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The inhibitory effects of iberiotoxin and 4-aminopyridine on the relaxation induced by β_1- and β_2-adrenoceptor activation in rat aortic rings

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1 In rat aortic rings contracted by phenylephrine, the relaxation induced by isoprenaline was partly inhibited by iberiotoxin, (ibTX), tetraethylammonium, 4-aminopyridine (4-AP) and 1,9-dideoxyforskolin, but not by glibenclamide.

2 In the presence of 4-AP, 1,9-dideoxyforskolin failed to inhibit further the relaxant response to isoprenaline. Cromakalim-induced relaxation was inhibited by glibenclamide.

3 In the absence of endothelium, ibTX and 4-AP still inhibited the relaxant response to isoprenaline.

4 The inhibitory effect of ibTX on the relaxant response to isoprenaline was eliminated by pretreatment with ICI-118,551, a β_2-adrenoceptor antagonist, but not by atenolol, a β_1-adrenoceptor antagonist.

5 The inhibitory effect of 4-AP on the relaxation induced by isoprenaline was abolished by atenolol, but not by ICI-118,551.

6 The inhibitory effect of ibTX on the isoprenaline-induced relaxation in the presence of atenolol was completely abolished by MDL 12,330A, an adenylate cyclase inhibitor. Further, the inhibitory effect of 4-AP on the isoprenaline-induced relaxation in the presence of ICI-118,551 was markedly reduced by MDL 12,330A.

7 The relaxation induced by dibutyryl cyclic AMP was partly inhibited by 4-AP but not by ibTX. However, in the presence of KT5720, an inhibitor of cyclic AMP-dependent protein kinase, ibTX failed to inhibit further the relaxation induced by isoprenaline.

8 These results suggest that, in rat aortic rings, K_{Ca} channels are involved in the relaxation induced by isoprenaline. In addition, K_{Ca} channels are mainly activated by β_2-adrenoceptors through cyclic AMP-dependent pathways. Further, the inhibition of isoprenaline-relaxation by 4-AP may be related to the activation of β_1-adrenoceptors and cyclic AMP formation.

Keywords: Rat aortic rings; relaxation; β_1- and β_2-adrenoceptors; K_{Ca} channels; K_v channels; adenylate cyclase

Introduction

The increased adenosine 3′:5′-cyclic monophosphate (cyclic AMP) formation is thought to be the reason for the relaxation induced by activation of β-adrenoceptors (Rasmussen, 1986). It is generally held that the relaxation of smooth muscle induced by cyclic AMP is mediated through activation of cyclic AMP-dependent protein kinase (PKA) and subsequent phosphorylation of specific proteins (Hardman, 1984; Bennet *et al.*, 1989). One of the principal targets of activated PKA is reported to be Ca^{2+}-activated K channels (K_{Ca}) (Sadoshima *et al.*, 1988b; Kume 1989; Carl *et al.*, 1991). In addition, it was reported in tracheal smooth muscles that K_{Ca} channel activation by PKA is less potent than that by the α subunit of a stimulatory guanine nucleotide binding protein of adenylate cyclase (Kume *et al.*, 1994). It was also reported that there is a tighter coupling in tracheal smooth muscles between relaxation and K_{Ca} channel opening by β-adrenoceptor stimulation (Hiramatsu *et al.*, 1994).

Distribution of subtypes of β-adrenoceptors (β_1 and β_2) varies widely in animal organs and species and activation of either of the subtypes in the smooth muscles causes relaxation (Minneman & Molinoff, 1980). In canine saphenous vein, the β_2-adrenoceptor was reported to be involved in the relaxation (Tokudome & Taira, 1981), as in the case of hyperpolarization through the activation of ATP-sensitive K-channels (K_{ATP}) (Nakashima & Vanhoutte, 1995). In addition, the β_2-adrenoceptor was reported to be responsible for membrane hyperpolarization induced by isoprenaline in canine tracheal smooth muscle (Ito, 1988). It was originally thought that only β_2-adrenoceptors were involved in the relaxant responses of rat aorta (Cohen & Wiley, 1978). However, more recent findings suggest that rat aorta may contain both β_1- and β_2-adrenoceptors (O'Donnell & Wanstall, 1984). Since no studies have been reported on the relationship between K channels and β-adrenoceptor subtypes in rat aortic rings, this was examined in the present study.

Methods

Tissue preparations and recording of mechanical actions

Male Wistar rats weighing 150–170 g were killed by cervical dislocation under ether anaesthesia. The aortae were isolated, and excess fat and connective tissue were removed. Vessels were cut into rings of about 3 mm in length. Preparations were mounted in organ baths containing 20 ml of a modified Krebs solution of the following composition (mM): NaCl 120.3, KCl 4.8, $CaCl_2$ 1.2, $MgSO_4$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 24.2 and glucose 5.8, at pH 7.4. The tissue bath solution was maintained at 37°C and bubbled with a 95% 0_2 and 5% CO_2 mixture. Stainless steel hooks were put through the aortic ring, one attaching the muscle to a stainless steel rod and the other to a transducer adjusted to give an initial stretched tension of 2 g. Changes in isometric tension were recorded through force-displacement transducers (Grass FT-03) connected to a 6-channel Grass polygraph.

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Experimental procedure

The aortic rings were contracted by phenylephrine (PE, 3 × 10^{-7} M) before the addition of vasorelaxant agents. In the aortic rings without endothelium, the concentration of PE was adjusted to 3 × 10^{-8} M so that the magnitude of contraction was the same as that with endothelium. Similarly, in the aortic rings pretreated with a high concentration of 4-aminopyridine (4-AP) or MDL 12,330A, the concentration of PE was adjusted to 2 × 10^{-7} M or 10^{-6} M, respectively, to obtain a contraction similar in magnitude to the control tissue. The presence of endothelium was confirmed by the presence of acetylcholine (10^{-6} M)-induced relaxation (100%) in the aorta precontracted with PE (3 × 10^{-7} M). Endothelium was removed by rubbing with a small wooden stick moistened with Krebs solution. The absence of endothelium was confirmed by the absence of relaxation to acetylcholine (10^{-6} M).

Drugs

The following drugs were used: isoprenaline (Sigma Chemical Co., St. Louis, MO, U.S.A.), phenylephrine (Sigma), 4-AP (Sigma), MDL-12,330A (cis-N-(2-phenylcyclopentyl)-azacyclotridec-1-en-2-amine monohydrochloride) (Research Biochem. Int'l., Natick, MA, U.S.A.), glibenclamide (Upjohn, Kalamazoo, MI, U.S.A.), iberiotoxin (Research Biochem Int'l.), ICI-118,551 ((I)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1 methylethyl) amino]-2-butanol hydrochloride) (Research Biochem Int'l.), acetylcholine (Sigma), atenolol (Stuart Pharmaceuticals, Wilmington, DE, U.S.A.), 1,9-dideoxyforskolin (LC Laboratories, Woburn, MA, U.S.A.), KT5720 (LC Laboratories), cromakalim (Beecham Pharmaceuticals, England), tetraethylammonium (Sigma).

Analysis of data

The pD_2 value was calculated as the negative log of the concentration of isoprenaline which causes 50% of the isoprenaline relaxation. The data are presented as the mean ± s.e.mean and statistically analysed by Student's two-tailed *t* test, analysis of variance and Dunnett's test.

Results

In rat aortic rings contracted by PE (3 × 10^{-7} M), isoprenaline (10^{-9}–10^{-5} M) caused relaxations in a concentration-dependent manner (pD_2 7.11 ± 0.11, maximal relaxation: 79.5 ± 2.9%) (Figure 1). Pretreatment of the tissues with iberiotoxin (ibTX: 2 × 10^{-8} M), but not with glibenclamide (10^{-6} M), partially inhibited the relaxation induced by isoprenaline (Figure 1). In addition, pretreatment with tetraethylammonium (TEA: 10^{-3} M) partially inhibited the relaxation induced by isoprenaline (control pD_2 6.90 ± 0.14, maximal relaxation: 71.6 ± 1.8%; TEA pD_2 6.40 ± 0.11, maximal relaxation: 46.7 ± 1.0%, $n=4$). Glibenclamide (10^{-6} M) also inhibited the relaxant response to cromakalim (10^{-7}–3 × 10^{-5} M) (control pD_2 6.44 ± 0.11, glibenclamide pD_2 5.56 ± 0.10, $n=5$). Pretreatment with 4-AP (5 × 10^{-3} M) partially inhibited the relaxant response to isoprenaline (3 × 10^{-8}–10^{-6} M) in the aortic rings contracted by PE (2 × 10^{-7} M) (Figure 1). However, a lower concentration of 4-AP (10^{-3} M) did not affect the isoprenaline-induced relaxation. Pretreatment of the aortic rings with 1,9-dideoxyforskolin (3 × 10^{-5} M) also inhibited the relaxation induced by isoprenaline (control pD_2 7.16 ± 0.10, maximal relaxation: 78.2 ± 2.1%; 1,9-dideoxyforskolin pD_2 6.75 ± 0.13, maximal relaxation: 62.8 ± 0.15%, $n=5$). In addition, in the aortic rings pretreated with 4-AP (5 × 10^{-3} M), 1,9-dideoxyforskolin (3 × 10^{-5} M) did not further inhibit the relaxation induced by isoprenaline. In the aortic rings denuded of endothelium, isoprenaline (10^{-9}–3 × 10^{-6} M) still caused relaxations of the aortic rings contracted by PE (3 × 10^{-8} M). Isoprenaline-induced relaxation in the absence of endothelium (pD_2 6.94 ± 0.07, maximal relaxation: 77.7 ± 2.5%) was similar in degree to the relaxation in the presence of endothelium. Pretreatment with ibTX (2 × 10^{-8} M) or 4-AP (5 × 10^{-3} M) still inhibited the relaxation induced by isoprenaline (10^{-8}–3 × 10^{-6} M) in the absence of endothelium. Pretreatment of the aortic rings with ICI-118,551 (10^{-7} M) or atenolol (10^{-6} M) significantly inhibited the relaxation induced by isoprenaline (10^{-9} × 10^{-5} M) (control pD_2 6.88 ± 0.08, maximal relaxation: 89.7 ± 1.7%; ICI-118,551 pD_2 5.37 ± 0.07, maximal relaxation: 61.6 ± 3.8%; atenolol pD_2 6.14 ± 0.10, maximal relaxation: 91.8 ± 2.9%, $n=5$). The relaxation induced by isoprenaline (10^{-7}–10^{-4} M) in the presence of ICI-118,551 (10^{-7} M) was completely inhibited by pretreatment with 4-AP (5 × 10^{-3} M), but not by ibTX (2 × 10^{-8} M), in the aortic rings contracted by PE (3 × 10^{-7} M) (Figure 2). In the aortic rings pretreated with atenolol (10^{-6} M), ibTX (2 × 10^{-8} M) further inhibited the relaxation

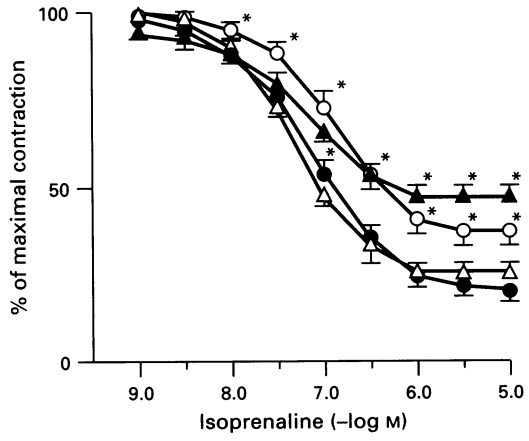

Figure 1 Effects of ibTX, 4-AP and glibenclamide on isoprenaline-induced relaxation in rat aortic rings. Some tissues were pretreated with ibTX (2 × 10^{-8} M) (○, $n=5$) or glibenclamide (10^{-6} M) (△, $n=5$) 20 min before the addition of PE. 4-AP (5 × 10^{-3} M) (▲, $n=5$) was added to the bath following the addition of PE. Maximal contractions induced by PE just before the addition of isoprenaline were taken as 100%. *Significantly different from the control (●, $n=5$) ($P<0.05$).

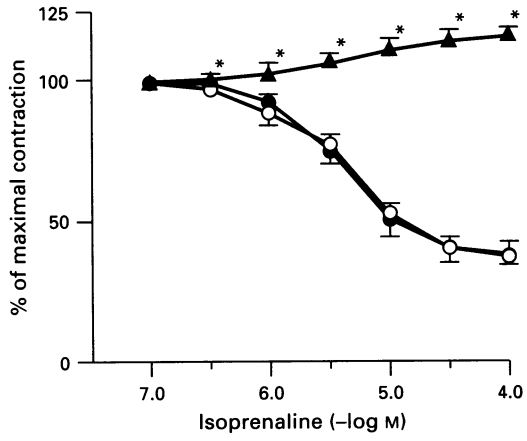

Figure 2 Effects of ibTX and 4-AP on the relaxation induced by isoprenaline in the presence of ICI-118,551 in rat aortic rings. The aortic rings were precontracted by PE in the presence of ICI-118,551 (10^{-7} M) before the addition of isoprenaline. Some tissues were pretreated with ibTX (2 × 10^{-8} M) (○, $n=6$) 20 min before the addition of PE. Other tissues were treated with 4-AP (5 × 10^{-3} M) following the addition of PE (▲, $n=6$). Maximal contractions induced by PE just before the addition of isoprenaline were taken at 100%. *Significantly differently from the tissues treated with ICI-118,551 alone (●, $n=6$) ($P<0.05$).

induced by isoprenaline (10^{-7}–10^{-4} M) (Figure 3). However, the relaxation induced by isoprenaline (10^{-8}–10^{-4} M) in the presence of atenolol (10^{-6} M) was not significantly affected by pretreatment with 4-AP (5×10^{-3} M) (Figure 3).

Combined pretreatment with atenolol (10^{-6} M) and MDL 12,330A (3×10^{-5} M) markedly inhibited the relaxation induced by isoprenaline (10^{-8}–10^{-4} M) (the maximal relaxation of less than 40%) (Figure 4) as compared to that with atenolol (10^{-6} M) alone (Figure 3). The inhibitory effect of the combined treatment on the isoprenaline-induced relaxation was not affected by the pretreatment with ibTX (2×10^{-8} M) (Figure 4). In tissues pretreated with ICI-118,551 (10^{-7} M), MDL 12,330A (3×10^{-5} M) slightly shifted the relaxation curve for isoprenaline (pD_2 4.85±0.06) (Figure 5) to the right as compared to that in the absence of MDL 12,330A (Figure 2). Pretreatment with 4-AP (5×10^{-3} M) in the presence of ICI-118,551 (10^{-7} M) and MDL 12,330A (3×10^{-5} M) further inhibited the relaxation induced by isoprenaline (10^{-5}–3×10^{-4} M) (Figure 5). In the aortic rings contracted by PE (3×10^{-7} M), dibutyryl cyclic AMP (10^{-5}–10^{-3} M) caused relaxations in a concentration-dependent manner (pD_2 3.73±0.03, $n=4$). Pretreatment with 4-AP (5×10^{-3} M) (pD_2 3.43±0.06, $n=4$), but not ibTX (2×10^{-8} M) (pD_2 3.73±0.03, $n=4$), partly inhibited the relaxant response to dibutyryl cyclic AMP. In the tissues pretreated with atenolol (10^{-6} M), KT5720 (5×10^{-7} M) partially inhibited the residual relaxation induced by isoprenaline (10^{-8}–10^{-4} M) (Figure 6). Pretreatment with ibTX (2×10^{-8} M) did not affect the residual relaxant response to isoprenaline in the presence of atenolol (10^{-6} M) and KT5720 (5×10^{-7} M (Figure 6).

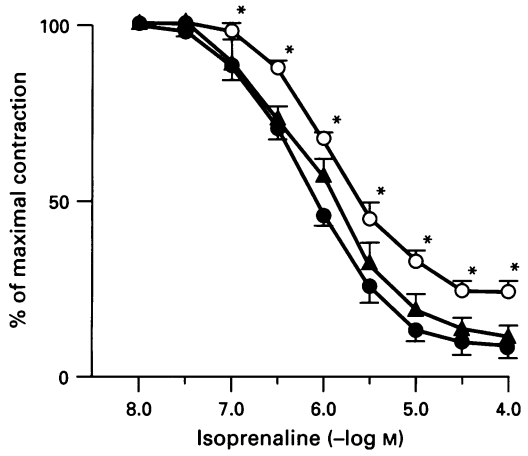

Figure 3 Effects of ibTX and 4-AP on the relaxation induced by isoprenaline in the presence of atenolol in rat aortic rings. The aortic rings were precontracted by PE in the presence of atenolol (10^{-6} M) before the addition of isoprenaline (●, $n=6$). Some tissues were pretreated with ibTX (2×10^{-8} M) (○, $n=6$) 20 min before the addition of PE. Other tissues were treated with 4-AP (5×10^{-1} M) following the addition of PE (▲, $n=6$). Maximal contractions induced by PE just before the addition of isoprenaline were taken as 100%. *Significantly different from the control (●) ($P<0.05$).

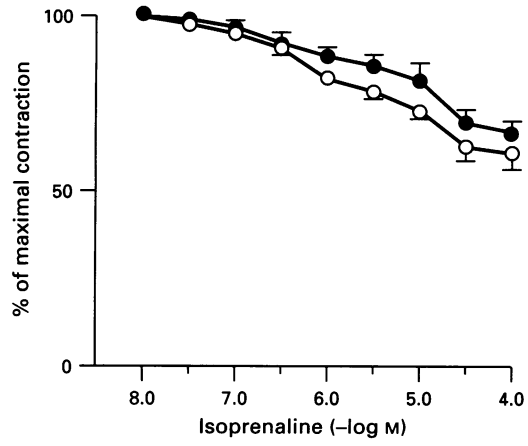

Figure 4 The effect of ibTX on the relaxation induced by isoprenaline in the presence of atenolol and MDL 12,330A in rat aortic rings. The aortic rings were precontracted by PE in the presence of atenolol (10^{-6} M) and MDL 12,330A (3×10^{-5} M) before the addition of isoprenaline (●, $n=6$). Some tissues were also pretreated with ibTX (2×10^{-8} M) (○, $n=6$) 20 min before the addition of PE. Maximal contractions induced by PE just before the addition of isoprenaline were taken as 100%.

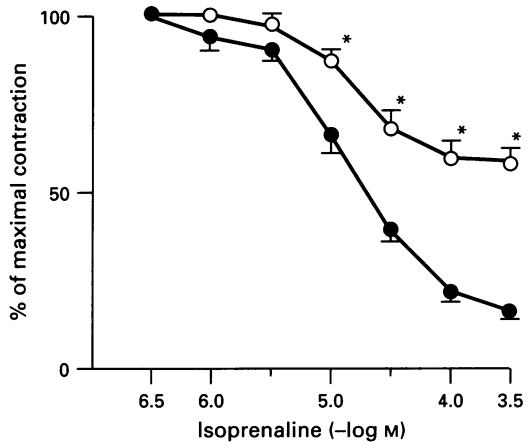

Figure 5 The effect of 4-AP on the relaxation induced by isoprenaline in the presence of ICI-118,551 and MDL 12,330A in rat aortic rings. The aortic rings were precontracted by PE in the presence of ICI-118,551 (10^{-7} M) and MDL 12,330A (3×10^{-5} M) before the addition of isoprenaline (●, $n=6$). Some tissues were also treated with 4-AP (5×10^{-3} M) following the addition of PE (○, $n=6$). Maximal contractions induced by PE just before the addition of isoprenaline were taken as 100%. *Significantly different from the control (●) ($P<0.05$).

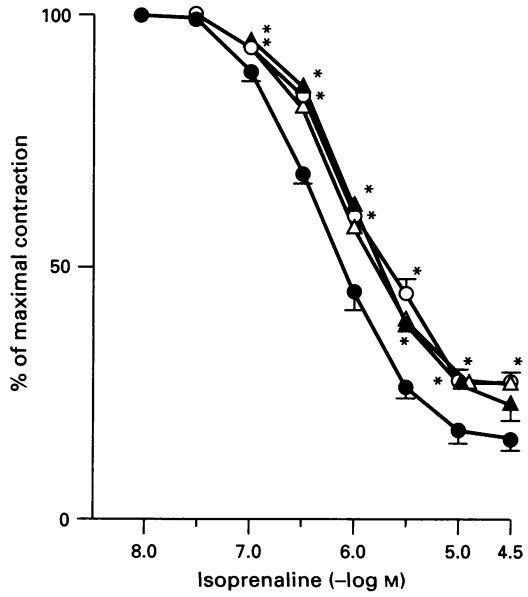

Figure 6 The effect of ibTX on the relaxation induced by isoprenaline in the presence of atenolol and KT5720 in rat aortic rings. The aortic rings were precontracted by PE in the presence of atenolol (10^{-6} M) before the addition of isoprenaline (●, $n=4$). Some tissues were additionally treated with ibTX (2×10^{-8} M), (○, $n=4$), KT5720 (5×10^{-7} M) (▲, $n=4$) or ibTX (2×10^{-8} M) plus KT5720 (5×10^{-7} M) (△, $n=4$) 20 min before the addition of PE. Maximal contractions just before the addition of isoprenaline were taken as 100%. *Significantly different from the control (●) ($P<0.05$).

Discussion

K_{Ca} channels are present in a variety of cells and they may play a role in relaxation of airway muscles (Kume *et al.*, 1989; Jones *et al.*, 1990), secretion from glands (Petersen & Maruyama, 1984), neurotransmitter release (Robitaille & Charlton, 1992; Stretton *et al.*, 1992) and repolarization of the action potential (Yoshida *et al.*, 1991). In aortic smooth muscles, K_{Ca} channels also seem to contribute to the membrane potential (Sadoshima *et al.*, 1988a,b; Shoemaker & Worrel, 1991). In the present study, isoprenaline-induced relaxations of rat aortic rings were inhibited by ibTX (Galvez *et al.*, 1990) and a low concentration of TEA, inhibitors of large conductance K_{Ca} channels. These results indicate that the activation of K_{Ca} channels may be involved in the relaxation mediated by β-adrenoceptors in rat aorta. Since a higher concentration of ibTX (10^{-7} M) did not cause further inhibition of the relaxation induced by isoprenaline (unpublished observation) than a lower concentration of ibTX (2×10^{-8} M), mechanisms other than K_{Ca} channel activation may also be involved in the isoprenaline-induced relaxation.

K_{ATP} channels have also been identified in vascular smooth muscle (Standen *et al.*, 1989). First discovered in cardiac cells (Noma, 1983), these channels are also present in excitable cells like pancreatic β-cells and other muscle types (Ashcroft & Ashcroft, 1990; Edwards & Weston, 1993) and in kidney (Wang *et al.*, 1990a,b; Tsuchiya *et al.*, 1992) and follicular cells of the *Xenopus* oocyte (Honore & Lazdunski, 1991; 1993). In smooth muscles, K_{ATP} channels are opened by various K^+ channel openers which induce smooth muscle relaxation (Quast, 1993; Edwards & Weston, 1994). The effects of these K^+ channel openers are inhibited by glibenclamide (Quast & Cook, 1989). It has been suggested that relaxation of the vascular smooth muscle by vasoactive intestinal peptide and by calcitonin gene-related peptide is due to activation of K_{ATP} channels (Nelson *et al.*, 1990a,b; Quayle *et al.*, 1994). In the present study, glibenclamide, an inhibitor of K_{ATP} channels, failed to affect the relaxation induced by isoprenaline. However, glibenclamide inhibited the relaxation induced by cromakalim, an activator of K_{ATP} channels. These results, therefore, indicate that the activation of K_{ATP} channels may not be involved in the relaxation mediated by β-adrenoceptor activation in rat aorta.

Voltage-dependent K^+ (K_v) channels have been identified in a variety of smooth muscles. It has been reported that, in smooth muscle cells from the rabbit pulmonary artery and portal vein, 4-AP, an inhibitor of K_v channels, at 5×10^{-3} M markedly inhibited the outward K^+ current through K_v channels (Okabe *et al.*, 1987; Beech & Bolton, 1989). In smooth muscle cells from the rabbit coronary and cerebral arteries, the outward K^+ current was inhibited by 4-AP at 10^{-2} M or 5×10^{-3} M by about 50 ~ 70% (Volk *et al.*, 1991; Robertson & Nelson, 1994). In canine renal arteries, 4-AP at 10^{-3} M inhibited the outward K^+ current by about 50% (Gelbrand & Hume, 1992), whereas in canine airway smooth muscle cells it inhibited it more than 90% (Boyle *et al.*, 1992). In guinea-pig portal veins, the outward K^+ current was inhibited by 4-AP at 5×10^{-3} M by 67% (Pfründer & Kreye, 1992). The outward K^+ current in cerebral arterial muscle cells from mongrel cats was inhibited by 4-AP at 10^{-2} M by about 50% (Bonnet *et al.*, 1991). Therefore, the degree to which they are sensitive to 4-AP appears to vary from tissue to tissue. The results in the present study also indicated that 4-AP at 5×10^{-3} M, but not at 10^{-3} M, partially inhibits isoprenaline-induced relaxation. The isoprenaline-induced relaxation was also inhibited by 1,9-dideoxyforskolin, an inhibitor of K_v channels without inhibitory effect on adenylate cyclase (Hoshi *et al.*, 1988). Since 1,9-dideoxyforskolin did not further inhibit the residual relaxation induced by isoprenaline in the presence of 4-AP, it is likely that the inhibitory effect of 4-AP on the isoprenaline-induced relaxation is, at least in part, due to inhibition of K_v channels.

It has been reported that the endothelium may play a role in the relaxation induced by β-adrenoceptor agonists in rat aorta (Kamata *et al.*, 1989; Gray & Marshall, 1992), canine coronary arteries (Rubanyi & Vanhoutte, 1985) and rat mesenteric arteries (Graves & Poston, 1993). However, others have suggested that the endothelium is not involved in β-adrenoceptor agonist-induced relaxation in rat aorta (Konishi & Su, 1983; Moncada *et al.*, 1991). The results in the present study indicate that the relaxation induced by isoprenaline is not dependent on the presence of endothelium in rat aortic rings. Further, since the removal of endothelium did not affect the inhibitory effect of ibTX or 4-AP, a possible activation of K_{Ca} or K_v channels mediated by β-adrenoceptors is probably independent of endothelium.

It has been reported that β-adrenoceptor activation leads to activation of K_{Ca} channels in cultured smooth muscle cells of rat aortae (Sadoshima *et al.*, 1988a), in tracheal smooth muscles of guinea-pigs (Kume *et al.*, 1989; 1992; 1994; Jones *et al.*, 1990; Murray *et al.*, 1991; Hiramatsu *et al.*, 1994) and man (Miura *et al.*, 1992). However, there is no report of a relationship between β-adrenoceptor subtypes and K channels in rat aortic rings. Therefore, this was examined further in the present study. The inhibition of β_1-adrenoceptors by atenolol, an inhibitor of β_1-adrenoceptors (Giudicelli *et al.*, 1973), apparently did not affect the inhibitory effect of ibTX on the relaxation induced by isoprenaline. This suggests that the activation of K_{Ca} channels mediated by isoprenaline is not related to β_1-adrenoceptors but may be due to activation of β_2-adrenoceptors. In fact, the inhibition of β_2-adrenoceptors by ICI-118,551, an inhibitor of β_2-adrenoceptors (Bilski *et al.*, 1983), completely eliminated the inhibitory effect of ibTX on the relaxation induced by isoprenaline. These results suggest that the activation of β_2-adrenoceptors, but not β_1-adrenoceptors, is responsible for the activation of K_{Ca} channels. However, β_2-adrenoceptors do not seem to be involved in the activation of K_v channels in rat aortic rings, since 4-AP completely inhibited the relaxation induced by isoprenaline in the presence of ICI-118,551. In addition, in the presence of ICI-118,551 and 4-AP, isoprenaline caused small contractions. It has been reported (Kamata *et al.*, 1989) that isoprenaline at high concentrations causes an increase in the resting tension of rat aortic strips through the activation of α-adrenoceptors. It is, therefore, possible in the present study that the decreased relaxant response to isoprenaline due to activation of β-adrenoceptors may have potentiated the contractile response to isoprenaline due to activation of α-adrenoceptors. It is likely that β_1-adrenoceptors may be involved in the activation of K_v channels, since atenolol attenuated the inhibitory effect of 4-AP.

The mechanisms of activation of these K channels by β_1 and β_2-adrenoceptor subtypes were further examined by use of MDL 12,330A, an inhibitor of adenylate cyclase (Hunt & Evans, 1980; Lippe & Ardizzone, 1991). The activation of K_{Ca} channels by β_2-adrenoceptors is apparently a cyclic AMP-dependent process, since MDL 12,330A abolished the effect of ibTX. The involvement of a cyclic AMP-dependent process in the possible activation of K_v channels by β_1-adrenoceptors is also indicated by the fact that MDL 12,330A markedly reduced the inhibitory effect of 4-AP. It has been reported that MDL 12,330A inhibits phosphodiesterase activity at higher concentrations (Hunt & Evans, 1980). In the present study, a higher concentration of PE in the presence of MDL 12,330A was required to induce contraction similar in degree to that in the absence of MDL 12,330A. This may suggest that MDL 12,330A at the concentration used in the present study inhibits phosphodiesterase activities. However, the effect of MDL 12,330A is apparently to reduce the relaxation induced by isoprenaline. This may suggest that the combined effect of MDL 12,330A (adenylate cyclase and phosphodiesterase inhibition) on the isoprenaline-induced relaxation is to reduce cyclic AMP levels. The involvement of a cyclic AMP-dependent process in the possible activation of K channels by β-adrenoceptors is also substantiated by the results that 4-AP attenuated the relaxation induced by dibutyryl cyclic AMP. The absence of inhibitory effect of ibTX on the relaxation

induced by dibutyryl cyclic AMP may suggest that either cyclic AMP is not responsible for the activation of K_{Ca} channels or some form of functional compartmentalization of cyclic AMP exists within the cells as discussed by Hayes & Brunton (1982). However, since the inhibitory effects of ibTX can be eliminated by pretreatment with KT5720, an inhibitor of cyclic AMP-dependent protein kinase (Kase *et al.*, 1987), it is likely that cyclic AMP-dependent processes are involved in the possible activation of K channels by β-adrenoceptors.

These results, therefore, suggest that, in rat aortic rings, isoprenaline may activate K_{Ca} and K_v channels. In addition, β_1 and β_2-adrenoceptor subtypes in rat aorta may be involved in the activations of K_v and K_{Ca} channels respectively. Further, the activations of these K channels by β_1 and β_2-subtypes are most likely cyclic AMP-dependent.

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