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Thromboxane A_2 modulates cyclic AMP relaxation and production in human internal mammary artery

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Abstract

Two forms of thromboxane A_2 (TP) receptors, TPα and TPβ receptors, have recently been cloned. These receptors regulate adenylate cyclase activity in two opposite ways: TPα receptors activate, whereas TPβ receptors inhibit adenylate cyclase and cAMP generation. The aim of this study was to examine the effects of the thromboxane A_2 analogue, U46619 (9,11-dideoxy-9α,11 α-methanoepoxy-prostaglandin $F_{2\alpha}$), on forskolin-induced relaxation and cAMP accumulation in human internal mammary artery (IMA) and saphenous vein (SV). In organ baths, IMA rings precontracted with U46619 (3.10^{-9} and 3.10^{-8} M) were less sensitive to forskolin than rings precontracted with methoxamine (3.10^{-6} M). In contrast, the sensitivity to forskolin was similar in SV rings contracted with the same concentrations of these agonists. U46619 reduced significantly the ten-fold increase in cAMP induced by forskolin in IMA but not in SV rings. Sensitivity and maximal relaxation in response to sodium nitroprusside were not altered in either IMA or SV. In summary, stimulation of TP receptors with the thromboxane A_2 analogue, U46619, inhibited the cAMP pathway of relaxation through the inhibition of cAMP synthesis in human IMA but not in SV. It is suggested that thromboxane A_2 may play a role in the control of muscle tone in IMA both by its potent contractile effect and by its inhibitory effect on the cAMP pathway of relaxation.

Keywords: Thromboxane A_2; TP receptor; Forskolin; cAMP; Mammary artery, internal; Saphenous vein

1. Introduction

Thromboxane A_2, generated by the cyclo-oxygenase metabolism of arachidonic acid, is one of the most powerful platelet activators and vascular smooth muscle constrictors. Thromboxane A_2 exerts its effects on platelets and vascular smooth muscles by binding to surface receptors that are shared with the precursor endoperoxide, prostaglandin H_2 (Parise et al., 1984), and thus are termed thromboxane A_2/prostaglandin H_2 receptors (TP receptors) (Coleman et al., 1984, 1994). Compelling pharmacological and biochemical evidences have pointed to the existence of TP receptor isoforms not only among animal species and among tissues within the same species but, in some instances, within the same cell (Dorn, 1989,1991; Takahara et al., 1990; Furci et al., 1991; Masuda et al., 1991). Consistent with this evidence, two forms of TP receptors have recently been cloned (Hirata et al., 1991; Raychowdhury et al., 1994). The two forms are generated by an alternative splicing mechanism from a single gene and differ only in their carboxy-terminal domains (Raychowdhury et al., 1994). Recently, the two isoforms of TP receptors expressed in Chinese hamster ovary cells have been shown to exhibit similar ligand binding characteristics and phospholipase C activation, but with opposite regulation of adenylate cyclase activity (Hirata et al., 1996). TPα receptors activated adenylate cyclase and cAMP generation, whereas TPβ receptors inhibited adenylate cyclase, as demonstrated by the inhibition of the forskolin-induced cAMP generation in cells expressing

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TPβ receptors, but not in cells expressing TPα receptors (Hirata et al., 1996). Stimulation of vascular smooth muscles with TP receptors produces vascular smooth muscle contraction through the activation of phospholipase C (Dorn and Becker, 1993). However, the effect of TP receptor stimulation on adenylate cyclase activity in vascular smooth muscle remains unknown. The aim of our study was therefore to examine the effects of U46619 (a stable thromboxane A_2 analogue) on the relaxation and cAMP production induced by forskolin in two types of human vascular smooth muscles, the internal mammary artery (IMA) and the saphenous vein (SV).

2. Materials and methods

2.1. Preparation of blood vessels

Human IMAs and SVs were harvested from 51 patients undergoing coronary bypass surgery. The vessel segments discarded during the operation were immediately placed in oxygenated Krebs solution maintained at 4°C and transferred to the laboratory within 2 h. The Krebs solution had the following composition (mM): NaCl (118), KCl (4.7), $CaCl_2$ (2.5), $MgSO_4$ (1.0), KH_2PO_4 (1.0), $NaHCO_3$ (25) and glucose (11). The blood vessels were dissected free of connective tissue, cut into 3-mm (IMA) or 5 mm (SV) length rings. No attempt was made to remove the endothelium. The number of rings taken from each artery and vein varied from 2 to 6 and 2 to 8, respectively. This study conforms with the principles outlined in the declaration of Helsinki.

The rings were mounted between two stainless-steel wires in organ baths filled with 6 ml of Krebs solution maintained at 37°C and gassed with a mixture of 95% oxygen and 5% carbon dioxide. The lower wire was fixed to a micrometer (Mitutoyo, Japan) in which incremental levels of tension could be applied to the rings. The upper wire was attached to an isometric force displacement transducer (UF-1 Pioden Controls, Canterbury, UK) through which changes in isometric forces were continuously displayed on recorders (Linseis L200E, Bioblock, Illkirch, France).

Each vessel was equilibrated and unstretched for 30 min, and then stretched in progressive steps to determine the wall tension–internal circumference exponential curve as previously described for human IMA and SV segments (He et al., 1989; Stanke et al., 1998a,b). The internal circumference of each ring corresponding to a transmural pressure of 100 mm Hg (IMA) or 20 mm Hg (SV) was determined from its wall tension–internal circumference exponential curve. The distance between the wires was then adjusted to get an internal circumference corresponding to 90% of the internal circumference the vessels would have at a transmural pressure of 100-mm Hg for IMA or 20 mm Hg for SV. Following this normalisation procedure, each ring was stabilised for 60 min more, with Krebs solution changed every 15 min. The rings were then challenged twice with KCl (9.10^{-2} M) at a 10-min interval, to ensure that responses were reproducible. After a further 60-min equilibration period, concentration–response curves for the agonists used in the present experiments were obtained.

As metabolites of the cyclooxygenase and nitric oxide pathways are known to produce relaxation and to alter cyclic nucleotide levels in vascular smooth muscle, all experiments were conducted in the presence of N_G-nitro-L-arginine (L-NNA, 10^{-4} M, 15 min) (Mülsch and Busse, 1990) and indomethacin (10^{-6} M, 30 min) (Cryer and Feldman, 1998) to inhibit the endogenous release of nitric oxide and prostanoids, respectively (Ortiz et al., 1992; Pussard et al., 1995).

2.2. Relaxation

Forskolin, sodium nitroprusside and 8-Br-cAMP concentration–relaxation curves were performed in IMA and SV contracted with either U46619 (3.10^{-9} or 3.10^{-8} M) or methoxamine (3.10^{-6} M), an α_1-adrenoceptor agonist. All the concentration–relaxation curves were constructed by cumulative addition of increasing concentrations of agonist (0.5-log increments) to the organ bath. Only one curve was made from each ring. At the end of each experiment, maximal relaxation of the preparation was determined after addition of papaverine (10^{-4} M). Data for the concentration–relaxation curves were expressed as percentages of the papaverine-induced maximal relaxation.

2.3. cAMP determinations

cAMP was measured in IMA and SV ring preparations that were not under tension. The rings were placed in glass tubes containing 6 ml of Krebs solution at 37°C and gassed with a mixture of 95% oxygen and 5% carbon dioxide for a 60-min equilibration period during which Krebs solution was changed every 15 min. All experiments were conducted in the presence of L-NNA (10^{-4} M, 15 min), indomethacin (10^{-6} M, 30 min) and of the phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine (IBMX, 10^{-4} M, 30 min).

U46619 (10^{-8} and 3.10^{-8} M) was added to the preparations for 15 min. The effect of U46619 on basal cAMP levels was studied in one concentration (10^{-8} M). The effect of U46619 on stimulated cAMP levels was studied in two concentrations (10^{-8} and 3.10^{-8} M) by addition of forskolin (10^{-5} M) for 15 min after the incubation period with U46619. The results were compared to the cAMP levels stimulated by forskolin in the absence of U46619. Reactions were terminated by rapidly plunging the tissue samples into liquid N_2. Frozen samples were stored at −80°C. After homogenisation, the extraction was performed in 1 ml of sodium phosphate buffer, with 6%

Table 1
Methoxamine- and U46619-induced contractions in human IMA and SV
Values are expressed as means ± 95% confidence intervals (given in parenthesis). E_{max}: maximal contraction expressed in gram.

	Methoxamine			U46619	
	IMA	SV		IMA	SV
n	6	6	*n*	6	6
pD_2	5.5 (5.1–5.9)	5.6 (5.2–6.0)	pD_2	8.6 (8.35–8.85)	8.7 (8.4–9.0)
E_{max} (g)	3.1 (2.5–3.7)	2.8 (2.2–3.4)	E_{max} (g)	4.5 (3.7–5.3)	3.9 (3.3–4.5)

Table 2
Relaxation induced by forskolin in human internal mammary artery and saphenous vein preparations precontracted with methoxamine or U46619
Tension is the pre-contraction value induced by the vasoconstrictor. Values are expressed as means ± 95% confidence intervals (given in parenthesis). $pD_2 = -\log(EC_{50})$ and E_{max} = maximal relaxation (percentage of maximal relaxation induced by papaverine 10^{-4} M).

	n	Tension (g)	pD_2 (sensitivity)	E_{max}
Internal mammary artery				
Methoxamine (3.10^{-6} M)	6	2.2 (1.4–3.0)	6.56 (6.38–6.74)	97 (95–99)
U46619 (3.10^{-9} M)	6	2.5 (1.3–3.7)	6.12[a] (6.00–6.24)	94 (91–97)
U46619 (3.10^{-8} M)	6	3.3 (1.9–4.7)	5.80[ab] (5.52–6.08)	79 (60–98)
Saphenous vein				
Methoxamine (3.10^{-6} M)	6	1.9 (1.1–2.7)	6.07 (5.63–6.51)	78 (67–89)
U46619 (3.10^{-9} M)	6	2.2 (1.0–3.4)	6.13 (5.57–6.69)	84 (77–91)
U46619 (3.10^{-8} M)	6	3.6 (2.4–4.8)	6.35 (6.04–6.65)	90 (85–95)

[a] $P < 0.05$ compared with methoxamine contracted preparations.
[b] $P < 0.05$ compared with U46619 (3.10^{-9} M)-contracted preparations (ANOVA and Bonferroni's test).

trichloracetic acid and IBMX 10^{-4} M, for 24 h at 4°C. Each extraction sample was placed in a polypropylene tube. Precipitated protein was separated from the soluble extract by centrifugation at $10\,000 \times g$ for 30 min at 4°C. Trichloracetic acid was removed from the supernatant with four successive water ethyl ether extractions (5 ml). After the last ether extraction, the tubes were placed in a temperature-controlled bath at 70°C (5 min) to eliminate all traces of ether. The concentration of cAMP in the tissue extracts was determined after acetylation, using a commercially available enzyme–immunoassay kit (Cayman, Ann Arbor, USA). Precipitates were used for protein determination by the method of Bradford (1976) using bovine serum albumin as standard.

2.4. Data analysis

The relaxant responses were evaluated in terms of maximum response (E_{max}) and concentration of agonist inducing 50% of maximal relaxation (EC_{50}). EC_{50} was determined from each curve by a logistic, curve-fitting equation and expressed as $pD_2(-\log EC_{50})$. cAMP values were expressed as pmol of nucleotide per mg of protein. All values were expressed as means ± 95% confidence intervals. Data were analysed with unpaired *t*-test to compare two means. More than two means were compared using an analysis of variance (and Bonferroni's test as post-hoc test). Values of $P < 0.05$, corrected for the number of comparisons made, were considered significant.

2.5. Drugs

The drugs used were: U46619, methoxamine, sodium nitroprusside, forskolin, 8-Br-cAMP, indomethacin, L-NNA and IBMX from Sigma (Saint Quentin Fallavier, France).

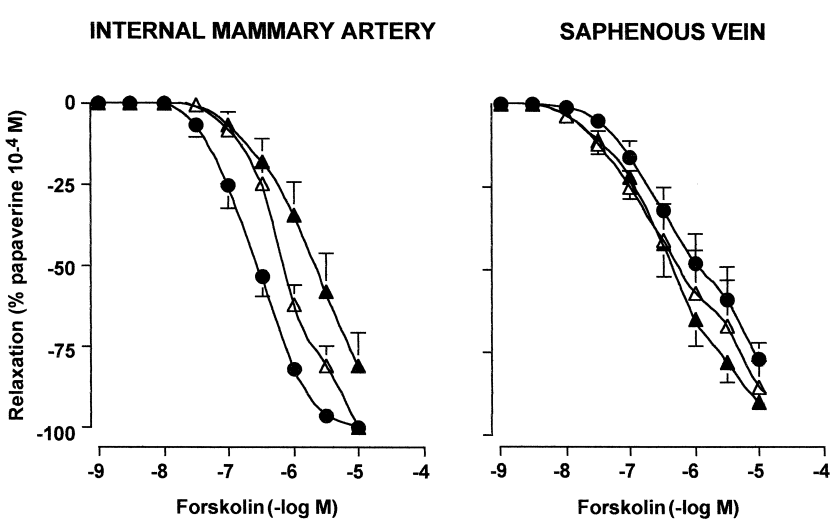

Fig. 1. Concentration-dependent relaxant effects of forskolin on methoxamine 3.10^{-6} M (●), U46619 3.10^{-9} M (△) and U46619 3.10^{-8} M (▲)-contracted IMAs (a) and SVs (b). Points are means ± SEM ($n = 6$ in all groups).

Table 3
Relaxation induced by 8-bromo-cyclicAMP in human internal mammary artery and saphenous vein preparations precontracted with methoxamine or U46619
Tension is the pre-contraction value induced by the vasoconstrictor. Values are expressed as means ± 95% confidence intervals (given in parenthesis). $pD_2 = -\log (EC_{50})$ and E_{max} = maximal relaxation (percentage of maximal relaxation induced by papaverine 10^{-4} M).

	n	Tension (g)	pD_2 (sensitivity)	E_{max}
Internal mammary artery				
Methoxamine (3.10^{-6} M)	6	2.0 (1.6–2.4)	4.91 (4.44–5.37)	67 (49–82)
U46619 (3.10^{-9} M)	6	2.8 (1.8–3.8)	5.01 (4.65–5.35)	78 (69–87)
Saphenous vein				
Methoxamine (3.10^{-6} M)	6	1.7 (0.9–2.5)	4.87 (4.40–5.30)	85 (79–91)
U46619 (3.10^{-9} M)	6	2.3 (1.3–3.3)	5.02 (4.25–5.79)	74 (69–79)

Table 4
Relaxation induced by sodium nitroprusside in human internal mammary artery and saphenous vein preparations precontracted with methoxamine or U46619
Tension is the pre-contraction value induced by the vasoconstrictor. Values are expressed as means ± 95% confidence intervals (given in parenthesis). $pD_2 = -\log (EC_{50})$ and E_{max} = maximal relaxation (percentage of maximal relaxation induced by papaverine 10^{-4} M).

	n	Tension (g)	pD_2 (sensitivity)	E_{max}
Internal mammary artery				
Methoxamine (3.10^{-6} M)	6	1.7 (1.1–2.3)	6.72 (6.45–6.99)	86 (80–92)
U46619 (3.10^{-9} M)	6	2.8 (1.8–3.8)	6.66 (6.35–6.97)	82 (72–92)
U46619 (3.10^{-8} M)	6	3.2 (2.2–4.2)	6.61 (6.35–6.87)	88 (80–96)
Saphenous vein				
Methoxamine (3.10^{-6} M)	6	1.9 (1.1–2.7)	7.06 (6.46–7.66)	98 (96–100)
U46619 (3.10^{-9} M)	6	2.5 (1.5–3.5)	6.98 (6.14–7.82)	94 (88–100)
U46619 (3.10^{-8} M)	6	3.4 (2.2–4.6)	6.7 (6.38–7.02)	91 (84–98)

Stock solutions of the drugs were held frozen (−20°C) in aliquots and were freshly dissolved in distilled water to the appropriate concentrations, expressed as final molar concentrations in the organ bath. The cAMP enzyme–immunoassay kits were purchased from Cayman (Ann Arbor, USA). The water-saturated ethyl ether and trichloracetic acid were obtained from Prolabo (Fontenay sous Bois, France).

3. Results

3.1. Contractions induced with U46619 and methoxamine

A first series of experiments were carried out in 24 vessel segments to obtain concentration–contraction curves for U46619 and for methoxamine and to determine the concentrations required to induce a contraction equivalent to 50% of the maximal contraction induced by the agent (EC_{50} expressed as $pD_2(-\log EC_{50})$). Concentration–contraction curves were made by cumulative addition of increasing concentrations of agonist (0.5-log increments) to the organ-bath. The pD_2 values and the maximal effects in response to U46619 and methoxamine in both IMA and SV are presented in Table 1. The pretreatment with L-NNA and indomethacin did not alter basal tone.

3.2. Relaxing effects of forskolin

In rings of IMA precontracted with equi-effective concentrations of U46619 (3.10^{-9} M) and methoxamine

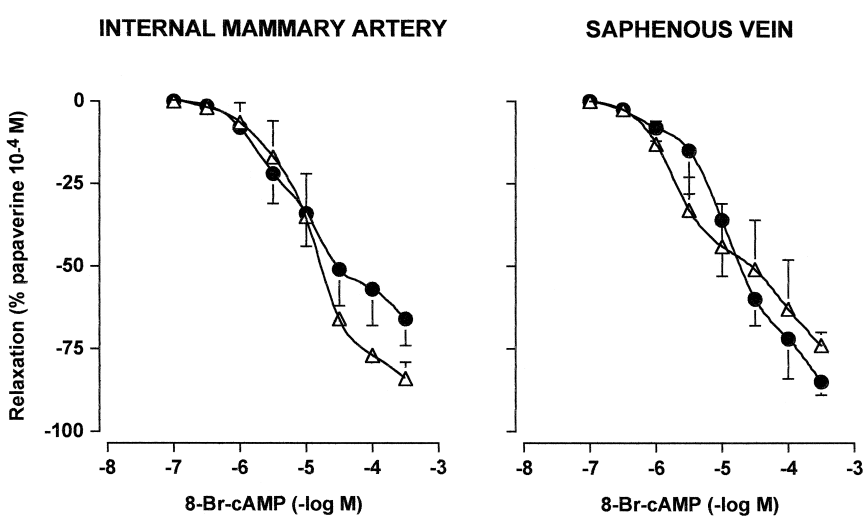

Fig. 2. Concentration-dependent relaxant effects of 8-Br-cyclicAMP on methoxamine 3.10^{-6} M (●) and U46619 3.10^{-9} M (△)-contracted internal mammary arteries (a) and saphenous veins (b). Points are means ± SEM ($n = 6$ in all groups).

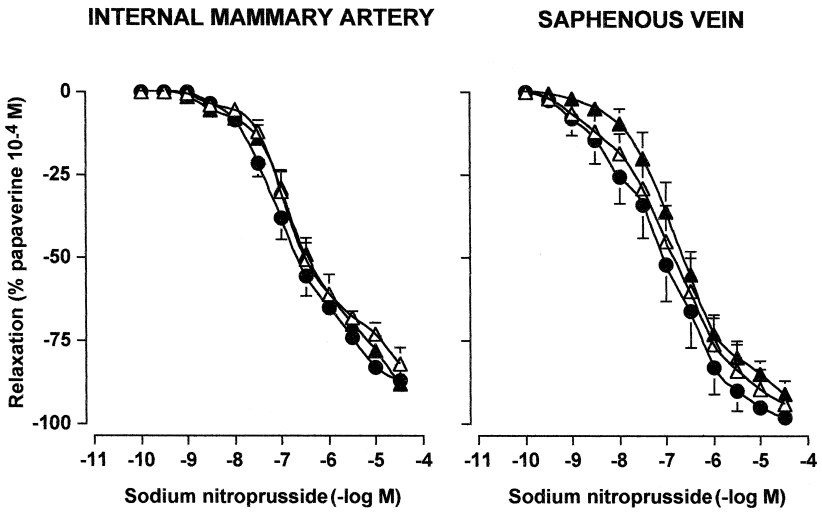

Fig. 3. Concentration-dependent relaxant effects of sodium nitroprusside on methoxamine 3.10^{-6} M (●), U46619 3.10^{-9} M (△) and U46619 3.10^{-8} M (▲)-contracted IMAs (a) and SVs (b). Points are means ± SEM ($n = 6$ in all groups).

(3.10^{-6} M), forskolin produced complete relaxations. A significant rightward shift of the forskolin concentration–response curve was however observed in U46619-precontracted IMA rings (Fig. 1a). In addition, in rings precontracted with a higher concentration of U46619 (3.10^{-8} M), the rightward shift of the concentration–response curve was larger than in rings precontracted with methoxamine or U46619 (3.10^{-9} M) (Table 2, Fig. 1a). In contrast to the results obtained with IMA, forskolin produced similar relaxations in rings of SV precontracted with either methoxamine or U46619 (Table 2, Fig. 1b).

3.3. Relaxing effects of 8-Br-cAMP

8-Br-cAMP (up to 3.10^{-4} M) did not produce complete relaxations of IMA and SV precontracted with methoxamine or U46619 (Table 3, Fig.2). The relaxations were however independent of the agonist used to raise the tone, and were similar in both arteries and veins (Fig. 2, Table 3).

3.4. Relaxing effects of SNP

Table 4 shows that the nitric oxide (NO) donor sodium nitroprusside acting through activation of guanylate cyclase produced similar relaxations in IMA contracted with either methoxamine or U46619. Thus, in contrast with the results obtained with the adenylate cyclase activator forskolin with IMA, the relaxant response to sodium nitroprusside was independent of the agonist employed to induce tone. In addition, the relaxant response to sodium nitroprusside in SV was similarly independent of the agonist used to raise the tone (Fig. 3).

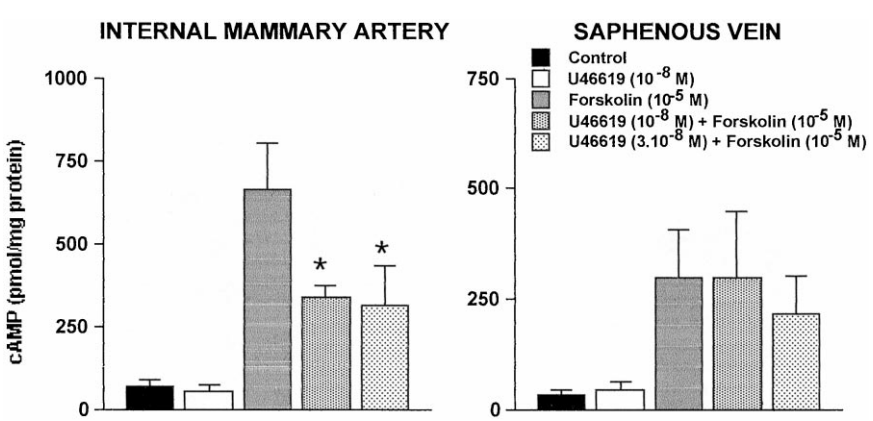

Fig. 4. Effect of U46619 on the cAMP levels in controls and forskolin-stimulated preparations. Each bar represents the mean ± SEM ($n = 6$ in all groups). * indicates cAMP levels significantly different ($P < 0.01$) from forskolin alone.

3.5. cAMP determinations

U46619 (10^{-8} M) did not modify the cAMP levels in either artery or vein segments compared with those of the control. Forskolin caused approximately a 10-fold increase in cAMP levels in IMA and SV compared with controls. U46619 (10^{-8} and 3.10^{-8} M) significantly reduced forskolin-induced cAMP production in IMA, whereas U46619 did not alter significantly the cAMP production in SV (Fig. 4). The inhibition of forskolin-induced cAMP production was 48% and 50% in IMA in presence of U46619, 10^{-8} and 3.10^{-8} M, respectively, and the inhibition was 27% in SV in the presence of 3.10^{-8} M U46619. No inhibition was found in SV in the presence of 10^{-8} M U46619.

4. Discussion

In the present study, we have demonstrated that the thromboxane A_2–mimetic U46619 inhibited both the forskolin-induced relaxation and the forskolin-induced production of cAMP in human IMA. In SV, U46619 did not alter either the forskolin-induced relaxation or cAMP production. In addition, U46619 did not alter the relaxant responses to sodium nitroprusside in either IMA or SV suggesting that U46619 exerts inhibitory effect on the cAMP pathway of relaxation but not on the NO/cyclicGMP pathway of relaxation.

In preliminary experiments, we have found that U46619 (3.10^{-8} M) reduced the relaxant responses to forskolin and to dobutamine in IMA with respect to the responses obtained in norepinephrine-contracted artery (data not shown). In the present study, the relaxant responses after contraction with U46619 were compared with those obtained after contraction with methoxamine, a selective α_1-adrenoceptor agonist. The choice of methoxamine as a constrictor instead of norepinephrine was made to rule out any possible interference of α_2-adrenoceptor and ß-adrenoceptor stimulation with the relaxation induced by forskolin or SNP. It is well known that vascular α_2-adrenoceptors and β-adrenoceptors mediate their actions through inhibition of adenylate cyclase and stimulation of adenylate cyclase, respectively (Bylund et al., 1995). In addition, stimulation of endothelial α_2-adrenoceptors in piglet pulmonary arteries induces a rise in cGMP concentration and the relaxation of the preparation, whereas stimulation of α_1-adrenoceptor with methoxamine does not enhance the effect on cGMP concentration and has no relaxant effect (Pepke-Zaba et al., 1993). Moreover, to preclude any endogenous participation of the endothelium to contractile and relaxant responses, vascular preparations were pretreated with indomethacin and L-NNA. This pretreatment allowed the evaluation of the direct effects of U46619 and methoxamine on forskolin, 8-Br-cAMP and sodium nitroprusside in the two types of human vessels.

The relaxation caused by forskolin was inhibited in IMA contracted with U46619 as compared to the relaxation in IMA contracted with methoxamine. Since the magnitude of precontraction may influence the relaxant responses (Hisajima et al., 1986; Asano et al., 1988), it was important that accurate comparison be made by assessing relaxant responses on preparations contracted to an equivalent magnitude. In IMA segments contracted with a concentration of U46619 (3.10^{-9} M), which produced a contractile response similar to that induced by methoxamine (3.10^{-6} M), the relaxant response to forskolin was reduced (as indicated by the rightward shift of the concentration–response curve), as compared to that of the preparations contracted with methoxamine. Therefore, a greater contractile response to U46619 did not explain the reduced relaxant response to forskolin in IMA. In addition, the inhibitory effect of U46619 was concentration-dependent, since a higher concentration (3.10^{-8} M) induced a larger rightward shift of the concentration–response curve and a reduction in the maximal response to forskolin. Since similar levels of contraction induced by U46619 in SV had no inhibitory effect on forskolin-induced relaxation, this result also suggests that the magnitude of the contraction did not explain the inhibitory effect of U46619 in IMA.

In addition, the absence of inhibitory effects of U46619 on forskolin-induced relaxation in SV, which has a similar responsiveness to the contractile effect of U46619, indicated a vessel-type specificity of the U46619 inhibitory effect on the relaxation in response to forskolin. Tissue specificity of the inhibitory effect of U46619 has also been shown for the relaxant response to sodium nitroprusside in piglet pulmonary arteries, since this inhibitory effect was not observed in piglet mesenteric or coronary arteries or in rat pulmonary arteries (Perez-Vizcaino et al., 1997). In connection with tissue specificity, endothelin-1 inhibited the relaxant responses to sodium nitroprusside in human pulmonary and mammary veins, but not in arteries of these vascular beds (Yang et al., 1989; Pussard et al., 1995). In terms of selectivity for the cyclic nucleotides-mediated pathways of relaxation, U46619 did not exert inhibitory effects on the relaxant responses to SNP, an agent acting through the NO/cGMP pathway, in either IMA or SV indicating that U46619 did not modulate this pathway of relaxation in these two vessel preparations.

The mechanism of the inhibitory effect of U46619 on the cAMP relaxation pathway was investigated with a membrane permeable and more stable cAMP analogue, 8-Br-cAMP, and by measurement of cAMP production. Relaxations in response to 8-Br-AMPc were not altered in either IMA or SV, suggesting that U46619 actions on forskolin relaxation are not associated with intracellular mechanisms induced by cAMP and leading to vascular relaxation. The results of the relaxation in response to forskolin after U46619 stimulation and the data obtained from the cAMP measurements are somewhat difficult to compare since relaxations and biochemical measurements

were not obtained from the same preparations and under the same experimental conditions (the vessels were not stretched in an organ bath). However, U46619 significantly altered both the relaxation and the increase in cAMP level induced by forskolin in IMA, whereas U46619 did not alter the forskolin-induced relaxation and cAMP level in SV. The forskolin-induced cAMP level in SV was weakly and non significantly altered only with the high concentration of U46619 (3.10^{-8} M). Since the cAMP measurements were made in presence of the phosphodiesterase inhibitor, IBMX, these results suggest strongly that TP receptor stimulation by U46619 induced, in human IMA, the inhibition of cAMP synthesis through inhibition of adenylate cyclase activity.

Two forms of TP receptors, termed TPα and TPβ, have recently been cloned (Hirata et al., 1991; Raychowdhury et al., 1994). These two forms are generated from a single gene by an alternative splicing mechanism and differ only in their carboxy-terminal domains (Raychowdhury et al., 1994). The two isoforms of TP receptors have been shown to exhibit similar ligand binding characteristics and phospholipase C activation but opposite regulation of adenylate cyclase activity (Hirata et al., 1996). TPα receptors activated adenylate cyclase and cAMP generation, whereas TPβ receptors inhibited adenylate cyclase as demonstrated by the inhibition of the forskolin-induced cAMP generation in cells expressing TPβ receptors but not in cells expressing TPα receptors (Hirata et al., 1996). Polymerase chain reaction analyses on human tissues have shown that both TPα and TPβ receptors are expressed in placental tissues and platelets, whereas endothelial cells express only TPβ (Raychowdhury et al., 1994; Hirata et al., 1996; Kinsella et al., 1997). The inhibitory effect of U46619 on forskolin-induced relaxation and cAMP production in the presence of indomethacin and L-NNA in human IMA suggests that the vascular smooth muscle of this vascular bed expresses the TPβ receptors. This hypothesis is supported by a recent study showing that both thromboxane A_2 receptor isoforms were found in vascular smooth muscle cells (Becker et al., 1999). However, there is evidence for the existence in platelets and in smooth muscle of TP receptor subtypes exhibiting different ligand binding characteristics, i.e., TP receptor subtypes different from TPα and TPβ receptors, from work in both animal tissues (Mais et al., 1988; Morinelli et al., 1989; Masuda et al., 1991) and human tissues (Krauss et al., 1996). Therefore, the possibility that a TP receptor subtype, different from TPα and TPβ receptors, is involved in the inhibitory effect of U46619 on forskolin-induced relaxation in human IMA cannot be ruled out.

In summary, these data suggest that thromboxane A_2 may play a role in the control of muscle tone in human IMAs, both through its potent contractile effect and through its modulating effect on the cAMP pathway of relaxation. This modulatory effect on the cAMP pathway was not observed in SV. In addition, thromboxane A_2 does not modulate the cGMP pathway of relaxation in either human IMAs or SVs.

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