}$ values, agonist maximum responses as the % of the 80 mM KCl response, and the KCl response in g tension. $n = 24$, 6 and 9 for R, IR and NR phenotypes, respectively.

sed subsequently). The results of SQ29548 against agonist responses in seven of the eight R aortae (which were designated R_1) are shown in Figure 4. SQ29548 (3×10^{-7} M) had no effect on histamine responses (Figure 4b). However, the concentration-response curves of U46619, $PGF_{2\alpha}$, and PGE_2 (Figure 4a, c and d, respectively) were shifted to the right approximately 40 fold. The pK_B values of SQ29548 against U46619, $PGF_{2\alpha}$ and PGE_2 (8.18 ± 0.11; 8.02 ± 0.12; 7.94 ± 0.07; respectively) were not significantly different ($P > 0.25$). The effects of SQ29548 against agonist responses in aortic strips derived from IR aortae are shown in Figure 5. At 10^{-6} M, SQ29548 had no significant effect on any of the agonist responses in IR aortae. Based on the apparent pK_B values of approximately 8, this concentration of SQ29548 would be expected to shift concentration-response curves for agonists interacting with a TxA_2/PGH_2 receptor by 100 fold. Since the affinity of a competitive antagonist is not affected by variations in efficacy

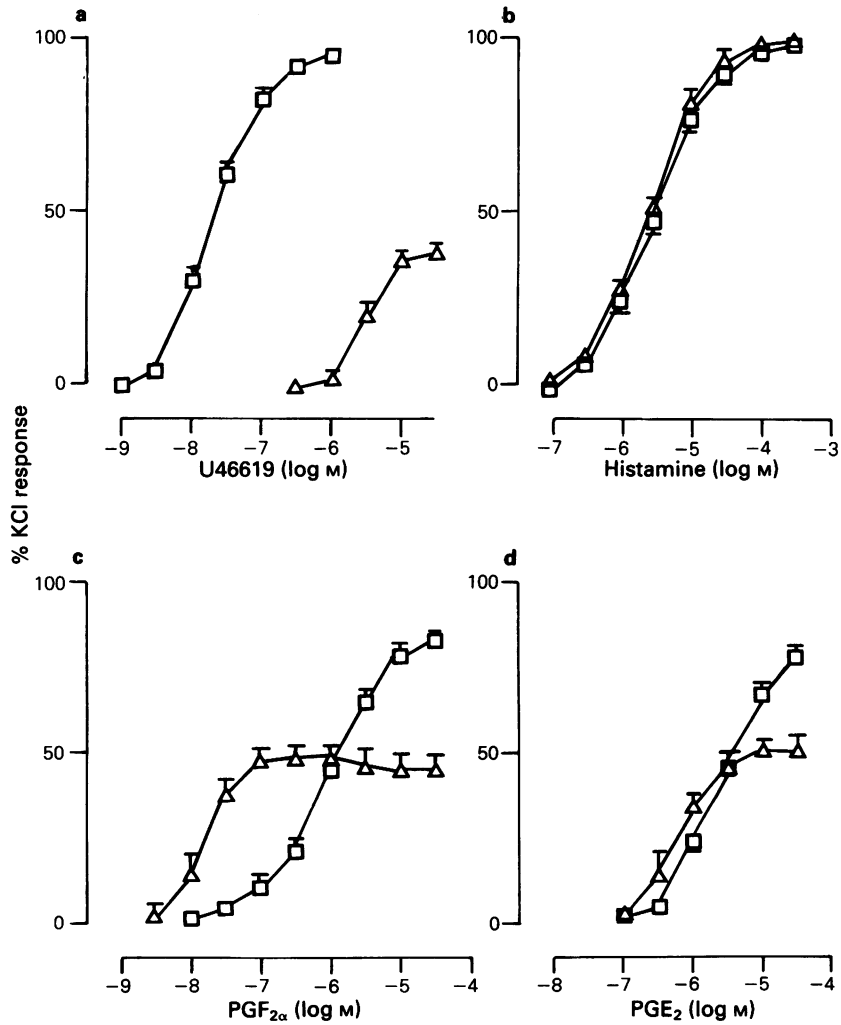

Figure 3 The effects of (a) U46619, (b) histamine, (c) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and (d) PGE_2 in U46619 responsive (R; □) and U46619 intermediate responsive (IR; △) rabbit aortic strips. Results are expressed as the % of the 80 mM KCl response. $n = 24$ and 6 for R and IR, respectively. Vertical lines show s.e. when it is larger than the symbol representing the point.

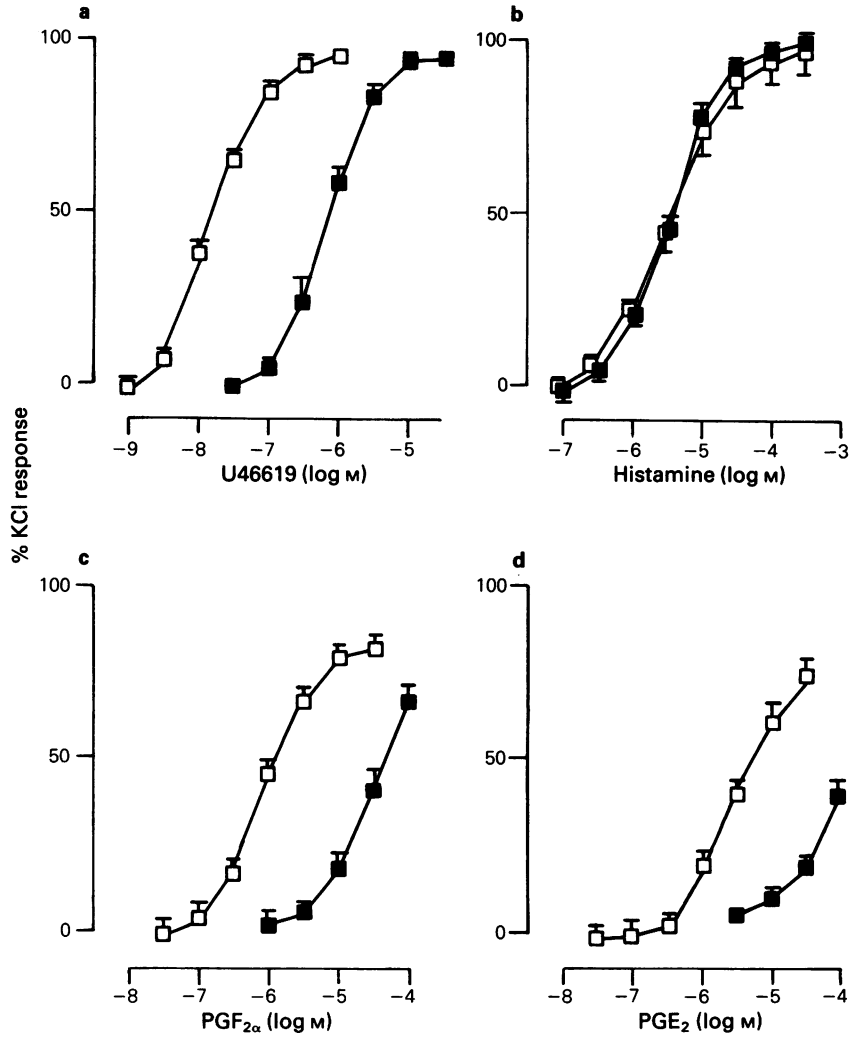

Figure 4 The responses to (a) U46619, (b) histamine, (c) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and (d) PGE_2 in U46619 responsive (R) rabbit aortic strips in the absence (□) and presence (■) of 3×10^{-7} M SQ29548. These tissues were ultimately subclassified as R_1 phenotypes (see text). Results are expressed as the % of the 80 mM KCl response. $n = 7$. The pK_B values for SQ29548 against U46619, $PGF_{2\alpha}$ and PGE_2 (8.18 ± 0.11, 8.02 ± 0.12, and 7.94 ± 0.07, respectively) were not significantly different ($P > 0.25$). Vertical lines show s.e. when it is larger than the symbol representing the point.

(Furchgott, 1972), these results suggest that the prostanoid agonist responses in IR are not mediated by a TxA_2/PGH_2 receptor.

As discussed previously, aortic strips from one of the eight R aortas in the SQ29548 experiments exhibited unusual responses. In this aorta, a substantial portion of the contractile effects of $PGF_{2\alpha}$ and PGE_2 were resistant to inhibition by SQ29548, whereas the effects of U46619 appeared to be competitively inhibited by SQ29548. In addition, the potency of $PGF_{2\alpha}$ appeared to be substantially greater (10 to 30 fold) compared to the other aortas in these experiments. A retrospective examination of the data of each aorta used in the experiments described in Figure 2 revealed that in a subgroup of 4 of the 24 R $PGF_{2\alpha}$ appeared to exhibit a greater potency than in

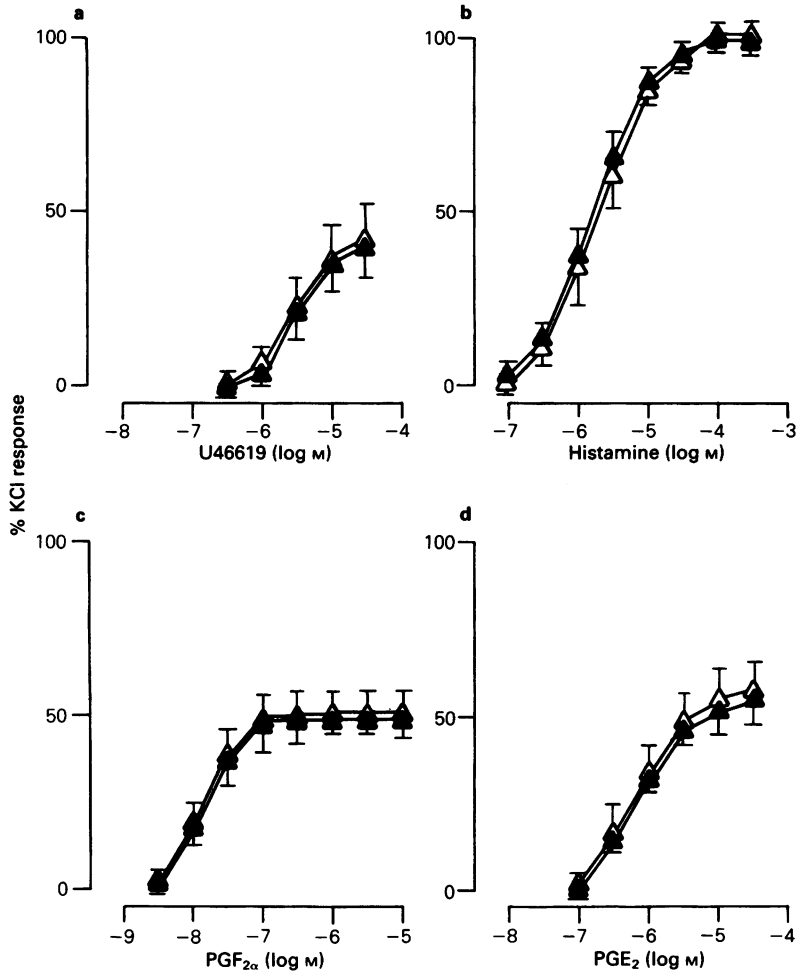

Figure 5 The responses to (a) U46619, (b) histamine, (c) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and (d) PGE_2 in IR rabbit aortic strips in the absence (△) and presence (▲) of 10^{-6} M SQ29548. Results are expressed as the % of the 80 mM KCl response. $n = 3$. Vertical lines show s.e.

the remaining 20 strips ($EC_{50} \approx 1$ μM for $n = 20$ and $EC_{50} \approx 0.1$ μM for $n = 4$). It was hypothesized that a greater potency of $PGF_{2\alpha}$ may be a unique characteristic of aortae that exhibit an unusual profile of inhibitory effects to SQ29548. Accordingly, a prospective study was commenced in which $PGF_{2\alpha}$, in addition to U46619, was used to select aortae. A single strip from each aorta was exposed to 10^{-7} M $PGF_{2\alpha}$ followed by 10^{-6} M U46619. The differential effects of these compounds identified four phenotypic responses: (1) R_1 responded weakly, if at all, to $PGF_{2\alpha}$ and was maximally contracted to U46619; (2) in R_2, $PGF_{2\alpha}$ produced a submaximal contraction and the subsequent addition of U46619 resulted in a maximal contraction; (3) in IR, $PGF_{2\alpha}$ produced a submaximal contraction and the subsequent addition of U46619 produced little, if any, further contraction; and (4) NR failed to respond to either $PGF_{2\alpha}$ or U46619. Single concentrations of $PGF_{2\alpha}$ and U46619 were used to evaluate aortic phenotypes because a considerable amount of time was saved in comparison to constructing full $PGF_{2\alpha}$ or U46619 concentration-response curves. In selected experiments, we confirmed that this method of phenotype selection reliably predicted the

four phenotypes R_1, R_2, IR and NR, based on comparing phenotype designations from full concentration-response curves to $PGF_{2\alpha}$ and U46619 on aortic strips from these aortae. Using this process, R_2 aortae were selected and tested for agonist responses in the presence and absence of SQ29548. Aortae that were not R_2 were used for studies unrelated to the present investigation.

When agonist responses in R_1 and R_2 were compared (Figure 6 and Table 2), the potency of histamine was identical in both groups (Figure 6b); the potencies of U46619 (Figure 6a) and PGE_2 (Figure 6d) were slightly greater (2 fold or less) in R_2, whereas the potency of $PGF_{2\alpha}$ was markedly greater in R_2 as compared to R_1 (approximately 10 fold; Figure 6c). In addition, the sinistral displacement of the $PGF_{2\alpha}$ concentration-response curve in R_2 was non-parallel in nature and the potency difference of $PGF_{2\alpha}$ was greater at lower concentrations. In R_2 the $PGF_{2\alpha}$ concentration-response curve was biphasic with an inflection point at 3×10^{-7} M $PGF_{2\alpha}$. Thus, the differential effects of $PGF_{2\alpha}$ clearly identified the subgroups of R_1

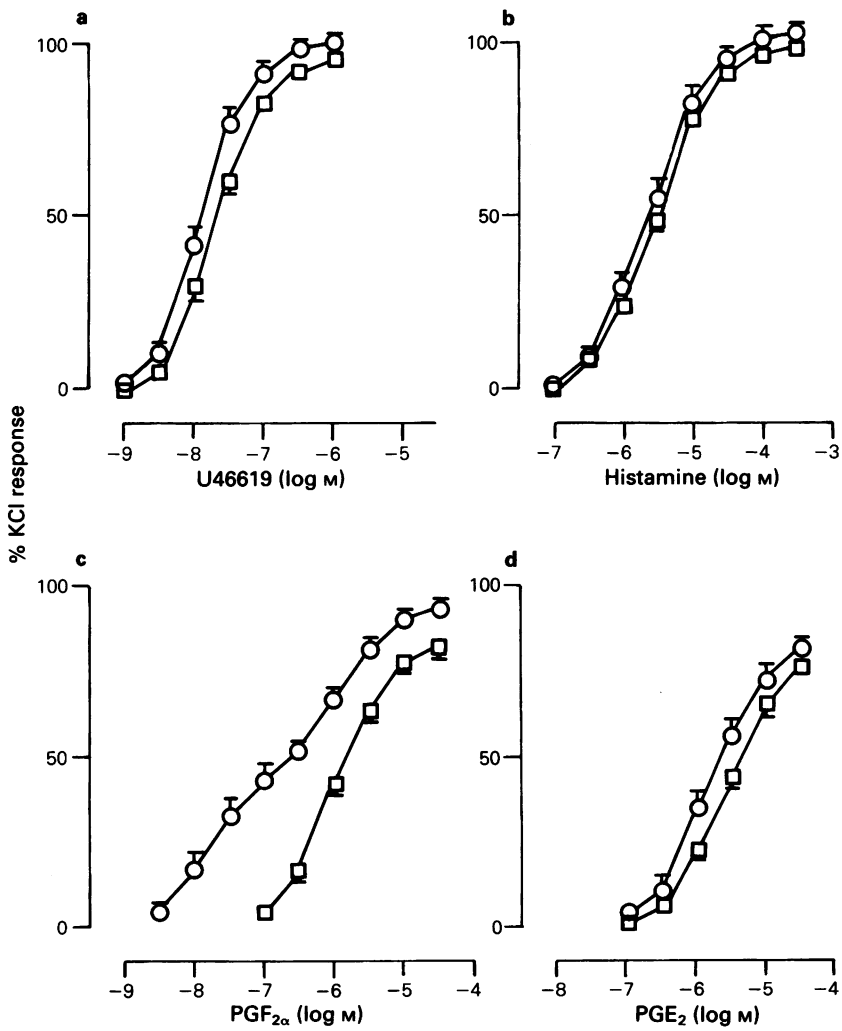

Figure 6 The effects of (a) U46619, (b) histamine, (c) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and (d) PGE_2 in R_1 (□) and R_2 (○) rabbit aorta phenotypes. Results are expressed as the % of the 80 mM KCl response. $n = 20$ for R_1 and 9 for R_2. Vertical lines show s.e. when it is larger than the symbol representing the point.

Table 2 Agonist responses in R_1 and R_2 rabbit aorta phenotypes

	R_1	R_2
U46619		
$EC_{50,KCl}$ (μM)	0.025 (0.020–0.031)	0.015 (0.009–0.022)
EC_{50} (μM)	0.019 (0.016–0.023)	0.014 (0.010–0.020)
Maximum response (% KCl)	95 (92–99)	100 (95–105)
KCl response (g)	5.5 (5.1–5.9)	4.7 (4.1–5.3)
Histamine		
$EC_{50,KCl}$ (μM)	3.3 (2.3–4.7)	2.6 (1.4–4.9)
EC_{50} (μM)	3.0 (2.4–3.6)	2.6 (1.7–4.1)
Maximum response (% KCl)	99 (95–103)	103 (96–110)
KCl response (g)	5.6 (5.1–6.1)	4.8 (4.2–5.4)
$PGF_{2\alpha}$		
$EC_{50,KCl}$ (μM)	2.0 (1.5–2.8)	0.18 (0.072–0.44)
EC_{50} (μM)	1.0 (0.83–1.2)	0.12 (0.059–0.26)
Maximum response (% KCl)	82 (78–86)	93 (88–98)
KCl response (g)	5.6 (5.2–6.0)	4.9 (4.3–5.5)
PGE_2		
$EC_{50,KCl}$ (μM)	5.1 (3.8–7.0)	2.3 (1.4–3.7)
EC_{50} (μM)	2.2 (1.8–2.8)	1.3 (0.91–1.8)
Maximum response (% KCl)	76 (72–80)	81 (74–8)
KCl response	5.4 (5.0–5.7)	4.6 (4.2–5.1)

The data shown represent means and 95% confidence limits (numbers in parentheses) of EC_{50} values, $EC_{50,KCl}$ values, agonist maximum responses as the % of the 80 mM KCl response, and the KCl response in g tension. $n = 20$ and 9 for R_1 and R_2 phenotypes, respectively.

and R_2 aortae which were initially designated R using only U46619 as the test agonist.

The effects of SQ29548 on agonist responses in R_2 are shown in Figure 7. Histamine responses were unaltered by SQ29548 (Figure 7b), and the displacement of U46619 concentration-response curves was similar in R_1 (Figure 4a) and R_2 (Figure 7a), resulting in pK_B values of 8.18 ± 0.11 and 8.34 ± 0.10, respectively ($P > 0.05$). In contrast to the competitive displacement of $PGF_{2\alpha}$ and PGE_2 concentration-response curves by SQ29548 in R_1 (Figure 4c and 4d, respectively), a substantial portion of the contractile effects of $PGF_{2\alpha}$ and PGE_2 was resistant to SQ29548 inhibition in R_2 (Figure 7c and d, respectively), resulting in biphasic concentration-response curves. Specifically, lower concentrations of $PGF_{2\alpha}$ and PGE_2 were resistant to SQ29548, whereas higher concentrations were sensitive to SQ29548. Thus, SQ29548 accentuated the biphasic nature of $PGF_{2\alpha}$ in R_2 which was somewhat apparent in the absence of the antagonist, and unmasked biphasic responses to PGE_2 in R_2. SQ29548 produced an apparently competitive shift of the $PGF_{2\alpha}$ concentration-response curve at higher $PGF_{2\alpha}$ concentrations in R_2, resulting in an approximate dose-ratio of 30, very similar to the dose-ratio obtained for $PGF_{2\alpha}$ in R_1. In addition, the SQ29548-resistant portions of the $PGF_{2\alpha}$ and PGE_2 concentration-respose curves in R_2 were very similar to the $PGF_{2\alpha}$ and PGE_2 concentration-response curves in IR, which were also resistant to SQ29548 inhibition.

Frequency distribution of R_1, R_2, IR and NR in a population of male New Zealand white rabbits

An estimate of the frequency of phenotypic variation in the population was made as a first step in determining the relative importance of genetic and environmental factors in causing individual variations in aortic prostanoid responses. In an attempt to increase the sample size without utilizing large numbers of rabbits solely for typing the prostanoid sensitivity of the aorta, when experiments utilizing the aorta were conducted which were unrelated to the present study, a single strip was tested for prostanoid sensitivity whereas the remaining strips were utilized for other purposes. For these experiments, a single concentration-response curve to $PGF_{2\alpha}$ could adequately differentiate the four phenotypes. In some of the earlier experiments only U46619 was used to identify phenotypes, which resulted in identifying only three (R, IR, and NR) of the four phenotypes. In all aortae tested ($n = 92$), the frequency distribution was: R, 69% ($n = 64$); IR, 11% ($n = 10$); and NR, 20% ($n = 18$). In a subgroup of these aortae in which U46619 and/or $PGF_{2\alpha}$ were used to designate phenotypes ($n = 63$), the frequency distribution was: R_1, 54% ($n = 34$); R_2, 19% ($n = 12$); IR, 6% ($n = 4$); and NR, 21% ($n = 13$).

Discussion

The rabbit aorta has been a useful experimental model tissue in prostanoid research because it exhibits a relatively greater sensitivity to thromboxane (i.e., RCS) and thromboxane mimetics than the other prostaglandins (e.g., Piper & Vane, 1969; Bunting *et al.*, 1976a; Coleman *et al.*, 1980; 1984; Jones *et al.*,

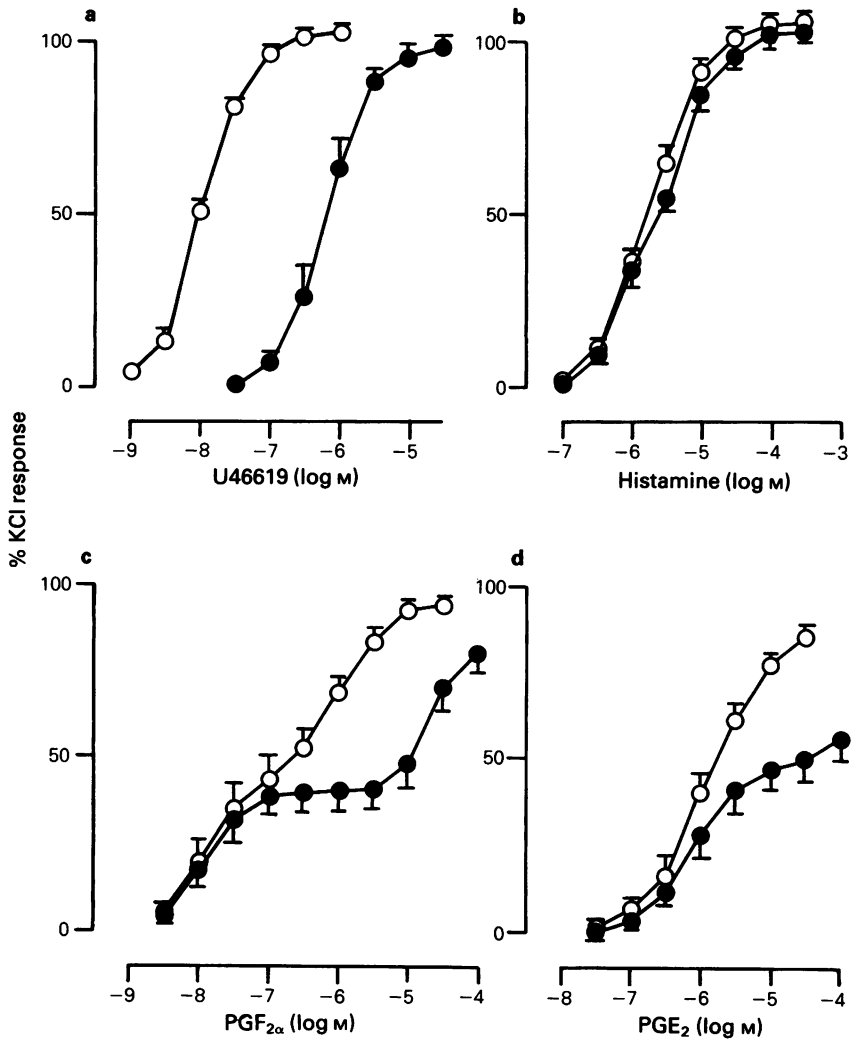

Figure 7 The responses to (a) U46619, (b) histamine, (c) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and (d) PGE_2 in the R_2 rabbit aorta phenotype in the absence (○) and presence (●) of 3×10^{-7} M SQ29548. Results are expressed as the % of the 80 mM KCl response. $n = 6$. Vertical lines show s.e. when it is larger than the symbol representing the point.

1982). Based on the rank order of prostanoid agonist potency and inhibitor selectivity, Coleman *et al.* (1984) have proposed that the rabbit aorta contains a single TxA_2/PGH_2 prostanoid receptor. However, in the original description of RCS by Piper & Vane (1969), it was implied that prostanoid responses may be variable in the rabbit aorta because aortae from two female rabbits failed to show a RCS-like response. Cunard *et al.* (1985) and Karanian *et al.* (1981a, b) have provided evidence suggesting that there may be a gender difference in the functional expression of TxA_2/PGH_2-receptors in rat vasculature. In preliminary experiments we found that aortae from three out of four female rabbits were R_1 and one aorta was IR (unpublished observations). This suggests that aortae from females are not invariably non-responsive. However, a population study of female rabbits would be required to determine if there are significant sex differences in the relative frequency of phenotypes.

In the present study, the observation of occasional

non-responsiveness of aortic strips from male rabbits to the TxA_2 mimetic, U46619, prompted us to make a prospective study of the frequency and selectivity of this phenomenon. In the entire population of male rabbits studied, aortic strips with three apparently distinct levels of responsiveness to U46619 were described: R, IR and NR. In addition, R could be subdivided into R_1 and R_2 based on a greater potency to $PGF_{2\alpha}$ in R_2 than in R_1. The four phenotypes, R_1, R_2, IR and NR, were characterized with respect to: (1) rank orders of prostanoid agonist potency, (2) intrinsic activity, and (3) agonist inhibition by the TxA_2/PGH_2 receptor antagonist, SQ29548. Table 3 summarizes these findings.

A working classification of prostanoid receptor nomenclature has recently been proposed (Kennedy *et al.*, 1982; Coleman *et al.*, 1984) based on rank orders of agonist potency and the relative selectivity of antagonists. It was proposed that prostanoid receptors be termed P-receptors and that the prostanoid agonist to which they are most sensitive be indicated by a preceding letter. According to this nomenclature, a TxA_2/PGH_2 receptor (TP-receptor) exhibits the greatest sensitivity to the TxA_2 mimetic, U46619, and agonist responses are inhibited by TxA_2/PGH_2 receptor antagonists. The $PGF_{2\alpha}$-selective receptor (designated an FP-receptor) exhibits the greatest sensitivity to $PGF_{2\alpha}$, and agonist responses are resistant to inhibition by TxA_2/PGH_2 receptor antagonists.

Based on the criteria of Coleman *et al.* (1984), we propose that the results obtained in the present investigation can be explained by postulating the variable presence of TP- and FP-receptor subtypes in the rabbit aorta. In R_1, the rank order of agonist potency was U46619 > $PGF_{2\alpha}$ > PGE_2 and all prostanoid responses were equally inhibited by SQ29548. According to receptor theory (Furchgott, 1972), these observations are consistent with the notion that all prostanoid agonists interact with a single TP-receptor subtype in R_1 aortae. These findings are similar to those of Jones *et al.* (1982) where it was shown that $PGF_{2\alpha}$ was a partial agonist in the rabbit aorta and that the thromboxane receptor antagonist EP 045 could antagonize the effects of both U46619 and $PGF_{2\alpha}$. In IR, the intrinsic activities of the prostanoid agonists were less than those found in R_1, and the rank order of agonist potency was $PGF_{2\alpha}$ > PGE_2 > U46619. Prostanoid agonist responses in IR were resistant to inhibition by SQ29548. These observations suggest that the prostanoid responses in IR aortae are not mediated by a TP-receptor because $PGF_{2\alpha}$ is at least 200 times more potent than the TxA_2 mimetic U46619, and the prostanoid agonist responses were not inhibited by the TP-receptor antagonist, SQ29548. We make the tentative proposal that IR aortae contain an FP-prostanoid receptor subtype or subtypes based on the criteria described by Coleman *et al.* (1984), i.e., the presence of an FP-receptor is suggested when $PGF_{2\alpha}$ is the most potent prostanoid agonist, and the responses are resistant to inhibition by a TP-receptor antagonist. Clearly, further studies are required to substantiate this proposal. However, a complete pharmacological characterization of prostanoid agonist responses in IR aortae would be difficult because they occur so infrequently in the population (approximately 6%), and there are no selective FP-receptor antagonists currently available. Preliminary studies indicated that PGD_2 was less potent than U46619 in R phenotypes and less potent than $PGF_{2\alpha}$ in IR aortae. This tends to rule out the possibility that a DP-receptor subtype, i.e., a

Table 3 Summary of prostanoid agonist effects in R_1, R_2, IR and NR rabbit aorta phenotypes

Phenotype	*Rank order of agonist potency*	*Rank order of intrinsic activity*	*Maximum intrinsic activity*[a]	*Inhibition by SQ29548*[b]	*Putative receptor subtypes*[c]
R_1	U46619 > $PGF_{2\alpha}$ > PGE_2	U46619 > $PGF_{2\alpha}$ > PGE_2	0.95	+ U46619 + $PGF_{2\alpha}$ + PGE_2	TP
R_2	U46619 > $PGF_{2\alpha}$ > PGE_2	U46619 > $PGF_{2\alpha}$ > PGE_2	1.0	+ U46619 ± $PGF_{2\alpha}$ ± PGE_2	TP + FP
IR	$PGF_{2\alpha}$ > PGE_2 > U46619	$PGF_{2\alpha}$ > PGE_2 > U46619	0.50	− U46619 − $PGF_{2\alpha}$ − PGE_2	FP
NR	$PGF_{2\alpha}$ > PGE_2 > U46619	$PGF_{2\alpha}$ > PGE_2 > U46619	0.10	ND	FP (low efficacy)

[a]Mean intrinsic activity of agonist that exhibited the highest intrinsic activity in each phenotype. [b]+, sensitive to SQ29548 inhibition; ±, partial resistance to SQ29548 inhibition; −, resistant to SQ29548 inhibition; ND, not determined. [c]TP = thromboxane receptor and FP = $PGF_{2\alpha}$-selective receptor.

PGD_2-selective receptor (Coleman *et al.*, 1984), is involved.

In R_2, the rank orders of agonist potency and intrinsic activity were similar to those of R_1. However, $PGF_{2\alpha}$ was significantly more potent in R_2, and concentration-response curves for $PGF_{2\alpha}$ and PGE_2 were biphasic in the presence of SQ29548. Biphasic concentration-response curves in the presence of an antagonist suggest that the agonist may be interacting with more than one receptor (Ariens *et al.*, 1964). The responses to low concentrations of $PGF_{2\alpha}$ and PGE_2 were resistant to inhibition by SQ29548 in R_2, whereas responses to higher concentrations were inhibited by SQ29548. Since the SQ29548-resistant responses were similar to those found in IR and the SQ29548-sensitive responses resembled those of R_1, we propose that R_2 contains both a TP- and putative FP-receptor subtype. SQ29548 inhibited the effects of U46619 similarly in both R_1 and R_2. This probably resulted because the displacement of the U46619 concentration-response curve by SQ29548 in R_2 was not sufficient to reach concentrations of U46619 where U46619-FP-receptor interactions were significant. In NR, the intrinsic activities of the prostanoid agonists were smaller than those found in IR, and the rank order of agonist potency was $PGF_{2\alpha} > PGE_2 >$ U46619. Based on these observations, we make the tentative proposal that NR contains a low efficacy FP-receptor subtype.

Thus, the four phenotypes could result from the independent regulation of the functional expression of TP- and FP-receptor subtypes with: (1) R_2 containing both the TP- and FP-receptor subtypes in a fully functional state; (2) R_1 containing only the functional TP-receptor; (3) IR containing only the functional FP-receptor; and (4) NR containing only a low efficacy FP-receptor system. In the limited number of aortae in which all four phenotypes were determined ($n = 63$), the TP-receptor was functionally expressed in approximately 73% of the population ($R_1 + R_2$), whereas the putative FP-receptor was functionally expressed in approximately 25% of the population ($R_2 + IR$). Although we hypothesize that prostanoid responses in IR and NR are mediated by an FP-receptor system, it should be emphasized that the lack of a selective FP-receptor antagonist makes this suggestion tentative. Furthermore, the results of the present investigation cannot determine whether the putative FP-receptors of IR and NR are identical and differ only in efficacy, or whether the responses are mediated by unique FP-receptor subtypes.

SQ29548 has been characterized as a selective and competitive thromboxane receptor antagonist that exhibited a pA_2 value of approximately 9 in the guinea-pig trachea (Ogletree *et al.*, 1985). The apparent affinity of SQ29548 was approximately 10 fold less in the present study using the rabbit aorta. It has been consistently found that TP-receptor affinities for other thromboxane antagonists, e.g., EP045 and AH19437, in the rabbit aorta were approximately 10 fold less than values obtained in other tissues, e.g., rat aorta, dog saphenous vein, and guinea-pig trachea (Jones *et al.*, 1982; Coleman *et al.*, 1984). Since the dissociation constants for SQ29548 were not derived from a Schild analysis in the present study, it is not known whether a non-competitive interaction (revealed as a Schild plot slope different from 1) is causing an under- or over-estimation of the actual receptor affinity. However, if one assumes that non-competitive interactions were not significant in the present study, the results suggest that SQ29548, like other TP-receptor antagonists, exhibits a lower apparent affinity in the rabbit aorta than in other TP-receptor-containing tissues.

The results of the present investigation have demonstrated that there are individual variations in the functional expression of a TP-receptor (TxA_2/PGH_2-receptor), as well as individual variations in the functional expression of a non-TP prostanoid receptor or receptors (tentatively proposed to be an FP-receptor) in aortae from male rabbits. Several factors could account for variations of the functional expression of a receptor activity. Some possibilities include changes in receptor number or affinity, changes in receptor-effector coupling, changes in a second messenger system, or changes in tissue degradative or uptake processes. The results of the present investigation cannot differentiate between these possibilities. It is also not possible to determine from these studies the relative importance of genetic or environmental factors in causing the individual variations. Therefore, although many questions remain concerning the mechanistic basis for the individual variations, the hypothesis presented in this study should provide a framework for future investigations.

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