

Effect of calcitonin gene-related peptide-induced preconditioning on attenuated endothelium-dependent vasorelaxation induced by lysophosphatidylcholine¹

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KEY WORDS calcitonin gene-related peptide; lysophosphatidylcholines; phenylephrine; vascular endothelium; thoracic aorta

AIM: To study the effects of calcitonin gene-related peptide (CGRP)-induced preconditioning on the inhibition of endothelium-dependent vasorelaxation to acetylcholine (ACh) by lysophosphatidylcholine (Lys) in the isolated rabbit and rat thoracic aortas.

METHODS: Endothelium-dependent relaxation to ACh was studied in the aortic rings precontracted with phenylephrine $0.1 \mu\text{mol} \cdot \text{L}^{-1}$ in the absence or presence of Lys. **RESULTS:** On the rabbit aortic rings, Lys $5 \text{ mg} \cdot \text{L}^{-1}$ impaired vasodilator responses to ACh. Pretreatment with CGRP $0.1 \mu\text{mol} \cdot \text{L}^{-1}$ for 5 min attenuated the inhibition of vasodilator responses to ACh by Lys. The effect of CGRP was blocked by 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7), an inhibitor of protein kinase C (PKC) (% relaxations to ACh $1 \mu\text{mol} \cdot \text{L}^{-1}$ were 88 ± 4 , 28 ± 10 , 65 ± 13 , and 25 ± 10 for control, Lys, Lys + CGRP, and Lys + CGRP + H-7, respectively). The same effects of CGRP were shown in the rat aortic rings, and the effect of CGRP was also abolished by H-7 (% relaxations to ACh $1 \mu\text{mol} \cdot \text{L}^{-1}$ was 84 ± 10 , 55 ± 11 , 76 ± 11 , and 50 ± 14 for control, Lys, Lys + CGRP, and Lys + CGRP + H-7, respectively). **CONCLUSION:** CGRP-induced preconditioning protected the endothelium against injury elicited by Lys, the effect of CGRP is related to the activation of PKC.

Calcitonin gene-related peptide (CGRP), a 37-amino acid peptide, possesses numerous physiological properties, several of which have been thought to be beneficial to the ischemic myocardium¹⁻³.

Pretreatment with CGRP also attenuated the inhibition of endothelium-dependent vasorelaxation to acetylcholine (ACh) by lysophosphatidylcholine (Lys)⁽⁴⁾. CGRP-induced preconditioning exerted a preconditioning-like cardioprotection in the isolated rat heart^(1,2). In the present study the protective effects of CGRP-induced preconditioning on endothelial function injured by Lys were evaluated.

MATERIALS AND METHODS

Reagents Lys, CGRP, phenylephrine, ACh, and 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7) were bought from Sigma Chemical Co.

Organ bath experiment Experiments were performed on the thoracic aortas from Sprague-Dawley ♂ rats (210 ± 5 g, $n = 30$) and New Zealand ♂ rabbits (2.2 ± 0.2 kg, $n = 25$). They were anesthetized with iv sodium pentobarbital $30 \text{ mg} \cdot \text{kg}^{-1}$. The aorta was cut into rings of 4 mm long, suspended in 5 mL Krebs' solution at 37°C gassed with 95% O_2 + 5% CO_2 . A resting tension of 6 g was applied on rabbit aorta and 2 g on rat aorta. The rings were equilibrated for 60 min and then precontracted with KCl $40 \text{ mmol} \cdot \text{L}^{-1}$.

After a maximal response to KCl was obtained, the rings were washed repeatedly with Krebs' solution and equilibrated again for 30 min. To measure vasodilator responses, rings were contracted with phenylephrine to 40% - 50% of the maximal contraction. After the contractions were stabilized, a cumulative concentration-response curve to ACh $0.01 - 1 \mu\text{mol} \cdot \text{L}^{-1}$ or CGRP $0.003 - 0.1 \mu\text{mol} \cdot \text{L}^{-1}$ was obtained. For Lys, the rings were exposed to Lys $5 \text{ mg} \cdot \text{L}^{-1}$ for 30 min. For the studies of the effect of CGRP-induced preconditioning on the inhibition of vasodilator responses to ACh by Lys, rings were pretreated with CGRP $0.1 \mu\text{mol} \cdot \text{L}^{-1}$ for 10 min, followed by a 10-min incubation with CGRP-free Krebs' solution before the treatment with Lys. Rings were exposed to H-7 $5 \text{ mmol} \cdot \text{L}^{-1}$ for 5 min and then exposed to CGRP in the presence of H-7 for 10 min, followed by incubation with fresh Krebs' solution before the treatment with Lys. For H-7, rings were exposed to H-7 $5 \text{ mmol} \cdot \text{L}^{-1}$ for 15 min.

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

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RESULTS

The aortic rings contracted on exposure to phenylephrine $0.1 \mu\text{mol} \cdot \text{L}^{-1}$, the active tension generated was $3.9 \pm 1.0 \text{ g}$ in the rabbit aortas ($n = 6$) and $1.3 \pm 0.5 \text{ g}$ in the rat aortas ($n = 6$). Pretreatment with Lys $5 \text{ mg} \cdot \text{L}^{-1}$ evoked a slight increase in constrictor responses to phenylephrine, the tension was $4.3 \pm 0.5 \text{ g}$ in the rabbit aortas ($P > 0.05$, $n = 6$) and $1.4 \pm 0.5 \text{ g}$ in the rat aortas ($P > 0.05$, $n = 6$). Pretreatment with H-7 ($5 \text{ nmol} \cdot \text{L}^{-1}$) did not affect the vasoconstrictor responses to phenylephrine; the tension was $3.9 \pm 0.7 \text{ g}$ in the rabbit aortas ($P > 0.05$, $n = 6$) and $1.2 \pm 0.2 \text{ g}$ in the rat aortas ($P > 0.05$, $n = 6$).

In the presence of phenylephrine $0.1 - 1 \mu\text{mol} \cdot \text{L}^{-1}$, ACh caused a concentration-dependent relaxation in the rabbit and rat aortas. Vasodilator responses to ACh $0.01 - 1 \mu\text{mol} \cdot \text{L}^{-1}$ were reduced in the presence of Lys $5 \text{ mg} \cdot \text{L}^{-1}$ ($P < 0.05$). After pretreatment with CGRP for 10 min, the inhibition of vasodilator responses to ACh $0.01 - 1 \mu\text{mol} \cdot \text{L}^{-1}$ by Lys was attenuated. The protective effect of CGRP was abolished in the presence of H-7 $5 \text{ nmol} \cdot \text{L}^{-1}$. H-7 itself had no effect on vasorelaxation responses to ACh in the rabbit and rat aortas ($P > 0.05$) (Tab 1).

CGRP treatment alone caused a slight vasorelaxation in the rabbit and rat aortas (Tab 2).

Tab 1. Effect of calcitonin gene-related peptide (CGRP) on inhibition by lysophosphatidylcholine (Lys) of endothelium-dependent vasorelaxation to ACh in aorta. Tissues were precontracted with phenylephrine $0.1 - 1 \mu\text{mol} \cdot \text{L}^{-1}$. $n = 6$, $\bar{x} \pm s$. ^a $P > 0.05$, ^b $P < 0.05$, ^c $P < 0.01$ vs control; ^d $P > 0.05$, ^e $P < 0.05$, ^f $P < 0.01$ vs Lys; ^g $P > 0.05$, ^h $P < 0.05$, ⁱ $P < 0.01$ vs Lys + CGRP.

ACh/ $-\lg \text{ mol} \cdot \text{L}^{-1}$	Control	H-7	Lys	Lys + CGRP	Lys + CGRP + H-7
Relaxation of isolated rat aorta/%					
8	15 ± 5	20 ± 7	8 ± 6 ^a	11 ± 5 ^d	3 ± 3 ^b
7.5	34 ± 9	41 ± 13	12 ± 8 ^c	22.9 ± 2.3 ^f	9 ± 5 ⁱ
7	60 ± 10	64 ± 16	24 ± 12 ^c	41 ± 8 ^e	20 ± 10 ⁱ
6.5	77 ± 11	82 ± 9	40 ± 11 ^c	63 ± 12 ^f	39 ± 11 ⁱ
6	84 ± 10	92 ± 6	55 ± 11 ^c	76 ± 11 ^e	50 ± 14 ⁱ
Relaxation of isolated rabbit aorta/%					
8	2 ± 3	5.0 ± 1.0	1.0 ± 0.8 ^a	2 ± 3 ^d	0 ± 0 ^g
7.5	12 ± 5	12 ± 4	5 ± 3 ^b	10 ± 6 ^d	1.5 ± 1.6 ^h
7	33 ± 8	30 ± 6	10 ± 5 ^c	22 ± 12 ^e	5.0 ± 2.3 ⁱ
6.5	65 ± 10	66 ± 7	17 ± 4 ^c	44 ± 19 ^f	14 ± 7 ⁱ
6	88 ± 4	79 ± 2	28 ± 10 ^c	65 ± 13 ^f	25 ± 10 ⁱ

Tab 2. Vasodilator responses to CGRP in aorta. Tissues were precontracted with phenylephrine $0.1 - 1 \mu\text{mol} \cdot \text{L}^{-1}$. $\bar{x} \pm s$.

CGRP/ $-\lg \text{ mol} \cdot \text{L}^{-1}$	Relaxation of isolated thoracic aorta/%	
	Rat ($n = 6$)	Rabbit ($n = 5$)
8.5	6.0 ± 1.1	3.0 ± 0.4
8	13.0 ± 1.6	5.0 ± 0.4
7.5	17.0 ± 1.6	7.0 ± 0.4
7	22.0 ± 1.7	10.0 ± 0.6

DISCUSSION

In the present study, preconditioning with CGRP significantly attenuated the inhibition of endothelium-dependent vasodilation to ACh by Lys in the rabbit and rat aortas. These results, along with previous observation that CGRP-induced preconditioning reduced myocardial damage due to ischemia-reperfusion in the isolated rat heart^[1,2], suggest that preconditioning with CGRP possess a protective effect on not only the myocardium tissue, but also endothelial cells. The mechanisms responsible for the protective effect of CGRP-induced preconditioning are not clear. Studies in cultured endothelial cells showed that the protective effect of anoxic or pharmacological preconditioning was mediated by the PKC pathway^[5]. In adult mammalian ventricular cardiomyocytes, CGRP increased the activation of PKC^[6]. Recently, our

studies showed that the protective effect of CGRP-induced preconditioning against myocardial damage due to endothelia-1 was negated by H-7, suggesting the protective effect of CGRP-induced preconditioning is related to the PKC pathway in the rat heart^[4]. In the present study, the protective effect of CGRP-induced preconditioning against endothelial cell injury elicited by Lys was abolished in the presence of H-7 in the isolated rabbit and rat aorta. The present results suggest that the protective effect of CGRP-induced preconditioning against endothelial cell damages is also involved in the PKC pathway in the rabbit and rat aorta. However, further studies measuring activity of PKC had to be done to establish this hypothesis for the protective effect of CGRP-induced preconditioning on the endothelial cells.

In conclusion, CGRP-induced preconditioning protects endothelial cells against damages elicited by Lys and the effect of CGRP is related to the activation of PKC in the isolated rabbit and rat aortas.

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降钙素基因相关肽预适应对溶血磷脂酰胆碱抑制内皮依赖性舒张的作用¹

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关键词 降钙素基因相关肽; 溶血磷脂酰胆碱类; 苯福林; 血管内皮; 胸主动脉

目的: 研究降钙素基因相关肽(CGRP)诱导预适应对溶血磷脂酰胆碱(Lys)抑制内皮依赖性舒张的作用。方法: 用苯福林收缩兔与大鼠离体胸主动脉环, 观察 Lys 对乙酰胆碱(ACh)所致内皮依赖性舒张的影响。结果: CGRP 预处理兔和大鼠离体胸主动脉环显著减轻 Lys 对 ACh 舒血管效应的抑制, 其作用可被蛋白激酶 C (PKC) 抑制剂 H-7 所取消。结论: CGRP 诱导预适应对所致内皮细胞损伤具有拮抗作用, 此作用与激活 PKC 有关。

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