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*Original Research*

# Role of calcitonin gene-related peptide in prostaglandins-mediated ischemic preconditioning in guinea pig hearts[1]

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**KEY WORDS** myocardial reperfusion injury; prostaglandins; calcitonin gene-related peptide; endothelin-1; heart function tests

## ABSTRACT

**AIM**: To examine the role of calcitonin gene-related peptide (CGRP) in ischemic preconditioning induced by prostaglandins in isolated guinea pig hearts. **METHODS**: The isolated guinea pig hearts were perfused in a Langendorff model. The heart rate, coronary flow, left ventricular pressure, and its first derivatives ($\pm dp/dt_{max}$) were recorded and the calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) and 6-keto-$PGF_{1\alpha}$ were measured. **RESULTS**: Endothelin-1 (200 pmol in 1 mL K-H buffer) reduced the left ventricular developed pressure and its first derivatives ($\pm dp/dt_{max}$), heart rate, and coronary flow. Preconditioning with two cycles of 5-min global ischemia and 5-min reperfusion attenuated endothelin-1-induced myocardial injury, and concentrations of both CGRP and 6-keto-$PGF_{1\alpha}$ in the coronary effluent were markedly raised in the preconditioning periods. Pretreatment with capsaicin, which depletes endogenous CGRP, abolished the elevated level of CGRP concomitantly with loss of the cardioprotection induced by ischemic preconditioning. $CGRP_{8-37}$ (100 nmol/L), a selective $CGRP_1$ receptor antagonist, also abolished the protective effects of ischemic preconditioning. After pretreatment with indometacin (10 μmol/L), an inhibitor of cyclooxygenase, the protective effects of ischemic preconditioning were abolished and the release of 6-keto-$PGF_{1\alpha}$ was no longer elevated. Pretreatment with indometacin abolished the elevated level of CGRP in the coronary effluent. **CONCLUSION**: Endogenous prostaglandins are involved in the protective effects of ischemic preconditioning, and the beneficial effects of prostaglandins are mediated by CGRP in the guinea pig heart.

## INTRODUCTION

In recent years, a great deal of interest has been focused on the phenomenon of ischemic preconditioning and the mechanisms by which its potent cardioprotective effect occurs. This fascinating phenomenon has stimulated numerous studies to determine potential triggers and/or mediators of this myocardial protection. Increasing evidence has shown that some endogenous chemical mediators, such as prostaglandins, adenosine, nitric oxide, and kinins, are involved in the mediation of ischemic preconditioning[1]. Recently, we and others have shown that endogenous calcitonin gene-related peptide (CGRP), a principal transmitter in capsaicin-sensitive sensory nerves, may play an important role in the mediation of ischemic preconditioning in rat hearts[2~4]. However, the contribution of CGRP to the cardioprotection of ischemic preconditioning in guinea pig heart has not been determined thus far.

Given that ischemia can undoubtedly stimulate the release of multiple endogenous chemical mediators, understanding the relationship of these substances in ischemic preconditioning appears formidable. Interactions among some endogenous chemical mediators have been shown pre- or post-junctionally. Bradykinin can modulate peptidergic neurotransmission by stimulation of prostaglandins production[5]. Recent evidence suggests that prostaglandins stimulate the release of endogenous CGRP[6,7]. To our knowledge the possible modulation by prostaglandin of CGRP-mediated ischemic preconditioning has not been tested to date.

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For these reasons, the present study has two objectives. The first is to examine whether endogenous CGRP is involved in the protective effects of ischemic preconditioning in guinea pig hearts. The second objective is to further explore whether the protective effects of prostaglandin-mediated ischemic preconditioning are related to CGRP.

## MATERIALS AND METHODS

**Reagents** Endothelin-1, $CGRP_{8-37}$(human), indometacin, and capsaicin were purchased from the Sigma Chemical Co (St Louis, MO, USA). RIA kits for measurement of CGRP (rat, alpha) and 6-keto-prostaglandin $F_{1\alpha}$, the stable metabolite of prostacyclin, were obtained from Dongya Immunity Technology Institute (Beijing, China).

**Preparation of the isolated heart** Male guinea pigs (weighing 220 – 280 g, obtained from Hunan Medical University Animal Center) were used. Animals were anesthetized with sodium pentobarbital (60 mg/kg, ip). The heart was rapidly excised into 4 ℃ Krebs-Henseleit (K-H) solution. Retrograde, nonrecirculating perfusion was performed in a Langendorff model, at a constant perfusion pressure (80 $cmH_2O$). The heart was perfused with K-H buffer (pH 7.4, 37 ℃), saturated with 95 % $O_2$ and 5 % $CO_2$. The K-H buffer solution had the following composition (mmol/L): 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 water-filled latex balloon was inserted in the left ventricle. The balloon was adjusted to a left ventricular end-diastolic pressure of 3 – 5 mmHg. The left ventricular developed pressure, its first derivatives ($\pm dp/dt_{max}$), and heart rate were continuously monitored. The resulting electrical signals were digitized by MacLab analogue to digital converter and recorded by a Power Macintosh 7220 computer. Coronary flow was measured by timed collection. $CGRP_{8-37}$ was diluted in the K-H buffer. Indometacin was initially dissolved in ethanol and further diluted in K-H buffer to proper final concentration. For endothelin-1, a single bolus dose of endothelin-1 (200 pmol) in 1 mL K-H buffer was given into the perfusion system through the sidearm.

**Radioimmunoassay** Coronary effluent during 5 min was collected before the first cycle of ischemia or during the two cycles of reperfusion in ischemic preconditioning, and the samples were divided in half, desalted using Sep-Pak Cartridge $C_{18}$ and lyophilized. Calcitonin gene-related peptide like immunoreactivity (CGRP-LI) and 6-keto-prostaglandin $F_{1\alpha}$, the stable metabolite of prostacyclin, in the samples were measured by radioimmunoassay (RIA) kits.

**Experimental protocols** The experiment was divided into 11 groups (Fig 1). All hearts were equilibrated for 20 min. (1) The control group was perfused with K-H buffer solution throughout the experiment. (2) The ischemic preconditioned group experienced two cycles of 5 min of global ischemia and 5 min of reperfusion. (3) The endothelin-1-treated group received a single bolus dose of endothelin-1 (200 pmol). (4) The ischemic preconditioning plus endothelin-1 group experienced two cycles of 5 min of global ischemia and 5 min of reperfusion before endothelin-1 treatment. (5) The $CGRP_{8-37}$ plus endothelin-1 group received $CGRP_{8-37}$ (100 nmol/L) for 15 min prior to endothelin-1 treatment. (6) The $CGRP_{8-37}$ plus ischemic preconditioning and endothelin-1 group received $CGRP_{8-37}$ (100 nmol/L) for 5 min prior to brief ischemia and continuing through the ischemic preconditioning periods before endothelin-1 treatment. (7) The capsaicin plus endothelin-1 group was pretreated with capsaicin (50 mg/kg) before endothelin-1 treatment. Capsaicin (dissolved in a vehicle containing 10 % Tween 80, 10 % ethanol and 80 % saline) was administered by sc injection 4 d before the experiments[8]. (8) The vehicle plus ischemic preconditioning and endothelin-1 group received vehicle 4 d before the experiments. (9) The capsaicin plus ischemic preconditioning and endothelin-1 group received capsaicin 4 d before the experiments. (10) The indometacin plus endothelin-1 group received indometacin (10 μmol/L) for 30 min before the endothelin-1 treatment. (11) The indometacin plus ischemic preconditioning and endothelin-1 group received indometacin (10 μmol/L) for 20 min prior to brief ischemia and continuing the ischemic preconditioning periods before endothelin-1 treatment.

**Statistics** Data were expressed as $\bar{x} \pm s$. Statistical analyses were performed using ANOVA and the Newman-Keuls tests.

## RESULTS

**Effect of $CGRP_{8-37}$ or capsaicin on the cardioprotection of ischemic preconditioning** In the control group, continuously perfused guinea pig hearts were observed for 70 min. There were no changes incoronary flow, left ventricular developed pressure,

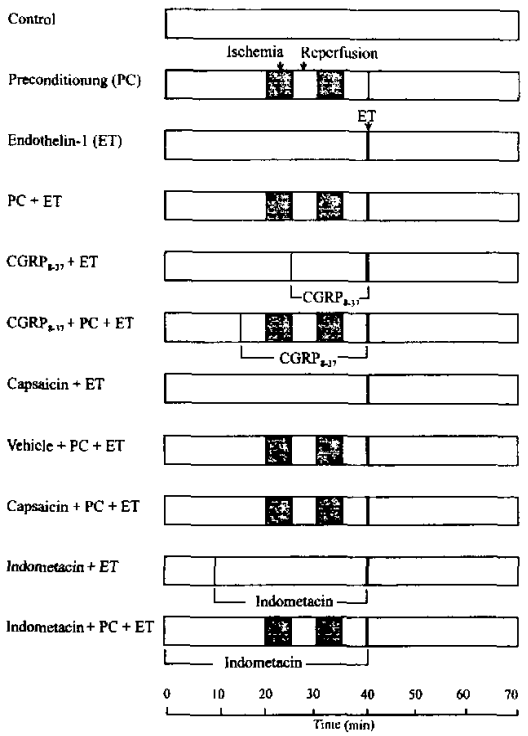


**Fig 1. Experimental protocol. Endothelin-1: 200 pmoL in 1 mL K-H buffer. Preconditioning: two cycles of 5-min global ischemia and 5-min reperfusion; $CGRP_{8-37}$: $CGRP_{8-37}$ was dissolved in K-H buffer; capsaicin or vehicle: animals were pretreated with capsaicin (50 mg/kg sc) or vehicle 4 d before the experiments. Indometacin: indometacin 10 μmol/L was perfused. The gray block: 5-min global ischemia.**

$\pm dp/dt_{max}$, coronary flow, and heart rate. Two cycles of 5 min of ischemia and 5 min of reperfusion alone did not affect the cardiac function either. Endothelin-1 (200 pmol) depressed cardiac function, as shown by the reduction of left ventricular developed pressure, $\pm dp/dt_{max}$, coronary flow, and heart rate. Preconditioning induced by two cycles of 5 min of global ischemia and 5 min of reperfusion improved the recovery of heart function (Tab 1).

To examine the role of endogenous CGRP in the protection conferred by ischemic preconditioning, $CGRP_{8-37}$ and capsaicin were used. The protective effects of ischemic preconditioning were abolished in the presence of $CGRP_{8-37}$ (100 nmol/L), a selective $CGRP_1$ receptor antagonist. After pretreatment with capsaicin (50 mg/kg, sc) to deplete sensory nerve transmitter content, the protection of ischemic preconditioning was also abolished (Tab 1). $CGRP_{8-37}$ or capsaicin treatment alone had no effect on endothelin-1-induced myocardial injury.

The content of CGRP-LI in the coronary effluent was increased during two cycles of reperfusion after brief ischemia in the isolated guinea pig heart. After treatment of capsaicin, the concentration of CGRP-LI in coronary effluent was no longer raised (Fig 2).

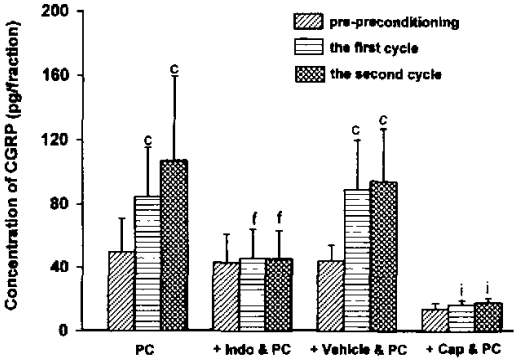


**Fig 2. Effect of ischemic preconditioning on the release of CGRP. PC: ischemic preconditioning; + Indo & PC: perfusion with indometacin (10 μmol/L) for 20 min and preconditioning-treatment in the presence of indometacin; + Vehicle & PC: ischemic preconditioning after pretreatment with vehicle; and + Cap & PC: ischemic preconditioning after pretreatment with capsaicin. $n = 5-8$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* pre-preconditioning; $^fP < 0.01$ *vs* PC at corresponding fractions; $^iP < 0.01$ *vs* + Vehicle & PC at corresponding fractions.**

**Effect of indometacin on the cardioprotection of ischemic preconditioning** The cardioprotection afforded by ischemic preconditioning was also abolished by pretreatment with indometacin, an inhibitor of cyclooxygenase. Indometacin treatment alone had no effect on endothelin-1-induced myocardial injury (Tab 1). Concentrations of prostacyclin in the coronary effluent were markedly increased during reperfusion after short-term ischemia. After pretreatment with indometacin, the elevated level of prostacyclin was absent (Fig 3).

**Effect of indometacin on the release of CGRP** To test whether prostaglandins are involved in the protective effects of CGRP-mediated ischemic preconditioning, indometacin was used. Pretreatment with indometacin to inhibit cyclooxygenase abolished the elevated level of CGRP induced by short-term ischemia concomitantly with loss of cardioprotection induced by ischemic preconditioning. Indometacin itself did not affect the basal release of

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**Tab 1. Effect of $CGRP_{8-37}$, capsaicin, or indometacin on the cardioprotection of ischemic preconditioning.** $\bar{x} \pm s$. [a] $P > 0.05$, [c] $P < 0.01$ *vs* control. [d] $P > 0.05$, [f] $P < 0.01$ *vs* endothelin-1. [g] $P > 0.05$, [i] $P < 0.01$ *vs* Endothelin-1 + ischemic preconditioning.

| | $n$ | Time after endothelin-1 treatment/min | | | | |
|---|---|---|---|---|---|---|
| | | Pretreatment | 5 | 10 | 20 | 30 |
| Left ventricular pressure/kPa | | | | | | |
| Control | 6 | 9.9 ± 1.3 | 10.0 ± 1.0 | 10.0 ± 0.7 | 9.6 ± 1.0 | 9.6 ± 1.3 |
| Ischemic preconditioning (PC) | 5 | 10.5 ± 0.6 | 9.9 ± 0.6[a] | 9.9 ± 0.6[a] | 9.7 ± 0.9[a] | 9.5 ± 0.9[a] |
| Endothelin-1 | 5 | 9.5 ± 0.9 | 2.3 ± 1.5[c] | 2.4 ± 0.9[c] | 2.5 ± 1.5[c] | 3.5 ± 0.9[c] |
| + PC | 8 | 10.4 ± 0.8 | 7.5 ± 1.5[f] | 8.5 ± 0.8[f] | 9.6 ± 0.8[f] | 9.7 ± 0.8[f] |
| + $CGRP_{8-37}$ | 6 | 9.5 ± 0.7 | 2.0 ± 2.0[d] | 2.5 ± 0.7[d] | 3.3 ± 0.7[d] | 4.1 ± 1.0[d] |
| + $CGRP_{8-37}$ & PC | 5 | 10.8 ± 1.2 | 1.3 ± 0.6[i] | 1.9 ± 0.6[i] | 3.1 ± 1.2[i] | 4.3 ± 1.8[i] |
| + Capsaicin | 6 | 9.9 ± 0.7 | 2.1 ± 0.3[d] | 2.4 ± 0.3[d] | 3.6 ± 0.7[d] | 4.3 ± 0.7[d] |
| + Vehicle & PC | 5 | 9.7 ± 0.6 | 7.6 ± 0.9[g] | 8.0 ± 0.6[g] | 8.3 ± 0.6[g] | 8.5 ± 0.9[g] |
| + Capsaicin & PC | 6 | 9.9 ± 0.7 | 1.3 ± 0.3[i] | 2.3 ± 0.4[i] | 3.2 ± 1.3[i] | 3.9 ± 1.3[i] |
| + Indometacin | 5 | 9.7 ± 0.9 | 2.3 ± 0.9[d] | 2.5 ± 0.3[d] | 2.9 ± 0.6[d] | 3.5 ± 0.6[d] |
| + Indometacin & PC | 6 | 10.2 ± 0.7 | 1.9 ± 0.7[i] | 2.4 ± 0.7[i] | 3.3 ± 1.3[i] | 4.1 ± 1.3[i] |
| $+dp/dt_{max}$/kPa·s$^{-1}$ | | | | | | |
| Control | 6 | 172 ± 26 | 178 ± 18 | 174 ± 20 | 175 ± 21 | 171 ± 20 |
| Ischemic preconditioning (PC) | 5 | 165 ± 38 | 163 ± 31[a] | 166 ± 34[a] | 167 ± 36[a] | 166 ± 25[a] |
| Endothelin-1 | 5 | 170 ± 16 | 30 ± 24[c] | 30 ± 25[c] | 35 ± 24[c] | 68 ± 20[c] |
| + PC | 8 | 169 ± 18 | 127 ± 33[f] | 148 ± 23[f] | 168 ± 17[f] | 175 ± 11[f] |
| + $CGRP_{8-37}$ | 6 | 143 ± 26 | 26 ± 8[d] | 31 ± 12[d] | 41 ± 14[d] | 63 ± 17[d] |
| + $CGRP_{8-37}$ & PC | 5 | 192 ± 25 | 27 ± 15[i] | 20 ± 11[i] | 47 ± 30[i] | 73 ± 41[i] |
| + Capsaicin | 6 | 163 ± 21 | 32 ± 4[d] | 37 ± 7[d] | 51 ± 13[d] | 61 ± 1[-d] |
| + Vehicle & PC | 5 | 187 ± 24 | 148 ± 16[g] | 165 ± 16[g] | 169 ± 17[g] | 173 ± 15[g] |
| + Capsaicin & PC | 6 | 175 ± 19 | 30 ± 5[i] | 46 ± 19[i] | 65 ± 33[i] | 79 ± 30[i] |
| + Indometacin | 5 | 182 ± 13 | 27 ± 10[d] | 30 ± 10[d] | 36 ± 13[d] | 69 ± 19[d] |
| + Indometacin & PC | 6 | 170 ± 26 | 31 ± 11[i] | 16 ± 5[i] | 37 ± 29[i] | 55 ± 24[i] |
| $-dp/dt_{max}$/kPa·s$^{-1}$ | | | | | | |
| Control | 6 | 171 ± 39 | 177 ± 36 | 170 ± 40 | 168 ± 36 | 167 ± 36 |
| Ischemic preconditioning (PC) | 5 | 200 ± 62 | 183 ± 21[a] | 192 ± 19[a] | 187 ± 22[a] | 184 ± 23[a] |
| Endothelin-1 | 5 | 163 ± 24 | 30 ± 20[c] | 30 ± 19[c] | 34 ± 20[c] | 39 ± 14[c] |
| + PC | 8 | 177 ± 32 | 126 ± 45[f] | 139 ± 35[f] | 164 ± 25[f] | 169 ± 19[f] |
| + $CGRP_{8-37}$ | 6 | 191 ± 36 | 22 ± 7[d] | 33 ± 10[d] | 37 ± 6[d] | 47 ± 10[d] |
| + $CGRP_{8-37}$ & PC | 5 | 201 ± 20 | 26 ± 5[i] | 27 ± 21[i] | 54 ± 36[i] | 77 ± 43[i] |
| + Capsaicin | 6 | 199 ± 34 | 124 ± 6[d] | 33 ± 8[d] | 39 ± 11[d] | 53 ± 13[d] |
| + Vehicle & PC | 5 | 210 ± 24 | 18 ± 20[g] | 150 ± 33[g] | 173 ± 36[g] | 177 ± 31[g] |
| + Capsaicin & PC | 6 | 181 ± 16 | 18 ± 4[i] | 26 ± 10[i] | 43 ± 23[i] | 55 ± 24[i] |
| + Indometacin | 5 | 165 ± 24 | 29 ± 10[d] | 31 ± 10[d] | 35 ± 11[d] | 44 ± 13[d] |
| + Indometacin & PC | 6 | 170 ± 27 | 20 ± 6[i] | 22 ± 17[i] | 32 ± 14[i] | 48 ± 16[i] |
| Coronary flow (mL/min) | | | | | | |
| Control | 6 | 13.0 ± 3.2 | 13.4 ± 2.9 | 12.9 ± 2.7 | 13.0 ± 2.9 | 13.0 ± 2.9 |
| Ischemic preconditioning (PC) | 5 | 12.8 ± 1.1 | 13.2 ± 1.6[a] | 13.0 ± 1.3[a] | 12.8 ± 1.3[a] | 12.9 ± 1.8[a] |
| Endothelin-1 | 5 | 10.4 ± 1.3 | 2.9 ± 0.2[c] | 3.0 ± 0.7[c] | 3.3 ± 0.4[c] | 3.7 ± 1.1[c] |
| + PC | 8 | 12.6 ± 1.4 | 9.5 ± 2.0[f] | 10.7 ± 1.1[f] | 11.6 ± 0.8[f] | 11.7 ± 1.1[f] |
| + $CGRP_{8-37}$ | 6 | 11.7 ± 1.7 | 2.5 ± 0.5[d] | 3.1 ± 0.2[d] | 3.6 ± 0.5[d] | 4.0 ± 0.5[d] |
| + $CGRP_{8-37}$ & PC | 5 | 12.8 ± 2.5 | 2.7 ± 0.9[i] | 3.5 ± 0.9[i] | 4.3 ± 0.4[i] | 4.7 ± 0.4[i] |
| + Capsaicin | 6 | 12.1 ± 1.0 | 3.4 ± 1.0[d] | 3.7 ± 0.7[d] | 3.9 ± 0.7[d] | 4.0 ± 0.7[d] |
| + Vehicle & PC | 5 | 10.9 ± 2.2 | 9.3 ± 3.1[g] | 10.2 ± 3.1[g] | 10.3 ± 3.1[g] | 10.0 ± 2.9[g] |
| + Capsaicin & PC | 6 | 10.6 ± 2.0 | 2.0 ± 0.7[i] | 2.2 ± 1.0[i] | 2.6 ± 1.2[i] | 2.7 ± 0.5[i] |
| + Indometacin | 5 | 11.0 ± 1.3 | 2.8 ± 0.4[d] | 2.9 ± 0.9[d] | 3.4 ± 1.1[d] | 3.6 ± 1.1[d] |
| + Indometacin & PC | 6 | 10.8 ± 2.2 | 3.5 ± 0.7[i] | 3.8 ± 0.7[i] | 3.8 ± 0.5[i] | 3.6 ± 0.7[i] |

(to be continued)

| | n | Time after endothelin-1 treatment/min | | | | |
|---|---|---|---|---|---|---|
| | | Pretreatment | 5 | 10 | 20 | 30 |
| Heart rate/beats·s$^{-1}$ | | | | | | |
| Control | 6 | 214 ± 15 | 222 ± 12 | 224 ± 20 | 220 ± 17 | 226 ± 12 |
| Ischemic preconditioning (PC) | 5 | 214 ± 11 | 223 ± 9[a] | 223 ± 7[a] | 222 ± 4[a] | 221 ± 4[a] |
| Endothelin-1 | 5 | 248 ± 29 | 98 ± 34[c] | 103 ± 31[c] | 110 ± 40[c] | 117 ± 31[c] |
| + PC | 8 | 225 ± 20 | 220 ± 20[f] | 223 ± 25[f] | 225 ± 23[f] | 221 ± 20[f] |
| + $CGRP_{8-37}$ | 6 | 228 ± 22 | 98 ± 44[d] | 146 ± 24[d] | 164 ± 27[d] | 178 ± 29[d] |
| + $CGRP_{8-37}$ & PC | 5 | 229 ± 13 | 104 ± 11[i] | 103 ± 4[i] | 141 ± 42[i] | 160 ± 49[i] |
| + Capsaicin | 6 | 214 ± 12 | 107 ± 12[d] | 128 ± 24[d] | 136 ± 24[d] | 153 ± 22[d] |
| + Vehicle & PC | 5 | 231 ± 20 | 245 ± 40[g] | 247 ± 40[g] | 244 ± 38[g] | 241 ± 36[g] |
| + Capsaicin & PC | 6 | 239 ± 27 | 99 ± 12[i] | 123 ± 42[i] | 169 ± 61[i] | 169 ± 51[i] |
| + Indometacin | 5 | 231 ± 18 | 105 ± 51[d] | 122 ± 54[d] | 164 ± 58[d] | 170 ± 54[d] |
| + Indometacin & PC | 6 | 247 ± 22 | 100 ± 32[i] | 109 ± 7[i] | 168 ± 51[i] | 173 ± 54[i] |

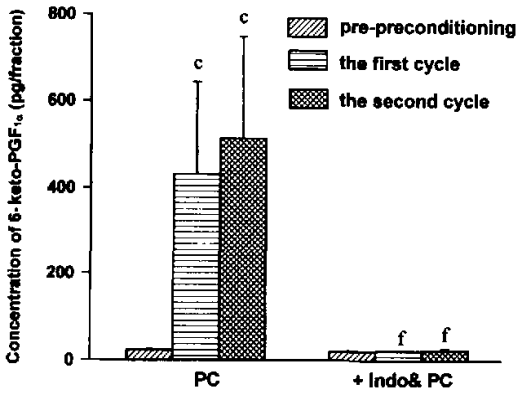


**Fig 3. Effect of ischemic preconditioning on the content of prostacyclin in coronary effluent. PC: ischemic preconditioning; + Indo & PC: perfusion with indometacin (10 μmol/L) for 20 min and treatment with ischemic preconditioning in the presence of indometacin. $n = 6-8$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* pre-preconditioning; $^fP < 0.01$ *vs* PC at corresponding fractions.**

CGRP (Tab 1, Fig 2).

## DISCUSSION

It has been suggested that ischemic preconditioning is capable of protecting the myocardium against a variety of harmful events or factors, such as ischemia-reperfusion[9], calcium paradox[10], lysophosphatidylcholine[11] and free radicals[12]. It has been reported that endogenous endothelin-1 is accumulated during myocardial ischemia[13] and that administration of endothelin-1 causes myocardial injury[14], suggesting endothelin-1 may be a contributor to myocardial injury during ischemia. In the present study, ischemic preconditioning also attenuated endothelin-1-induced myocardial injury. A similar effect has also been seen in a variety of animals[15]. These results suggest that ischemic preconditioning provide a protection of the myocardium against different deleterious factors.

CGRP, the principal transmitter in cardiac sensory nerves, is present in the hearts of humans and animals[16]. Clinical studies have shown that the plasma concentration of CGRP is markedly raised in patients with acute myocardial infarction[17]. Recently, the ischemia-related outflow of CGRP has been found in coronary artery bypass grafting without cardiopulmonary bypass[18]. CGRP release stimulated by ischemia is also seen in isolated guinea pig hearts[16]. Pretreatment with capsaicin aggravates the myocardial infarction due to ischemia-reperfusion in the porcine heart[19]. These results suggest that the elevated level of CGRP during ischemia probably constitutes a compensatory response.

Previous investigations have shown that in the rat heart, the protective effect of ischemic preconditioning is abolished by $CGRP_{8-37}$[2,3]. Others have also reported that pretreatment with capsaicin to deplete endogenous CGRP abolishes pacing-induced preconditioning in the rat[4]. Our recent work has shown that the protection of the heart by brief ischemia of the small intestine in rabbits is mediated by CGRP[20]. In the present study, preconditioning induced by two cycles of 5 min of global ischemia and 5 min of reperfusion caused an increase in the release of CGRP concomitantly with an improvement in the recovery of cardiac function, and the protective effects of ischemic preconditioning were abolished by $CGRP_{8-37}$, a selective antagonist of $CGRP_1$ receptors[21], or capsaicin in the isolated guinea pig heart. The elevat-

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ed level of CGRP due to short-term ischemia was also absent in the hearts pretreated with capsaicin. These findings suggest that endogenous CGRP may be involved in the mediation of ischemic preconditioning in different animal species.

Prostacyclin, a major product of cyclooxygenase pathway which is activated during ischemia, possesses multiple physiological properties, several of which are thought to be beneficial to the ischemic myocardium[22]. It has been reported that the cardioprotection of ischemic preconditioning is abolished in the presence of sodium meclofenamate, an inhibitor of cyclooxygenase in the dog[23]. However, involvement of prostaglandins in ischemic preconditioning varies considerably across species. Inhibition of prostaglandin formation has no effect on the cardioprotection of ischemic preconditioning in the rat heart and rabbit heart[24,25]. In the present study, the cardioprotection of ischemic preconditioning was abolished by pretreatment with indometacin, suggesting that in the guinea pig heart ischemic preconditioning involves the activation of cyclooxygenase pathway.

Ischemia can stimulate the release of multiple endogenous chemical mediators. It is probably that these endogenous chemical substances mediate the cardioprotection of ischemic preconditioning via interactions among them. There is evidence to suggest that the role of adenosine in the protection of the heart by ischemic preconditioning is involved in the activity of adrenoceptors in dogs[26]. Involvement of catecholamines in ischemic preconditioning has been shown to be related to stimulation of adenosine release in rat[27]. As mentioned above, prostaglandins can stimulate the release of CGRP[6,7]. We speculate that involvement of prostaglandin in ischemic preconditioning may be related to stimulation of CGRP release. The results of the present study showed that pretreatment with indometacin abolished the elevated level of CGRP in the coronary effluent concomitantly with the loss of cardioprotection induced by ischemic preconditioning in the guinea pig. This suggests that the cardioprotection of ischemic preconditioning may be mediated by CGRP via prostaglandin pathways. However, further work is needed before a definitive conclusion can be drawn about this matter.

In conclusion, the present study suggests that prostaglandins are involved in the cardioprotection of ischemic preconditioning and that the beneficial effects of prostaglandins are mediated by CGRP in the guinea pig heart.

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## 降钙素基因相关肽在前列腺素介导豚鼠心脏缺血预适应中的作用[1]

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**关键词** 心肌再灌注损伤；前列腺素；降钙素基因相关肽；内皮素-1；心脏功能试验

**目的**：研究降钙素基因相关肽与前列腺素在豚鼠心脏缺血预适应中的相互作用. **方法**：采用 Langendorff 方法灌注豚鼠离体心脏. 记录心率、冠脉流量、左室内压以及最大变化速率，并测定冠脉流出液中降钙素基因相关肽(CGRP)与 6-酮-$PGF_{1\alpha}$的释放量. **结果**：内皮素-1 (200 pmoL)引起心功能下降，表现为冠脉流量、心率、左室内压及其最大变化速率降低. 缺血预适应可明显减轻内皮素-1 引起的心脏损伤，同时预适应期间 CGRP 与 6-酮-$PGF_{1\alpha}$的释放量明显增加. 应用辣椒素耗竭内源性 CGRP 后，缺血预适应的保护作用被取消. 选择性 $CGRP_1$ 受体拮抗剂 $CGRP_{8-37}$ 100 nmol/L 也能取消缺血预适应的保护作用. 环氧化酶抑制剂吲哚美辛(10 μmol/L)可取消缺血预适应的保护作用，同时缺血预适应促进 CGRP 与 6-酮-$PGF_{1\alpha}$释放的作用也被取消. **结论**：前列腺素参与了缺血预适应对豚鼠心脏的保护作用，前列腺素的作用是由 CGRP 所介导.

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