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Protective effects of nitroglycerin-induced preconditioning mediated by calcitonin gene-related peptide in rat small intestine

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Abstract

Previous studies of myocardium have shown that ischemic preconditioning could be mimicked by nitroglycerin through stimulating the release of calcitonin gene-related peptide (CGRP). The present study examined whether nitroglycerin could also provide a preconditioning stimulus in the peripheral vascular bed (the anse intestinalis of rat), and whether endogenous CGRP is involved in this process. The model of in situ perfusion was prepared with rat small intestine. One hour of ischemia and 15 min of reperfusion caused a significant impairment of intestinal morphology and an increase in the release of both lactate dehydrogenase and malondialdehyde. Pretreatment with nitroglycerin, 10^{-7}, 3×10^{-7}, 10^{-6} M for 5 min produced a significant improvement of intestinal tissue morphology and a decrease in the release of both lactate dehydrogenase and malondialdehyde. However, the protection afforded by nitroglycerin was abolished by CGRP-(8-37), a selective CGRP acceptor antagonist. Pretreatment with capsaicin, which specifically depletes the transmitter content of sensory nerves, also abolished the protection by nitroglycerin. In addition, the content of CGRP-like immunoreactivity in the effluent was increased during nitroglycerin perfusion. On the other hand, the results from the in vivo experiment showed that nitroglycerin (i.v. 0.13 mg/kg) injected 5 min before prolonged ischemia could provide significant protection against the injury caused by 30-min ischemia and 1-h reperfusion in the rat small intestine, but would also cause a significant increase in the levels of CGRP in the plasma. All these findings suggest that nitroglycerin-induced preconditioning is related to stimulation of CGRP release in the rat small intestine.

Keywords: Nitroglycerin; Preconditioning; CGRP (calcitonin gene-related peptide); CGRP-(8-37); Capsaicin; Small intestine

1. Introduction

Ischemic preconditioning has been extensively studied in the heart. There is much recent evidence that this phenomenon is a common one (Liu et al., 1996; Liauw et al., 1996; Loke and Woodman, 1996). Both our and Liu et al.'s (1996) work proved that ischemic preconditioning also occurs in the rat small intestine (Yang et al., 1999). Research on small intestinal ischemic preconditioning both shows its importance in the protection against the mucosal and microvascular dysfunction associated with intestinal ischemia–reperfusion, and throws light on the mechanism responsible for the protective effects of ischemic preconditioning. In addition, if we can explore some new drugs related to this mechanism that induce a protective effect similar to that of ischemic preconditioning, it will have further clinical significance.

Drug preconditioning is defined as substituting drug for the ischemia to induce the preconditioning effect. Many drugs have been shown to mimic the protective roles through stimulating the release of some endogenous, active substance. Calcitonin gene-related peptide (CGRP) is one of the important neurotransmitter in capsaicin-sensitive sensory nerves. Recently, it has been found that some drugs, which evoke the release of CGRP, could induce ischemic preconditioning in the heart, while the antagonist or depletion can abolish the drug's protective effects (Xiao et al., 1996; Li et al., 1996). Sensory nerves are widely distributed in the gastrointestinal tract. Our earlier work provided preliminary that CGRP mediates the protective effect of ischemic preconditioning in the rat small intestine (Yang et al., 2000). Nitroglycerin is one of the most useful antiangina drugs. There is new evidence to suggest that

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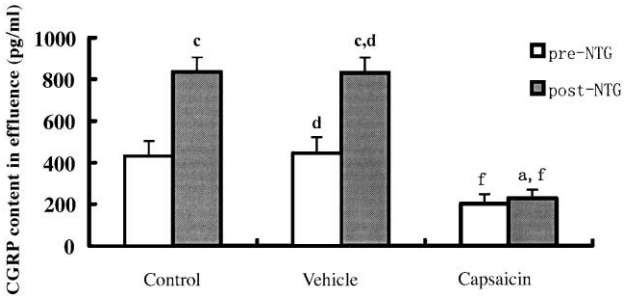

Fig. 1. Effect of nitroglycerin on the release of calcitonin gene-related peptide. Control: preparations were exposed to nitroglycerin (10^{-7} M) for 5 min. Capsaicin or vehicle was given s.c. injection 4 days before perfusion with nitroglycerin. Means ± S.E.M. ($N = 6–7$) $^{a}P > 0.05$, $^{c}P < 0.01$ vs. pre-NTG; $^{d}P > 0.05$, $^{f}P < 0.01$ vs. control.

nitroglycerin can induce heart early or delayed protection. There is a report that nitroglycerin can stimulate the release of CGRP (Booth et al., 1997). More recently, it was reported that nitroglycerin could imitate ischemic preconditioning through stimulating calcitonin gene-related peptide (CGRP) release in the myocardium (Hu et al., 1999). We now examined whether nitroglycerin can also induce the protective effects of preconditioning in the rat small intestine (Fig. 1).

2. Materials and methods

2.1. In situ perfusion of the rat small intestine

Male wistar rats weighing 220–260 g were fasted for 24 h before the experiment with access to water only. The animals were anesthetized with urethane (1 g/kg, i.p.). After a midline laparotomy, heparin sodium (600 U/kg, i.v.) was given, then superior mesenteric artery and super mesenteric vein were exposed and mounted onto a cannula attached to a perfusion apparatus (Yeo et al., 1989). At a constant perfusion pressure of 85 cm H_2O, the small intestine was perfused with Krebs–Henseleit (K–H) buffer saturated with 95% O_2 and 5% CO_2 (37 °C, pH 7.4). The buffer had the following composition (mmol/l): NaCl 118; KCl 4.7; KH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 25; $CaCl_2$ 2.5; D-glucose 5.5; and gelation 10 g/l^{-1}. After the perfusion was started, the rat was killed by exsanguination from the abdominal aorta.

2.2. Surgical procedure for in vivo experiment

After laparotomy, the super mesenteric artery was exposed. In the mean time, systemic arterial pressure was measured with an arterial pressure recorder connected to a carotid artery cannula so as to monitor the condition of the animal. The animal was allowed to stabilize for 15 min before the study was continued, and the super mesenteric artery was occluded when needed.

2.3. Lactate dehydrogenase, protein measurement

Lactate dehydrogenase, a kind of cytoplasmic enzyme, was measured to monitor the tissue lesion. The protein level in the effluent was tested to monitor microvascular permeability. Lactate dehydrogenase activity in serum or in the effluent of superior mesenteric vein was measured spectrophotometrically. The spectrophotometric enzyme assay was performed with a special assay kit. Measurement of enzyme activity was based on the oxidation of lactate and the rate of increase in absorbance at 340 nm. The activity of lactate dehydrogenase was expressed as units per liter serum or effluent. The protein content in the effluent was measured with a method based on the reaction of brilliant blue G with protein in an acid-alcohol medium to form a blue-colored protein dye complex. The color measured at 595 nm is proportional to the protein concentration.

2.4. Morphologic observation and malondialdehyde measurement

At the end of the reperfusion, small intestine tissue was obtained approximately 15 cm from the ileocecal junction. The tissue was sectioned into three segments. (1) Biopsy specimens were rapidly placed in 10% formalin buffer (pH 7.4). For the perfusion experiment, the degree of intestinal injury was graded on a scale, from 0 to VIII (Chiu et al., 1970; Haglind et al., 1980). In in vivo experiment, the hemorrhagic degree of the mucosa was evaluated (Li et al., 1989). (2) About 1-g sample was used to measure the wet weight/dry weight ratio so as to monitor intestine edema. (3) Remaining tissue was used to assay malondialdehyde levels. These tissues were homogenized in 10-vol. saline solution. After centrifugation, the supernatants were used to measure malondialdehyde contents with the thiobarbituric acid method. The results are expressed as nmol per milligram protein. The extent of lipid peroxidation was determined by measuring the malondialdehyde concentration in the intestine.

2.5. Myeloperoxidase activity measurement

Myeloperoxidase activity was measured, especially for the small intestine tissue from the in vivo experiment. Intestine tissue samples were homogenized in 0.5% hexadecyltrimethyl ammonium bromide (HTAB), dissolved in 50 mM potassium phosphate buffer pH 6. Homogenates were centrifuged for 30 min at 12,000 × *g* and 4 °C. The supernatants were decanted and added to 0.167 mg/ml *O*-dianisidine dihydrochloride and 0.0005% H_2O_2 in 50 mM phosphate buffer pH 6. The change in absorbance was

Table 1
Lactate dehydrogenase release, protein level, content of malondialdehyde, and intestinal WW/DW in different groups

Group	N	Dose (mol/l)	Intestinal tissue		Effluent	
			WW/DW (mol/l)	MDA (nmol/mg pro wt)	LDH (U/l)	PR(g/l)
CON	6		4.39 ± 0.17	0.26 ± 0.02	10.88 ± 0.84	80.96 ± 3.19
I/R	7		6.68 ± 0.14^{c}	0.96 ± 0.07^{c}	121.86 ± 5.24^{c}	103.35 ± 3.14^{c}
PC	6		$5.43 \pm 0.16^{c,f}$	$0.40 \pm 0.03^{b,f}$	$70.93 \pm 4.19^{c,f}$	$87.84 \pm 3.79^{a,f}$
NTG I	6	10^{-7}	$5.02 \pm 0.06^{c,f}$	$0.40 \pm 0.04^{b,f}$	$48.57 \pm 5.16^{c,f}$	$80.98 \pm 5.51^{a,f}$
NTG II	7	3×10^{-7}	$5.53 \pm 0.15^{c,f}$	$0.38 \pm 0.02^{b,f}$	$64.63 \pm 4.96^{c,f}$	$81.80 \pm 3.49^{a,f}$
NTG III	6	10^{-6}	$5.23 \pm 0.18^{c,f}$	$0.43 \pm 0.06^{b,f}$	$65.25 \pm 5.46^{c,f}$	$86.56 \pm 2.05^{a,f}$
CGRP	7		$4.45 \pm 0.18^{a,f,i}$	$0.25 \pm 0.03^{a,f,h}$	$41.04 \pm 3.41^{c,f,g}$	$80.02 \pm 3.74^{a,f,g}$
CGRP-(8-37) + NTG	6		$5.72 \pm 0.17^{c,f,i}$	$0.55 \pm 0.06^{c,f,h}$	$108.70 \pm 6.85^{c,d,i}$	$98.90 \pm 3.92^{c,d,I}$
Vehicle + NTG	6		$4.96 \pm 0.09^{b,f,g}$	$0.35 \pm 0.04^{a,f,g}$	$57.69 \pm 6.79^{c,f,g}$	$80.18 \pm 3.40^{a,f,g}$
Capsaicin + NTG	6		$5.75 \pm 0.14^{c,f,i}$	$0.72 \pm 0.05^{c,f,i}$	$110.61 \pm 8.48^{c,d,i}$	$97.42 \pm 1.99^{c,d,I}$

CON: control group, I/R: ischemia and reperfusion group, PC: preconditioning group, NTG: nitroglycerin-treated group, CGRP: CGRP-treated group, CGRP-(8-37) + NTG: CGRP-(8-37)-treated group, Capsaicin + NTG: pretreatment with capsaicin group, Vehicle + NTG: pretreatment with vehicle group. Means ± S.E.M. $^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ vs. control; $^{d}P > 0.05$, $^{f}P < 0.01$ vs. I/R; $^{g}P > 0.05$, $^{h}P < 0.05$, $^{i}P < 0.01$ vs. NTG.

measured with a spectrophotometer at 460 nm. One unit of myeloperoxidase activity is defined as that quantity of enzyme degrading 1 μM of peroxide per minute at 25 °C. Myeloperoxidase activity was used as an index of polymorphonuclear neutrophil accumulation in the tissues since it correlates closely with the number of neutrophils in small intestine tissues.

2.6. Radioimmunoassay

After 5-min of nitroglycerin perfusion, a sample was collected from the effluent of the superior mesenteric vein for measurement of CGRP release. As for the in vivo experiment, CGRP in the plasma was measured at the end of the experiment. CGRP-like immunoreactivity was assayed with a radioimmunoassay kit with antisera raised against rat CGRP, ^{125}I-labelled CGRP and rat CGRP standard.

2.7. Experimental protocol

2.7.1. In situ perfusion experiment

All intestines except those from the control group were allowed an initial 10-min stabilization period before being subjected to 60-min ischemia and 15-min reperfusion.

Six groups of animals were studied to evaluate the small intestine protective effects of pretreatment with nitroglycerin. The control group was perfused with K–H buffer solution throughout the experiment. The ischemia–reperfusion group underwent 1-h ischemia and 15-min reperfusion. The preconditioning group was subjected to three 5-min cycles of ischemia and reperfusion before the prolonged 60-min occlusion. For the nitroglycerin-treated groups, the intestines were perfused with nitroglycerin at concentrations of 10^{-7}, 3×10^{-7}or 10^{-6} M for 5 min and then washed out for 10 min with nitroglycerin-free K–H buffer solution before the 60-min ischemia period.

Table 2
Grade of intestinal mucosal damage in different groups

Group	Grade of mucosal damage (number of animals)									Mean grade
	0	I	II	III	IV	V	VI	VII	VIII	
CON	5	1	0	0	0	0	0	0	0	0.17
I/R	0	0	0	0	0	0	0	5	2	7.29^{c}
PC	0	0	2	2	2	0	0	0	0	$3.00^{a,d}$
NTG I	0	2	3	1	0	0	0	0	0	$1.33^{a,e}$
NTG II	0	1	3	2	1	0	0	0	0	$2.43^{a,d}$
NTG III	0	0	1	4	1	0	0	0	0	$3.00^{a,d}$
CGRP	0	0	2	3	2	0	0	0	0	$3.43^{a,d}$
CGRP-(8-37) + NTG	0	0	0	0	0	3	2	1	0	$5.67^{b,d}$
Vehicle + NTG	0	1	3	2	0	0	0	0	0	$2.17^{a,d}$
Capsaicin + NTG	0	0	0	0	0	2	2	2	1	$6.28^{b,d}$

CON: control group, I/R: ischemia and reperfusion group, PC: preconditioning group, NTG: nitroglycerin-treated group, CGRP: CGRP-treated group, CGRP-(8-37) + NTG: CGRP-(8-37)-treated group, Capsaicin + NTG: pretreatment with capsaicin group, Vehicle + NTG: pretreatment with vehicle group ($N = 6$–7). $^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ vs. control; $^{d}P > 0.05$, $^{e}P < 0.05$ vs. I/R.

A second series of experiments was designed to evaluate the role of CGRP in nitroglycerin-induced preconditioning. The preparations from the CGRP-(8-37)-treated group were exposed to CGRP-(8-37) (10^{-7} M) for 5 min, then to CGRP-(8-37) and nitroglycerin(3×10^{-7} M)together for 5 min, followed by a 10-min wash-out period before the 60-min ischemia. For the study on the effect of capsaicin on the protection provided by nitroglycerin, preparations were exposed to nitroglycerin (3×10^{-7} M) after pretreatment with capsaicin. Capsaicin (50 mg/kg) or vehicle was given s.c. injection 4 days before experiments. For the CGRP-treated group, the small intestine was perfused with CGRP (10^{-7} M) for 5 min, and then washed out for 10 min with buffer solution before the 60-min ischemia.

2.7.2. In vivo experiment

Four groups of animals were used to study the small intestine protective effects of pretreatment with nitroglycerin in the anesthetized rat. The control group was la-

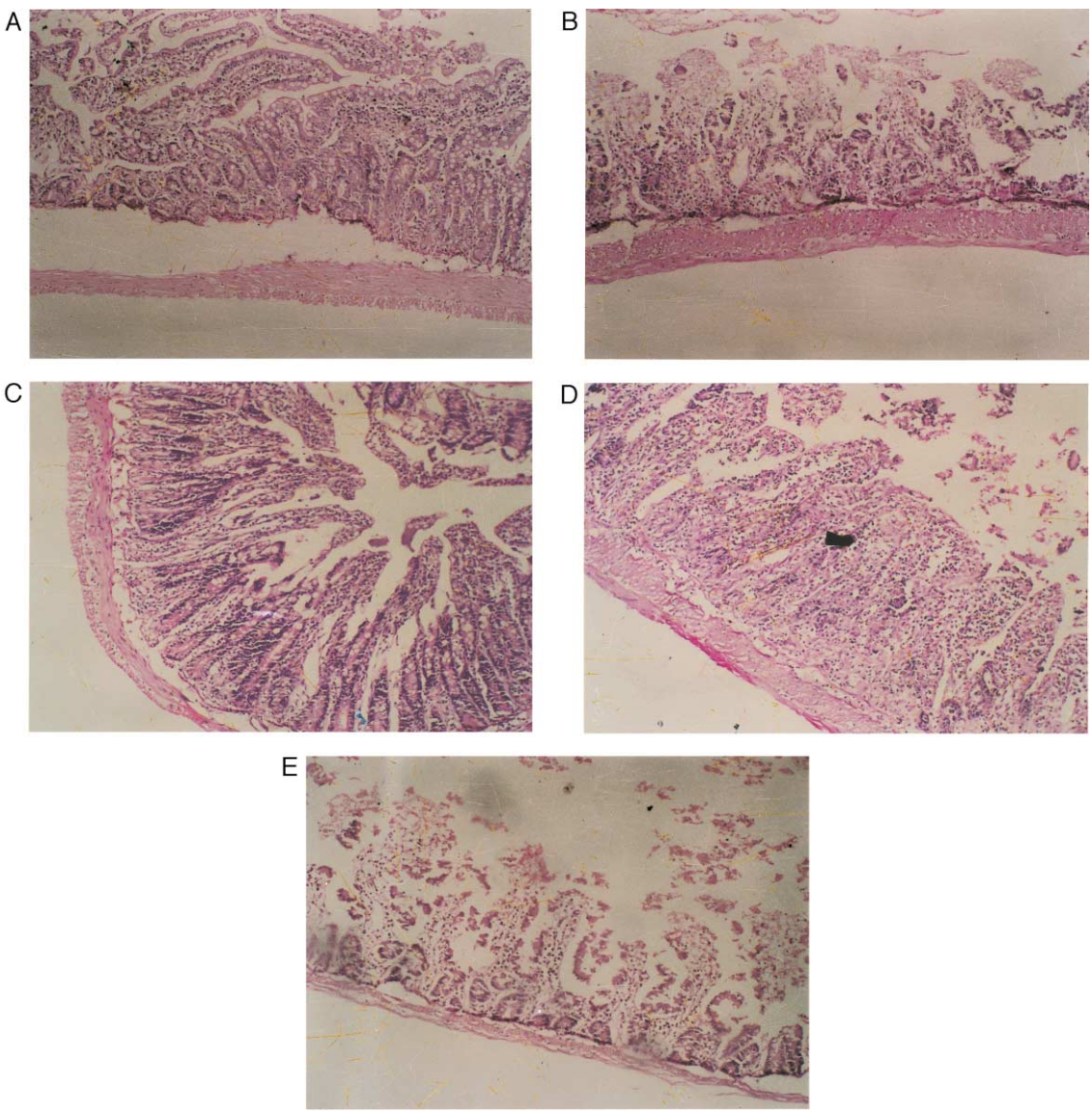

Fig. 2. Representative example of small intestine mucosal injury from in vitro experiment. HE stain, ×100. (A) The mesenteric vascular bed after 75 min of perfusion. Note the normal cellar morphology, lack of interstitial edema, and intact villus tips (control group). (B) The mesenteric vascular bed suffered 60 min of ischemia and 15 min of reperfusion. Note the villous destruction with involvement of the crypt layer and appearance of mucosal necrosis (ischemia–reperfusion group). (C) Preparations were exposed to nitroglycerin (10^{-7} M) for 5 min before the mesenteric vascular bed suffered ischemia and reperfusion. Note mainly intact structure of the mucosa (nitroglycerin-treated group). (D) Preparations were exposed to CGRP-(8-37)(10^{-7} M) for 5 min, then to CGRP-(8-37) and nitroglycerin(3×10^{-7} M)together for 5 min, followed by a 10-min wash-out period before the mesenteric vascular bed suffered ischemia and reperfusion. Note digestion and disintegration of lamina propria with ulceration (CGRP-(8-37)-treated group). (E) Preparations were exposed to nitroglycerin (3×10^{-7} M) after pretreatment with capsaicin. Capsaicin (50 mg/kg) was given s.c. injection 4 days before experiments. Note the loss of villi tissues and destruction of the crypt layer (capsaicin-treated group).

paratomised without clipping of the super mesenteric artery. In the case of the ischemia–reperfusion group, the rats were subjected to 30-min occlusion of the super mesenteric artery followed by 60-min reperfusion. The preconditioning group was same as the ischemia–reperfusion group, but with a previous three-cycle occlusion of the super mesenteric artery for 5-min ischemia and 5-min reperfusion. For the nitroglycerin-treated group, nitroglycerin (0.13 mg/kg i.v.) was given 5 min before 30-min ischemia and 60-min reperfusion.

2.8. Reagents

CGRP, CGRP-(8-37), capsaicin, *O*-dianisidine and hexadecyltrimethyl ammonium bromide were purchased from Sigma. Nitroglycerin was purchased from Guangzhou Pharmaceutical Factory. CGRP, CGRP-(8-37) and nitroglycerin were dissolved in K–H buffer solution. Capsaicin was dissolved in a vehicle containing 10% Tween 80, 10% ethanol, and 80% saline. The radioimmunoassay kit was obtained from Dongya Immunity Technology Institution. Supplies for the lactate dehydrogenase and malondialdehyde assay were obtained from Beijing Zhongsheng High-Tech Bioengineering, Beijing, China.

2.9. Statistics

One-way analysis of variance and Newman–Keuls tests were used to compare the group differences. Wilcoxon rank sum test was used to evaluate the morphological appearance of tissues. The significance level was chosen as $P < 0.05$.

3. Results

3.1. Effects of nitroglycerin on perfused small intestine

As shown in Table 1, 60 min of ischemia and 15 min of reperfusion caused a significant increase in the protein level and lactate dehydrogenase activity in the effluent. Malondialdehyde content in the small intestine was also elevated. In the mean time, the intestine appeared seriously edematous (wet weigh/dry weigh rising). Pretreatment with nitroglycerin 10^{-7}, 3×10^{-7} or 10^{-6} M for 5 min caused a significant decrease in all these levels ($P < 0.01$). Preconditioning had a similar protective effect on small intestine tissue. However, there was no difference between the preconditioning group and nitroglycerin-treated groups ($P > 0.05$).

Results from morphological observations showed that ischemia and reperfusion caused severe mucosal lesioning. Pretreatment with nitroglycerin 10^{-7} M led to less pronounced mucosal damage than in the ischemia–reperfusion group ($P < 0.05$) (Table 2, Fig. 2).

3.2. The role of CGRP in the nitroglycerin-induced preconditioning

Involvement of the protective effects of nitroglycerin-induced preconditioning in activation of sensory nerves was tested with capsaicin. After pretreatment with capsaicin to deplete the transmitter in sensory nerves, the protective effects of nitroglycerin were abolished (Tables 1 and 2; Fig. 2).

To further explore the mediation of CGRP in the protective effects of nitroglycerin, CGRP and CGRP-(8-37) were used. Pretreatment with CGRP produced a protective effect similar to that of nitroglycerin. However, CGRP-(8-37), a selective CGRP receptor antagonist, abolished the protective effects of nitroglycerin (Tables 1 and 2; Fig. 2).

As shown in Fig. 1, the content of CGRP-like immunoreactivity in the effluent was significantly increased during nitroglycerin perfusion. However, after pretreatment with capsaicin, nitroglycerin only caused a slight increase in the effluent content of CGRP-like immunoreactivity. The levels of CGRP-like immunoreactivity were significantly lower in the effluent from the capsaicin plus nitroglycerin-treated group than in those of the nitroglycerin-treated group.

3.3. Effects of nitroglycerin on in *vivo* small intestine

As shown in Table 3, ischemic reperfusion caused a significant increase in serum lactate dehydrogenase.

Table 3
Protective effect of nitroglycerin-induced preconditioning on I/R injury in in vivo rat small intestine

Group	*N*	Serum	Plasma	Tissue	
		LDH (U/l)	CGRP (pg/ml)	MDA (nmol/mg pro wt)	MPO (U/100 g)
CON	7	462.80 ± 23.74	771.21 ± 43.63	0.19 ± 0.03	0.73 ± 0.07
I/R	6	753.74 ± 34.04^{c}	364.17 ± 48.29^{c}	0.58 ± 0.04^{c}	2.54 ± 0.28^{c}
PC	7	$631.33 \pm 26.84^{c,f}$	$580.30 \pm 46.42^{b,e}$	$0.39 \pm 0.05^{c,f}$	$0.79 \pm 0.07^{a,f}$
NTG	7	$638.31 \pm 33.18^{c,e}$	$736.14 \pm 68.97^{a,f}$	$0.37 \pm 0.05^{c,f}$	$0.65 \pm 0.13^{a,f}$

CON: control group, I/R: ischemic and reperfusion group, PC: preconditioning group, NTG: nitroglycerin-treated group. Means ± S.E.M. $^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ vs. control; $^{e}P < 0.05$, $^{f}P < 0.01$ vs. I/R.

Myeloperoxidase activity and the malondialdehyde level of the tissue were elevated, while plasma CGRP-like immunoreactivity was significantly decreased. Pretreatment with nitroglycerin caused a significant increase in plasma CGRP and had marked protective effects against the injury from ischemic reperfusion.

As shown in Fig. 3, mucosal tissue samples from control group appeared normal. The ischemia–reperfusion group had severe mucosa hemorrhagic lesions. Pretreatment with nitroglycerin produced less pronounced mucosal damage than in the ischemia–reperfusion group ($P < 0.05$) (Table 4).

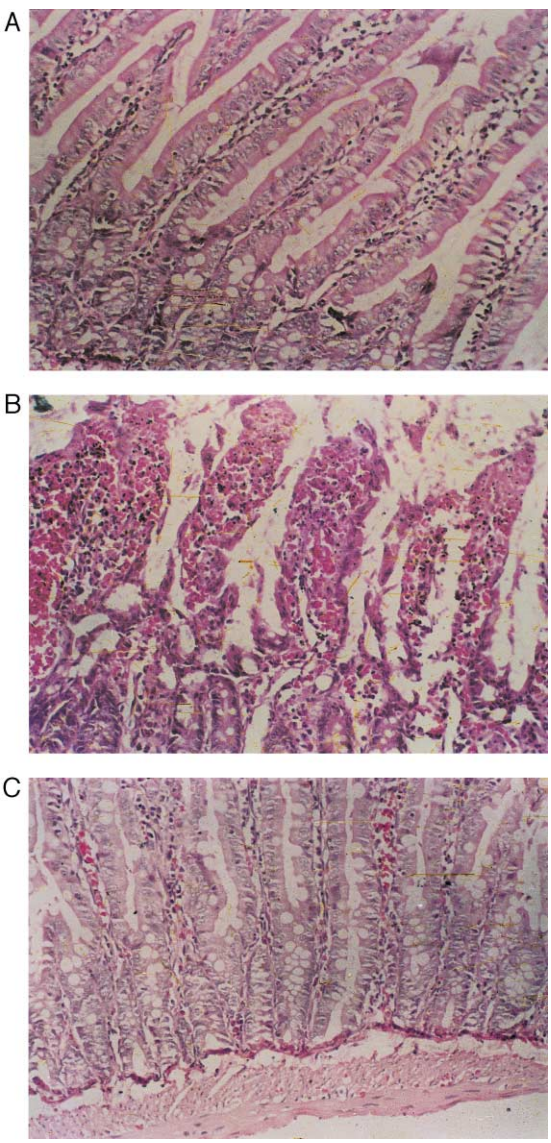

Fig. 3. Representative example of small intestine mucosal hemorrhage from in vivo experiment. HE stain, ×200. (A) The animals suffered sham operation. Note the normal mucosal villi (control group). (B) The mesenteric vascular bed suffered 30-min occlusion followed by 60-min reperfusion. Note disintegration of lamina propria; hemorrhage and ulceration (ischemia–reperfusion group). (C) Nitroglycerin (0.13 mg/kg i.v.) was given 5 min before 30-min ischemia and 60-min reperfusion. Note the normal mucosal villi, and scattered hemorrhage area (nitroglycerin-treated group).

Table 4
Effect of nitroglycerin-induced preconditioning on hemorrhage of intestinal mucosa

Group	Hemorrhagic degree of mucosa					Mean grade
	0	I	II	III	IV	
CON	4	3	0	0	0	0.42
I/R	0	0	2	3	2	2.85[c]
PC	0	3	2	2	0	1.86[a,d]
NTG	0	4	3	0	0	1.42[a,e]

CON: control group, I/R: ischemia and reperfusion group, PC: preconditioning group, NTG: nitroglycerin-treated group ($N = 6–7$). [a]$P > 0.05$, [c]$P < 0.01$ vs. control; [d]$P > .05$, [e]$P < 0.05$ vs. I/R.

4. Discussion

The major new findings of the present study were: (1) previous perfusion with nitroglycerin reduces the tissue injury from ischemia and reperfusion in rat small intestine. (2) Nitroglycerin produces its protective effects via stimulation of CGRP release, because nitroglycerin increased the level of CGRP-like immunoreactivity in the effluent, and because CGRP-(8-37) or capsaicin abolished the protective effects of nitroglycerin. In addition, a 5-min previous perfusion of CGRP produced protective effects similar to those of nitroglycerin.

Nitroglycerin is one of the most useful drugs in the short-term management of acute ischemic coronary syndromes. It is now known to cause an increased CGRP level in plasma (Fanciullacci et al., 1995). Our in vivo experiment confirmed this result. The mechanism of the nitroglycerin-induced release of CGRP is, however, still unclear. In recent years, following research on nitric oxide (NO), nitroglycerin has been classified as a kind of NO donor drug. There is a report that the release of CGRP from the capsaicin nerve depends on NO (Hughes and Brain, 1994). Our experiments showed that nitroglycerin significantly increases the release of CGRP from the effluent, but that the protective effect of nitroglycerin was abolished by CGRP-(8-37) or pretreatment with capsaicin, which depletes the transmitter content of sensory nerves. We have to consider the issue: what kind of role did NO play in this process? Did it act only as a bridge? Or did it produce some protection of preconditioning directly? Some reports suggest that NO is not a mediator in the ischemic preconditioning, while it causes remarkable vasodilatation, and inhibits thrombocyte aggregation. So its only importance is as a substance maintaining the functional stability of the circulatory system. Vegh, however, reported that the

protection of preconditioning may be related to the activity of the NO system, because L$^{\omega}$-nitro-L-arginine methyl ester (L-NAME), a selective nitric oxide synthase antagonist, can abolish the protective effects of preconditioning (Vegh et al., 1993). The preconditioning induced by nitroglycerin presented us with a problem: what kind of relationship exists between CGRP, NO and preconditioning? This complex relationship appears to be the question we will look into.

At present, the mechanism responsible for ischemic or drug preconditioning is still unclear. Growing evidence suggests that endogenous chemical mediators are involved in the process (Parratt, 1993, 1995; Schultz et al., 1995). CGRP, a 37-amino acid peptide, is widely distributed in the central and peripheral nervous systems. In the early 1990s, Li et al. (1996) found that CGRP is involved in the ischemic preconditioning in the rat heart (Peng et al., 1996). Recently, there have been reports that CGRP mediates the heat stress preconditioning and drug preconditioning in the myocardium (Song et al., 1999; Zhou et al., 1998). There is also research into the role of CGRP in the ischemic preconditioning of other organs. For example, CGRP participates in protection of ischemic preconditioning in rat hindlimbs (Zhou et al., 1998) and CGRP is involved in early and delayed cardioprotection induced by brief ischemia of the small intestine (Tang et al., 1999). Our previous work also showed that CGRP mediates the protection by ischemic preconditioning in rat small intestine (Yang et al., 2000). All these data suggest that CGRP may be the common mediator in the protection by preconditioning. The present study also showed that nitroglycerin–induced preconditioning in the rat small intestine was closely related to the release of endogenous CGRP. The final mechanism of preconditioning has not been explained yet. A new and convincing body of data has suggested that protein kinase C is the first element of the complex kinase cascade (Downey and Cohen, 1997), which is activated during the prolonged ischemia in preconditioning, and that mitochondrial adenosine 5′-triphosphate-sensitive K^+ channels may be the final mediator of protection for ischemic preconditioning (Baines et al., 1999). Evidence shows that CGRP can induce the translocation and activation of protein kinase C, so we postulate that the way CGRP participates in the protection by preconditioning may be as follows: CGRP first combines with its receptor, and then protein kinase C is activated. Current evidence indicates that this cascade ultimately leads to the activation of p38 mitogen-activated protein kinase or the activation of Jun N-terminal/stress-activited protein kinase or the activation of both by tyrosine kinase. Finally, these protein kinases, which can be activated by stress, lead to opening of the mitochondrial adenosine 5′-triphosphate-sensitive K^+ channel, and the latter may be the final mediator of protection for ischemic preconditioning (Liu et al., 1999; Fryer et al., 1999). Someone suggested that CGRP is not the only mediator in the preconditioning; many other endogenous active substances also participate in the process. For example, Ouyang et al. (1999) pointed out that the preconditioning induced by CGRP is not as strong as that induced by ischemia. As a result, we postulate that CGRP and some other endogenous substances may all act as signal substances to activate the initial period in the chain of preconditioning.

Another interesting phenomenon we encountered is that the protective effects of nitroglycerin-induced preconditioning appeared to be concentration-independent. There was no significant difference between the three dosages for protection against the small intestinal ischemic reperfusion injury. It has been suggested that ischemic or pharmacological preconditioning protects against reperfusion by triggering signal transduction pathways (Laslay and Mentzer, 1992; Mosca et al., 1994; Tosaki et al., 1995). This may rely on sparking a threshold. Once endogenous protective mechanisms have been triggered, the protective effects will appear fully. Thus, choosing the proper dosage to stimulate the preconditioning will reach the more effective protection.

In addition to the in vitro experiment, we also studied the effects of nitroglycerin on small intestinal ischemic–reperfusion injury in vivo in the rat. We assayed myeloperoxidase activity, a specific marker for neutrophil infiltration. The result showed that nitroglycerin caused a significant decrease in myeloperoxidase activity in the small intestine tissues. It is well known that inflammatory factors are often involved in the injury caused by ischemia and reperfusion. Neutrophil infiltration in the tissue is one of the important traits of inflammation. It often aggravates ischemic lesioning in the tissue, and speeds up lipid peroxidation. The present in vivo experiment showed that nitroglycerin caused a significant decrease in neutrophil infiltration. In the mean time, the malondialdehyde level in the intestinal tissue, an indicator of lipid peroxidation (Parks et al., 1982), also decreased. On the other hand, nitroglycerin-induced preconditioning decreased the lactate dehydrogenase activity in the serum just as did ischemic preconditioning, and attenuated intestinal edema. Elevation of serum lactate dehydrogenase activity is known to occur in many diseases including the injury of ischemia–reperfusion (Erickson and Morales, 1961; Cohen et al., 1964). It may also be looked upon as a strong indicator of intestinal injury. Thus, nitroglycerin can provide an important protective effect against ischemic reperfusion injury in the in vivo rat small intestine. The mechanism may be related to CGRP in the plasma, because we found that the protective effects of ischemic and nitroglycerin-induced preconditioning are positively related to the level of CGRP in plasma.

In summary, the present results from in vitro and in vivo experiments suggest that nitroglycerin induces preconditioning-like protective effects on the rat small intestine. They also suggest that the effects of nitroglycerin are due to stimulation of endogenous CGRP release in rat mesenteric vascular bed.

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