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Calcitonin gene-related peptide-induced preconditioning protects against ischemia-reperfusion injury in isolated rat hearts

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Abstract

Our previous work has suggested that the calcitonin gene-related peptide (CGRP) receptor antagonist CGRP-(8–37) can abolish the protective effect of ischemic preconditioning in the isolated rat heart. Therefore we tested the hypothesis that CGRP- or capsaicin-induced preconditioning protects against ischemia-reperfusion injury in the isolated perfused rat heart. Thirty minutes of global ischemia and 30 min of reperfusion caused a significant cardiac contractile dysfunction, ventricular arrhythmia, and an increased release of creatine phosphate kinase. Pretreatment with CGRP or capsaicin, which evokes release of CGRP from cardiac sensory nerves, for 5 min produced a significant improvement of cardiac function, a reduction in the incidence of ventricular arrhythmia, and a decrease in the release of creatine phosphate kinase. However, the cardioprotection provided by CGRP- or capsaicin-induced preconditioning was abolished by CGRP-(8–37) and ruthenium red, respectively. These findings suggest that CGRP- or capsaicin-induced preconditioning protects against ischemic myocardial injury. The present results also suggest that CGRP may be an endogenous myocardial protective substance in the rat.

Keywords: Heart, rat; Preconditioning; CGRP (calcitonin gene-related peptide); CGRP-(8–37); Capsaicin; Ruthenium red

1. Introduction

Ischemic preconditioning was first described by Murry and his colleagues in 1986 (Murry et al., 1986), and this phenomenon has been confirmed in various animals (Schott et al., 1990; Liu et al., 1991; Liu and Downey, 1992; Yao and Gross, 1994) and in humans (Alkhulaifi et al., 1994). The mechanism underlying preconditioning remains unknown. There is now an increasing amount of evidence which suggests the involvement of endogenous myocardial protective substances in ischemic preconditioning (Parratt, 1993, 1994). Calcitonin gene-related peptide (CGRP), a principal transmitter in capsaicin-sensitive sensory nerves, is present in the hearts of animals and humans (Wharton et al., 1986; Franco-Cereceda, 1988). It has been shown that CGRP possesses numerous physiological properties, several of which are thought to be beneficial to the ischemic myocardium (Ren et al., 1993; Li et al., 1995).

It has been shown that capsaicin, which evokes CGRP release from sensory nerves, protects against myocardial injury induced by ischemia-reperfusion in the isolated perfused rat heart (D'Alonzo et al., 1995). Recently, we showed that in the isolated rat heart, the CGRP receptor antagonist CGRP-(8–37) can abolish the cardioprotection of ischemic preconditioning (Xiao et al., 1996). These studies suggest that CGRP may play an important role in mediation of ischemic preconditioning. The present study was designed to further explore whether CGRP is an endogenous myocardial protective substance, and more specifically to answer the following questions: (1) does treatment with CGRP result in a preconditioning-like effect in the rat model of ischemia-reperfusion? and (2) does capsaicin, which evokes the release of CGRP, possess a similar beneficial effect?

2. Materials and methods

2.1. Isolated perfused heart

Male Sprague-Dawley rats weighing 200–250 g were anesthetized with ether. The hearts were rapidly excised and placed in cold Krebs-Henseleit buffer solution (4°C). Retrograde, nonrecirculating perfusion through the aorta was performed according to the modified Langendorff

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procedure (Srimani et al., 1990). The hearts were allowed to beat spontaneously and were perfused with Krebs-Henseleit buffer, saturated with 95% O_2 and 5% CO_2, at a constant perfusion pressure (100 cm H_2O). The Krebs-Henseleit buffer solution had the following composition (mM): 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 and its first derivative (LV dp/dt) were recorded by a polyphysiological recorder (Nihon Kohden). Coronary flow was measured and samples of coronary effluent after 5 min of reperfusion were collected for measurement of creatine phosphate kinase. An epicardial electrocardiogram recording (ECG) was made throughout the experiment, and ECG was analyzed for heart rate, as well as the incidence of ventricular fibrillation and ventricular tachycardia.

2.2. Creatine phosphate kinase measurement

The creatine phosphate kinase activity in the coronary effluent was measured spectrophotometrically. Supplies for creatine phosphate kinase assay were obtained from Baoding Chemical Co., Baoding, P.R. China.

2.3. Experimental protocols

The hearts were equilibrated for 10 min before each experiment was started. The experiment was divided into 8 groups. (1) The control group was perfused with Krebs-Henseleit buffer solution throughout the experiment. (2) The ischemia-reperfusion group experienced 30 min of global ischemia and 30 min of reperfusion. (3) The CGRP group received CGRP (5×10^{-9} M) for 5 min, followed by a 10-min wash-out period before the 30-min ischemia. (4) The CGRP-(8–37) group received CGRP-(8–37) (10^{-7} M) for 10 min. (5) The CGRP plus CGRP-(8–37) group received CGRP-(8–37), beginning 5 min prior to CGRP and continuing both CGRP-(8–37) and CGRP for 5 min, followed by a 10 min wash-out period before the 30-min ischemia. (6) The capsaicin group received capsaicin (3×10^{-6} M) for 5 min, followed by a 10 min wash-out period before the 30-min ischemia. (7) The ruthenium red group received ruthenium red (5×10^{-6} M) for 10 min. (8) The capsaicin plus ruthenium red group received ruthenium red (5×10^{-6} M) 5 min prior to capsaicin and then received both capsaicin and ruthenium red for 5 min, followed by a 10-min wash-out period before the 30-min ischemia.

2.4. Reagents

CGRP (rat), CGRP-(8–37) (human), capsaicin, and ruthenium red were purchased from Sigma. All drugs were dissolved in Krebs-Henseleit buffer solution, except capsaicin which was initially dissolved in ethanol and further diluted in Krebs-Henseleit buffer solution to proper final concentration. The creatine phosphate kinase assay kit was obtained from Baoding Chemical Co., Baoding, P.R. China.

2.5. Statistics

All values are expressed as means ± S.E.M. The significance of differences in cardiac function and creatine phosphate kinase was determined by analysis of variance and the Newman-Keuls test. The incidence of ventricular fibrillation and ventricular tachycardia was compared by two-tailed Fisher's exact probability test. The level of significance was chosen as $P < 0.05$.

3. Results

3.1. Effect of CGRP

In the control group, continuously perfused rat hearts were observed for 90 min. There were no changes in heart rate, coronary flow, left ventricular pressure, and LV dp/dt_{max} (Tables 1–4). Thirty minutes of global ischemia and 30 min of reperfusion caused a decrease in cardiac function (heart rate, coronary flow, left ventricular pressure, and LV dp/dt_{max}) (Tables 1–4). All hearts in the

Table 1
Effect of CGRP or capsaicin on the left ventricular pressure (kPa) during reperfusion

	n	Preischemia	Reperfusion (min)			
			5	10	20	30
Control	8	10.7 ± 0.1	10.6 ± 0.1	10.5 ± 0.1	10.6 ± 0.2	10.6 ± 0.3
CGRP-(8–37)	5	10.4 ± 0.5	10.6 ± 0.4	10.8 ± 0.3	11.0 ± 0.4	11.0 ± 0.5
Ruthenium red (RR)	5	10.6 ± 0.4	11.0 ± 0.3	10.9 ± 0.3	10.6 ± 0.6	10.6 ± 0.5
Ischemia-reperfusion	8	10.0 ± 0.3	4.6 ± 0.2 [a]	5.5 ± 0.3 [a]	6.6 ± 0.4 [a]	7.4 ± 0.3 [a]
+CGRP	7	11.4 ± 0.1	11.5 ± 0.2 [b]	11.5 ± 0.2 [b]	11.3 ± 0.2 [b]	11.2 ± 0.1 [b]
+CGRP + CGRP-(8–37)	5	10.3 ± 0.3	4.6 ± 0.7	6.0 ± 1.1	6.8 ± 1.2	7.3 ± 1.2
+Capsaicin	5	10.6 ± 0.1	11.0 ± 0.2 [b]	11.1 ± 0.2 [b]	11.1 ± 0.2 [b]	10.9 ± 0.3 [b]
+Capsaicin + RR	7	11.3 ± 0.2	5.8 ± 0.5	6.4 ± 0.5	7.0 ± 0.5	7.0 ± 0.5

[a] $P < 0.01$ as compared with control; [b] $P < 0.01$ as compared with ischemia-reperfusion. Values are means ± S.E.M.

ischemia-reperfusion group developed ventricular arrhythmia, both ventricular fibrillation and ventricular tachycardia (Table 5). Reperfusion after 30 min of ischemia also caused a significant increase in the release of creatine phosphate kinase (Fig. 1).

Pretreatment with CGRP (5×10^{-9} M) for 5 min caused a significant improvement of cardiac function (heart rate, coronary flow, left ventricular pressure, LV dp/dt_{max}) (Tables 1–4), a reduction in the incidence of ventricular arrhythmia (Table 5), and a decrease in the release of creatine phosphate kinase during reperfusion (Fig. 1). However, the protective effects of CGRP were abolished in the presence of CGRP-(8–37) (10^{-7} M), a selective CGRP receptor antagonist (Tables 1–5; Fig. 1). CGRP-(8–37) itself had no effect on cardiac function and creatine phosphate kinase release in the isolated rat heart.

3.2. Effect of capsaicin

To explore the beneficial effect of endogenous CGRP on the ischemic myocardium, capsaicin, which evokes the release of CGRP from cardiac sensory nerves, was used. Exposure to a brief infusion of capsaicin (3×10^{-6} M) prevented cardiac dysfunction (Tables 1–4), ventricular arrythmia (Table 5), and creatine phosphate kinase release during reperfusion. As has been shown previously, the

Table 2
Effect of CGRP or capsaicin on the maximal rate of left ventricular pressure rise (LV dp/dt_{max}, kPa · s $^{-1}$) during reperfusion

	n	Preischemia	Reperfusion (min)			
			5	10	20	30
Control	8	271 ± 8	268 ± 9	267 ± 9	255 ± 8	256 ± 8
CGRP-(8–37)	5	264 ± 9	266 ± 9	270 ± 7	270 ± 8	267 ± 9
Ruthenium red (RR)	5	263 ± 14	264 ± 9	265 ± 7	268 ± 9	267 ± 9
Ischemia-reperfusion	8	266 ± 5	93 ± 9 [a]	126 ± 10 [a]	169 ± 10 [a]	197 ± 5 [a]
+ CGRP	7	287 ± 4	304 ± 5 [b]	300 ± 5 [b]	295 ± 4 [b]	300 ± 5 [b]
+ CGRP + CGRP-(8–37)	5	265 ± 11	112 ± 10	160 ± 20	188 ± 16	205 ± 15
+ Capsaicin	8	265 ± 5	281 ± 12 [b]	292 ± 8 [b]	282 ± 6 [b]	284 ± 7 [b]
+ Capsaicin + RR	7	268 ± 4	123 ± 14	140 ± 14	159 ± 14	167 ± 13

[a] $P < 0.01$ as compared with control; [b] $P < 0.01$ as compared with ischemia-reperfusion.

Table 3
Effect of CGRP or capsaicin on the coronary flow (ml · min^{-1}) during reperfusion

	n	Preischemia	Reperfusion (min)			
			5	10	20	30
Control	8	13.5 ± 0.5	13.3 ± 0.5	13.1 ± 0.6	12.5 ± 0.7	12.4 ± 0.4
CGRP-(8–37)	5	12.6 ± 1.0	11.8 ± 0.7	12.0 ± 0.7	11.8 ± 0.8	11.7 ± 0.7
Ruthenium red (RR)	5	12.8 ± 1.2	11.8 ± 1.2	11.4 ± 0.8	11.3 ± 0.6	10.9 ± 0.8
Ischemia-reperfusion	8	12.6 ± 0.5	7.7 ± 0.6 [a]	7.5 ± 0.6 [a]	7.3 ± 0.6 [a]	7.1 ± 0.6 [a]
+ CGRP	7	12.9 ± 0.6	14.2 ± 0.4 [b]	14.9 ± 0.4 [b]	11.6 ± 0.5 [b]	10.3 ± 0.4 [b]
+ CGRP & CGRP-(8–37)	5	11.8 ± 0.9	9.8 ± 0.7	9.0 ± 0.6	8.6 ± 0.8	8.0 ± 0.9
+ Capsaicin	8	11.0 ± 0.3	14.4 ± 0.5 [b]	14.6 ± 0.5 [b]	11.7 ± 0.5 [b]	10.4 ± 0.4 [b]
+ Capsaicin + RR	7	11.3 ± 0.3	6.3 ± 0.6	6.1 ± 0.5	5.5 ± 0.6	5.4 ± 0.5

[a] $P < 0.01$ as compared with control; [b] $P < 0.01$ as compared with ischemia-reperfusion.

Table 4
Effect of CGRP or capsaicin on the heart rate (beats · min^{-1}) during reperfusion

	n	Preischemia	Reperfusion (min)			
			5	10	20	30
Control	8	310 ± 7	300 ± 6	298 ± 8	296 ± 9	295 ± 10
CGRP-(8–37)	5	305 ± 18	299 ± 13	300 ± 13	299 ± 14	299 ± 12
Ruthenium red (RR)	5	295 ± 14	297 ± 9	300 ± 8	307 ± 18	300 ± 15
Ischemia-reperfusion	8	300 ± 7	199 ± 13 [a]	223 ± 10 [a]	238 ± 5 [a]	243 ± 4 [a]
+ CGRP	7	319 ± 4	269 ± 14	309 ± 13 [b]	290 ± 7 [b]	293 ± 7 [b]
+ CGRP + CGRP-(8–37)	5	300 ± 11	211 ± 14	226 ± 21	248 ± 10	260 ± 13
+ Capsaicin	8	311 ± 7	274 ± 11 [b]	301 ± 11 [b]	279 ± 11 [b]	274 ± 9 [b]
+ Capsaicin + RR	7	323 ± 5	179 ± 7	211 ± 6	227 ± 8	248 ± 5

[a] $P < 0.01$ as compared with control; [b] $P < 0.01$ as compared with ischemia-reperfusion.

Table 5
Effect of CGRP or capsaicin on the incidence of ventricular fibrillation and ventricular tachycardia

	n	Ventricular fibrillation	Ventricular tachycardia
Control	8	0	0
CGRP-(8–37)	5	0	0
Ruthenium red (RR)	5	0	0
Ischemia-reperfusion	8	8 [a]	8 [a]
+CGRP	7	1 [b]	1 [b]
+CGRP&CGRP-(8–37)	5	5	5
+Capsaicin	8	1 [b]	1 [b]
+Capsaicin+RR	7	7	7

[a] $P < 0.01$ as compared with control; [b] $P < 0.01$ as compared with ischemia-reperfusion.

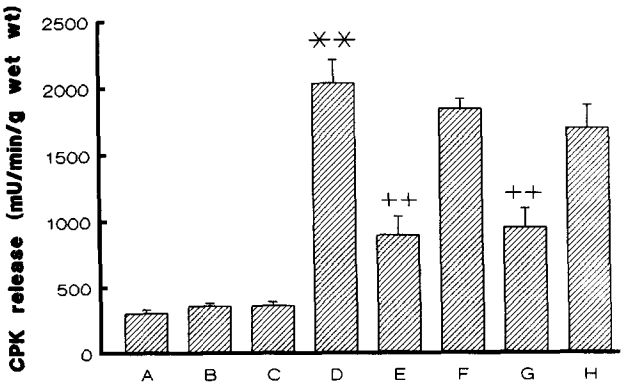

Fig. 1. Effect of CGRP or capsaicin on activity of creatine phosphate kinase during reperfusion. A: control; B: CGRP-(8–37); C: ruthenium red; D: ischemia-reperfusion; E: +CGRP; F: +CGRP in the presence of CGRP-(8–37); G: +capsaicin; H: +capsaicin in the presence of ruthenium red. Values are means±S.E.M. ($n = 5$–8) ** $P < 0.01$ as compared with control; ++ $P < 0.01$ as compared with ischemia-reperfusion.

cardiostimulatory actions of capsaicin are blocked by ruthenium red (Franco-Cereceda et al., 1991). In the present study, the protective effect of capsaicin-induced preconditioning was completely abolished in the presence of ruthenium red (5×10^{-6} M). However, treatment with ruthenium red alone had no effect on the cardiac function and creatine phosphate kinase release in the isolated rat heart (Tables 1–5; Fig. 1).

4. Discussion

This is the first study to demonstrate that exposure to a brief infusion of CGRP or capsaicin can result in a preconditioning-like protection in the isolated rat heart. These results support the hypothesis that CGRP may be an endogenous myocardial protective substance.

A consistent and convincing body of work has indicated that endogenous chemical mediators play a central role in ischemic preconditioning (Parratt, 1993, 1994). Several chemical mediators such as adenosine, catecholamine, bradykinin, prostaglandin(s), and nitric oxide have been suggested to participate in the mediation of preconditioning. However, adenosine-induced preconditioning or reversal of the effect of adenosine receptor antagonist on preconditioning has been shown in the hearts of the rabbit (Liu et al., 1991), pig (Schott et al., 1990), and dog (Yao and Gross, 1994), but not the rat (Cave et al., 1993). α-Adrenoceptor agonists can substitute for preconditioning ischemia in rabbit hearts (Bankwala et al., 1994), but pretreatment with reserpine to deplete endogenous catecholamines does not affect preconditioning-induced cardioprotection in the rat (Weselcouch et al., 1995a). The antiarrhythmic effects of ischemic preconditioning in anesthetized dogs are prevented by methylene blue, an inhibitor of guanylate cyclase (Vegh et al., 1992), but endogenous nitric oxide is not correlated with the beneficial effect of ischemic preconditioning in the rat heart (Weselcouch et al., 1995b). These investigations suggest that in the rat the mediators of ischemic preconditioning in the heart may differ from those of other species.

Our recent work showed that in the isolated rat heart, the CGRP receptor antagonist CGRP-(8–37) abolished preconditioning-induced protection, including the significant improvement of cardiac function, the reduction in the incidence of reperfusion ventricular arrhythmias, and the decrease in the release of creatine phosphate kinase during reperfusion (Xiao et al., 1996). We postulate that CGRP may be an endogenous myocardial protective substance. It has been documented that capsaicin-sensitive sensory nerves are present in the hearts of animals and humans, and that CGRP is a principal transmitter. The release of CGRP is regulated by multiple factors. Myocardial ischemia, even a brief ischemic period of 5 min, causes a significant increase in the release of CGRP in isolated guinea pig hearts (Franco-Cereceda, 1988; Franco-Cereceda et al., 1994), and the increase in release of CGRP from the heart has been shown to be related to low pH and stimulation of prostacyclin formation during ischemia (Franco-Cereceda et al., 1994).

According to the hypothesis proposed by Parratt (1993) about 'endogenous myocardial protective substances', pharmacological preconditioning induced by CGRP or capsaicin was observed in the isolated rat heart. In the present study, pretreatment with CGRP for 5 min produced a significant protective effect on the ischemic myocardium, as shown by the enhanced postischemic myocardial function, the reduced incidence of ventricular arrhythmia, and the attenuated release of creatine phosphate kinase. These results suggest that treatment with CGRP may provide preconditioning stimuli.

More recently, it has been shown that pretreatment with capsaicin reduces the incidence of ventricular tachycardia and ventricular fibrillation during ischemia or reperfusion in the isolated perfusion rat heart (D'Alonzo et al., 1995). It is of interest that capsaicin-induced preconditioning also protected the ischemic myocardium. The effect of cap-

saicin was abolished by ruthenium red which inhibits capsaicin-induced cardiostimulatory actions. These findings indicate that CGRP, either endogenous or exogenous, may cause preconditioning-like protection.

The mechanisms responsible for the beneficial effect of CGRP on the ischemic myocardium remain unclear. Early studies have shown that treatment with CGRP protects the ischemic myocardium in the rabbits and rat models of ischemia-reperfusion (Yu and Dong, 1993), and a similar beneficial effect is also seen in cultured myocytes (Ren et al., 1993). It has been proposed that the effect of CGRP on the myocardium may be due to a reduction of calcium overload and inhibition of lipid peroxidation.

A number of studies have suggested that myocardial protective substances, endogenous or exogenous, cause cardioprotection by activation of protein kinase C (Parratt, 1994). It has recently been shown that, in adult mammalian ventricular cardiomyocytes, CGRP increases the activation of the protein kinase C (Bell et al., 1995). We postulate that the cardioprotection of CGRP-induced preconditioning may be due to the activation of the protein kinase C pathway. However, further studies measuring the activity of protein kinase C and using selective inhibitors need to be done to establish this hypothesis for the cardioprotection of CGRP-induced preconditioning.

In summary, the results presented in this study suggest that (1) CGRP produces a preconditioning-like cardioprotection, and (2) CGRP may be an endogenous myocardial protective substance.

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References

Alkhulaifi, A.M., D.M. Yollon and W.B. Pugsley, 1994, Preconditioning the human heart during aorto-coronary bypass surgery, Eur. J. Cardiothorac. Surg. 8, 270.

Bankwala, Z., S.L. Hale and R.A. Kloner, 1994, α-Adrenoceptor stimulation with exogenous norepinephrine or release of endogenous catecholamines mimics ischemic preconditioning, Circulation 90, 1023.

Bell, D., K.D. Schlüter, X.J. Zhou, B.J. McDermott and H.M. Piper, 1995, Hypertropic effects of calcitonin gene-related peptide (CGRP) and amylin on adult mammalian ventricular cardiomyocytes, J. Mol. Cell. Cardiol. 27, 2433.

Cave, A.C., C.S. Collis, J.M. Downey and D.J. Hearse, 1993, Improved functional recovery by ischaemic preconditioning is not mediated by adenosine in the globally ischaemic isolated rat hearts, Cardiovasc. Res. 27, 663.

D'Alonzo, A.J., G.J. Grover, R.B. Darbenzio, T.A. Hess, P.G. Sleph, S. Dzwonczyk, J.L. Zhu and J.C. Sewter, 1995, In vitro effects of capsaicin: antiarrhythmic and antiischemic activity, Eur. J. Pharmcol. 273, 269.

Franco-Cereceda, A., 1988, Calcitonin gene-related peptide and tachykinins in relation to local sensory control of cardiac contractility and coronary vascular tone, Acta Physiol. Scand. 133 (Suppl. 596), 3.

Franco-Cereceda, A., Y.P. Lou and J.M. Lundberg, 1991, Ruthenium-red inhibits CGRP release by capsaicin and resiniferatoxin but not by ouabain, bradykinin or nicotine in guinea-pig heart: correlation with effects on cardiac contractility, Br. J. Pharmacol. 104, 305.

Franco-Cereceda, A., G. Källner and J.M. Lundberg, 1994, Cyclooxygenase products released by low pH have capsaicin-like actions on sensory nerves in the isolated guinea pig heart, Cardiovasc. Res. 28, 365.

Li, Y.J., Y.J. Li, C.F. Peng, N.S. Li, J.M. You and H.W. Deng, 1995, Calcitonin gene-related peptide protects against endothelial cell damage due to oxidised low-density lipoprotein, Med. Sci. Res. 23, 253.

Liu, Y. and J.M. Downey, 1992, Preconditioning protects against infarction in the rat heart, J. Mol. Cell. Cardiol. 24 (Suppl. III), S5.

Liu, G.S., J. Thornton, D.M. Van Winkle, A.W.H. Stanley, R.A. Olsson and J.M. Downey, 1991, Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart, Circulation 84, 350.

Murry, C.E., R.B. Jennings and K.A. Reimer, 1986, Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium, Circulation 74, 1124.

Parratt, J., 1993, Endogenous myocardial protective (antiarrhythmic) substances, Cardiovasc. Res. 27, 693.

Parratt, J.R., 1994, Protection of the heart by ischaemic preconditioning: mechanisms and possibilities for pharmacological exploitation, Trends Pharmacol. Sci. 15, 19.

Ren, Y.S., T.G. Ma, H.B. Wang and S.Q. Yu, 1993, Protective effects of calcitonin gene-related peptide (CGRP) on myocardial cell injury and calcium and magnesium contents following severe hypoxia and simulated reperfusion, Med. Sci. Res. 21, 177.

Schott R.J., S. Rohmann, E.R. Braun and W. Schaper, 1990, Ischemic preconditioning reduces infarct size in swine myocardium, Circ. Res. 66, 1133.

Srimani, B.N., R.M. Engelman, R. Jones and D.K. Das, 1990, Protective role of intracoronary fatty acid binding protein in ischemic and reperfused myocardium, Circ. Res. 66, 1535.

Vegh, A., J.G. Papp, L. Szekeres and J. Parratt, 1992, The local intracoronary administration of methylene blue prevents the pronounced antiarrhythmic effect of ischaemic preconditioning, Br. J. Pharmacol. 107, 910.

Weselcouch, E.O., A.J. Baird, P.G. Sleph, S. Dzwonczyk, H.N. Murry and G.J. Grover, 1995a, Endogenous catecholamines are not necessary for ischaemic preconditioning in the isolated perfused rat heart, Cardiovasc. Res. 29, 126.

Weselcouch, E.O., A.J. Baird, P. Sleph and G.J. Grover, 1995b, Inhibition of nitric oxide synthesis does not affect ischemic preconditioning in isolated perfused rat hearts. Am. J. Physiol. 268, H242.

Wharton, J., S. Gulbenkian, P.K. Mulderry, M.A. Ghatei, G.P. McGregor, S.R. Bloom and J.M. Polak, 1986, Capsaicin induces a depletion of calcitonin gene-related peptide (CGRP)-immunoreactive nerves in the cardiovascular system of the guinea pig and rat, J. Auton. Nerv. Syst. 16, 289.

Xiao, Z.S., Y.J. Li and H.W. Deng, 1996, Role of calcitonin gene-related peptide in ischemic preconditioning of isolated rat hearts, Acta Pharmacol. Sin. (in press).

Yao, Z.H. and G.J. Gross, 1994, A comparison of adenosine-induced cardioprotection and ischemic preconditioning in dogs: Efficacy, time course, and role of K_{ATP} channels, Circulation 89, 1229.

Yu, Y. and L.W. Dong, 1993, Effect of CGRP on experimental myocardial infarction, Chin. J. Pathophysiol. 9, 137.