

Cardioprotective effect of bradykinin-induced preconditioning mediated by calcitonin gene-related peptide in isolated rat heart¹

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KEY WORDS calcitonin gene-related peptide; bradykinin; capsaicin; heart function tests; heart; creatine kinase

ABSTRACT

AIM: To study the mediation of calcitonin gene-related peptide (CGRP) in the cardioprotective effect of bradykinin-induced preconditioning in heart. **METHODS:** The isolated rat hearts were perfused in a Langendorff mode. The cardiac function and creatine kinase (CK) were measured. **RESULTS:** Pretreatment with bradykinin for 5 min caused an improvement of heart function and a decrease of CK release during reperfusion [CK was (0.18 ± 0.06), (1.07 ± 0.14), and (0.37 ± 0.15) $U \cdot \min^{-1} \cdot g^{-1}$ /(wet wt) for control, ischemia-reperfusion, and bradykinin, respectively, $P < 0.01$], and the effect of bradykinin was abolished in the presence of icatibant acetate (Hoe140 $1 \mu\text{mol} \cdot L^{-1}$) or CGRP₈₋₃₇ ($0.1 \mu\text{mol} \cdot L^{-1}$) [CK was (0.37 ± 0.15), (1.01 ± 0.23), and (1.07 ± 0.23) $U \cdot \min^{-1} \cdot g^{-1}$ (wet wt) for bradykinin, Hoe140, and CGRP₈₋₃₇, respectively, $P < 0.01$]. Pretreatment with capsaicin also abolished the protection of bradykinin [CK was (0.30 ± 0.04) and (1.14 ± 0.12) $U \cdot \min^{-1} \cdot g^{-1}$ (wet wt) for vehicle and capsaicin, respectively, $P < 0.01$]. **CONCLUSION:** The cardioprotective effect of bradykinin-induced preconditioning was related to stimulation of

CGRP release in the rat.

INTRODUCTION

Endogenous bradykinin might be involved in the mediation of ischemic preconditioning, and exogenous bradykinin could mimic the cardioprotection of ischemic preconditioning^[1,2]. Our recent work showed that the calcitonin gene-related peptide (CGRP) played an important role in the mediation of ischemic preconditioning in the rat^[3,4].

Bradykinin stimulated CGRP release from cardiac sensory nerves^[5]. The present study was to examine whether the cardioprotective effect of bradykinin-induced preconditioning was mediated by endogenous CGRP in the heart.

MATERIALS AND METHODS

Reagents CGRP₈₋₃₇, capsaicin, bradykinin, and icatibant acetate (Hoe140) were purchased from Sigma. All drugs were dissolved in Krebs-Henseleit (K-H) buffer solution, except capsaicin was dissolved in a vehicle containing 10% Tween 80, 10% ethanol, and 80% saline. The creatine kinase (CK) assay kit was obtained from Baoding Chemical Co.

Isolated heart preparation Sprague-Dawley rats (Laboratory Animal Center, Hu-nan Medical University, Grade II, Certificate No 20-011) (\uparrow , $n = 46$, $245 \text{ g} \pm s 25 \text{ g}$) were anesthetized with ether. The hearts were excised and perfused under 9.8 kPa with gassed (95% O₂ and 5% CO₂) K-H buffer solution (37 °C, pH 7.4), according to the modified Langendorff procedure^[6]. The K-H buffer solution had the following composition ($\text{mmol} \cdot L^{-1}$): NaCl

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119.0; NaHCO₃ 25.5; KCl 4.3; KH₂PO₄ 1.2; MgSO₄ 1.2; CaCl₂ 2.5; and glucose 11.0.

A water-filled latex balloon was inserted in left ventricle and connected to a polyphysiologic recorder for examination of the left ventricular pressure (LVP) and its first derivative (LV dp/dt_{max}). An epicardial electrocardiogram recording (ECG) throughout the experiment was analyzed for heart rate (HR). Coronary flow (CF) was measured by timed collection of coronary effluent.

Creatine kinase measurement Samples of coronary effluent after 5 min of reperfusion were collected for measurement of CK release to monitor myocardial injury. CK activity was assayed by spectrophotometry.

Experimental protocols The hearts were equilibrated for 10–30 min before each experiment. In the control group, the heart was perfused with K-H buffer solution throughout the experiment. In the ischemia-reperfusion group, the heart was subjected to 30 min of global ischemia and 30 min of reperfusion. In the preconditioned group, the heart was exposed to 3 cycles of 5 min of ischemia and 5 min of reperfusion before long-term ischemia. In the bradykinin-induced preconditioning group, the heart was exposed to bradykinin (0.5 μmol·L⁻¹) for 5 min, and then washed out for 5 min with bradykinin-free K-H buffer before long-term ischemia. For the studies on the effect of Hoe140 or CGRP₈₋₃₇ on the protective effect of bradykinin, the hearts were pretreated with Hoe140 (1 μmol·L⁻¹) or CGRP₈₋₃₇ (0.1 μmol·L⁻¹) for 5 min, and then exposed to bradykinin (0.5 μmol·L⁻¹) in the presence of Hoe140 or CGRP₈₋₃₇ for 5 min. In the case of capsaicin, the hearts were treated with capsaicin (50 mg·kg⁻¹) by sc injection 4 d before experiments. In the vehicle-treated group, the rats were injected with vehicle 4 d before experiments.

Statistics Data were expressed as $\bar{x} \pm s$.

Statistical analyses were performed using ANOVA and the Newman-Keuls tests.

RESULTS

In the control group, continuously perfused rat hearts were observed for 100 min. There were no changes in CF, HR, LVP, and LV dp/dt_{max}. Thirty minutes of global ischemia and 30 min of reperfusion caused a decrease in cardiac functions (CF, HR, LVP, and LV dp/dt_{max}) (Tab 1).

Reperfusion after 30 min of ischemia also increased the release of CK (Tab 2).

Ischemic preconditioning induced by 3 cycles of 5-min ischemia and 5-min reperfusion improved the recovery of heart function, as shown in enhancement of CF, HR, LVP, and LV dp/dt_{max}, and reduction of CK release (Tab 1, 2).

Pretreatment with bradykinin (0.5 μmol·L⁻¹) for 5 min also caused an improvement of cardiac functions (CF, HR, LVP, and LV dp/dt_{max}) and a decrease in the release of CK during reperfusion, and the protective effects of bradykinin were abolished by Hoe140 (1 μmol·L⁻¹) or CGRP₈₋₃₇ (0.1 μmol·L⁻¹).

Pretreatment with capsaicin to deplete transmitters in sensory nerves abolished the protective effect of preconditioning with bradykinin. Vehicle treatment alone had no effect on the cardioprotective effect of bradykinin (Tab 1, Tab 2).

DISCUSSION

There was considerable evidence to suggest that endogenous myocardial protective substances might play a central role in ischemic preconditioning^[7]. Our previous studies showed that in the isolated rat heart, the protective effect of ischemic preconditioning against ischemia-reperfusion injury was abolished by the CGRP receptor antagonist CGRP₈₋₃₇^[3]. Pretreatment with capsaicin, which evoked CGRP release from sensory nerves, could mimic the protective effect

Tab 1. Effect of bradykinin on cardiac functions in isolated rat hearts. ^aP > 0.05, ^cP < 0.01 vs control. ^dP > 0.05, ^fP < 0.01 vs ischemia-reperfusion. ^eP > 0.05, ⁱP < 0.01 vs + bradykinin. ^jP > 0.05, ^lP < 0.01 vs + vehicle + bradykinin.

	n	Preischemia	Reperfusion/min			
			5	10	20	30
Coronary flow/mL·min ⁻¹						
Control	7	10.3 ± 1.0	10.2 ± 0.9	10.2 ± 0.9	10.1 ± 0.7	9.9 ± 0.7
Ischemia-reperfusion	7	10.6 ± 1.2 ^a	5.7 ± 1.6 ^c	6.3 ± 1.1 ^c	6.3 ± 0.9 ^c	6.2 ± 0.8 ^c
+ Preconditioning	6	10.4 ± 1.0 ^d	9.7 ± 1.6 ^f	10.3 ± 1.4 ^d	10.1 ± 1.3 ^f	9.9 ± 1.2 ^f
+ Bradykinin (BK)	5	10.7 ± 2.0 ^d	9.9 ± 2.0 ^f	10.1 ± 1.9 ^f	10.5 ± 2.0 ^f	10.3 ± 2.2 ^f
+ Hoe140 + BK	5	10.8 ± 1.1 ^e	5.3 ± 0.6 ⁱ	5.9 ± 0.5 ⁱ	6.1 ± 0.3 ⁱ	5.9 ± 0.4 ⁱ
+ CGRP ₈₋₃₇ + BK	5	10.3 ± 0.9 ^e	6.4 ± 2.2 ⁱ	6.6 ± 1.6 ⁱ	6.4 ± 1.1 ⁱ	6.2 ± 0.9 ⁱ
+ Vehicle + BK	5	9.6 ± 0.3 ^e	8.8 ± 1.0 ^e	9.6 ± 0.7 ^e	9.8 ± 0.5 ^e	9.7 ± 0.3 ^e
+ Capsaicin + BK	6	9.8 ± 0.5 ⁱ	4.3 ± 0.4 ^l	4.5 ± 0.4 ^l	4.8 ± 0.4 ^l	4.8 ± 0.6 ^l
Left ventricular pressure/kPa						
Control	7	11.6 ± 0.7	11.3 ± 0.4	11.3 ± 0.4	11.1 ± 0.7	11.3 ± 0.7
Ischemia-reperfusion	7	11.0 ± 0.7 ^a	3.0 ± 0.7 ^c	4.1 ± 0.8 ^c	5.5 ± 0.7 ^c	6.0 ± 0.5 ^c
+ Preconditioning	6	11.3 ± 0.7 ^d	8.9 ± 1.2 ^f	10.1 ± 1.1 ^f	11.0 ± 1.1 ^f	11.7 ± 1.1 ^f
+ Bradykinin (BK)	5	11.4 ± 1.1 ^d	8.1 ± 2.2 ^f	9.1 ± 2.0 ^f	9.8 ± 1.7 ^f	9.5 ± 1.2 ^f
+ Hoe140 + BK	5	11.0 ± 1.0 ^e	3.6 ± 0.9 ⁱ	4.6 ± 0.7 ⁱ	4.8 ± 0.5 ⁱ	5.3 ± 0.6 ⁱ
+ CGRP ₈₋₃₇ + BK	5	11.5 ± 0.5 ^e	3.8 ± 1.3 ⁱ	5.2 ± 1.7 ⁱ	5.9 ± 1.9 ⁱ	6.2 ± 1.2 ⁱ
+ Vehicle + BK	5	11.8 ± 0.8 ^e	6.7 ± 0.8 ^e	9.3 ± 1.6 ^e	10.4 ± 1.5 ^e	10.6 ± 1.7 ^e
+ Capsaicin + BK	6	11.6 ± 1.0 ⁱ	3.0 ± 0.4 ^l	3.3 ± 1.1 ^l	4.6 ± 1.4 ^l	4.8 ± 1.3 ^l
Left ventricle dp/dt _{max} (kPa · s ⁻¹)						
Control	7	308 ± 30	299 ± 22	300 ± 29	295 ± 27	295 ± 29
Ischemia-reperfusion	7	318 ± 27 ^a	53 ± 24 ^c	86 ± 30 ^c	134 ± 24 ^c	161 ± 24 ^c
+ Preconditioning	6	297 ± 13 ^d	192 ± 42 ^f	215 ± 32 ^f	251 ± 19 ^f	262 ± 34 ^f
+ Bradykinin (BK)	5	295 ± 35 ^d	172 ± 56 ^f	198 ± 37 ^f	229 ± 48 ^f	238 ± 42 ^f
+ Hoe140 + BK	5	289 ± 19 ^e	70 ± 8 ⁱ	85 ± 10 ⁱ	99 ± 18 ⁱ	123 ± 24 ⁱ
+ CGRP ₈₋₃₇ + BK	5	298 ± 19 ^e	88 ± 43 ⁱ	99 ± 45 ⁱ	157 ± 21 ⁱ	158 ± 26 ⁱ
+ Vehicle + BK	5	288 ± 25 ^e	158 ± 30 ^e	238 ± 44 ^e	254 ± 43 ^e	247 ± 47 ^e
+ Capsaicin + BK	6	278 ± 21 ⁱ	36 ± 15 ^l	78 ± 30 ^l	86 ± 41 ^l	101 ± 54 ^l
Heart rate/beat · min ⁻¹						
Control	7	306 ± 4	304 ± 5	304 ± 5	301 ± 5	298 ± 5
Ischemia-reperfusion	7	296 ± 2 ^a	157 ± 5 ^c	183 ± 4 ^c	200 ± 2 ^c	208 ± 2 ^c
+ Preconditioning	6	310 ± 4 ^d	263 ± 7 ^f	284 ± 4 ^f	284 ± 5 ^f	287 ± 4 ^f
+ Bradykinin (BK)	5	304 ± 5 ^d	239 ± 13 ^f	258 ± 10 ^f	260 ± 9 ^f	269 ± 9 ^f
+ Hoe140 + BK	5	302 ± 9 ^e	128 ± 6 ⁱ	190 ± 10 ⁱ	195 ± 12 ⁱ	205 ± 11 ⁱ
+ CGRP ₈₋₃₇ + BK	5	322 ± 6 ^e	155 ± 21 ⁱ	192 ± 11 ⁱ	216 ± 9 ⁱ	227 ± 4 ⁱ
+ Vehicle + BK	5	316 ± 34 ^e	231 ± 23 ^e	230 ± 23 ^e	242 ± 16 ^e	238 ± 10 ^e
+ Capsaicin + BK	6	296 ± 11 ⁱ	171 ± 24 ^l	191 ± 13 ^l	195 ± 13 ^l	194 ± 7 ^l

of ischemic preconditioning in the rat heart^[4,8], and that exogenous CGRP could substitute for ischemic preconditioning and prevent myocardial injury due to ischemia-reperfusion, endothelin-1 or oxygen free radicals^[4,9,10]. Recently, other investigators reported that in the rat pretreated with capsaicin to destroy sensory nerves, the cardioprotective effect of pacing-induced pre-

conditioning was abolished^[11]. These findings suggested that CGRP might be a key mediator in the protective effect of ischemic preconditioning in the rat heart.

Interactions of peptides with autacoids, including bradykinin, were shown to occur prejunctionally^[5]. There was also evidence to suggest that bradykinin evoked CGRP release

**Tab 2. Effect of bradykinin (BK) on release of creatine kinase during reperfusion. $\bar{x} \pm s$.
^c $P < 0.01$ vs control. ^f $P < 0.01$ vs ischemia-reperfusion. ^g $P > 0.05$, ⁱ $P < 0.01$ vs +BK.
^l $P < 0.01$ vs +Vehicle +BK.**

Group	Rat hearts	CK release/ U · min ⁻¹ · g ⁻¹ (wet wt)
Control	7	0.18 ± 0.06
Ischemia-reperfusion	7	1.07 ± 0.14 ^c
+ Preconditioning	6	0.32 ± 0.09 ^f
+ BK	5	0.37 ± 0.15 ^f
+ Hoe140 + BK	5	1.01 ± 0.23 ⁱ
+ CGRP ₈₋₃₇ + BK	5	1.07 ± 0.23 ⁱ
+ Vehicle + BK	5	0.30 ± 0.04 ^g
+ Capsaicin + BK	6	1.14 ± 0.12 ^l

from the guinea-pig atria^[5]. In the present study, the cardioprotective effect of bradykinin-induced preconditioning was negated in the presence of CGRP₈₋₃₇, a selective CGRP receptor antagonist. This suggested that the cardioprotective effect of preconditioning with bradykinin was mediated by endogenous CGRP released from sensory nerves in the isolated rat heart.

Capsaicin-sensitive sensory nerves contained a number of peptides, including CGRP, substance P, and neurokinin A, and capsaicin selectively stimulated release of these peptides^[12]. As described above, among these peptides CGRP was shown to mediate the protection of ischemic preconditioning^[3,4]. In the present study, pretreatment of capsaicin to deplete neurotransmitters in sensory nerves abolished the cardioprotective effect of bradykinin-induced preconditioning, as assessed by the contractile function and CK release. These results suggested that capsaicin-sensitive sensory nerves were involved in the effect of bradykinin, in further support of the conclusion that the cardioprotective effect of bradykinin-induced preconditioning was secondary to the release of endogenous CGRP in the rat heart.

In summary, the results of this study

suggested that the cardioprotective effect of bradykinin-induced preconditioning was related to stimulation of CGRP release in the rat.

REFERENCES

- 1 Wall TM, Sheehy R, Hartman JC. Role of bradykinin in myocardial preconditioning. *J Pharmacol Exp Ther* 1994; 270: 681-9.
- 2 Starkopf J, Bugge E, Ytrehus K. Preischemic bradykinin and ischemic preconditioning in functional recovery of the globally ischemic rat heart. *Cardiovasc Res* 1997; 33: 63-70.
- 3 Xiao ZS, Li YJ, Deng HW. Ischemic preconditioning mediated by calcitonin gene-related peptide in isolated rat hearts. *Acta Pharmacol Sin* 1996; 17: 445-8.
- 4 Li YJ, Xiao ZS, Peng CF, Deng HW. Calcitonin gene-related peptide-induced preconditioning protects against ischemia-reperfusion injury in isolated rat hearts. *Eur J Pharmacol* 1996; 311: 163-7.
- 5 Geppetti P, Maggi CA, Perretti F, Frilli S, Manzini S. Simultaneous release by bradykinin of substance P and calcitonin gene-related peptide immunoreactivities from capsaicin-sensitive structures in guinea-pig heart. *Br J Pharmacol* 1988; 94: 288-90.
- 6 Srimani BN, Engelman RM, Jones R, Das DK. Protective role of intracoronary fatty acid binding protein in ischemic and reperfused myocardium. *Circ Res* 1990; 66: 1535-43.
- 7 Parratt JR. Protection of the heart by ischemic preconditioning: mechanisms and possibilities for pharmacological exploitation. *Trends Pharmacol Sci* 1994; 15: 19-25.
- 8 D'alonzo AJ, Grover GJ, Darbenzio RB, Hess TA, Sleph PG, Dzwonczyk S, *et al.* *In vitro* effects of capsaicin: antiarrhythmic and antiischemic activity. *Eur J Pharmacol* 1995; 272: 269-78.
- 9 Peng CF, Li YJ, Deng HW, Xiong Y. The protective effects of ischemia and calcitonin gene-related peptide-induced preconditioning on myocardial injury by endothelin-1 in the isolated rat

heart.
Life Sci 1996; 59: 1507-14.

10 Tao ZW, Li YJ, Deng HW.
Attenuation of myocardial injury due to oxygen free radicals (OFR) by pretreatment with OFR or calcitonin gene-related peptide.
Acta Pharmacol Sin 1997; 18: 312-6.

11 Ferdinandy P, Csont T, Csonka C, Török M, Dux M, Németh J, *et al*.
Capsaicin-sensitive sensory innervation is involved in pacing-induced preconditioning in the rat hearts: role of nitric oxide and CGRP?
Naunyn-Schmiedebergs Arch Pharmacol 1997; 356: 356-63.

12 Franco-cereceda A.
Calcitonin gene-related peptide and tachykinins in relation to local sensory control of cardiac contractility and coronary vascular tone.
Acta Physiol Scand 1988; 133 Suppl 596: 1-63.

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降钙素基因相关肽介导缓激肽预处理
对离体大鼠心脏的保护作用¹

R972

RB54.1

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CGRP

关键词 降钙素基因相关肽; 缓激肽; 辣椒素;
心功能测定; 心脏; 肌酸激酶

10 心解保护

目的: 研究降钙素基因相关肽 (CGRP) 在缓激肽预处理保护离体大鼠心脏中的调节作用。方法: Langendorff 法灌流心脏, 观测心功能与肌酸激酶 (CK) 释放。结果: 缓激肽显著改善再灌时心功能并减少 CK 释放。缓激肽的作用可被缓激肽受体拮抗剂 Hoe140 ($1 \mu\text{mol} \cdot \text{L}^{-1}$) 或 CGRP 受体拮抗剂 CGRP₈₋₃₇ ($0.1 \mu\text{mol} \cdot \text{L}^{-1}$) 所取消预先用辣椒素处理也能取消缓激肽的作用。结论: 缓激肽诱导预处理的心脏保护作用与促进 CGRP 的释放有关。

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