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Endothelium- and cytochrome P-450-dependent relaxation induced by isoproterenol in rat aortic rings

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Abstract

In rat aortic rings, the mechanism of endothelium-dependent relaxation induced by isoproterenol is examined. Pretreatment with (±)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol (ICI-118,551), a β_2-adrenoceptor antagonist, or atenolol, a β_1-adrenoceptor antagonist, partly inhibited the relaxing response to isoproterenol. The relaxing response to isoproterenol in the presence of ICI-118,551 or atenolol was markedly inhibited by removal of endothelium. In the aorta pretreated with ICI-118,551 or atenolol, residual relaxing response to isoproterenol was also inhibited by 2-methyl-1,2-di-3-pyridyl-1-propanone (metyrapone), α-naphthoflavone or 8-methoxypsoralen, cytochrome P-450 monoxygenase inhibitors, and methylene blue, but not by indomethacin, a cyclooxygenase inhibitor, 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA861), a 5-lipoxygenase inhibitor, N^G-nitro-L-arginine (NOARG), a nitric oxide synthase inhibitor, Zn protoporphyrin IX, a heme oxygenase inhibitor, or yohimbine, a α_2-adrenoceptor antagonist. In the aorta denuded of endothelium, metyrapone did not affect the residual relaxing response to isoproterenol in the presence of atenolol. These results suggest that the cytochrome P-450 system may be involved in the endothelium-dependent relaxation induced by isoproterenol through β_1- and β_2-adrenoceptor activation.

Keywords: Isoproterenol; β_1-Adrenoceptor; β_2-Adrenoceptor; Endothelium; Relaxation; Cytochrome P450; Aortic ring, rat

1. Introduction

Relaxation induced by isoproterenol is thought to be due to activation of adenylate cyclase and an increase in cAMP levels in the smooth muscle cell (Kukovetz et al., 1981; Heeson and De Mey, 1990). Some reports suggested that the removal of endothelium attenuates the relaxation induced by isoproterenol in rat aortas (Kamata et al., 1989) and canine coronary arteries (Rubanyi and Vanhoutte, 1985). The inhibition of nitric oxide (NO) synthesis was also reported to attenuate the isoproterenol-induced relaxation in rat aortas (Gray and Marshall, 1991) and rat mesenteric arteries (Graves and Poston, 1993). In addition, indomethacin was reported to potentiate the relaxation induced by isoproterenol in canine coronary arteries (Rubanyi and Vanhoutte, 1985). More specifically, the vasodilation caused by β_2-adrenoceptor activation was attenuated by a NO-synthesis inhibitor in rat mesenteric arteries (Graves and Poston, 1993) and hindquarter vascular bed of conscious rats (Gardiner et al., 1991). However, Moncada et al. (1991) reported that the removal of endothelium or an inhibitor of NO synthase had no effect on the relaxation induced by isoproterenol in rat aortas. Similarly, the removal of endothelium had no effect on the isoproterenol-induced relaxation in femoral arteries and aortas from normotensive rats (Konishi and Su, 1983). In canine arteries and veins, De Mey and Vanhoutte (1982) reported that the relaxation induced by isoproterenol was not affected by the removal of endothelium. Therefore, it is not clear at this time whether the endothelium is involved in the relaxation induced by β-adrenoceptor activation. Recently, in a preliminary study in rat aortas, we found that the removal of endothelium attenuated the relaxation induced by either β_1- or β_2-adrenoceptor activation. In this study, the mechanisms of endothelium-dependent vasorelaxation induced by β_1- and β_2-adrenoceptor activation are investigated in rat aortic rings.

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2. Materials and methods

2.1. Mechanical response

Male Wistar rats weighing 150–170 g were killed by cervical dislocation under ether anesthesia. The aortas were isolated, and excess fat and connective tissue were removed. Vessels were cut into rings of about 3 mm 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% O_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.

The aortic rings were contracted by phenylephrine (3×10^{-7} M) before the addition of vasorelaxing agents. In the aortic rings without endothelium, the concentration of phenylephrine 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 N^G-nitro-L-arginine (NOARG), methylene blue, α-naphthoflavone or 8-methoxypsoralen, the concentration of phenylephrine was adjusted to 3×10^{-8} M, 3×10^{-8} M, 2×10^{-6} M or 2×10^{-6} M, respectively, to obtain the contraction similar in magnitude to the control tissue. Tissues were pretreated by various inhibitors for 10 min before the addition of phenylephrine. The presence of endothelium was confirmed by the presence of acetylcholine (10^{-6} M)-induced relaxation (100%) in the aorta precontracted with phenylephrine (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 by acetylcholine (10^{-6} M).

2.2. Chemicals

The following drugs were used: isoproterenol (Sigma Chemical Co., St. Louis, MO, USA), phenylephrine (Sigma), (±)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol (ICI-118,551) (Research Biochemicals International, Natick, MA, USA), acetylcholine (Sigma), atenolol (Stuart Pharmaceuticals, Wilmington, DE, USA), yohimbine (Sigma), indomethacin (Sigma), 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA861) (Takeda Pharmaceuticals, Japan), 2-methyl-1,2-di-3-pyridyl-1-propanone (metyrapone) (Sigma), α-naphthoflavone (Sigma), 8-methoxypsoralen (Sigma), methylene blue (Fisher Scientific Company, Fair Lawn, NJ, USA), N^G-nitro-L-arginine (LC Laboratories, Woburn, MA, USA), Zn protoporphyrin IX (Research Biochemicals).

2.3. Statistical analysis

The pD_2 value was calculated as the negative log of the concentration of isoproterenol which causes 50% of the isoproterenol-induced relaxation. The data were presented as the mean ± S.E.M. and statistically analyzed using Student's *t*-test.

3. Results

In rat aortic rings contracted by phenylephrine (3×10^{-7} M), isoproterenol (10^{-9}–10^{-5} M) caused relaxation in a concentration-dependent manner (Fig. 1). Removal of endothelium significantly reduced the relaxing response to isoproterenol (10^{-9}–3×10^{-7} M) (Fig. 1). In the aortic rings contracted by phenylephrine (3×10^{-7} M), pretreatment with ICI-118,551 (10^{-7} M) markedly inhibited the maximal relaxing response to isoproterenol (10^{-8}–3×10^{-5} M) (Fig. 2A) while pretreatment with atenolol (10^{-6} M) also significantly inhibited the relaxing response to isoproterenol (10^{-7}–10^{-5} M) (Fig. 2B). Removal of endothelium almost completely inhibited the relaxing response to isoproterenol (10^{-7}–3×10^{-5} M) in the aortic rings pretreated with ICI-118,551 (10^{-7} M) (Fig. 2A). Similarly, removal of endothelium markedly reduced the relaxing response to isoproterenol (3×10^{-8}–10^{-5} M) in the aortic rings pretreated with atenolol (10^{-6} M) (Fig. 2B). Pretreatment with yohimbine (10^{-7} M), however, had no significant effect on the relaxing response to isoproterenol either in the presence of ICI-118,551 (10^{-7} M) (ICI-118,551, pD_2 5.67 ± 0.11, maximal relaxation 24.7 ± 1.6%; ICI-118,551 plus yohimbine, pD_2 5.76 ± 0.09, max-

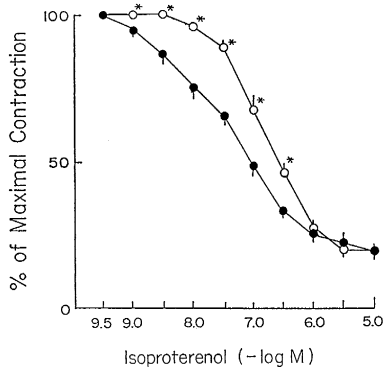

Fig. 1. The effect of endothelium removal on the relaxation induced by isoproterenol in rat aortic rings. Rat aortic rings precontracted by phenylephrine were relaxed by isoproterenol (●). Endothelium was removed from some tissues (○). Maximal contractions induced by phenylephrine just before the addition of isoproterenol were taken as 100%. Values are mean ± S.E.M. of 4 experiments. * Significantly different from the control (●) ($P < 0.05$).

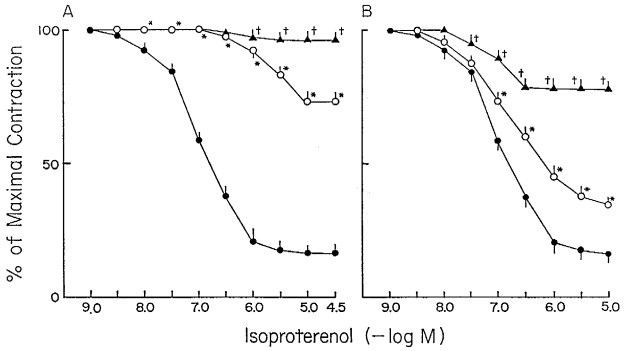

Fig. 2. The effects of ICI-118,551, atenolol and endothelium removal on the relaxation induced by isoproterenol in rat aortic rings. Rat aortic rings precontracted by phenylephrine (3×10^{-7} M) were relaxed by isoproterenol (10^{-9}–10^{-5} M) (●). Some tissues were pretreated by (A) ICI-118,551 (10^{-7} M) (○) or (B) atenolol (10^{-6} M) (○). Other tissues denuded of endothelium were also pretreated by (A) ICI-118,551 (10^{-7} M) (▲) or (B) atenolol (10^{-6} M) (▲). Maximal contractions induced by phenylephrine just before the addition of isoproterenol were taken as 100%. Values are mean ± S.E.M. of 4 experiments. * Significantly different from the control without any treatment (●) ($P < 0.05$). + Significantly different from the group treated with ICI-118,551 or atenolol (○) ($P < 0.05$).

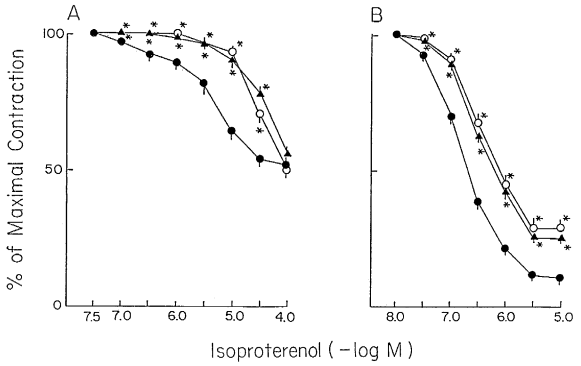

Fig. 4. The effects of α-naphthoflavone and 8-methoxypsoralen on the relaxation induced by isoproterenol in the presence of ICI-188,551 or atenolol. Rat aortic rings precontracted by phenylephrine were relaxed by isoproterenol (●) in the presence of (A) ICI-188,551 (10^{-5} M) or (B) atenolol (10^{-6} M). Some tissues were additionally treated with α-naphthoflavone (10^{-5} M) (○) or methoxypsoralen (10^{-5} M) (▲). Maximal contractions induced by phenylephrine just before the addition of isoproterenol were taken as 100%. Values are mean ± S.E.M. of 4 experiments. * Significantly different from the control (●) ($P < 0.05$).

imal relaxation 26.7 ± 2.5%; $n = 5$) or atenolol (10^{-6} M) (atenolol, pD_2 6.91 ± 0.10, maximal relaxation 69.7 ± 1.7%; atenolol plus yohimbine, pD_2 6.86 ± 0.09, maximal relaxation 70.1 ± 2.9%; $n = 5$). In the aortic rings pretreated with ICI 118,551 (10^{-7} M) (Fig. 3A) or atenolol (10^{-6} M) (Fig. 3B), additional treatment with indomethacin (10^{-6} M) or AA861 (10^{-5} M) did not significantly affect the relaxing response to isoproterenol (Fig. 3A,B) while additional treatment with metyrapone (10^{-3} M) completely (Fig. 3A) or partly (Fig. 3B) inhibited the relaxing response to isoproterenol. In addition, pretreatment of the aortas by α-naphthoflavone (10^{-5} M) or 8-methoxypsoralen (10^{-5} M) significantly inhibited the residual relaxing response to isoproterenol in the presence of ICI-118,551 (10^{-7} M) (Fig. 4A) or in the presence of atenolol (10^{-6} M) (Fig. 4B). Further, in the aortic rings pretreated with ICI-118,551 (10^{-7} M), additional treatment with methylene blue (10^{-6} M), but not NOARG (10^{-4} M) or Zn protoporphyrin IX (10^{-6} M), almost completely inhibited the relaxing response to isoproterenol (10^{-7}–3×10^{-5} M) (Fig. 5A). Similarly, in the aortic rings pretreated with atenolol (10^{-6} M), additional treatment with methylene blue (10^{-6} M), but not NOARG (10^{-4} M) or Zn protoporphyrin IX (10^{-6} M), partly

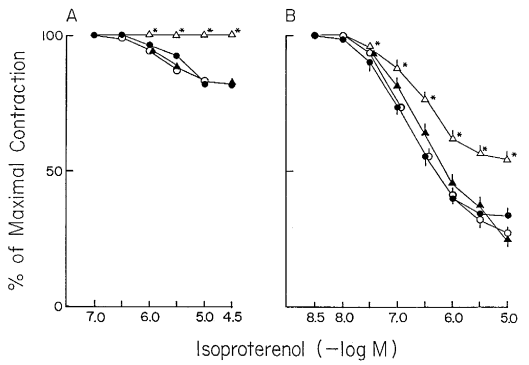

Fig. 3. The effects of indomethacin, AA861 and metyrapone on the relaxation induced by isoproterenol in the presence of ICI-188,551 or atenolol. Rat aortic rings precontracted by phenylephrine (3×10^{-7} M) were relaxed by isoproterenol (●) in the presence of (A) ICI-118,551 (10^{-7} M) or (B) atenolol (10^{-6} M). Some tissues were additionally treated with indomethacin (10^{-6} M) (○), AA861 (10^{-5} M) (▲) or metyrapone (10^{-3} M) (△). Maximal contractions induced by phenylephrine just before the addition of isoproterenol were taken as 100%. Values are mean ± S.E.M. of 4–8 experiments. * Significantly different from the control (●) ($P < 0.05$).

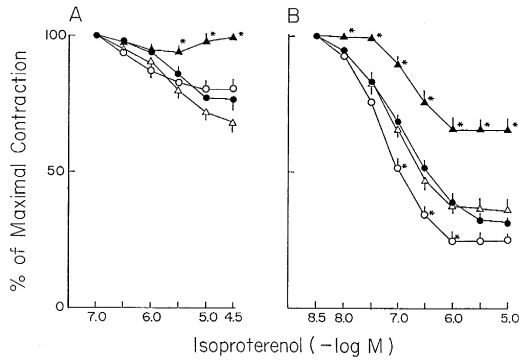

Fig. 5. The effects of methylene blue, NOARG and Zn protoporphyrin IX on the relaxation induced by isoproterenol in the presence of ICI-188,551 or atenolol. Rat aortic rings precontracted by phenylephrine (3×10^{-7} M) were relaxed by isoproterenol (●) in the presence of (A) ICI-118,551 (10^{-7} M) or (B) atenolol (10^{-6} M). Some tissues were additionally treated with NOARG (10^{-4} M) (○), methylene blue (10^{-6} M) (▲) or Zn protoporphyrin IX (10^{-6} M) (△). Maximal contractions induced by phenylephrine just before the addition of isoproterenol were taken as 100%. Values are mean ± S.E.M. of 4–8 experiments. * Significantly different from the control ($P < 0.05$).

inhibited the relaxing response to isoproterenol (3×10^{-9}–10^{-5} M) (Fig. 5B). In the endothelium-denuded aortic rings pretreated with atenolol, the relaxing response to isoproterenol (10^{-6} M) (pD_2 6.76 ± 0.10, maximal relaxation $23.3 \pm 2.7\%$, $n = 5$) was not significantly affected by treatment with metyrapone (10^{-3} M) (pD_2 6.65 ± 0.09, maximal relaxation $22.8 \pm 3.3\%$, $n = 5$).

4. Discussion

The present study in rat aortic rings demonstrates that the endothelium contributes to the vasorelaxation induced by isoproterenol. Specifically, the endothelium contributes to the relaxation induced by the activation of both β_1- and β_2-adrenoceptors, since the relaxing response to isoproterenol in the presence of ICI 118,551, a β_2-adrenoceptor antagonist (Bilski et al., 1983), or atenolol, a β_1-adrenoceptor antagonist (Giudicelli et al., 1973), was inhibited by the removal of endothelium. Results in the present study also indicate that the relaxation induced by the β_1-adrenoceptor activation is apparently more dependent on the presence of endothelium than the relaxation induced by the β_2-adrenoceptor activation, since the relaxation induced by the β_1-adrenoceptor activation can be inhibited almost completely by the removal of endothelium while the relaxation induced by the β_2-adrenoceptor activation is only partly inhibited by the removal of endothelium. Therefore, the mechanism of the endothelium-dependent relaxation induced by β_1- and β_2-adrenoceptor activations was further examined. A possible involvement of α_2-adrenoceptors in the relaxation induced by isoproterenol can be ruled out by the result that yohimbine, an α_2-adrenoceptor antagonist, failed to affect the isoproterenol-induced relaxation. In addition, it is not likely that arachidonic acid metabolites through cyclooxygenase and 5-lipoxygenase pathways are involved in the relaxation induced by β_1- and β_2-adrenoceptor activations, since indomethacin, a cyclooxygenase inhibitor, and AA861, a 5-lipoxygenase inhibitor (Tamaki et al., 1992), failed to affect the isoproterenol-induced relaxation. A possible involvement of cytochrome P-450 monooxygenase in the relaxation induced by the activation of both β_1- and β_2-adrenoceptors is suggested by the result that metyrapone, α-naphthoflavone and 8-methoxypsoralen, inhibitors of cytochrome P-450 monooxygenase (Hildebrandt, 1972; Coceani et al., 1988; Corriu et al., 1996), significantly inhibited the isoproterenol-induced residual relaxation in the aortic rings pretreated with the inhibitors of β_1- and β_2-adrenoceptor. It has been reported that, in rabbit aorta, inhibitors of cytochrome P-450 inhibit endothelium-dependent relaxations induced by metacholine and arachidonic acid (Singer et al., 1984; Pinto et al., 1986). It has also been reported that, in rat and rabbit aortas, the functional cytochrome is located mainly in the endothelium and uses arachidonic acid as a substrate (Pinto et al., 1986; Escalante et al., 1989). In addition, it was shown that epoxyeicosatrienoic acids, cytochrome P-450-derived arachidonic acid metabolites, relaxed cat cerebral arteries, porcine and bovine coronary arteries (Gebremedhin et al., 1992; Rosolowsky and Marshall, 1993; Hecker et al., 1994). Therefore, it is conceivable that the activity of cytochrome P-450 may also be involved in the endothelium-dependent relaxation induced by β_1- and β_2-adrenoceptor activation. The results in the present study demonstrated that in the absence of endothelium metyrapone does not affect the relaxing response to β_2-adrenoceptor activation. This suggests that in rat aortic rings the activation of cytochrome P-450 may be involved in the endothelium-dependent relaxation induced by β_2-adrenoceptor activation. The effect of metyrapone on the relaxation induced by β_1-adrenoceptor activation in the absence of endothelium could not be examined in the present study since either the removal of endothelium or metyrapone almost completely inhibited the relaxation induced by β_1-adrenoceptor activation. Since the relaxation induced by β_1-adrenoceptor activation is almost totally dependent on the presence of endothelium and cytochrome P-450 activity, it may be reasonable to assume that cytochrome P-450 is also involved in the endothelium-dependent relaxation induced by β_1-adrenoceptor activation. However, a further study is necessary to clarify the involvement of cytochrome P-450 in the endothelium-dependent relaxation induced by β_1-adrenoceptor activation.

The results in the present study also suggest that an increase in carbon monoxide through heme oxygenase or nitric oxide through nitric oxide synthase may not be involved in the relaxation induced by β_1- and β_2-adrenoceptor activation, since Zn protoporphyrin IX, an inhibitor of heme oxygenase (Verma et al., 1993) or NOARG, an inhibitor of nitric oxide synthase (Ishii et al., 1990), failed to affect the relaxation induced by β_1- and β_2-adrenoceptor activation. The results also indicated that the relaxation induced by β_1- and β_2-adrenoceptor activation can be inhibited markedly by methylene blue. It has been reported that methylene blue acts as a direct inhibitor of nitric oxide synthase (Mayer et al., 1993). However, in the present study, nitric oxide synthase may not be involved in the inhibitory effect of methylene blue since NOARG did not affect the relaxation induced by isoproterenol as described above. It was also reported that, under aerobic conditions, reduced forms of methylene blue are readily reoxidized by molecular oxygen thereby generating superoxide anion (McCord and Fridovich, 1970). Therefore, superoxide-mediated inactivation of nitric oxide (Wolin et al., 1990) may be the reason for the effect of methylene blue in the present study. However, it has been reported that the inhibitory effect of methylene blue was not significantly affected by superoxide dismutase (Mayer et al., 1993). In addition, hemoglobin (5×10^{-6} M) did not affect the vasorelaxation induced by isoproterenol in the presence of ICI-118,551 (10^{-7} M) or atenolol (10^{-6} M) (unpublished data). These results suggest that the inactivation of nitric

oxide may not be the reason for the effect of methylene blue in the present study. Further, formation of cGMP via guanylate cyclase may not be involved in the effect of methylene blue in the present study, since we have recently reported that isoproterenol failed to affect the level of cGMP in rat aortas (Ito et al., 1996). Therefore, a further study is necessary to clarify the mechanism of the inhibitory effect of methylene blue on the relaxation induced by isoproterenol.

In conclusion, the present study suggests that the relaxations induced by β_1- and β_2-adrenoceptor activation are partly dependent on the presence of endothelium in rat aortic rings. In addition, cytochrome P-450 monooxygenase may be involed in the endothelium-dependent relaxation induced by β_1- and β_2-adrenoceptor activation.

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