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Different mechanisms mediate β adrenoceptor stimulated vasorelaxation of coronary and femoral arteries[1]

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KEY WORDS adrenergic beta-receptors; nitric oxide; coronary artery; femoral artery; vascular endothelium

ABSTRACT

AIM: To determine whether different mechanisms mediate vasorelaxation of swine coronary and femoral arteries induced by β adrenoceptor stimulation. **METHODS**: The organ-bath was used to observe the tension changes in rings of coronary and femoral arteries to different concentrations of isoprenaline (ISO) after endothelium removal, nitric-oxide synthase (NOS) inhibition by *L*-NMMA and β_1 and/or β_2 adrenoceptor antagonization. **RESULTS**: Endothelium removal, NOS inhibition, β_2 adrenoceptor antagonization did not change the vasorelaxation induced by ISO in coronary artery, however, abolished the vasorelaxation in femoral artery. β_1 Adrenoceptor antagonization did not change the tension of femoral artery, but abolished the ISO induced vasorelaxation of coronary artery. **CONCLUSIONS**: Vasorelaxing effect of ISO in swine coronary artery is mediated through β_1 adrenoceptor and not through *L*-arginine/nitric oxide pathway, however, in femoral artery, it is mediated through β_2 adrenoceptor and *L*-arginine/nitric oxide pathway.

INTRODUCTION

Stimulation of β adrenoceptors on vascular smooth muscle results in vasorelaxation through activation of adenylyl cyclase[1]. Vascular endothelial cells also may express β adrenoceptor[2,3], although their physiologic function, if any, remains unclear. Evidence is accumulating, however, that the vascular endothelium may facilitate or mediate β adrenergic relaxation. We have previously reported that β adrenoceptor-mediated relaxation is endothelium dependent in human umbilical vein[4]. This is in agreement with other reports that have demonstrated *in vitro* that removal of the endothelium or inhibition of nitric-oxide synthases (NOS) impairs relaxation to isoprenaline in systemic vessels[5,6]. In contrast, two independent studies of isolated canine coronary arteries have suggested that β adrenoceptor-mediated vasorelaxation was endothelium-independent. It seems that different mechanisms mediate vasorelaxation by β adrenoceptor stimulation in coronary and peripheral arteries[7,8]. In the present study, we simultaneously examined *in vitro* whether stimulation of β adrenoceptor in swine coronary and femoral arteries caused endothelium-dependent vasorelaxation, whether this occured through activation of the *L*-arginine/NO pathway, and receptor subtype involved.

MATERIALS AND METHODS

Arterial rings Six healthy mongrel pigs (purchased from the Experiment Animal Center of Railway Ministry of People's Republic of China), either sex, age 4 – 6 months and body weight (60 ± 10) kg, were anesthetized with sodium pentobarbital 30 mg/kg ip. Heart was isolated, through middle incision, after blocking arteries and veins to and from the heart. The middle part of femoral artery was also isolated from leg of the same pig. Hearts and femoral arteries were incubated in Krebs buffer of the following composition (mmol/L): NaCl 125, KCl 4.8, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 11, edetic acid 0.3, $CaCl_2$ 2.5, pH 7.4 (gassed with 95 % O_2 + 5 % CO_2). In Krebs solution, coronary arteries were isolated from heart. Connective and adipose tissue was carefully cleared from coronary and femoral arteries.

Arteries were then cut into 2 – 3 mm rings and mounted in 3 mL organ baths containing Krebs buffer bubbled with 95 % O_2 + 5 % CO_2. Resting tension was set to 2 g, and viability was confirmed by repeated contractions to KCl 45 mmol/L. Once stable and repro-

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ducible contractions were obtained, rings were contracted with prostaglandin $F_{2\alpha}$ 0.5 – 0.75 μmol/L, the concentration which elicited approximately 70 % of the tension induced by KCl. The presence of functional endothelium was confirmed by the demonstration of > 60 % relaxation in response to bradykinin 1 μmol/L.

Protocol Following washout, rings were contracted once again with the same concentration of prostaglandin $F_{2\alpha}$ and relaxant concentration-effect curves were determined to ISO (concentration range 1 nmol/L – 1 mmol/L, with incremental additions at 2-min intervals). After washout, rings were once again contracted with prostaglandin $F_{2\alpha}$ and repeat concentration-effect curves were determined to ISO, following preincubation with one of the following: NOS inhibitor *L*-NMMA 1 mmol·L^{-1}, high selective β_1 adrenoceptor antagonist CGP 20712A 300 nmol·L^{-1}[9], high selective β_2 adrenoceptor antagonist ICI 118551 100 nmol·L^{-1}[10]. Preliminary experiments showed that repeated exposures of these rings to ISO in the absence of any antagonists did not give rise to tachyphylaxis. Concentration-effect curves to ISO were determined simultaneously in rings from the same artery whose endothelium had been removed by gently passing a metal rod along the lumen.

Reagents Following drugs were used: ISO (Sigma), Bradykinin (Sigma), ICI 118551 (Zeneca), CGP 20712A (Ciba-Geigy), *L*-NMMA (Sigma), prostaglandin $F_{2\alpha}$ (Sigma). All drugs were dissolved in Krebs solution.

Statistical analysis All data were expressed as $\bar{x} \pm s$. Relaxant responses to increasing concentrations of ISO in arterial rings, in the absence or presence of antagonists, were compared by two-way ANOVA. Statistical significance was taken as $P < 0.05$ (two-sided). Curves for relaxant responses were fitted by nonlinear regression using GraphPad Prism version 2.01 (GraphPad Software, Inc).

RESULTS

ISO elicited a concentration-dependent vasorelaxation in precontracted femoral and coronary rings with intact endothelium (log EC_{50} – 6.2 ± 0.1, E_{max} 60.1 % ± 2.7 % in femoral arteries and – 6.1 ± 0.2, 40.1 % ± 3.8 % in coronary arteries). Relaxation to ISO was abolished by co-incubation with NOS inhibitor *L*-NMMA 1 mmol·L^{-1}, β_2 adrenoceptor blocker ICI 118551 100 nmol·L^{-1} or prior denudation of endothelium in femoral arteries, but not in coronary arteries. Relaxation to ISO was prevented by co-incubation with β_1 adrenoceptor blocker CGP 20712A 300 nmol·L^{-1} in coronary arteries, but not in femoral arteries (Fig 1).

DISCUSSION

Distribution of subtypes of β adrenoceptors (mainly β_1 and β_2) varies widely in animal and human organs and species. Previous studies have shown that activation of either of the subtypes in the smooth muscles causes vasorelaxation, however, recent studies reveal that endothelium-removal and NOS inhibition decreased or abolished vasorelaxation caused by β adrenoceptors in human and animal resistance arteries[5,6,11]. These studies suggest that β adrenoceptor-mediated vasorelaxation is partly or completely dependent on NOS activation, but studies regarding subtype (s) of β adrenoceptors which activate NOS and regarding mechanical differences present between coronary and other resistance arteries with respect to vasorelaxation by ISO are still rare.

Our present study shows that endothelium-removal, NOS inhibition abolish the vasorelaxation by ISO in femoral arteries, but have no effect on coronary arteries. This suggests that vasorelaxation by ISO in femoral arteries is mediated through activation of NOS or is endothelium-dependent, but in coronary arteries, ISO directly relaxes vascular smooth muscles.

We also show in the present study that β_2 adrenoceptor blockade can completely abolish the vasorelaxation by ISO in femoral arteries, and β_1 adrenoceptor antagonization prevents the vasorelaxation in coronary arteries. These findings combining with above results suggest that the vasorelaxation induced by ISO is through stimulation of endothelial β_2 adrenoceptor and which then activates NOS in femoral arteries, but in coronary arteries it is through direct stimulation of β_1 adrenoceptors on smooth muscle cells. These results are also in agreement with our recent report which demonstrates that endothelial β_2 adrenoceptor stimulation and cyclic AMP elevation activate the *L*-arginine/NO system, and give rise to vasorelaxation, in human umbilical vessels[12].

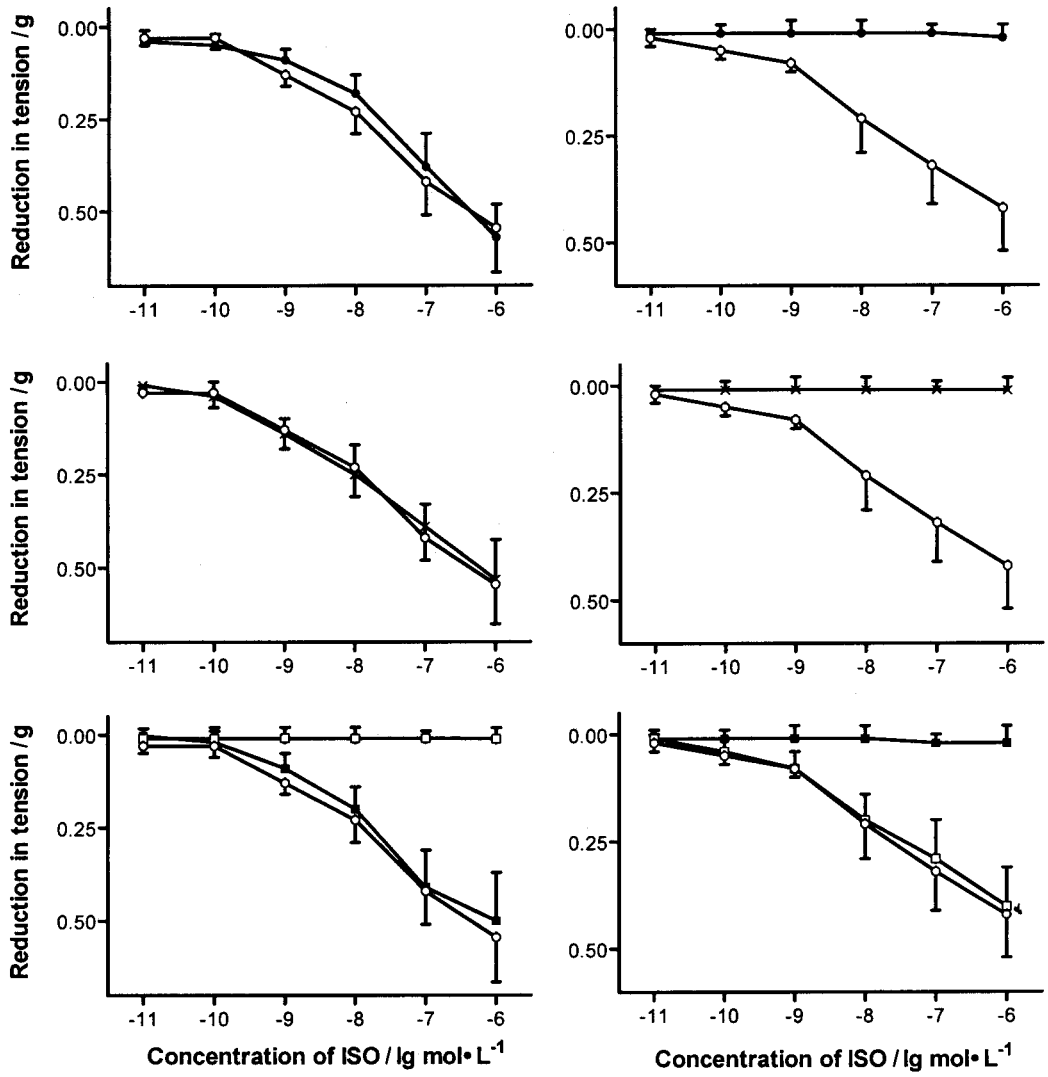

Fig 1. Relaxation induced by isoprenaline (ISO) in coronary artery (left side) and femoral artery (right side). n = 6 pigs. ○：ISO；●：ISO + endothelium removal；※：ISO + *L*-NMMA；■：ISO + ICI 118551；□：ISO + CGP 20712A.

REFERENCES

1 Kuriyama H, Ito Y, Suzuki H, Kitamura K, Itoh T. Factors modifying contraction-relaxation cycle in vascular smooth muscles. Am J Physiol 1982；243：H641－2.

2 Howell RE, Albeda SM, Daise ML, Levine EM. Characterization of β adrenergic receptors in cultured human and bovine endothelial cells. J Appl Physiol 1988；65：1251－7.

3 Molenaar P, Malta E, Jones CR, Buxton BF, Summers RJ. Autoradiographic localization and function of β adrenoceptors on the human internal mammary artery and saphenous vein. Br J Pharmacol 1988；95：225－33.

4 Xu B, Queen LR, Ferro A. β-Adrenoceptor-mediated vasorelaxation is largely mediated through the *L*-arginine/nitric oxide system in human umbilical vein. J Physiol (Lond) 1998；506：P30－1.

5 Graves J, Poston L. β-Adrenoceptor agonist mediated relaxation of rat isolated resistance arteries：a role for the endothelium and nitric oxide. Br J Pharmacol 1993；108：631－7.

6 Blankesteijn WM, Thien T. Effect of NG-monomethyl-*L*-arginine on the β-adrenoceptor- mediated relaxation of rat mesenteric resistance arteries. Life Sci 1993；52：PL135－9.

7 MacDonald PS, Dubbin PM, Dusting GJ. β-Adrenoceptors on endothelial cells do not influence release of relaxing factor in dog coronary arteries. Clin Exp Pharmacol 1987；14：525－34.

8 Bea ML, Ghaleh B, Giudicelli JF, Berdeaux A. Lack of importance of NO in β-adrenoceptor-mediated relaxation of large epicardial canine coronary arteries. Br J Pharmacol 1994；111：981－2.

9 Kaumann AJ. The β_1 adrenoceptor antagonist CGP 20712A unmasks β_2 adrenoceptors activated by (－) adrenaline in the rat sino-atrial node. Naunyn Schmiedeberg's Arch Pharmacol 1986；333：73－6.

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E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

10 Lemoine H，Ehle B，Kaumann AJ. Direct labelling of 1 β_2 adrenoceptors：comparisons of binding potency of ^{3}H-ICI 118551 and blocking potency of ICI 118551. Naunyn Schmiedeberg's Arch Pharmacol 1985；331：40－51.
11 Dawes M，Chowienczyk PJ，Ritter JM. Effects of inhibition of the *L*-arginine/nitric oxide pathway on vasodilation caused by β-adrenergic agonists in human forearm. Circulation 1997；95：2293－7.
12 Ferro A，Queen LR，Priest RM，Xu B，Ritter JM，Poston L，*et al*. Activation of nitric oxide synthase by β_2 adrenoceptors in human umbilical vein endothelium *in vitro*. Br J Pharmacol 1999；126：1872－80.

不同的机制介导β肾上腺素受体激动产生的冠状动脉和股动脉血管舒张

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关键词 β肾上腺素受体；一氧化氮；冠状动脉；股动脉；血管内皮

目的：研究β肾上腺素受体激动产生的猪冠状动脉和股动脉血管扩张是否通过不同的机制介导. 方法：利用器官组织浴血管环法观察冠状动脉和股动脉在给予不同浓度的异丙肾上腺素(ISO)后血管张力的变化，以及去除血管内皮，一氧化氮合成酶(NOS)抑制，β_1 和/或 β_2 受体阻断后对其的影响. 结果：无论冠状动脉或股动脉 ISO 均产生浓度依赖的血管舒张. 去除内皮，NOS 抑制，β_2 受体阻断不影响 ISO 产生的冠状动脉血管舒张，而完全消除了股动脉的血管舒张. β_1 受体阻断不影响 ISO 产生的股动脉血管舒张，但消除了冠状动脉的血管舒张. 结论：ISO 产生的猪冠状动脉血管舒张通过 β_1 受体介导，不通过 *L*-精氨酸/一氧化氮通路，而在股动脉通过 β_2 受体和 *L*-精氨酸/一氧化氮通路.

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