BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Jul; 16 (4): 357—360

Influence of endothelium on responses of isolated dog coronary artery to β-adrenoceptor agonists[1]

TANG Zi-Lue[2], WU Wen-Jian, XIONG Xiao-Ming
(*Department of Pharmacology, Hu-nan Medical University, Changsha 410078, China*)

AIM: To study the role of the endothelium in mediating the response of the coronary arteries to adrenoceptor agonists. **METHODS:** Endothelium intact and denuded dog epicardial coronary artery rings were used. The contractile responses were recorded on forcetransducer and a pen recorder. **RESULTS:** In all ring segments, norepinephrine (NE) and isoproterenol (Iso) produced concentration-dependent relaxation in the presence and absence of phentolamine. Endothelium-removal decreased this relaxation and depressed the maximal response. Inhibition of nitric oxide synthesis by N^{ω}-nitro-*L*-arginine-methyl ester induced the same influences as endothelium-removal. **CONCLUSION:** β-adrenoceptor agonists produce relaxation of dog coronary artery by both endothelium-dependent and independent mechanisms, endothelium-dependent relaxation induced by β-adrenoceptor agonists is mediated by nitric oxide.

KEY WORDS coronary vessels; vascular endothelium; norepinephrine; isoproterenol; phentolamine

There is controversy over the role of the endothelium in mediating the response of the coronary arteries to adrenoceptor agonists. Some reported that endothelium-removal decreased the relaxative action of coronary artery to catecholamines[1]. Functional α_2- and β_2-adrenoceptors on the endothelium of coronary arteries have been characterized in dogs and pigs[1,2]. Some reported that α-adrenergic activation caused minimal or no constriction in pig coronary microcirculation[3]. Norepinephrine (NE) predominantly dilates porcine coronary microvessels by β-adrenoceptor activation. Some failed to confirm adrenoceptor-mediated responses via the endothelium in the dog[4] and rabbit coronary arteries[5]. Some reported enhanced relaxation of dog coronary artery to β-adrenoceptor agonists after removal of endothelial cells[6]. The present study was to explore the role of functional β-adrenoceptor in dog coronary artery endothelium and the probable underlying mechanisms of endothelium dependent responses to β-adrenoceptor agonists. To determine the mechanisms of vasodilator responses of β-adrenoceptor agonists, an inhibitor of NO synthase was used.

MATERIALS AND METHODS

Vascular reactivity Mongrel dogs (15.0±2.5 kg) were anesthetized with iv sodium phentobarbital (120 mg kg^{-1}). The heart was excised and the left circumflex coronary artery was dissected free of the surrounding adherent myocardium. Ring segment approximately 4 mm in length was mounted horizontally and connected to a force transducer and a pen recorder. The bath contained 6 mL Krebs solution (NaCl 133; KCl 4.7; NaH_2CO_3 16.3; NaH_2PO_4 1.35; Glucose 7.8; $MgSO_4$ 0.61; $CaCl_2$ 1.89 mmol L^{-1}) gassed with 95% O_2+5% CO_2 at 37 ℃. The solution in the bath was changed every 20—30 min. The endothelium was either intact or removed by gently rubbing against the teeth of a pair of forceps. The

[1] Project supported by the National Natural Science Foundation of China, № 39200150.
[2] Correspondence to Dr TANG Zi-Lue.
Received 1994-06-20 Accepted 1994-08-15

effectiveness of removal of endothelium was functionally tested by acetylcholine 1 mmol L^{-1} which induced relaxation of coronary artery precontracted with KCl 25 mmol L^{-1}.

The ring segments were allowed to equilibrate for 15 min before progressively stretched to optimal tension 12 g[8], and were then allowed to equilibrate for at least 90 min. Before data collection, stimulation of the rings with prostaglandin $F_{2\alpha}$ (2 μmol L^{-1}) was repeated every 15 min for 2—4 times until a reproducible contraction was obtained. The relaxation was calculated as % of contraction to prostaglandin $F_{2\alpha}$.

Drugs Acetylcholine, phentolamine, noradrenaline, prostaglandin $F_{2\alpha}$, isoprenaline and N^{ω}-nitro-*L*-arginine-methyl ester (*L*-NAME) (all Sigma Chemical Co) were dissolved in Krebs solution, except that NE and Iso were dissolved in ascorbic acid (10 μmol L^{-1}). The drugs were added to the bath in volumes of 10—100 μL.

Statistics ANOVA was performed by using Newman-Keuls test.

RESULTS

Vasodilator responses to NE and Iso

Concentration-dependent relaxation response of the dog coronary artery to NE and Iso were obtained in vessels. After removal of the endothelium, the concentration-relaxation curves to NE and Iso were shifted to the right with a depression of maximal response (Fig 1).

Pretreated with phentolamine (10 μmol L^{-1}) for 5 min, concentration-relaxation to norepinephrine were significantly shifted to the left with an increased maximal response either with or without endothelium. Response curve was shifted to the right with a decreased maximal response in endothelium denuded preparations (Fig 1).

Role of nitric oxide (NO) in relaxation responses to NE and Iso Incubated with *L*-NAME (30 μmol L^{-1}) for 45 min attenuated the vasodilator responses to NE in the presence or absence of phentolamine on endothelium intact rings. But *L*-NAME failed to alter the relaxation response to NE on denuded vessels. Just as in NE responses, *L*-NAME reduced vasodilator responses to Iso only on dog coronary arteries with intact endothelium. Both NE and Iso relaxation-response were shifted to the right and showed no difference between endothelium intact and denuded groups in the presence of *L*-NAME (Fig 1).

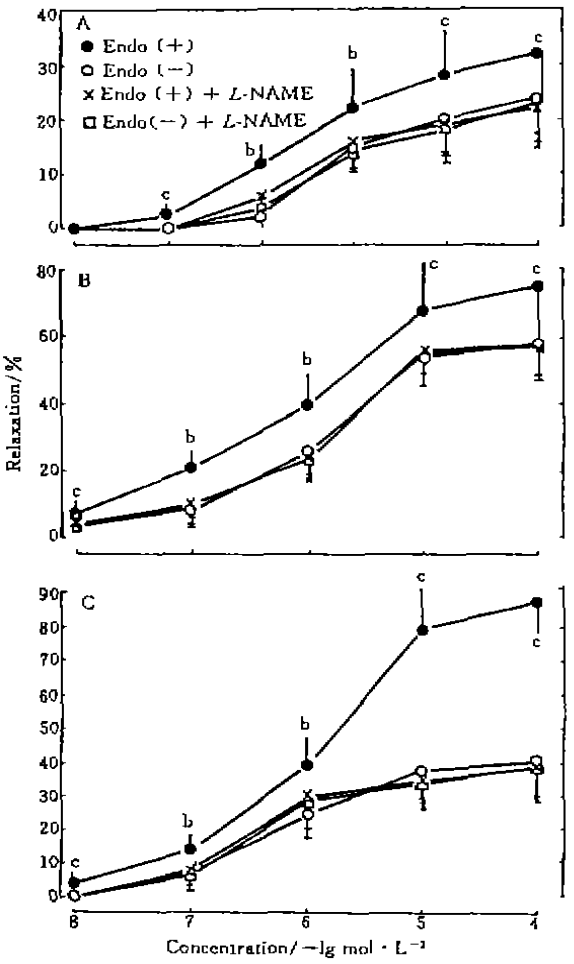

Fig 1. Relaxation to norepinephrine in the absence (A, $n=8$) and presence (B, $n=9$) of phentolamine (10 μmol L^{-1}) and to isoproterenol (C, $n=9$) in rings of dog coronary arteries. $\bar{x}\pm s$. $^{b}P<0.05$, $^{c}P<0.01$ *vs* endothelium denuded and *L*-NAME (30 μmol L^{-1})-pretreated rings.

DISCUSSION

There are β-adrenoceptors on coronary vascular endothelial cells[7]. Recent studies demonstrated the existence of β_1-receptors and β_2-receptors in the wall of coronary arteries. β_2-receptors predominated on coronary endothelium, which is an important vasodilative mechanism[8]. But other researchers have not been able to confirm a β-adrenoceptor mediated endothelium dependent relaxant response. One group has even observed an enhanced relaxation to β-receptor agonists after removal of the endothelial cells[6].

Our study demonstrated that catecholamines induced concentration-dependent relaxation on dog coronary arteries in the presence and absence of phentolamine, an alpha-receptors antagonist. These data indicated the existence of functional β-adrenoceptors on canine coronary artery endothelium. Since the responsiveness of the canine coronary artery to NE and Iso was reduced but not abolished by removal of endothelium, the present study suggested that the endothelium contribute, albeit not necessarily, to the relaxing action of these substances.

NO has been identified as an endothelium-derived relaxing factor (EDRF), first described by Furchgott as the factor responsible for the dilative effect of acetylcholine on precontracted isolated blood vessels[9]. EDRF also played an important role in the regulation of coronary vascular resistance[10]. To determine the relationship between adrenoceptor activation and release of EDRF in the endothelium, phentolamine has been used. The data showed the fact that influence of endothelium on catecholamine-induced vasodilation was not attenuated by phentolamine, failed to confirm the presence of founctional α-receptors in endothelium. Our results showed that the release of EDRF caused by β-receptor agonists was related to β-adrenoceptor activation. *L*-NAME can abolish the influences of endothelium on catecholmine-induced vasodilation. We imagine that catecholamines dilate the coronary arteries predominantly by activation of β-receptors on vascular smooth muscle cells, and the endothelium dependent relaxation is mediated by the release of EDRF. This study suggested that catecholamines predominantly dilate the dog coronary arteries, both by β-adrenoceptor activation and by stimulating the release of EDRF.

Our results and findings of other authors suggested the presence of functional β-adrenoceptor on coronary artery endothelium. As discussed above, vascular sympathetic tone may be modulated by adrenoceptor both on vascular smooth muscle cells and on endothelial cells. The mechanism of β-receptor related vasodilation on endothelium is related to the release of EDRF. The significance of this pattern of distribution of the receptors to β-adrenoceptor agonists remains to be further explored.

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357-360

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血管内皮对狗离体冠状动脉 β肾上腺素受体激动剂反应性的影响

唐子略、邹文建、熊小明 R965.2
(湖南医科大学药理教研室·长沙 410078, 中国)

目的: 研究内皮细胞对离体冠状动脉 β 肾上腺素受体激动剂反应性的影响. **方法**: 狗冠状动脉环离体实验, 生理记录仪记录血管张力. **结果**: 去甲肾上腺素(NE)和异丙肾上腺素(Iso)引起离体狗冠状动脉剂量依赖性舒张反应, 酚妥拉明加强 NE 的作用. 血管去内皮后, 对 NE 和 Iso 的反应减弱, 一氧化氮(NO)合成酶抑制剂 N^{ω}-硝基左旋精氨酸甲酯亦可减弱 NE 和 Iso 的作用. **结论**: β 肾上腺素受体激动剂对狗冠状动脉舒张作用部分依赖于内皮. 此作用由 NO 介导.

关键词 冠状血管; 血管内皮; 去甲肾上腺素; 异丙肾上腺素; 酚妥拉明

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Jul; 16 (4): 360—365

Thrombin-induced neuropeptide Y secretion from rat platelets[1]

CHEN Song-Hai, HAN Qi-De[2] (*Institute of Vascular Medicine, The Third Hospital, Beijing Medical University, Beijing 100083, China*)

AIM: To study the thrombin-induced secretion of platelet neuropeptide Y (NPY) in rats. **METHODS**: The platelet aggregation induced by ADP or thrombin was recorded by an aggregometer. NPY in platelet and plasma was measured by radioimmunoassay. The intracellular free Ca^{2+} ($[Ca^{2+}]_i$) was measured by Fura-2 fluorescent assay. **RESULTS**: Thrombin 0.75 or 2.5 kU L^{-1} increased $[Ca^{2+}]_i$ from 119±8 nmol L^{-1} to 530±60 or 1340±100 nmol L^{-1}, respectively together with the secretion of platelet NPY. Edetic acid 2 mmol L^{-1} almost abolished the thrombin-induced increases of $[Ca^{2+}]_i$ and reduced the NPY secretion by 56 % and 30 %, respectively. Neither $[Ca^{2+}]_i$ increase nor platelet NPY secretion induced by thrombin was affected by verapamil. The thrombin-induced NPY secretion was inhibited by 55 %—70 % by indometacin or creatine phosphate plus creatine phosphokinase. **CONCLUSION**: Thrombin-induced platelet NPY secretion was related to an Ca^{2+} influx

[1] Project supported by the National Natural Science Foundation of China. № 39270776 and the research grant from the State Educational Commission of China.
[2] Correspondence to Prof HAN Qi-De.
Received 1994-05-23 Accepted 1994-11-29