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Ischemic preconditioning mediated by calcitonin gene-related peptide in isolated rat hearts[1]

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KEY WORDS calcitonin gene-related peptide; myocardial reperfusion injury; heart function tests; creatine kinase

AIM: To study the mediating effect of calcitonin gene-related peptide (CGRP) in ischemic preconditioning in the isolated perfused rat heart. **METHODS**: Isolated rat hearts were subjected to 3 cycles of a 5-min ischemia and a 5-min reperfusion before a 30-min global ischemia followed by a 30-min reperfusion. **RESULTS**: Ischemic preconditioning caused an improvement of heart functions, reduced the incidence of ventricular arrhythmias and decreased the release of creatine kinase (CK) during reperfusion (CK activities = 0.30 ± 0.07, 2.03 ± 0.49, and 0.92 ± 0.40 U·min^{-1}/g wet wt for control, ischemia-reperfusion, and preconditioning, respectively). However, pretreatment with $CGRP_{8-37}$ (0.1 μmol·L^{-1}) abolished the improvement of cardiac contractivity, the reduction of the incidence of arrhythmias, and the inhibition of CK release by preconditioning (CK activities = 0.9 ± 0.4 *vs* 2.55 ± 0.32 U·min^{-1}/g wet wt, $P < 0.01$). **CONCLUSION**: CGRP is an endogenous myocardial protective substance that played an important role in mediation of ischemic preconditioning.

A brief ischemia ("ischemic preconditioning") followed by reperfusion renders the heart very resistant to a subsequent sustained period of myocardial ischemic injury in animals[1-4] and humans[5]. But, the mechanism underlying preconditioning (PC) is not clear[6]. PC may be due to the release of endogenous myocardial protective substances from the ischemic myocardium[7].

Capsaicin-sensitive sensory nerves are present in the hearts[8], and calcitonin gene-related peptide (CGRP), a principal transmitter in cardiac sensory nerves, is released from the heart during ischemia[9]. CGRP protected the myocardium against ischemia-reperfusion injury[10,11]. This study was to find whether CGRP participates in meditation of ischemic PC.

MATERIALS AND METHODS

Isolated heart preparation Wistar rats (Laboratory Animal Center, Hu-nan Medical University) (♂, $n = 35$, weighing 220 ± s 30 g) were anesthetized with ether. The heart was excised and perfused under 10 kPa with gassed (95 % O_2 and 5 % CO_2) Krebs-Henseleit (K-H) buffer solution (37 ℃, pH 7.4), according to the modified Langendorff procedure[12]. The K-H solution was composed of (mmol·L^{-1}): NaCl 119.0, $NaHCO_3$ 25.5, KCl 4.3, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, and glucose 11.0.

A polyethylene catheter was inserted into the left ventricle through the apex and connected to a pressure transducer. The left ventricular pressure (LVP) and its first derivative (LV dp/dt) were recorded by a polygraph recorder (Nihon Kohden). Coronary flow (CF) was measured by timed collection of coronary effluent. An epicardial electrocardiogram (ECG) was recorded continuously. The ECG was analyzed for heart rate (HR), as well as the incidence of ventricular fibrillation (VF) and ventricular tachycardia (VT).

Creatine kinase (CK) measurement Myocardial cellular injury was monitored by assaying the release of CK from hearts. CK was assayed using an assay kit obtained from Baoding Chemical Co, China.

Experimental protocol Hearts equilibrated for 10 min before experiment were divided into 5 groups. (1) The control group was perfused with K-H solution. (2) The $CGRP_{8-37}$ (Sigma, USA) group received $CGRP_{8-37}$ (0.1 μmol·L^{-1}) for 20 min. (3) The ischemia-reperfusion group (I-R) received a 30-min global ischemia and a 30-min reperfusion. (4) The PC group received 3 cycles of 5-min global ischemia and a 5-min reperfusion prior to a 30-min ischemia. (5) The PC + $CGRP_{8-37}$ group received $CGRP_{8-37}$ (0.1 μmol·L^{-1}), began at 5 min prior to PC episode of ischemia until the start of the 30-min ischemia.

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Statistics All values were expressed as $\bar{x} \pm s$. The significances of differences in heart function and CK were determined by ANOVA and the Newman-Keuls test. The incidences of VF and VT were compared by two-tailed Fisher's exact probability test.

RESULTS

In control group, continuously perfused rat hearts were observed for 90 min. There were no changes in HR, CF, LVP, and LV dp/dt_{max}. A 30-min ischemia and a 30-min reperfusion resulted in the reduced heart function, including the decrease of LVP and LV dp/dt_{max}, the reduction of CF, and the slowing of HR (Tab 1). PC improved the recovery of heart function, as seen in enhancement of LVP, LV dp/dt_{max}, CF and HR (Tab 1). However, the effects of PC were completely abolished by treatment with $CGRP_{8-37}$. PC markedly enhanced CF before ischemia-reperfusion (19.2 ± 1.7 *vs* 13.5 ± 1.5 mL·min^{-1} for PC and control, respectively), and this effect of PC was also abolished by $CGRP_{8-37}$. $CGRP_{8-37}$ itself had no effect on heart function and CK release as compared with control.

VF and VT occured in all hearts in the ischemia-reperfusion group. PC caused a reduction in the incidence of arrhythmias. However, $CGRP_{8-37}$ abolished the protective effect of PC as reappearance of arrhythmias (Tab 2).

Reperfusion after 30 min of ischemia caused an increase in release of CK (Tab 2). PC markedly reduced CK release. The inhibition of CK release by PC was also abolished in the presence of $CGRP_{8-37}$.

DISCUSSION

The present study demonstrated for the first

Tab 1. Effect of $CGRP_{8-37}$ (0.1 μmol·L^{-1}) on improvement of heart function by PC. $\bar{x} \pm s$.
$^aP>0.05$, $^cP<0.01$ *vs* control; $^fP<0.01$ *vs* I-R (ischemia-reperfusion); $^hP<0.05$, $^iP<0.01$ *vs* PC.

	Hearts	Before ischemia	Time of reperfusion/min 5	10	20	30
			Left ventricular pressure (kPa)			
Control	8	10.7 ± 0.2	10.6 ± 0.4	10.5 ± 0.3	10.6 ± 0.5	10.6 ± 0.8
$CGRP_{8-37}$	5	10.4 ± 0.5	10.6 ± 0.4^a	10.8 ± 0.3^a	11.0 ± 0.4^a	11.0 ± 0.5^a
I-R	8	10.0 ± 0.8	4.6 ± 0.5^c	5.5 ± 0.7^c	6.6 ± 1.0^c	6.4 ± 0.7^c
PC	7	11.0 ± 0.8	10.9 ± 0.7^f	11.2 ± 0.6^f	10.8 ± 0.6^f	11.0 ± 0.8^f
+ $CGRP_{8-37}$	5	10.3 ± 0.4	4.8 ± 1.4^h	6.1 ± 1.3^h	7.4 ± 1.3^h	8.4 ± 1.0^h
			Left ventricular dp/dt_{max} (kPa·s^{-1})			
Control	8	271 ± 22	268 ± 26	267 ± 25	255 ± 24	256 ± 23
$CGRP_{8-37}$	5	264 ± 18	266 ± 18^a	270 ± 14^a	270 ± 16^a	267 ± 18^a
I-R	8	266 ± 14	93 ± 25^c	126 ± 27^c	169 ± 28^c	197 ± 13^c
PC	7	269 ± 11	279 ± 16^f	288 ± 20^f	295 ± 22^f	297 ± 24^f
+ $CGRP_{8-37}$	5	244 ± 13	97 ± 31^h	142 ± 42^h	180 ± 40^h	211 ± 27^h
			Coronary flow (mL·min^{-1})			
Control	8	13.5 ± 1.5	13.3 ± 1.4	13.1 ± 1.8	12.5 ± 2.0	12.4 ± 1.0
$CGRP_{8-37}$	5	12.6 ± 1.0	11.8 ± 0.7^a	12.0 ± 0.7^a	11.8 ± 0.8^a	11.7 ± 0.7^a
I-R	8	12.6 ± 1.4	7.7 ± 1.7^c	7.5 ± 1.8^c	7.3 ± 1.6^c	7.1 ± 1.6^c
PC	7	19.2 ± 1.7^f	17.3 ± 2.5^f	16.9 ± 2.2^f	15.1 ± 1.5^f	14.1 ± 1.8^f
+ $CGRP_{8-37}$	5	13.8 ± 1.5^h	8.2 ± 2.4^h	8.6 ± 2.4^h	8.6 ± 2.4^h	8.6 ± 2.7^h
			Heart rate (beats·min^{-1})			
Control	8	310 ± 20	300 ± 18	298 ± 22	296 ± 25	295 ± 28
$CGRP_{8-37}$	5	305 ± 18	299 ± 13^a	300 ± 13^a	299 ± 14^a	299 ± 12^a
I-R	8	300 ± 19	199 ± 36^c	223 ± 27^c	238 ± 13^c	243 ± 10^c
PC	7	306 ± 22	274 ± 30^f	289 ± 32^f	283 ± 25^f	280 ± 19^f
+ $CGRP_{8-37}$	5	297 ± 17	216 ± 26^h	235 ± 10^h	235 ± 10^h	244 ± 11^f

Tab 2. Effect of $CGRP_{8-37}$ (0.1 μmol·L^{-1}) on inhibition of ventricular arrhythmias and CK release by PC. VF: ventricular fibrillation; VT: ventricular tachycardia. $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control. $^fP<0.01$ *vs* ischemia-reperfusion. $^bP<0.05$, $^fP<0.01$ *vs* PC.

	n	Ventricular arrhythmias		CK activity U·min^{-1}/g wet wt
		VF	VT	
Control	8	0	0	0.30±0.07
$CGRP_{8-37}$	5	0[a]	0[a]	0.35±0.05[a]
Ischemia-reperfusion	8	8[c]	8[c]	2.03±0.49[c]
(Preconditioning) PC	7	1[b]	1[f]	0.92±0.40[f]
PC + $CGRP_{8-37}$	5	5[b]	5[b]	2.55±0.32[c]

time that $CGRP_{8-37}$, a selective CGRP receptor antagonist, abolished the cardioprotective effect of ischemic PC. This suggested that endogenous CGRP plays an important role in cardioprotection of ischemic PC in the rat heart.

Endogenous chemical mediators, such as catecholamine, adenosine, bradykinin and nitric oxide, play a central role in ischemic PC[6,7]. However, there is evidence to suggest that adenosine, nitric oxide, or catecholamine may be not related with PC in the rat heart[13,14,15].

CGRP is a principal transmitter in cardiac sensory nerves. CGRP possesses numerous physiological properties, and several of which are thought to be beneficial to the ischemic myocardium[10,11]. Even a brief ischemic period (5 min) caused a significant increase of CGRP release in the guinea-pig heart[9]. According to the hypothesis proposed by Jim Parratt about "endogenous myocardial protective substances", the cardioprotection of ischemic PC mediated by them is abolished in the presence of selective antagonists[7]. In the present study, $CGRP_{8-37}$, a selective CGRP receptor antagonist, abolished the cardioprotection of ischemic PC as reappearance of cardiac dysfunction, ventricular arrhythmias, and increased release of CK. A possible explanation of our findings is that ischemic PC may trigger CGRP release from cardiac sensory nerves, resulting in cardiac protection. These results support the hypothesis that CGRP may be an endogenous myocardial protective substance.

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降钙素基因相关肽介导的离体大鼠心脏缺血预处置[1]

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关键词 降钙素基因相关肽；心肌再灌注损伤；心功能试验；肌酸激酶

目的：研究降钙素基因相关肽(CGRP)在离体大鼠心脏缺血预处置(PC)中的介导作用。方法：离体大鼠心脏缺血 5 min 后再灌 5 min，重复三次，然后缺血 30 min，再灌 30 min。结果：PC 显著改善再灌时心功能，降低室性心律失常发生率和减少心肌 CK 释放(对照、缺血再灌和预处置组 CK 活性分别为 0.30±0.07、2.03±0.49 和 0.92±0.4 $U \cdot min^{-1}/g$ wet wt)。CGRP 受体拮抗剂 $CGRP_{8-37}$ ($0.1\ \mu mol \cdot L^{-1}$)能取消 PC 所致的心功能改善，室性心律失常发生率降低和 CK 释放减少(预处置和 $CGRP_{8-37}$ 处理组 CK 活性分别为 0.92±0.4 和 2.55±0.32 $U \cdot min^{-1}/g$ wet wt^{-1}，$P<0.01$)。结论：CGRP 是一种内源性心肌保护物质，在大鼠心脏的 PC 中起重要作用

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Effect of captopril on intracellular pH in vascular smooth muscle cells[1]

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KEY WORDS thoracic aorta; cultured cells; inbred SHR rats; inbred WKY rats; hydrogen; sodium; angiotensin Ⅱ; captopril

AIM: To observe the effect of captopril (Cap) on intracellular pH (pH_i) in aortic smooth muscle cells (ASMC). **METHODS:** Cultured ASMC derived from rat and rabbit aortae were loaded with the fluorescent dye BCECF and pH_i was determined using digital image processing method. **RESULTS:** The pH_i of untreated SHR and WKY rats were 7.37±0.29 and 7.19±0.31, respectively. Oral Cap decreased pH_i (7.11±0.26, $P<0.05$) and exaggerated pH_i response to angiotensin Ⅱ (Ang-Ⅱ, $0.1\ \mu mol \cdot L^{-1}$) in ASMC of SHR rats *vs* WKY rats (0.14±0.05 *vs* 0.21±0.05 pH units, $P<0.01$). Cap *in vitro* had no effect on Ang-Ⅱ induced intracellular alkalinization *in* ASMC of rabbits. **CONCLUSION:** Oral Cap inhibits $Na^+ - H^+$ exchange activity in ASMC of SHR rats.

The $Na^+ - H^+$ antiport is involved in the pathogenesis of hypertension[1]. An augmented activity of this ion transport system is demonstrated in human hypertension and in inbred SHR rats[2,3].

Antihypertensive treatment with angiotensin-converting enzyme (ACE) inhibitor captopril (Cap) reduces cellular Na^+ and Ca^{2+} which are linked to $Na^+ - H^+$ exchanger in patients with essential hypertension and SHR rats[3-5]. Little is known about the effect of Cap on intracellular pH (pH_i) and its modulator $Na^+ - H^+$ exchanger[1] although the effect of enalapril on this antiport has been examined[2]. The present study was undertaken to determine whether oral Cap affected $Na^+ - H^+$ antiport in aortic smooth muscle cells (ASMC) of SHR rats. We also examined the effect of Cap *in*

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