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Effect of anisodamine on acetylcholine-induced endothelium-dependent vasorelaxation in cat isolated arteries

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Abstract: AIM To examine the effect of anisodamine on acetylcholine (ACh)-induced endothelium-dependent vasorelaxation. **METHODS** Using isometric-tension test, in isolated artery rings derived from cat femoral, renal, mesentary and coronary arteries, the effects of anisodamine on endothelium-dependent vasorelaxation induced by ACh were observed. **RESULTS** The endothelium-dependent vasorelaxation induced by ACh could be blocked by anisodamine in a concentration-dependent manner in the isolated preparations derived from cat mesentery, femoral and renal arteries, the value of IC_{50} were 0.236, 0.729 and 0.508 nmol·L^{-1}, respectively, against ACh 10 μmol·L^{-1}. The antagonism of anisodamine was fit for non-competitive mode. Moreover, anisodamine at concentration of 10 nmol·L^{-1} inhibited the endothelium-dependent coronary artery relaxation induced by ACh ($P < 0.05$). **CONCLUSION** Anisodamine has potent effect against ACh-induced endothelium-dependent vasorelaxation with the tissue specific property.

Key words: anisodamine; acetylcholine; endothelium, vascular; arteries; vasodilation

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The vascular endothelium plays a key role in the regulation of the arterial tone and tissue hemostasis[1]. The endothelial target-activated by acetylcholine (ACh) distributes in all kinds of arteries and can elicit endothelium-dependent vasorelaxation through stimulating the release of potent vasorelaxant endothelium-derived NO and/or endothelium-derived hyperpolarizing factor[2]. The endothelium-dependent vasorelaxation induced by ACh has been accepted widely as a marker of endothelium dysfunction at abnormality state including atherosclerosis, hypertension, diabetes and so on. The nature of endothelial target-activated by ACh suggested by most of studies was belong to muscarinic or muscarinic-like choline receptor[3].

Anisodamine, an atropine-like alkaloid isolated from the Chinese traditional herb *Anisodus tanguticus*, has higher selectivity in spasmolysis and less toxicity than atropine[4]. Little is known about the delicate mechanism of anisodamin, especially the action in endothelium[5].

In the present experiment, using isometric-tension test, the effects of anisodamine on ACh-induced endothelium-dependent vasorelaxation in isolated artery rings derived from cat different organs were examined.

1 MATERIALS AND METHODS

1.1 Drugs and animal

Anisodamine (Hangzhou Minsheng Pharmaceutical Company); ACh (Beijing Chemical Plant); norepinephrine (NE, Wuhan Pharmaceutical Company). Male cats weighting 2.6 – 3.6 kg provided by Experimental Animal Center of Academy of Military Medical Sciences.

1.2 Preparation of isolated arteries

Femoral artery, mesentery artery, renal artery and coronary artery of cats were quickly removed, cleaned and cut into 3 mm in length, then suspended in organ baths filled with Kreb′s buffer solution of the following composition (mmol·L^{-1}: NaCl 118, KCl 4.7, $CaCl_2$ 11.0, $NaHCO_3$ 25.0,

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glucose 11.1, $MgSO_4$ 11.2, EDTACa-Na_2 0.026, pH 7.4) at 37℃ and gassed with 95% O_2 and 5% CO_2, connected to a force transducer and recorders. The solution in the baths was changed every 15 min. The rings were equilibrated for 20 min before stretching them to approximate tones, and allowed to further equilibration for 60 min. Before data collection, the rings were stimulated with 1 $\mu mol \cdot L^{-1}$ NE once, then re-equilibrated with Kreb′s buffer solution for 40 min. All concentrations were expressed as final organ chamber concentrations.

1.3 Experimental protocol

Preliminary test indicated that when the rings were in resting state and in steady state (pre-contracted with 1 $\mu mol \cdot L^{-1}$ NE or 25 $mol \cdot L^{-1}$ KCl in coronary artery), administration of anisodamine 0.01, 0.1, 1, 10 $nmol \cdot L^{-1}$, respectively did not cause any changes in all tested arteries. In formal test, rings were pre-incubated with saline or differential concentration of anisodamine (0.01, 0.1, 1 and 10 $nmol \cdot L^{-1}$), then at steady state (pre-contracted with 1 $\mu mol \cdot L^{-1}$ NE or 25 $mol \cdot L^{-1}$ KCl in coronary artery), ACh (0.001, 0.01, 0.1, 1, 10 $\mu mol \cdot L^{-1}$) were added in cumulative manner.

1.4 Data analysis

The data were expressed as $\bar{x} \pm s$. Relaxation of vessel rings was expressed as percent of contracted plateau induced by NE or KCl. IC_{50} values of anisodamine for inhibiting relaxation induced by ACh and EC_{50} values of ACh inducing relaxation were calculated by Logit analysis. The antagonism mode was determined by parallel analysis. Difference between control and treatment by anisodamine was determined by paired t test.

2 RESULTS

In isolated mesentery, femoral and renal arteries pre-contracted with NE 1 $\mu mol \cdot L^{-1}$, ACh at concentration from 0.001 to 10 $\mu mol \cdot L^{-1}$ caused endothelium-dependent vasorelaxation in a concentration-dependent manner. In the presence of anisodamine 0.01 – 1.0 $nmol \cdot L^{-1}$, the concentration-response curves were shifted rightwards with the increased EC_{50} of ACh and the decreased slope (b), which indicated the decreased maximum effects (Tab 1). The results suggest that the

Tab 1. Effect of anisodamine on the EC_{50} values of acetylcholine (ACh) inducing endothelium-dependent vasorelaxation in cats

Artery	Anisodamine /$nmol \cdot L^{-1}$	$EC_{50} \pm L_{95}$ /$\mu mol \cdot L^{-1}$	$b \pm s_b$	r
Mesentery	0	0.44 ± 0.14	1.39 ± 0.41	0.922
	0.01	0.80 ± 0.68	0.61 ± 0.09	0.980
	0.1	2.46 ± 3.27	0.67 ± 0.11	0.972
	1.0	29.91 ± 21.4	0.44 ± 0.03	0.996
Femoral	0	0.04 ± 0.09	1.38 ± 0.36	0.939
	0.01	0.77 ± 0.76	0.42 ± 0.07	0.974
	0.1	0.92 ± 0.88	0.51 ± 0.08	0.977
	1.0	9.25 ± 20.9	0.58 ± 0.11	0.967
Renal	0	0.14 ± 0.62	1.91 ± 0.79	0.925
	0.1	1.49 ± 0.37	0.43 ± 0.03	0.998
	1.0	5.15 ± 1.35	0.40 ± 0.02	0.999

EC_{50}: 50% of the maximum vasorelaxation induced by ACh (0.001, 0.01, 0.1, 1, 10 $\mu mol \cdot L^{-1}$). $\bar{x} \pm s$, $n = 7$ for mesentery artery, $n = 6$ for femoral artery, $n = 5$ for renal artery. The parameters of the dose-response relationship were calculated by Logit analysis.

Tab 2. The IC_{50} values of anisodamine against acetylcholine-induced endothelium-dependent vasorelaxation in cats

Artery	ACh /$\mu mol \cdot L^{-1}$	$IC_{50} \pm L_{95}$ /$nmol \cdot L^{-1}$	$b \pm s_b$	r
Mesentery	0.1	0.004 ± 0.007	0.388 ± 0.068	0.985
	1.0	0.087 ± 0.088	0.426 ± 0.102	0.972
	10	0.236 ± 0.048	0.411 ± 0.017	0.998
Femoral	0.1	0.016 ± 0.099	0.544 ± 0.269	0.896
	1.0	0.122 ± 0.170	0.327 ± 0.069	0.958
	10	0.729 ± 0.355	0.453 ± 0.057	0.992
Renal	0.1	0.026 ± 0.010	0.250 ± 0.012	0.998
	1.0	0.167 ± 0.221	0.387 ± 0.083	0.978
	10	0.508 ± 1.187	0.366 ± 0.113	0.916

IC_{50}: 50% of the maximum inhibitory concentration. $\bar{x} \pm s$, $n = 7$ for mesentery artery, $n = 6$ for femoral artery, $n = 5$ for renal artery. The parameters of the dose-response relationship were calculated by Logit analysis.

antagonism of anisodamine is fit for non-competitive mode. The IC_{50} value of anisodamine is shown in Tab 2.

In isolated coronary artery pre-contracted with KCl 25 mol·L^{-1}, ACh at concentration from 1.0 to 100 nmol·L^{-1} induced endothelium-dependent vasorelaxation in a concentration-dependent manner (Fig 1), and this effect was no more further enhanced by 1 nmol·L^{-1} ACh. Anisodamine 10 nmol·L^{-1} could block the endothelium-depend vasorelaxation caused by ACh (Fig 1).

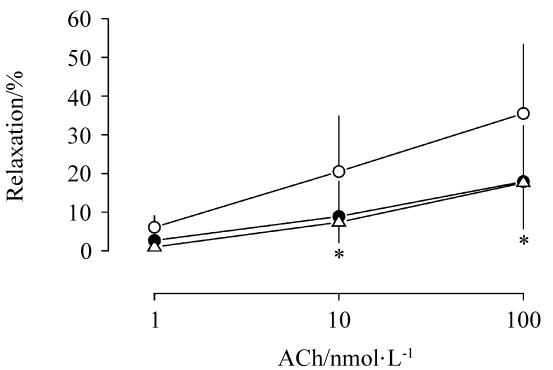

Fig 1. Effect of anisodamine on endothelium-dependent coronary vasorelaxation induced by acetylcholine. (○) normal control; (●) anisodamine 1 nmol·L^{-1}; (△) anisodamine 10 nmol·L^{-1}. $\bar{x} \pm s$, $n = 6$. $P < 0.05$, compared with control.

3 DISCUSSION

The present study demonstrates that anisodamine at 0.01 – 10 nmol·L^{-1} is able to antagonize ACh induced endothelium-dependent vasorelaxation in concentration-dependent manner in rat mesentery, femoral and renal arteries and the antagonism was fit for non-competitive mode. It is attractive that the IC_{50} value of anisodamine against ACh of 10 μmol·L^{-1} in mesentery artery is 0.236 nmol·L^{-1}, the least one among all IC_{50} values in Tab 2. This result may infer that the endothelial target-activated by ACh in mesentery artery endothelium having high sensitivity to anisodamine. Moreover, our results also showed that the minimum effective dose of anisodamine inhibiting endothelium-dependent vasorelaxation induced by ACh in femoral, renal and coronary arteries were at 0.01, 0.1 and 10 nmol·L^{-1} level, respectively. It may be the tissue difference of anisodamime action and be related to the tissue specific property of endothelial target-activated by ACh[6].

It is well known that higher concentrations of atropine and atropine-like anisodamine could induce vasodilation through blocking M_1 or M_3 muscarinic receptor in vascular smooth[7] and the endothelial target-activated by ACh could be described to muscarinic or muscarinic-like receptor. In present study, in order to rule out the possible interference from the muscarinic receptor on isolated artery smooth, preliminary test was done with anisodamine at test concentrations. When the rings were in resting state and in steady state (pre-contracted with NE or KCl), administration of anisodamine 0.01, 0.1, 1, 10 nmol·L^{-1} did not cause any changes in all tested arteries. However, in 1992, it was reported that anisodamine inhibited ACh-induced endothelium-dependent vasorelaxation in canine femoral artery at the concentration 1 – 100 μmol·L^{-1}[8]. It is 1000 times higher than the present results in cat femoral artery, which seemed due to nonspecific receptor effects or different experimental protocol.

Recent advances have established that excessive, prolonged production of NO contributes to tissue damage in many cardiovascular disease states[9-11]. Anisodamine may inhibit NO overproduction and protect vascular endothelium through blocking endothelial target-activated by ACh. The higher sensitivity of endothelial target-activated by ACh to anisodamine in mesentery artery may be better in the beneficial effect of anisodamine on improving microcirculation. For it is known that mesentery artery is closely related to microcirculation in viscera. The endothelium protective mechanism of anisodamine and the relationship to endothelial target-activated by ACh needs to be studied further.

In summary, anisodamine has potent effect against ACh-induced endothelium-dependent vasorelaxation in arteries of cats with the tissue specific property.

4 REFERENCES:

[1] Li K, Sirois P, Rouleau JL. Role of endothelial cells in cardiovascular function[J]. *Life Sci*, 1994, **54**(9):579 – 592.

[2] Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine[J]. *Nature*, 1980, **288**(5789):373 – 376.

[3] Fruchgott RF, Cherry PD. The muscarinic receptor of vascular endothelium that subserves vasodilation[J]. *Trends Pharmacol Sci*, 1984, (Suppl):45 – 48.

[4] Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences; Department of Pediatrics, Beijing Friendship Hospital; First Laboratory of an Institute of Chinese Academy of Medical Sciences. Pharmacologic effects of anisodamine[J]. *Chin Med J*(中华医学杂志), 1975, **1**(2):133 – 138.

[5] Su JY. Medical progress in China – cell protection mechanism of anti-shock action of anisodamine[J]. *Chin Med J*(中华医学杂志), 1992, **105**(12):976 – 979.

[6] Caulfield MP. Muscarinic receptors – characterization, coupling and function[J]. *Pharmacol Ther*, 1993, **58**(3):319 – 379.

[7] Eglen RM, Hegde SS, Watson N. Muscarinic receptor subtypes and smooth muscle function[J]. *Pharmacol Rev*, 1996, **48**(4):531 – 565.

[8] Guo HY, Loren RR, Vanhoutte PM. Anisodamine inhibits acetylcholine-induced endothelium dependent relaxation of canine femoral artery[J]. *Chin Med J*(中华医学杂志), 1992, **105**(8):666 – 670.

[9] Brady AJ. Nitric oxide, myocardial failure and septic shock[J]. *Int J Cardiol*, 1995, **50**(3):269 – 272.

[10] Wolfe TA, Dasta JF. Use of nitric oxide synthase inhibitors as a novel treatment for septic shock[J]. *Ann Pharmacother*, 1995, **29**(1):36 – 46.

[11] Bradley JR, Wilks D, Rubenstein D. The vascular endothelium in septic shock[J]. *J Infect*, 1994, **28**(1):1 – 10.

山莨菪碱对乙酰胆碱诱导的猫离体动脉内皮依赖性舒张反应的影响

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摘要：目的　观察山莨菪碱对乙酰胆碱(ACh)诱导的内皮依赖性血管舒张反应的影响。**方法**　采用猫离体血管功能实验，观察山莨菪碱对 ACh 诱发的内皮依赖性血管舒张反应的影响。**结果**　在猫肠系膜动脉、肾动脉和股动脉，山莨菪碱 0.01 ~ 1.0 nmol·L^{-1}能够浓度依赖地抑制 ACh 诱导的内皮依赖的血管舒张反应。山莨菪碱抑制 10 μmol·L^{-1} ACh 所诱导血管舒张的 IC_{50}分别为 0.236，0.729 和 0.508 nmol·L^{-1}，山莨菪碱的拮抗作用符合非竞争性拮抗模式。此外，10 nmol·L^{-1}山莨菪碱能有效拮抗 ACh 诱导的冠状动脉内皮依赖性舒张反应。**结论**　山莨菪碱能强效拮抗 ACh 诱发的内皮依赖性血管舒张反应，这种效应具有组织特异性的特点。

关键词： 山莨菪碱；乙酰胆碱；内皮，血管；动脉；血管舒张

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