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Inhibition of cardiac tumor necrosis factor-α production by calcitonin gene-related peptide-mediated ischemic preconditioning in isolated rat hearts

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Abstract

Previous investigations have demonstrated that calcitonin gene-related peptide (CGRP) plays an important role in the mediation of ischemic preconditioning in rats. In the present study, we examined signal transduction pathways of CGRP-mediated ischemic preconditioning. Thirty minutes of global ischemia and 40 min of reperfusion caused a dramatic decrease in myocardial function, and a significant increase in the release of cardiac creatine kinase in the coronary effluent and in the content of tumor necrosis factor-α (TNF-α) in myocardial tissues. However, ischemic preconditioning (three cycles of 5-min ischemia and 5-min reperfusion) or pretreatment with CGRP for 5 min dramatically improved the recovery of cardiac function, and reduced the release of cardiac creatine kinase and the TNF-α content. The effect of ischemic preconditioning was abolished by CGRP-(8-37), the selective CGRP receptor antagonist, and by capsaicin, which depletes sensory nerve neurotransmitter content, but was unaltered by treatment with glibenclamide, a blocker of the ATP-sensitive potassium (K_{ATP}) channel. The protective effects of exogenous CGRP-induced preconditioning were also not blocked by glibenclamide. These results suggest that the cardioprotective effects afforded by CGRP-mediated ischemic preconditioning are related to inhibition of cardiac TNF-α production, but not to activation of the K_{ATP} channel.

Keywords: Preconditioning; CGRP (calcitonin gene-related peptide); TNF-α (tumor necrosis factor-α); Glibenclamide; Heart, rat

1. Introduction

Brief periods of myocardial ischemia and reperfusion render the myocardium tolerant to subsequent sustained ischemia. This phenomenon, termed ischemic preconditioning (Murry et al., 1986), has been demonstrated in various animal species and in humans. The mechanism responsible for the protective effects of ischemic preconditioning is still incompletely understood. Evidence suggests that the protective effects of ischemic preconditioning are mediated by endogenous chemical substances including neurotransmitters and autacoids (Parratt, 1993). Calcitonin gene-related peptide (CGRP), the principal transmitter in capsaicin-sensitive sensory nerves, is widely distributed in cardiovascular tissues (Franco-Cereceda, 1988). Recently, we and others have shown that endogenous CGRP may play an important role in the mediation of ischemic preconditioning in rat hearts (Li et al., 1996; Ferdinandy et al., 1997), and that administration of exogenous CGRP can also induce preconditioning-like protection in rat hearts (Zhou et al., 1999).

It has been suggested that neurotransmitters and autacoids interact with G-protein-linked receptors, with subsequent activation of intracellular signal transduction, leading to the cardioprotection. The ATP-sensitive K^+ (K_{ATP}) channel is recognized as an end-effector in the signal transduction cascade of ischemic preconditioning (Gross, 1995). There is evidence that CGRP relaxes vascular smooth muscle and protects the gastric mucosa against ulcerogenic stimuli through the activation of the K_{ATP} channel (Nelson et al., 1990; Wellman et al., 1998; Doi et al., 1998). Therefore, we hypothesize that the cardioprotective effect of CGRP on the myocardium is also mediated by the activation of the K_{ATP} channel.

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Previous investigations have shown that tumor necrosis factor-α (TNF-α) may contribute to cardiac dysfunction and cardiomyocyte death in ischemia–reperfusion injury (Meldrum et al., 1998a; Gurevitch et al., 1997). Recently, the protective effects of ischemic preconditioning have been shown to be related to the inhibition of the production of myocardial TNF-α, and it has been postulated that TNF-α may be an ultimate effector of ischemic preconditioning (Meldrum et al., 1998b). It has been reported that endogenous CGRP inhibits TNF-α production in rat osteoblasts and vascular smooth muscle cells (Millet and Vignery, 1997; Li et al., 1997). It is not known, however, whether the cardioprotection provided by CGRP-mediated ischemic preconditioning also involves in the inhibition of TNF-α production.

Since the K_{ATP} channel is involved in the mediation of ischemic preconditioning and CGRP can activate the K_{ATP} channel, we examined whether the K_{ATP} channel is an important component of the coupling mechanism by which endogenous CGRP mediates the cardioprotection of ischemic preconditioning. Since myocardial injury due to ischemia–reperfusion is related to stimulation of TNF-α production and CGRP inhibits the generation of TNF-α, we explored whether the cardioprotective effects afforded by CGRP-mediated ischemic preconditioning are also related to the inhibition of myocardial TNF-α production.

2. Materials and methods

Animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication 85-23, revised 1985)

2.1. Perfusion of the isolated heart

Male Sprague–Dawley rats weighing 180–220 g were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). The hearts were excised rapidly and immersed in cold Krebs–Henseleit buffer solution (4°C). Each heart was attached to a Langendorff apparatus via the aorta for retrograde perfusion with Krebs–Henseleit buffer solution (mmol/l: NaCl, 1190; $NaHCO_3$, 25.0; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4.H_2O$, 1.2; $CaCl_2$, 2.5; and glucose, 11.0). The perfusate solution was equilibrated with 95% O_2 and 5% CO_2, maintained at 37°C and pH 7.4. Perfusion pressure was maintained at 80 cm H_2O.

A water-filled latex ballon connected to a pressure transducer was inserted into the left ventricle through the mitral valve (the left artiotomy), and the volume was adjusted to achieve a stable left ventricular end-diastolic pressure of 2–3 mm Hg during initial equilibration and this pressure was maintained throughout the experiments. Left ventricular pressure and heart rate were continuously monitored. The resulting electric signals were digitized by a MacLab analogue-to-digital converter and recorded by a Power Macintosh 7220 computer. Coronary flow was measured by timed collection of coronary effluent.

2.2. Experimental protocols

All hearts had an initial stabilization period of 15–20 min. The hearts were randomly divided into eight groups. The control group was perfused with Krebs–Henseleit solution throughout the experiment. The ischemia–reperfusion group experienced 30-min global ischemia and 40-min reperfusion. In the case of ischemic preconditioning, hearts were subjected to three cycles of 5-min global ischemia and 5-min reperfusion before 30-min ischemia and 40-min reperfusion. For CGRP-(8-37) and glibenclamide, hearts were perfused with CGRP-(8-37) (10^{-7} M) or glibenclamide (10^{-5} M) for 5 min, and then preconditioned with three cycles of 5-min global ischemia and 5-min reperfusion in the presence of CGRP-(8-37) or glibenclamide before 30-min ischemia and 40-min reperfusion. For the studies on the effect of capsaicin on the protective effects of ischemic preconditioning, rats were pretreated with capsaicin (50 mg/kg, s.c.) 4 days before the experiment (Song et al., 1999). In the case of CGRP-induced preconditioning, hearts were perfused with exogenous CGRP (5×10^{-9} M) for 5 min, followed by a 10-min wash-out period before 30-min ischemia and 40-min reperfusion. For studies on the effect of glibenclamide on the protective effects of CGRP-induced preconditioning, hearts were perfused with glibenclamide (10^{-5} M) (Asemu et al., 1999; Matsuda et al., 1999) for 5 min, and then perfused with glibenclamide and CGRP (5×10^{-9} M) together for 5 min, followed by a 10-min wash-out period before 30-min ischemia and 40-min reperfusion.

2.3. Measurement of creatine kinase

Myocardial injury was monitored by assaying creatine kinase. The activity of creatine kinase in the coronary effluent from the heart at 5 min of reperfusion was assayed spectrophotometrically (Linz et al., 1986).

2.4. Measurement of myocardial TNF-α

At 40 min of reperfusion, the left ventricular myocardium of every heart was washed with cold saline, and then excised and added to 10 volumes of cold isotonic homogenization buffer (mmol/l: phenylmethyl sulfonyl fluoride, 1.0; KH_2PO_4, 13.4 and K_2HPO_4, 86.6; pH 7.6). The individual tissue samples were homogenized with a tissue homogenizer and then centrifugated at 3000 rpm for 15 min at 4°C. The supernatant was stored at −70°C until assay.

The cardiac homogenate TNF-α content was determined by radioimmunoassay kits using antisera raised against rat TNF-α, ^{125}I-labelled TNF-α and rat TNF-α standard.

Table 1
Effect of ischemic preconditioning or CGRP treatment on cardiac function

		Reperfusion (min)					
	n	Pre-ischemia	5	10	20	30	40
Left ventricular pressure (mm Hg)							
Control	6	73.1 ± 3.2	75.1 ± 3.0	71.9 ± 3.9	72.7 ± 3.9	71.7 ± 3.9	72.5 ± 3.4
I/R	8	76.5 ± 4.0	11.0 ± 2.9[b]	17.6 ± 5.2[b]	22.6 ± 4.6[b]	37.4 ± 2.5[b]	40.2 ± 3.8[b]
+PC	7	79.5 ± 6.8	42.0 ± 5.0[d]	78.5 ± 3.9[d]	83.0 ± 7.5[d]	80.9 ± 7.1[d]	80.4 ± 4.5[d]
+PC and Glibenclamide	7	75.8 ± 3.3	32.0 ± 4.9	59.7 ± 4.7	66.6 ± 5.3	71.3 ± 5.8	72.2 ± 4.4
+PC and $CGRP_{8-37}$	6	84.8 ± 5.3	19.3 ± 6.4[f]	22.1 ± 7.5[f]	32.3 ± 6.8[f]	45.8 ± 6.9[f]	46.8 ± 5.4[f]
+PC and Capsaicin	7	84.1 ± 3.9	12.3 ± 4.5[f]	18.2 ± 3.9[f]	28.7 ± 4.1[f]	41.6 ± 4.3[f]	44.5 ± 3.6[f]
+CGRP	6	82.1 ± 4.0	37.3 ± 5.1[d]	54.4 ± 3.1[d]	73.1 ± 1.5[d]	72.2 ± 2.6[d]	73.2 ± 3.2[d]
+CGRP and Glibenclamide	6	83.2 ± 5.2	36.2 ± 3.2	58.2 ± 3.4	65.2 ± 3.2	70.4 ± 4.2	72.5 ± 4.2
$+dp/dt_{max}$ *(mm Hg / s)*							
Control	6	2488 ± 130	2465 ± 132	2510 ± 160	2501 ± 155	2485 ± 160	2526 ± 132
I/R	8	2546 ± 184	360 ± 85[b]	563 ± 139[b]	563 ± 139[b]	977 ± 66[b]	1022 ± 66[b]
+PC	7	2530 ± 195	1456 ± 163[d]	2484 ± 241[d]	2631 ± 225[d]	2641 ± 244[d]	2588 ± 212[d]
+PC and Glibenclamide	7	2713 ± 151	1246 ± 183	2185 ± 176	2360 ± 143	2404 ± 154	2412 ± 136
+PC and $CGRP_{8-37}$	6	2748 ± 169	543 ± 181[f]	602 ± 204[f]	877 ± 196[f]	1263 ± 179[f]	1265 ± 178[f]
+PC and Capsaicin	7	2563 ± 70	333 ± 98[f]	491 ± 69[f]	679 ± 63[f]	1050 ± 112[f]	1124 ± 102[f]
+CGRP	6	2904 ± 79	772 ± 96[c]	1354 ± 183[d]	1981 ± 75[d]	2107 ± 50[d]	2289 ± 88[d]
+CGRP and Glibenclamide	6	2847 ± 111	945 ± 110	1417 ± 172	1780 ± 131	2237 ± 107	2298 ± 114
$-dp/dt_{max}$ *(mm Hg / s)*							
Control	6	2031 ± 208	1858 ± 155	1908 ± 190	1899 ± 175	1891 ± 189	1978 ± 168
I/R	8	1961 ± 188	237 ± 50[b]	418 ± 67[b]	487 ± 69[b]	779 ± 98[b]	842 ± 56[b]
+PC	7	1922 ± 218	1292 ± 192[d]	1904 ± 185[d]	2001 ± 187[d]	2080 ± 218[d]	2022 ± 205[d]
+PC and Glibenclamide	7	2045 ± 128	935 ± 177	1472 ± 142	1574 ± 150	1690 ± 187	1788 ± 136
+PC and $CGRP_{8-37}$	6	2230 ± 183	438 ± 51[f]	498 ± 68[f]	763 ± 157[f]	1017 ± 145[f]	1103 ± 121[f]
+PC and Capsaicin	7	2129 ± 60	294 ± 85[f]	432 ± 68[f]	638 ± 62[f]	1052 ± 153[f]	1099 ± 78[f]
+CGRP	6	2195 ± 72	816 ± 112[d]	1198 ± 40[d]	1598 ± 48[d]	1769 ± 45[d]	1885 ± 88[d]
+CGRP and Glibenclamide	6	2158 ± 52	742 ± 68	1083 ± 51	1477 ± 53	1662 ± 48	1806 ± 79
Heart rate (beats / min)							
Control	6	297 ± 6	287 ± 8	295 ± 8	297 ± 6	299 ± 8	289 ± 7
I/R	8	299 ± 14	136 ± 8[b]	171 ± 21[b]	202 ± 19[b]	213 ± 16[a]	245 ± 18
+PC	7	323 ± 13	214 ± 16[d]	276 ± 26[d]	282 ± 15[c]	288 ± 12[c]	298 ± 14
+PC and Glibenclamide	7	320 ± 15	198 ± 24	262 ± 23	265 ± 24	272 ± 20	289 ± 15
+PC and $CGRP_{8-37}$	6	320 ± 13	137 ± 13[f]	186 ± 16[e]	196 ± 16[e]	248 ± 20	268 ± 19
+PC and Capsaicin	7	285 ± 4	133 ± 9[f]	184 ± 16[e]	205 ± 15[e]	254 ± 17	260 ± 18
+CGRP	6	312 ± 12	156 ± 20	254 ± 11[c]	279 ± 12[c]	288 ± 10[c]	297 ± 15
+CGRP and Glibenclamide	6	298 ± 9	144 ± 16	243 ± 7	272 ± 7	278 ± 7	287 ± 16
Coronary flow (ml / min)							
Control	6	10.3 ± 0.5	10.4 ± 0.5	10.4 ± 0.4	10.3 ± 0.4	9.8 ± 0.4	10.0 ± 0.5
I/R	8	10.2 ± 0.5	4.5 ± 0.4[b]	4.8 ± 0.4[b]	5.0 ± 0.3[b]	5.8 ± 0.4[b]	6.2 ± 0.4[b]
+PC	7	9.5 ± 0.5	6.8 ± 0.6[d]	8.3 ± 0.6[d]	8.8 ± 0.6[d]	9.0 ± 0.5[d]	9.5 ± 0.5[d]
+PC and Glibenclamide	7	9.5 ± 0.6	6.2 ± 0.4	7.5 ± 0.5	8.0 ± 0.5	8.0 ± 0.6	9.0 ± 0.4
+PC and $CGRP_{8-37}$	6	10.9 ± 0.5	5.0 ± 0.6[e]	5.2 ± 0.6[f]	5.7 ± 0.7[f]	6.0 ± 0.8[f]	6.1 ± 0.6[f]
+PC and Capsaicin	7	9.2 ± 0.7	3.9 ± 0.2[f]	4.2 ± 0.3[f]	4.5 ± 0.2[f]	4.9 ± 0.3[f]	5.2 ± 0.4[f]
+CGRP	6	10.3 ± 0.5	6.8 ± 0.6[c]	8.2 ± 0.6[d]	8.9 ± 0.6[d]	9.1 ± 0.5[d]	9.4 ± 0.4[d]
+CGRP and Glibenclamide	6	10.5 ± 0.6	6.9 ± 0.4	7.8 ± 0.3	8.4 ± 0.5	8.8 ± 0.5	9.0 ± 0.5

Values are means ± S.E.M., $n = 6–8$. I/R: ischemia–reperfusion; PC: preconditioning.
[a] $P < 0.05$ vs. control.
[b] $P < 0.01$ vs. control.
[c] $P < 0.05$ vs. I/R.
[d] $P < 0.01$ vs I/R.
[e] $P < 0.05$ vs. PC.
[f] $P < 0.01$ vs. PC.

2.5. Reagents

CGRP, CGRP-(8-37), capsaicin and dimethyl sulfoxide (DMSO) were purchased from Sigma. Glibenclamide was supplied from Research Biochemical International. Capsaicin was dissolved in a vehicle containing 10% Tween80, 10% ethanol and 80% saline. Glibenclamide was dissolved in 0.1% DMSO. Supplies for the creatine kinase assay were obtained from Beijing Zhongshen High-tech Bioengineering, Beijing, P. R. China. Radioimmunoassay kits for measurement of TNF-α were obtained from Dongya Immunity Technology Institution, Beijing, P.R. China.

2.6. Statistics

All values are expressed as means ± S.E.M. Statistical analysis was carried out by analysis of variance and the Newman–Keuls test. The level of significance was chosen as $P < 0.05$.

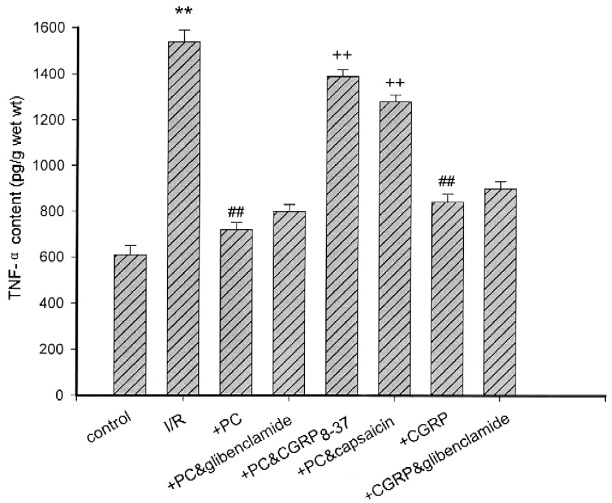

Fig. 2. Effect of ischemic preconditioning or CGRP treatment on the content of cardic TNF-α. I/R: ischemia–reperfusion; PC: preconditioning. Values are means ± S.E.M., $n = 6–8$. $^{**}P < 0.01$ vs. control; $^{\#\#}P < 0.01$ vs. I/R; $^{++}P < 0.01$ vs. PC.

3. Results

3.1. Cardiac function and creatine kinase release

30-min global ischemia and 40-min reperfusion caused a dramatic decrease in myocardial function (left ventricular pressure, dp/dt_{max}, heart rate and coronary flow). Ischemic preconditioning by three cycles of 5-min ischemia and 5-min reperfusion or preconditioning induced by exogenous CGRP enhanced the improvement of cardiac function. However, the protective effects afforded by ischemic preconditioning were abolished by CGRP-(8-37), the selective CGRP receptor antagonist, or by pretreatment with capsaicin, which depletes the neurotransmitter content in sensory nerves (Table 1).

Ischemia–reperfusion caused a significant increase in the release of creatine kinase. Ischemic preconditioning or CGRP-induced preconditioning dramatically reduced the release of creatine kinase, an effect which was also abolised by CGRP-(8-37) or capsaicin treatment (Fig. 1).

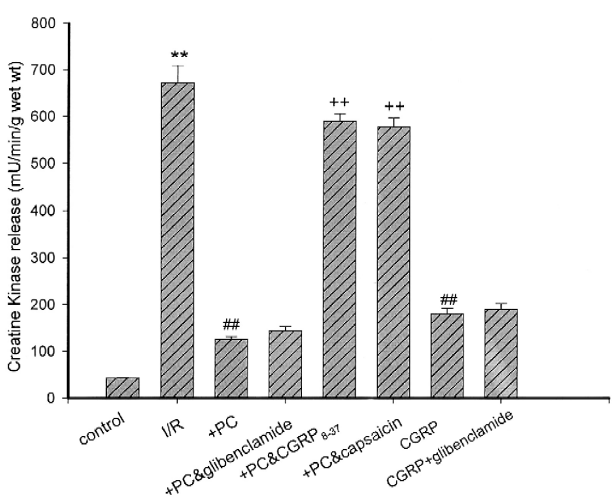

Fig. 1. Effect of ischemic preconditioning or CGRP treatment on creatine kinase release. I/R: ischemia–reperfusion; PC: preconditioning. Values are means ± S.E.M., $n = 6–8$. $^{**}P < 0.01$ vs. control; $^{\#\#}P < 0.01$ vs. I/R; $^{++}P < 0.01$ vs. PC.

3.2. Effect of glibenclamide on the protective effect of ischemic preconditioning

To examine the possible contribution of K_{ATP} opening by the preconditioned myocardium, glibenclamide, a blocker of the K_{ATP} channel, was used. The cardioprotection afforded by ischemic preconditioning or CGRP-induced preconditioning was unaltered in the presence of glibenclamide.

3.3. The myocardial TNF-α content

As shown in Fig. 2, ischemia–reperfusion caused a profound increase in the content of cardiac TNF-α. Ischemic preconditioning or CGRP-induced preconditioning significantly decreased the elevated level of cardiac TNF-α during ischemia–reperfusion. However, the effect of ischemic preconditioning was abolished by capsaicin or CGRP-(8-37) treatment, as shown by the reappearance of the elevated level of TNF-α elicited by ischemia–reperfusion. The inhibition of TNF-α production by ischemic preconditioning or CGRP-induced preconditioning was unaltered in the presence of glibenclamide.

4. Discussion

The results of this study further confirm the hypothesis that, the cardioprotective effects afforded by ischemic preconditioning are mediated by endogenous CGRP in rat hearts because the protective effects of ischemic preconditioning were abolished by CGRP-(8-37), a selective CGRP receptor antagonist, and by capsaicin which depletes the CGRP content in sensory nerves, prior to the experiment (Ferdinandy et al., 1997). Furthermore, administration of exogenous CGRP could also induce preconditioning. The main findings of the present investigation include: (1) the cardioprotection afforded by CGRP-mediated ischemic preconditioning is not related to the activation of K_{ATP} channels in rat hearts, and (2) CGRP may mediate the protective effect of ischemic preconditioning through the inhibition of myocardial TNF-α production.

It has been suggested that the K_{ATP} channel may be an end-effector in the signal transduction cascade of ischemic preconditioning. K_{ATP} channel blockers, such as glibenclamide or sodium 5-hydroxydecanoate, abolish ischemic preconditioning in large animal models such as the dog and pig, and K_{ATP} channel openers such as bimakalim mimic ischemic preconditioning, or lower the threshhold for induction of preconditioning (Schulz et al., 1994; Gross and Auchampach, 1992; Auchampach et al., 1992). However, the evidence for involvement of K_{ATP} channels in ischemic preconditioning is controversial in smaller animal species such as the rabbit (Thornton et al., 1993) and rat (Grover et al., 1993; Lu et al., 1993; Yake et al., 1995). As has been reported previously in the present study, the cardioprotection afforded by ischemic preconditioning or CGRP-induced preconditioning was unaltered in the presence of glibenclamide in rat hearts. This finding, as well as that of others, suggests that the cardioprotective effect of ischemic preconditioning is not related to the activation of K_{ATP} channels in the rat. As mentioned above, CGRP may be an endogenous myocardial protective substance in ischemic preconditioning. The present results also suggest that the cardioprotective effect of CGRP-mediated ischemic preconditioning is not due to the activation of the K_{ATP} channel.

TNF-α, a cytokine, is found in several tissues including cardiovascular tissues. It has been shown that the heart is a rich source of TNF-α (Giroir et al., 1992), and that the heart produces as much TNF-α per gram of tissue as either the liver or the spleen (Kapadia et al., 1995). TNF-α exerts complex pathophysiological effects. TNF-α, besides inducing the production of other active substances, causes calcium dyshomeostasis, cell apoptosis and direct cytotoxicity. Cardiac TNF-α is implicated as a mediator of cardiovascular disease such as acute myocardial infarction, chronic heart failure and atherosclerosis (Meldrum, 1998). Previous investigations have shown that ischemia–reperfusion causes an increase in the content of TNF-α in the heart. Recently, it has been reported that the protection provided by ischemic preconditioning against ischemia–reperfusion is related to the inhibition of TNF-α production, and it has been postulated that TNF-α may be an ultimate effector in signal transduction pathways of ischemic preconditioning (Meldrum et al., 1998b; Belosjorow et al., 1999). A similar phenomenon has been observed in the liver (Peralta et al., 1999), renal (Meldrum and Donnahoo, 1999) and skeletal muscle (Papanastasious et al., 1999). Based on the involvement of CGRP in the mediation of ischemic preconditioning, in the present study, we tested the effect of ischemic preconditioning on the content of cardiac TNF-α in the presence and absence of CGRP-(8-37) or capsaicin, as well as the effect of CGRP-induced preconditioning on the content of cardiac TNF-α. The present results were consistent with previous observations that ischemia–reperfusion causes a significant increase in the content of TNF-α (Meldrum et al., 1998a). The elevated level of TNF-α induced by ischemia–reperfusion was diminished by ischemic preconditioning or CGRP-induced preconditioning. However, the inhibition of cardiac TNF-α production by ischemic preconditioning was abolished by CGRP-(8-37) or capsaicin. These findings suggest that the protective effects of CGRP-mediated ischemic preconditioning are due to the inhibition of cardiac TNF-α production in rat hearts.

The mechanism responsible for the inhibition of TNF-α production by CGRP remains unclear. There is evidence to suggest that the generation of reactive oxygen metabolites during ischemia–reperfusion activates protein kinases which stimulate TNF-α production (Neumann et al., 1995). Previous studies have shown that CGRP decreases lipid peroxidation (Zhang et al., 1994). Thus, we hypothesize that CGRP inhibits the generation of TNF-α via a decrease in lipid peroxidation. However, the exact mechanism of the inhibition of TNF-α production by CGRP awaits further investigation.

In summary, the present results suggest that the cardioprotective effects afforded by CGRP-mediated ischemic preconditioning are due to inhibition of cardiac TNF-α production and not to activation of the K_{ATP} channel in the isolated rat heart.

Acknowledgements

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