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Involvement of calcitonin gene-related peptide in nitroglycerin induced improvement of preservation with cardioplegic solution[1]

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KEY WORDS nitroglycerin; calcitonin gene-related peptide; glyburide; myocardial reperfusion injury; heart function tests

ABSTRACT

AIM: To study improvement of preservation with cardioplegic solution induced by nitroglycerin was related to stimulation of calcitonin gene-related peptide (CGRP) release. **METHODS**: The isolated rat heart was arrested using St Thomas Hospital cardioplegic solution and then was reperfused with normothermic Krebs-Henseleit solution for 40 min after the 4-h hypothermic ischemic period. Heart rate, coronary flow, left ventricular pressure (LVP), and its first derivative ($\pm dp/dt_{max}$) were recorded, and the calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) and the release of creatine kinase (CK) were measured. **RESULTS**: Nitroglycerin (0.1 or 1 μmol/L) or CGRP (5 or 10 nmol/L) caused an improvement of cardiac function (LVP and $\pm dp/dt_{max}$) and a decrease in the release of creatine kinase during reperfusion. The protection induced by nitroglycerin was abolished by $CGRP_{8-37}$, the selective CGRP receptor antagonist, or pretreatment with capsaicin to deplete sensory nerves neurotransmitter content, but was unaltered by treatment with glibenclamide, the blocker of the ATP-sensitive potassium channel (K_{ATP}). The protection induced by exogenous CGRP was not also blocked by glibenclamide. Levels of CGRP-LI in the coronary effluent were significantly increased in the hearts treated with nitroglycerin. However, the elevated level of CGRP-LI by nitroglycerin was abolished by pretreatment with capsaicin. **CONCLUSION**: The improvement of preservation with cardioplegic solution induced by nitroglycerin was related to stimulation of CGRP release in the rat heart, and the effect is not related to the activation of the K_{ATP} channel.

INTRODUCTION

Nitroglycerin is a vasodilator currently used in the treatment of angina pectoris. It has been shown that the addition of nitroglycerin to the cardioplegic solution is also capable of enhancing protective effects on the ischemic myocardium[1].

Calcitonin gene-related peptide (CGRP), a principal transmitter in capsaicin-sensitive sensory nerves, is widely distributed in cardiovascular tissues[2]. CGRP has been shown to possess several properties that have been proposed to be responsible for its protection of the ischemic myocardium, including an increase in coronary blood flow, a reduction in lipid peroxides, and a direct protection of myocardial and endothelial cells[3,4].

Since previous investigations have shown that nitroglycerin can evoke the release of CGRP from vascular tissues and the heart[5,6], in the present study, we examined whether improvement of preservation with cardioplegic solution induced by nitroglycerin was related to stimulation of CGRP release.

MATERIALS AND METHODS

Reagents Capsaicin, $CGRP_{8-37}$, and dimethyl sulphoxide (Me_2SO) were purchased from Sigma Chemical Co (St Louis, USA). Nitroglycerin was purchased from Beijing Yiming Pharmaceutical Factory, China. Glibenclamide was supplied by Research Biochemicals International, Natick, USA. Capsaicin was dissolved in a vehicle containing 10 % Tween 80, 10 % ethanol, and 80 % saline. Glibenclamide was dissolved in Me_2SO which was further diluted in Krebs solution to the final concentration 0.1 %. Supplies for the creatine kinase assay were obtained from Beijing Zhongsheng High-tech

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Bioengineering Co, China. Radioimmunoassay kits for measurement of CGRP were obtained from Dongya Immunity Technology Institution, Beijing, China.

Preparation of the isolated heart Male Sprague-Dawley rats (Laboratory Animal Center, Hunan Medical University, Grade II, Certificate № 20-011) weighing 250 ~ 300 g were anesthetized by intraperitoneal administration of 60 mg/kg sodium pentobarbital. The hearts were excised rapidly and immersed in cold Krebs-Henseleit buffer solution (4 ℃). The heart was attached to a Langendorff apparatus via the aorta for retrograde perfusion with Krebs-Henseleit buffer solution (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). The perfusate was equilibrated with 95 % O_2 and 5 % CO_2, maintained at 37 ℃ and pH 7.4. Perfusion pressure was maintained at 90 cm H_2O.

A water-filled latex balloon connected to a pressure transducer was inserted into the left ventricle via the mitral valve. Left ventricular pressure (LVP), the first derivative of left ventricular pressure ($\pm dp/dt_{max}$), and heart rate (HR) were continuously monitored. Coronary flow (CF) was measured by timed collection of coronary effluent.

Creatine kinase assay The activity of creatine kinase (CK) in the coronary effluent from the heart at 5 min of reperfusion was assayed spectrophotometrically.

Radioimmunoassay Perfusate fraction (5 min) was collected during reperfusion, and acetic acid (final concentration 0.2 mol/L) was added. The samples were desalted using sep-pak C_{18} cartridge, lyophilized and stored at -20 ℃ until assay. The perfusate fraction samples were redissolved in appropriate buffer. CGRP-like immunoreactivity (CGRP-LI) of perfusate fraction was measured using antisera raised against rat CGRP, ^{125}I-labelled CGRP, and rat CGRP standard.

Experimental protocols All hearts had an initial stabilization period for 20 min, and received a 2-min infusion of St Thomas Hospital solution (4 ℃, 10 mL). The St Thomas Hospital solution had the following composition (in mmol/L): NaCl 110, KCl 16, $MgCl_2$ 16, $CaCl_2$ 1.2, $NaHCO_3$ 10. Hearts of all groups were immersed in respective cardioplegic solution maintained at 4 ℃ for 4 h, and then were reperfused with Krebs-Henseleit solution for 40 min (37 ℃).

The experiment was randomly divided into 13 groups. In control group, the cardioplegic solution used was the St Thomas Hospital solution. For nitroglycerin, nitroglycerin at the concentration of 0.1, 1, or 10 μmol/L was added to the St Thomas Hospital solution. For the studies on the effect of capsaicin on the protection afforded by nitroglycerin, preparations were exposed to nitroglycerin (1 μmol/L) after pretreatment with capsaicin. Capsaicin (50 mg/kg) or vehicle was administrated by sc injection 4 d before the experiment. For the studies on the effect of $CGRP_{8-37}$ or glibenclamide on the protection afforded by nitroglycerin, hearts were treated with nitroglycerin (1 μmol/L) in the presence of $CGRP_{8-37}$ (0.1 μmol/L) or glibenclamide (10 μmol/L). In the case of cardioprotection afforded by CGRP, exogenous CGRP (5 or 10 nmol/L) was added to cardioplegic solution. For the studies on the effect of glibenclamide on the protection induced by CGRP, hearts were treated with CGRP (10 nmol/L) in the presence of glibenclamide (10 μmol/L).

Statistics Data were expressed as $\bar{x} \pm s$. Statistical analysis was carried out by analysis of variance and the Newman-Keuls test. The level of significance was chosen as $P < 0.05$.

RESULTS

The basic values of cardiac function are listed in Tab 1 – 5. There were no significant differences in LVP, $\pm dp/dt_{max}$, HR, and CF in all groups. After hypothermic ischemia for 4 h, a decline in cardiac function (LVP and $\pm dp/dt_{max}$) and CF and an increase in the release of creatine kinase were shown during reperfusion. Nitroglycerin at the concentration of 0.1 μmol/L only caused a slight improvement of preservation with cardioplegic solution. Nitroglycerin at the concentration of 1 μmol/L significantly improved the recovery of cardiac function (LVP and $\pm dp/dt_{max}$) and reduced the release of creatine kinase during reperfusion after hypothermic ischemia. Nitroglycerin at the concentration of 10 μmol/L only reduced the release of creatine kinase, but had no effect on reperfusion-induced cardiac dysfunction (Tab 1 – 4, Tab 6).

To explore the possible contribution of endogenous CGRP, capsaicin and $CGRP_{8-37}$ were used. After pretreatment with capsaicin to deplete transmitters in sensory nerves or $CGRP_{8-37}$, a selective CGRP receptor antagonist, was added to the cardioplegic solution, the cardioprotective effects of nitroglycerin were observed to be abolished (Tab 1 – 4, Tab 6).

Levels of CGRP-LI in the coronary effluent were

Tab 1. Effect of nitroglycerin on left ventricular pressure (kPa) during reperfusion after a 4-h hypothermic ischemic period. STH: St Thomas Hospital solution. $\bar{x} \pm s$. [a]$P > 0.05$, [b]$P < 0.05$, [c]$P < 0.01$ *vs* **STH solution.** [d]$P > 0.05$, [f]$P < 0.01$ *vs* **NTG 1 μmol/L.** [g]$P > 0.05$ *vs* **CGRP 10 nmol/L.**

	n	Baseline	Reperfusion/min 10	20	30	40
STH solution	7	15.0±3.3	6.5±1.6	7.0±1.33	7.7±0.8	7.3±1.1
+ Me_2SO	6	13.4±2.4	8.1±1.2[a]	7.4±2.1[a]	7.7±2.0[a]	7.0±2.1[a]
+ Glibenclamide (GLI, 10 μmol/L)	6	14.6±3.3	7.4±1.3[a]	8.1±1.7[a]	7.0±1.7[a]	6.1±2.4[a]
+ Nitroglycerin (NTG, 0.1 μmol/L)	7	14.5±2.4	10.4±2.8[a]	10.1±1.7[a]	9.8±2.7[a]	9.7±2.8[a]
+ Nitroglycerin (1 μmol/L)	8	15.3±3.1	11.0±2.5[c]	12.0±2.4[c]	12.8±2.4[c]	12.1±2.0[c]
+ Nitroglycerin (10 μmol/L)	6	13.3±3.1	8.1±2.0[a]	7.8±2.4[a]	7.3±1.2[a]	7.4±1.1[a]
+ NTG 1 μmol/L & Vehicle	6	13.7±2.5	9.2±2.0[d]	11.4±1.9[d]	11.3±2.7[d]	10.8±2.7[d]
+ NTG 1 μmol/L & Capsaicin	8	13.3±3.2	7.7±2.4[d]	7.3±2.7[f]	7.3±2.7[f]	6.7±2.5[f]
+ NTG 1 μmol/L & $CGRP_{8-37}$	8	15.7±4.1	8.1±1.9[d]	8.0±1.3[f]	7.3±1.1[f]	6.8±0.8[f]
+ NTG 1 μmol/L & GLI	6	15.8±2.7	11.6±1.7[d]	12.1±1.2[d]	12.0±1.2[d]	11.8±0.8[d]
+ CGRP 5 nmol/L	6	15.8±1.9	10.5±3.5[a]	9.7±2.5[a]	9.3±2.7[a]	9.1±2.7[a]
+ CGRP 10 nmol/L	6	16.5±3.5	11.3±1.7[c]	11.7±1.3[c]	11.3±1.6[b]	11.3±1.7[c]
+ CGRP 10 nmol/L & GLI	6	15.2±2.1	0.5±1.2[g]	11.3±1.1[g]	10.9±0.5[g]	10.5±0.7[g]

Tab 2. Effect of nitroglycerin on $+dp/dt_{max}$ **(kPa·s^{-1}) during reperfusion after a 4-h hypothermic ischemic period. STH: St Thomas Hospital solution.** $\bar{x} \pm s$. [a]$P > 0.05$, [b]$P < 0.05$, [c]$P < 0.01$ *vs* **STH solution.** [d]$P > 0.05$, [f]$P < 0.01$ *vs* **NTG 1 μmol/L.** [g]$P > 0.05$ *vs* **CGRP 10 nmol/L.**

	n	Baseline	Reperfusion/min 10	20	30	40
STH solution	7	397±88	167±58	193±35	213±34	210±27
+ Me_2SO	6	390±67	241±30[a]	234±52[a]	268±51[a]	224±61[a]
+ Glibenclamide (GLI, 10 μmol/L)	6	389±100	182±33[a]	223±39[a]	195±48[a]	173±64[a]
+ Nitroglycerin (NTG, 0.1 μmol/L)	7	477±104	286±86[b]	324±64[c]	319±76[a]	321±75[b]
+ Nitroglycerin (1 μmol/L)	8	446±76	292±62[b]	346±72[c]	373±76[c]	359±45[c]
+ Nitroglycerin (10 μmol/L)	6	464±80	278±54[a]	285±60[a]	275±29[a]	282±47[a]
+ NTG 1 μmol/L & Vehicle	6	431±85	268±49[d]	368±61[d]	362±74[d]	348±72[d]
+ NTG 1 μmol/L & Capsaicin	8	414±121	205±103[d]	237±88[f]	240±93[f]	222±87[f]
+ NTG 1 μmol/L & $CGRP_{8-37}$	8	420±75	206±45[d]	227±33[f]	214±47[f]	200±44[f]
+ NTG 1 μmol/L & GLI	6	501±68	315±51[d]	373±48[d]	374±44[d]	369±41[d]
+ CGRP 5 nmol/L	6	488±51	290±97[b]	314±90[c]	308±91[a]	303±93[a]
+ CGRP 10 nmol/L	6	466±92	314±46[c]	340±39[c]	334±43[b]	333±41[b]
+ CGRP 10 nmol/L & GLI	6	471±70	280±16[g]	339±19[g]	347±25[g]	340±20[g]

significantly increased in the hearts treated with nitroglycerin. However, the elevated level of CGRP-LI by nitroglycerin was abolished by pretreatment with capsaicin (Tab 7).

CGRP at the concentration of 5 nmol/L only caused a slight improvement of preservation with cardioplegic solution. CGRP at the concentration of 10 nmol/L significantly improved the recovery of cardiac function (LVP and $\pm dp/dt_{max}$) and reduced the release of creatine kinase during reperfusion after hypothermic ischemia (Tab 1 -4, Tab 6).

To test whether K_{ATP} channel was involved in the protective effects of nitroglycerin, glibenclamide was used. The cardioprotective effects of nitroglycerin were not abolished in the presence of glibenclamide. The cardioprotection afforded by CGRP was also not affected by glibenclamide (Tab 1 - 4, Tab 6).

DISCUSSION

In the present study, the addition of nitroglycerin to cardioplegic solution significantly improved recovery of cardiac function and decreased the release of creatine kinase during reperfusion after 4 h of cold ischemia in rat

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Tab 3. Effect of nitroglycerin on $-dp/dt_{max}$ ($kPa \cdot s^{-1}$) during reperfusion after a 4-h hypothermic ischemic period. STH: St Thomas Hospital solution. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* STH solution. $^dP > 0.05$, $^fP < 0.01$ *vs* NTG 1 μmol/L. $^gP > 0.05$ *vs* CGRP 10 nmol/L.

	n	Baseline	Reperfusion/min 10	20	30	40
STH solution	7	328 ± 61	117 ± 41	136 ± 30	147 ± 19	141 ± 21
+ DMSO	6	311 ± 60	149 ± 21[a]	144 ± 39[a]	148 ± 34[a]	136 ± 41[a]
+ Glibenclamide (GLI, 10 μmol/L)	6	280 ± 77	128 ± 26[a]	154 ± 37[a]	132 ± 36[a]	117 ± 48[a]
+ Nitroglycerin (NTG, 0.1 μmol/L)	7	313 ± 63	177 ± 67[a]	196 ± 40[a]	188 ± 49[a]	184 ± 53[a]
+ Nitroglycerin (1 μmol/L)	8	309 ± 45	198 ± 42[b]	234 ± 55[c]	251 ± 48[c]	236 ± 40[c]
+ Nitroglycerin (10 μmol/L)	6	290 ± 53	158 ± 43[a]	151 ± 43[a]	144 ± 19[a]	146 ± 19[a]
+ NTG 1 μmol/L & Vehicle	6	313 ± 71	175 ± 45[d]	258 ± 49[d]	252 ± 55[d]	239 ± 54[d]
+ NTG 1 μmol/L & Capsaicin	8	323 ± 86	136 ± 41[d]	142 ± 57[f]	144 ± 61[f]	130 ± 53[f]
+ NTG 1 μmol/L & $CGRP_{8-37}$	8	337 ± 63	152 ± 36[d]	166 ± 29[d]	147 ± 22[f]	137 ± 25[f]
+ NTG 1 μmol/L & GLI	6	387 ± 69	249 ± 45[d]	290 ± 44[d]	282 ± 38[d]	273 ± 29[d]
+ CGRP 5 nmol/L	6	305 ± 36	174 ± 57[a]	183 ± 52[a]	178 ± 53[a]	173 ± 55[a]
+ CGRP 10 nmol/L	6	330 ± 70	209 ± 41[b]	228 ± 42[b]	223 ± 35[a]	219 ± 38[b]
+ CGRP 10 nmol/L & GLI	6	298 ± 40	173 ± 17[g]	220 ± 18[g]	219 ± 19[g]	209 ± 10[g]

Tab 4. Effect of nitroglycerin on coronary flow ($mL \cdot min^{-1}$) during reperfusion after a 4-h hypothermic ischemic period. STH: St Thomas Hospital solution. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* STH solution. $^dP > 0.05$, $^fP < 0.01$ *vs* NTG 1 μmol/L. $^gP > 0.05$ *vs* CGRP 10 nmol/L.

	n	Baseline	Reperfusion/min 10	20	30	40
STH solution	7	12.7 ± 1.1	9.3 ± 2.2	7.7 ± 1.2	7.1 ± 1.1	6.9 ± 1.0
+ DMSO	6	12.6 ± 2.5	11.2 ± 2.4[a]	9.0 ± 1.7[a]	7.9 ± 1.0[a]	7.3 ± 1.7[a]
+ Glibenclamide (GLI, 10 μmol/L)	6	12.1 ± 2.5	10.6 ± 3.1[a]	9.9 ± 3.6[a]	9.3 ± 3.1[a]	8.4 ± 3.4[a]
+ Nitroglycerin (NTG, 0.1 μmol/L)	7	13.4 ± 2.2	11.8 ± 2.9[a]	10.5 ± 3.3[a]	9.6 ± 3.8[a]	9.0 ± 3.8[a]
+ Nitroglycerin (1 μmol/L)	8	13.0 ± 1.3	15.7 ± 2.2[b]	14.0 ± 2.2[c]	13.8 ± 2.2[c]	13.3 ± 1.8[a]
+ Nitroglycerin (10 μmol/L)	6	12.1 ± 2.3	12.1 ± 2.9[a]	8.3 ± 1.7[a]	7.3 ± 1.3[a]	6.8 ± 1.2[a]
+ NTG 1 μmol/L & Vehicle	6	12.4 ± 1.9	15.9 ± 1.0[d]	13.3 ± 0.3[d]	13.1 ± 0.6[d]	12.7 ± 0.2[d]
+ NTG 1 μmol/L & Capsaicin	8	13.6 ± 2.4	10.3 ± 3.2[d]	7.3 ± 2.2[f]	6.7 ± 2.2[f]	5.9 ± 2.1[d]
+ NTG 1 μmol/L & $CGRP_{8-37}$	8	12.4 ± 1.4	10.0 ± 2.1[d]	8.2 ± 1.8[e]	7.3 ± 1.7[f]	6.5 ± 1.6[d]
+ NTG 1 μmol/L & GLI	6	14.7 ± 2.4	15.4 ± 3.8[d]	14.1 ± 2.7[d]	12.5 ± 2.3[d]	11.5 ± 2.2[d]
+ CGRP 5 nmol/L	6	14.5 ± 2.9	14.1 ± 3.4[a]	12.4 ± 2.4[a]	11.3 ± 2.7[a]	10.7 ± 2.7[a]
+ CGRP 10 nmol/L	6	12.5 ± 1.7	17.6 ± 5.6[c]	15.9 ± 4.6[c]	14.6 ± 5.4[c]	13.7 ± 5.7[b]
+ CGRP 10 nmol/L & GLI	6	13.2 ± 3.1	19.2 ± 6.0[g]	18.4 ± 6.2[g]	17.3 ± 6.8[g]	16.3 ± 7.0[g]

hearts. Potentiation by nitroglycerin of preservation with cardioplgic solution is in agreement with previous observations in the rat model of ischemia-reperfusion[7] as well as in the neonatal lamb models of ischemia-reperfusion[1].

Nitroglycerin is one of the most useful drugs in therapy of acute ischemic coronary syndromes. The vasodilatory response to nitroglycerin is ascribed to the release of nitric oxide, which stimulates guanylyl cyclase and increases cyclic GMP levels[8].

It has been reported that nitroglycerin activates sensory nerves fibres to release CGRP from vascular tissues in both the central nervous system and the periphery[5,6]. Our recent work has shown that the cardioprotection of nitroglycerin-induced preconditioning is also related to stimulation of CGRP release[9]. In the present study, the addition of nitroglycerin to cardioplegic solution significantly attenuated ischemia-induced myocardial damages concomitantly with an increase in the content of CGRP-LI in the coronary effluent. The protection afforded by nitroglycerin was abolished by pretreatment with capsaicin, which depletes the transmitters content of sensory nerves, or $CGRP_{8-37}$, a selective CGRP receptor antagonist. Pretreatment with capsaicin also abolished the elevated

Tab 5. Effect of nitroglycerin on heart rate (beats·min^{-1}) during reperfusion after a 4-h hypothermic ischemic period. STH: St Thomas Hospital solution. $\bar{x} \pm s$.

	n	Baseline	Reperfusion/min 10	20	30	40
STH solution	7	275 ± 22	249 ± 56	256 ± 28	246 ± 26	240 ± 22
+ Me_2SO	6	266 ± 29	291 ± 62	290 ± 33	278 ± 36	284 ± 35
+ Glibenclamide (GLI, 10 μmol/L)	6	285 ± 42	215 ± 54	248 ± 54	241 ± 66	242 ± 78
+ Nitroglycerin (NTG, 0.1 μmol/L)	7	275 ± 27	209 ± 66	241 ± 24	240 ± 24	248 ± 17
+ Nitroglycerin (1 μmol/L)	8	265 ± 43	238 ± 65	254 ± 43	252 ± 33	246 ± 39
+ Nitroglycerin (10 μmol/L)	6	264 ± 25	284 ± 37	258 ± 34	239 ± 24	237 ± 23
+ NTG 1 μmol/L & Vehicle	6	310 ± 39	301 ± 39	289 ± 40	273 ± 41	287 ± 38
+ NTG 1 μmol/L & Capsaicin	8	288 ± 39	281 ± 65	285 ± 63	278 ± 65	276 ± 66
+ NTG 1 μmol/L & $CGRP_{8-37}$	8	287 ± 22	270 ± 39	281 ± 31	281 ± 34	270 ± 35
+ NTG 1 μmol/L & GLI	6	293 ± 26	255 ± 64	266 ± 48	258 ± 47	256 ± 47
+ CGRP 5 nmol/L	6	276 ± 28	259 ± 76	302 ± 32	294 ± 28	291 ± 27
+ CGRP 10 nmol/L	6	288 ± 14	302 ± 41	293 ± 29	292 ± 26	290 ± 28
+ CGRP 10 nmol/L & GLI	6	295 ± 25	259 ± 36	271 ± 41	282 ± 36	281 ± 34

Tab 6. Effects of nitroglycerin on the activity of CK in coronary effluent. $\bar{x} \pm s$. $^cP < 0.01$ *vs* Baseline. $^aP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* STH solution. $^gP > 0.05$, $^hP < 0.05$, $^iP < 0.01$ *vs* NTG 1 μmol/L. .

	n	CK/u·min^{-1}·g^{-1} wet wt
Baseline	7	0.11 ± 0.05
STH solution	7	3.04 ± 0.54^c
+ Me_2SO	6	3.02 ± 0.82^d
+ Glibenclamide (GLI, 10 μmol/L)	6	3.17 ± 0.81^d
+ Nitroglycerin (NTG, 0.1 μmol/L)	7	1.70 ± 0.63^f
+ Nitroglycerin (1 μmol/L)	8	1.00 ± 0.43^f
+ Nitroglycerin (10 μmol/L)	6	1.84 ± 0.81^e
+ NTG 1 μmol/L & Vehicle	6	1.16 ± 0.44^g
+ NTG 1 μmol/L & Capsaicin	8	2.58 ± 0.51^i
+ NTG 1 μmol/L & $CGRP_{8-37}$	8	2.22 ± 0.49^h

Tab 7. Effect of nitroglycerin on the CGRP-LI level in the coronary effluent. $\bar{x} \pm s$. $^aP > 0.05$ *vs* Baseline. $^fP < 0.01$ *vs* STH solution. $^iP < 0.01$ *vs* NTG 1 μmol/L.

	n	CGRP/pg·fraction^{-1}
Baseline	7	219 ± 29
STH solution	7	173 ± 13^a
+ Nitroglycerin (NTG 0.1 μmol/L)	7	336 ± 93^f
+ Nitroglycerin (1 μmol/L)	8	385 ± 92^f
+ Nitroglycerin (10 μmol/L)	6	350 ± 75^f
+ NTG 1 μmol/L & Capsaicin	8	188 ± 29^i

release of CGRP induced by nitroglycerin. These results support the hypothesis that improvement of preservation with cardioplegic solution induced by nitroglycerin is due to the stimulation of CGRP release.

CGRP, a predominant neurotransmitter in capsaicin-sensitive sensory nerves, is present in cardiovascular tissues. Studies have shown that the release of CGRP from cardiac sensory nerves is regulated by multiple factors and both heat and ischemia cause a significant increase in its release[10,11]. Myocardial ischemia, even a brief ischemia of 5 min, causes a significant increase of CGRP release in the isolated guinea-pig[2] and rat heart[11]. The cardioprotection afforded by brief, remote organ ischemia, such as in the small intestine, is at least partially related to the activation of capsaicin-sensitive sensory nerves[12]. Acute application of capsaicin to stimulate the release of CGRP significantly attenuates myocardial injury due to ischemia-reperfusion[13]. Pretreatment with capsaicin to deplete neurotransmitters in sensory nerves aggravates myocardial infarction in the porcine heart[14]. Studies in clinics have shown that the plasma concentration of CGRP is markedly elevated in patients with acute myocardial infarction[15] and the cardioprotection of ischemic preconditioning is also associated with the release of CGRP[16]. It has been postulated that the elevated level of CGRP during ischemia probably constitutes a compensatory response[14]. Exogenous administration of CGRP has been shown to attenuate the incidence of reperfusion-induced ventricular arrhythmias[13] and to protect the cultured myocytes against hypoxia injury[3] and the endothelial cell against ischemia-reperfusion injury[17]. Recently, CGRP has been shown to enhance preservation with cardioplegic solution in the rat heart[18]. These findings suggest that CGRP, endogenous or exogenous, possesses a protective effect on the ischemic myocardial

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and endothelial cells.

The mechanisms underlying CGRP effect have not yet been elucidated. Endogenous mediators including neurotransmitters bind to specific receptors and then activate the endogenous protective mechanisms via complex signal pathways which are related to the activation of protein kinase or K_{ATP} channels[19]. There is evidence to suggest that pretreatment with K_{ATP} channel openers produce myocardial protection during global ischemia with cardioplegic solution, and is also able to mimic ischemic preconditioning[20-22]. K_{ATP} channel blockers have been observed to abolish ischemic preconditioning in large animal models such as the dog and pig[21,22]. It has been demonstrated that CGRP increases the activation of K_{ATP} channels. Using the patch-clamp technique, extracellular application of CGRP activated K_{ATP} channels in cultured smooth muscle cells[23]. However, the evidence for involvement of K_{ATP} channel in such protective mechanisms is controversial in smaller animal species such as the rabbit and rat[24,25]. In the present study, the cardioprotection afforded by nitroglycerin was unaltered in the presence of glibenclamide in rat hearts. The protection by exogenous CGRP was also not affected by glibenclamide. These results suggest that the cardioprotection of CGRP-mediated nitroglycerin is not due to the activation of the K_{ATP} channel in the rat heart.

In summary, the present study suggests that improvement of preservation with cardioplegic solution induced by nitroglycerin is related to stimulation of CGRP release in the rat heart, and the effect is not due to the activation of K_{ATP} channels.

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降钙素基因相关肽介导硝酸甘油增强心停搏液的保护作用[1]

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关键词 硝酸甘油;降钙素基因相关肽;格列苯脲;心肌再灌注损伤;心脏功能实验

目的:研究硝酸甘油增强心停搏液的保护作用与促进降钙素基因相关肽释放的关系. **方法**: 在 St Thomas Hospital 心停搏液条件下, 离体心脏低温缺血 4 h 后再灌 40 min, 记录心率、冠脉流量及心功能, 并测定灌注液中降钙素基因相关肽(CGRP)的浓度及肌酸激酶(CK)的释放量. **结果**: 硝酸甘油(0.1 或 1 μmol/L)改善心功能,降低 CK 释放,同时促进 CGRP 的释放. CGRP(5 或 10 nmol/L)也改善心功能及降低 CK 释放. 预先用辣椒素耗竭感觉神经递质后. 硝酸甘油的心肌保护和升高灌注液中 CGRP 浓度作用消失. 选择性 CGRP 受体拮抗剂 $CGRP_{8-37}$也能取消硝酸甘油的心肌保护作用. 格列苯脲对硝酸甘油和 CGRP 的心保护作用均无影响. **结论**: 硝酸甘油增强心停搏液的保护作用是通过内源性 CGRP 所介导,其保护作用与 ATP 敏感的钾通道无关.

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