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酪氨酸激酶参与 α_{1A}-肾上腺素受体介导的灌流大鼠后肢血管床收缩反应[1]

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关键词 alpha-1 肾上腺素受体；酪氨酸激酶；后肢；erbstatin；槲皮素；氟化钠；钒酸盐；十四酰乙酸盐

目的：研究酪氨酸激酶是否参与 α_{1A}-肾上腺素受体引起血管平滑肌收缩的信号传导. **方法**：灌流大鼠后肢血管床标本，观察酪氨酸激酶抑制剂对去甲肾上腺素(NE)引起收缩反应的影响. **结果**：酪氨酸激酶抑制剂 tyrphostin 和 genistein 均显著抑制 NE 引起的收缩反应，但对 KCl 引起的收缩反应无影响；酪氨酸磷酸酶抑制剂 Na_3VO_4 显著加强 NE 引起的收缩反应；tyrphostin 和 genistein 对蛋白激酶 C 激动剂 phorbol 12-myristate 13-acetate 引起的收缩反应均无影响，但均抑制 G 蛋白激动剂 NaF 引起的收缩反应. **结论**：Tyrphostin 和 genistein 敏感的酪氨酸激酶参与 α_{1A}-肾上腺素受体介导的大鼠后肢血管床收缩反应.

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Mediation of calcitonin gene-related peptide in protection of ischemic preconditioning in rat hindlimbs[1]

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KEY WORDS calcitonin gene-related peptide; capsaicin; acetylcholine; phenylephrine; norepinephrine; vasodilation; reperfusion injury

AIM: To study modulation of calcitonin gene-related peptide (CGRP) in the protective effect of ischemic preconditioning on endothelial cells. **METHODS**: Rat hindlmbs were subjected to ischemia for 2 h, and endothelium-dependent vasorelaxation to acetylcholine (ACh) was examined in rat hindlimbs. **RESULTS**: Two hours of ischemia elicited no effect on vasoconstrictor responses to norepinephrine, but markedly impaired vasodilator responses to ACh. Ischemic preconditioning induced by 5-min aortic occlusion and 10-min blood reperfusion prevented the impairment of vasorelaxation to ACh due to long-term ischemia. The protection of ischemic preconditioning was abolished by repeated pretreatments with capsaicin to deplete CGRP. Acute application of capsaicin to evoke CGRP release or CGRP caused an ischemic preconditioning-like protection. **CONCLUSION**: Capsaicin-sensitive sensory nerves are involved in the protective effect of ischemic preconditioning on endothelial cells in the rat hindlimbs, and CGRP can mimic the protective effect of ischemic preconditioning in blood vessels.

Ischemic preconditioning showed protection on not only the ischemic myocardium, but also endothelial cells. It was postulated the cardioprotection of ischemic preconditioning might be

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secondary to the protection of endothelial cells[1]. Endogenous chemical mediators were suggested to play a pivotal role in the mediation of ischemic preconditioning[2]. Our studies showed that calcitonin gene-related peptide (CGRP), a principal transmitter in capsaicin-sensitive sensory nerves, was involved in the protection of ischemic preconditioning in the rat hearts[3].

According to the presence of capsaicin-sensitive sensory nerves in vascular tissues and the protective effects of exogenous CGRP on endothelial cells[4], the present study examined whether the protective effect of ischemic preconditioning on endothelial cells was mediated by endogenous CGRP in rat hindlimbs.

MATERIALS AND METHODS

Reagents Phenylephrine, acetylcholine (ACh), norepinephrine, CGRP, and capsaicin were obtained from Sigma. All drugs were dissolved in Krebs' solution, except that capsaicin was dissolved in a vehicle containing 10 % Tween 80, 10 % ethanol, and 80 % saline.

Perfusion of rat hindlimbs Sprague-Dawley rats (♂, $n = 47$, 220 ± s 24 g) were anesthetized with ip sodium pentobarbital 30 mg·kg^{-1}. The abdominal aorta was quickly cannulated adjacent to the iliac bifurcation and the vena cava was sectioned to permit the perfusate to escape as previously described[5]. The hindlimbs were perfused with Krebs' solution (37 ℃, saturated with 95 % O_2 + 5 % CO_2). The perfusion pressure was recorded by a pressure transducer and physiologic recorder. After perfusion with Krebs' solution in hindlimbs was commenced, the rat was killed by an intracardiac injection of KCl 4 mol·L^{-1}.

Experimental protocols Phenylephrine was administered by switching the perfusion solution to solution containing drug at the concentration indicated. For measurement of vasoconstrictor or vasodilator responses to norepinephrine and ACh respectively, boluses doses (100 μL) of them were given. Vasodilator responses to ACh were examined in the presence of phenylephrine. In the ischemic group, the hindlimbs were subjected to 2-h ischemia followed by reperfusion with Krebs' solution. In the preconditioned group, the hindlimbs were subjected to a single preconditioning episode of min aortic occlusion and 10-min blood reperfu before long-term ischemia.

In the case of CGRP-induced preco tioning, rats were treated with iv CGRP 8 kg^{-1} 10 min before long-term ischemia. For studies on the mediation of endogenous CGR ischemic preconditioning, rats were treated capsaicin by sc injection. For acute applica of capsaicin to protect endothelial cells, the were treated with a single dose of capsaicin 50 ·kg^{-1} 3 h before beginning of each experim and then the hindlimbs were subjected to ischemia before examining vasorelaxation to A For repeated administration of capsaicin deplete neurotransmitters in sensory nerves, received capsaicin 50 mg·kg^{-1} followed b second injection of 50 mg·kg^{-1} 24 h later. rule out a direct effect of capsaicin on endoth cells, after repeated capsaicin injection for 4 the animals were again injected with capsaicin mg·kg^{-1} 3 h before beginning of each exp ment[6], and then the hindlimbs were subje to a brief episode of preconditioning before l term ischemia.

Control rats were injected with veh alone.

Statistics All values were expressed $\bar{x} \pm s$ and analyzed with ANOVA.

RESULTS

Effects of ischemic or CGRP-indu preconditioning Basal perfusion press during perfusion with Krebs' solution at constant rate of 12 mL·min^{-1} in rat hindli was (4.0 ± 0.5) kPa. Norepinephrine (6 - μmol ·L^{-1}) evoked a concentration-depend vasoconstriction, and the effects were influenced by long-term ischemia (Tab 1).

Tab 1. Vasoconstrictor responses to norepinephrine (increase in pressure/kPa). $n = 6$ rats.

Norepinephrine/μmol·L^{-1}	Control	Ischemia
6	0.4 ± 0.1	0.4 ± 0.1
18	1.0 ± 0.1	1.0 ± 0.1
60	1.3 ± 0.3	1.3 ± 0.1
180	2.1 ± 0.3	2.0 ± 0.2
600	5.3 ± 1.0	5.2 ± 1.1

Phenylephrine (5 – 30 $\mu mol \cdot L^{-1}$) was added to increase vascular tone. The active tension generated was (9.7 ± 1.2), (10.0 ± 1.2), (10.7 ± 1.9), and (8.7 ± 0.9) kPa for control, ischemia, preconditioning, and CGRP respectively. Under these conditions, ACh (0.04 – 4.00 $\mu mol \cdot L^{-1}$) caused a concentration-dependent vasorelaxation. Vasodilator responses to ACh in the rats subjected to 2-h ischemia were decreased. However, ischemic preconditioning induced by 5-min ischemia and 10-min reperfusion with blood markedly reduced the inhibition of vasodilator responses to ACh by long-term ischemia. A similar protection was observed in the rats pretreated with CGRP (Tab 2).

Effects of capsaicin Pretreatment with capsaicin 3 h before experiment reduced the inhibition of vasodilator responses to ACh by ischemia in the rat hindlimbs (Tab 2).

After repeated pretreatments with capsaicin to deplete CGRP, the protective effect of ischemic preconditioning on endothelial function was abolished, as shown by the reappearance of inhibition of vasodilator responses to ACh by ischemia (Tab 2).

DISCUSSION

The present results confirmed previous observations that ischemic preconditioning improved the impairment of vasodilator responses to ACh due to ischemia in the rat hindlimbs[5]. A similar protection had also been seen in coronary arteries and the cultured endothelial cells. It was suggested that the protection of ischemic preconditioning was mediated by stimulation of endogenous chemical mediator release[1,7].

CGRP is widely distributed in cardiovascular tissues, and myocardial ischemia, even a brief ischemic period of 5 min, causes a significant increase in the release of CGRP in guinea pig hearts[8]. CGRP possessed a beneficial effect on the myocardium and endothelial cells[3,4]. Our previous investigations showed that the cardioprotection of ischemic preconditioning was mediated by endogenous CGRP in the rat heart[9]. Results of the present study revealed that acute application of capsaicin induced an ischemic preconditioning-like protection, while repeated pretreatments with capsaicin abolished the protection of ischemic preconditioning in rat hindlimbs, suggesting that CGRP also participates in the mediation of . ischemic preconditioning in blood vessels. Recently, we also found that acute application of capsaicin to stimulation of CGRP release from sensory nerves attenuated endothelial cell damages elicited by lysophosphatidylcholine (LPC)[10]. These studies suggest that CGRP may be an endogenous cardiovascular protective substance.

The protective effects of pharmacological preconditioning on the myocardium and endothelial cells have been documented[7]. Our recent studies showed that CGRP-induced preconditioning protected the ischemic myocardium, and pretreatment with CGRP also reduced the attenuated endothelium-depedent vasorelaxation by LPC[11]. In the present study, pretreatment with CGRP also improved the impairment of vasodilator responses to ACh by ischemia in the rat hindlimbs . These results

Tab 2. Vasodilator responses to acetylcholine (relaxation %). Rat hindlimbs were precontracted with phenylephrine (5 – 30 $\mu mol \cdot L^{-1}$). Cap-R: repeated treatment with capsaicin. $n = 5$ rats.
$^cP < 0.01$ *vs* control. $^fP < 0.01$ *vs* ischemia. $^iP < 0.01$ *vs* preconditioning. $^lP < 0.01$ *vs* vehicle.

	Acetylcholine/$\mu mol \cdot L^{-1}$				
	0.04	0.12	0.4	1.2	4
Control	13.0 ± 1.1	18.0 ± 1.6	28.0 ± 1.7	38.0 ± 3.0	44.0 ± 2.4
Ischemia	9.0 ± 0.4^c	11.0 ± 2.7^c	15.0 ± 3.4^c	19.0 ± 2.0^c	25.0 ± 3.4^c
+ Vehicle	9.0 ± 0.1^c	13.0 ± 1.2^c	18.0 ± 1.5^c	24.0 ± 1.7^c	31.0 ± 2.5^c
+ Preconditioning (PC)	14.0 ± 1.4^f	19.0 ± 1.7^f	25.0 ± 2.4^f	33.0 ± 2.9^f	41.0 ± 2.3^f
+ PC + Cap-R	9.0 ± 1.3^i	13.0 ± 1.3^i	18.0 ± 1.4^i	25.0 ± 2.6^i	29.0 ± 2.6^i
+ Capsaicin	13.0 ± 1.1^l	20.0 ± 1.0^l	27.0 ± 3.0^l	30.0 ± 3.2^l	38.0 ± 2.8^l
+ CGRP	12.0 ± 2.0^f	19.0 ± 1.0^f	27.0 ± 1.5^f	34.0 ± 2.3^f	39.0 ± 2.7^f

suggest that CGRP, endogenous or exogenous, can protect against endothelial cell damages due to a variety of harmful factors.

In conclusion, the present study suggests that (1) capsaicin-sensitive sensory nerves are involved in the protection of ischemic preconditioning in rat hindlimbs; and (2) CGRP can mimic the protective effect of ischemic preconditioning in blood vessels.

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降钙素基因相关肽介导大鼠后肢缺血预适应的保护作用[1]

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关键词 降钙素基因相关肽；辣椒素；乙酰胆碱；苯福林；去甲肾上腺素；血管舒张；再灌注损伤

目的：研究降钙素基因相关肽(CGRP)介导缺血预适应对血管内皮的保护. **方法**：大鼠后肢缺血 2 h 后，观察乙酰胆碱诱导血管内皮依赖性舒张反应. **结果**：缺血不影响去甲肾上腺素的缩血管效应，但能显著削弱乙酰胆碱的舒血管效应. 缺血预适应能阻止长时间缺血对乙酰胆碱舒血管效应的抑制作用，这种保护作用可被反复应用辣椒素耗竭 CGRP 所取消. 急性应用辣椒素促进 CGRP 释放或外源性应用 CGRP 均可产生预适应样的保护作用. **结论**：大鼠后肢缺血预适应对内皮细胞的保护与辣椒素敏感的感觉神经有关；CGRP 能模拟缺血预适应保护血管.

Corrigendum

Acta Pharmacologica Sinica 1998 May; 19 (3): 269. The chemical structure of artemisinin should be