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Ischemic preconditioning mediated by activation of K_{ATP} channels in rat small intestine[1]

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KEY WORDS small intestine; glyburide; cromakalim; ischemic preconditioning; potassium channels

ABSTRACT

AIM: To study whether the protective effects of ischemic preconditioning against rat small intestine ischemia/reperfusion injury could be mediated by K_{ATP} channel opener. **METHODS**: Preconditioning (Pc) was induced by 3 cycles of 8-min superior mesenteric artery (SMA) occlusion and 10-min reperfusion before prolonged ischemia. Cromakalim (Cro 75 $\mu g \cdot kg^{-1}$) and glibenclamide (Gli 8 $mg \cdot kg^{-1}$) were injected iv 10 min before prolonged ischemia and Pc, respectively. **RESULTS**: Compared with ischemic reperfusion (IR) group, Pc before prolonged ischemia (Pc + IR) decreased LDH release [(380 ± 55) *vs* (559 ± 49) $U \cdot L^{-1}$, $P < 0.05$], attenuated intestinal edema [wet weight/dry weight (WW/DW), 5.6 ± 0.6 *vs* 6.34 ± 0.29, $P < 0.05$], ameliorated intestinal histological damage (grading scale, 3.4 *vs* 5.7, $P < 0.01$), and improved reperfusion-induced hypotension. These effects of Pc were mimicked by Cro [LDH, (298 ± 40) *vs* (559 ± 49) $U \cdot L^{-1}$, $P < 0.05$; WW/DW, 5.6 ± 0.4 *vs* 6.34 ± 0.29, $P < 0.05$; grading scale, 3.6 *vs* 5.7, $P < 0.01$] and abolished in the presence of Gli [LDH, (624 ± 44) *vs* (559 ± 49) $U \cdot L^{-1}$; WW/DW, 6.6 ± 0.6 *vs* 6.34 ± 0.29; grading scale, 5.7 *vs* 5.7; $P > 0.05$] compared with IR group, respectively. **CONCLUSION**: Ischemic preconditioning on the rat small intestine is mediated by activation of K_{ATP} channels.

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INTRODUCTION

Single or mutiple brief periods of ischemia protected the myocardium from infarction after a subsequent more prolonged ischemic insult[1]. This phenomenon was described as preconditioning (Pc) and received attention because of its marked cardioprotective effects against various types of myocardial ischemia/reperfusion injuries[1,2]. Ischemic preconditioning also prevented intestinal injury induced by ischemic reperfusion[3]. Evidences from myocardium suggested that activation of myocardial K_{ATP} channel was involved in this effect[4,5]. K_{ATP} channel was present in smooth muscle cells of mesenteric arteries and activation of this channel markedly increased mesenteric blood flow[6]. In the present study the relationship between ischemic preconditioning and K_{ATP} channels of the rat small intestine was evaluated.

MATERIALS AND METHODS

Reagents Cromakalim (Cro) and glibenclamide (Gli) were from Sigma Chemical Co. Lactate dehydrogenase (LDH) assay kit was bought from Beijing Zhongsheng Biological Co.

Surgical procedure Wistar rats (♂, $n = 50$, 280 - 320 g, provided by Laboratory Animal Center of Shanxi Medical University (Grade Ⅱ, Certificate No LSDZ 521) were fasted for 24 h before the experiments with access to water. The rats were anesthetized with urethane 1 $g \cdot kg^{-1}$, ip. Systemic arterial pressure was measured with an arterial pressure recorder that was connected to a carotid artery cannula. A midline laparotomy was performed and superior mesenteric artery (SMA) was exposed. After gently clearing the surrounding fat and connective tissue, heparin sodium 600 $U \cdot kg^{-1}$ was iv injected.

The rats were divided into 6 groups. Control

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group: only laparotomy was performed without clipping of SMA; ischemic reperfusion (IR) group: rats were subjected to 30-min occlusion of SMA followed by 60-min reperfusion; preconditioning (Pc) + ischemic reperfusion (Pc + IR) group, the same as IR group, but with previous 3-cycle occlusion of SMA for 8-min and 10-min reperfusion; Cro group: Cro 75 $\mu g \cdot kg^{-1}$, a selective K_{ATP} channel opener was injected iv before ischemic reperfusion damage; Gli group: Gli 8 $mg \cdot kg^{-1}$, a selective K_{ATP} channel blocker, was injected iv 10 min before Pc followed by prolonged ischemia, 5 mL 10 % glucose was injected iv along with Gli and during the reperfusion period; Pc group: no other treatment was made but Pc.

A small intestinal tissue was obtained approximately 10 cm to the ileocecal junction at the end of reperfusion or Pc. The tissue was sectioned in 2 segments. The biopsy specimens were rapidly placed in buffered (pH 7.4) 10 % formalin. About 1-g samples were used for analysis of wet weight/dry weight (WW/DW). At the same time, blood samples were withdrawn from the femoral artery and centrifuged (4 ℃, 3000 × g) for 10 min. The supernatants were preserved at −20 ℃ for analysis of LDH.

Experimental protocol The degree of intestinal injury was evaluted on a grading scale, Grade 0 − Ⅴ[7] and Ⅵ − Ⅷ[8]. Systemic blood pressure was recorded before Pc, after 30-min ischemia and 60-min reperfusion, before or after iv Cro and Gli. Intestinal edema was evaluted by DW/WW measurement. LDH level of serum was analysed by assay kit.

Statistics Data were expressed as $\bar{x} \pm s$. Between groups differences were compared by ANOVA. Significant differences between morphological appearance of tissue was determined by the Wilcoxon rank sum test.

RESULTS

Pc or iv Cro decreased LDH release [(380 ± 55), (298 ± 40) *vs* IR (559 ± 49) $U \cdot L^{-1}$, $P < 0.05$] and improved the intestinal edema [(WW/DW, 5.6 ± 0.6, 5.6 ± 0.4 *vs* IR (6.34 ± 0.29), $P < 0.05$]. Gli abolished these effects of Pc [WW/DW, Gli (6.6 ± 0.6) *vs* IR (6.34 ± 0.29), $P > 0.05$; LDH, Gli (624 ± 44) *vs* IR (559 ± 49) $U \cdot L^{-1}$, $P > 0.05$] (Tab 1).

Tab 1. LDH release and intestinal wet weight/dry weight (WW/DW). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^dP > 0.05$, $^eP < 0.05$ *vs* IR. IR: Ischemic reperfusion. Pc: preconditioning. Cro: cromakalim. Gli: glibenclamide.

Group	*n*	WW/DW	LDH/$U \cdot L^{-1}$
Control	6	5.6 ± 0.6	276 ± 31
IR	6	6.34 ± 0.29[b]	559 ± 49[c]
Pc + IR	7	5.6 ± 0.6[e]	380 ± 55[c]
Cro	6	5.5 ± 0.4[e]	298 ± 40[c]
Gli	7	6.6 ± 0.6[d]	624 ± 44[d]
Pc	7	5.6 ± 0.5[a]	271 ± 21[a]

Pc itself did not result in blood pressure change. Pc or Cro improved hypotension induced by reperfusion as compared with IR group [(Pc + IR, from (11.4 ± 1.7) to (11.7 ± 1.7) kPa; Cro, from (13.8 ± 1.3) to (11.4 ± 0.7) kPa *vs* IR, from (14.0 ± 0.9) to (10.0 ± 2.3) kPa, $P < 0.05$) (Tab 2).

Tab 2. Mean arterial pressure changes during Pc, occlusion, and reperfusion (kPa). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* baseline within individual group.

Group	*n*	Procedure baseline	Precondition-ing	30 min of occlusion	60 min of reperfusion
Control	6	11.8 ± 2.3		11.8 ± 2.0	12.1 ± 1.6
IR	6	14.0 ± 0.9		14.2 ± 1.3	10.0 ± 2.3[b]
Pc + IR	7	11.4 ± 1.7	10.8 ± 1.5	13.0 ± 0.9	11.7 ± 1.7[a]
Cro	6	13.8 ± 1.3		13.4 ± 1.6	11.4 ± 0.7[a]
Gli	6	12.1 ± 1.3	12.1 ± 1.7	13.6 ± 2.3	12.2 ± 1.6
Pc	7	12.4 ± 1.7	11.6 ± 1.6		

The specimen taken from the control group or Pc group showed a normal mucosa in all the rats. IR group revealed severe mucosal lesion. Pc or pretreatment with Cro before ischemic reperfusion (Pc + IR or Cro group) produced a less pronounced mucosal damage compared with IR group (grading scale, 3.4, 3.6 *vs* 5.7, $P < 0.01$). This effect was abolished in the presence of Gli (Tab 3, Fig 1).

DISCUSSION

Ischemic preconditioning has marked cardioprotective effects[1,2,4,5]. Our present results along with

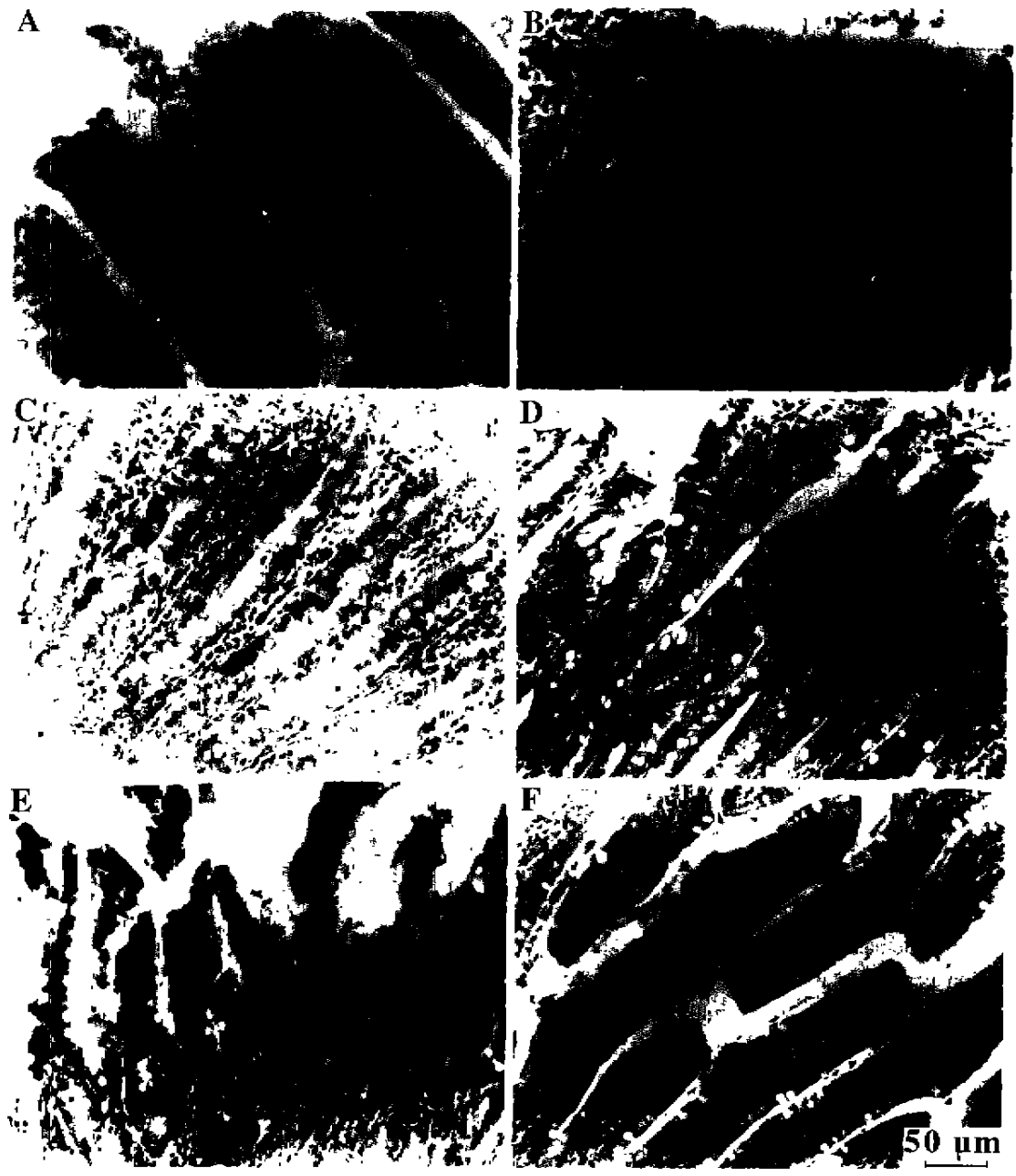

Fig 1. Representative examples of small intestinal mucosal injury. HE stain, × 200.
A) Control; B) IR group; C) Pc + IR group; D) Cro group; E) Gli group; F) Pc group.

Hotter's work[3] suggest that ischemic preconditioning has similar protective effects against the rat small intestinal injury induced by ischemic reperfusion. We also found that the specific K_{ATP} channel antagonist abolished the beneficial effects of Pc and these effects were mimicked by Cro. These findings suggest that ischemic preconditioning might be a universal protective effect and intestine has a similar mechanism to myocardium.

Although the present study demonstrated the role of K_{ATP} channel opener in intestinal Pc, it is necessary to investigate which endogenous mediators are involved in this protective effect. The intestinal Pc was triggered by an initial transient increase in NO synthesis[3].

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Tab 3. Grade of intestinal mucosal damage in different groups. $^{a}P > 0.05$ *vs* **control.** $^{d}P > 0.05$, $^{f}P < 0.01$ *vs* **IR.**

Group	Grade of mucosal damage (number of rats)									$\bar{x}$
	0	Ⅰ	Ⅱ	Ⅲ	Ⅳ	Ⅴ	Ⅵ	Ⅶ	Ⅷ	
Control	4	2	0	0	0	0	0	0	0	0.3
IR	0	0	0	0	1	2	2	2	0	5.7
Pc + IR	0	0	1	2	4	0	0	0	0	3.4^{f}
Cro	0	0	0	3	4	0	0	0	0	3.6^{f}
Gli	0	0	0	0	0	3	2	1	0	5.7^{d}
Pc	2	3	1	0	0	0	0	0	0	0.7^{a}

NO itself appeared to activate K_{ATP} channels of vascular smooth muscle cells[9]. This suggested that NO might be an endogenous mediator that resulted in activation of the K_{ATP} channels and thus preconditioned small intestine which was resistant to subsequent more prolonged ischemia. However, further work is needed to investigate the relationship between K_{ATP} channels and NO, and other endogenous substances, such as calcitonin gene related peptide (CGRP), adenosine to get more direct evidence.

In the present study, the ability of Gli to affect insulin and glucose blood levels may also have a negative effect on Pc which can not be ruled out. Previous work showed that Pc protection occurred only in the presence of high glucose concentrations during the reperfusion period[10]. To avoid this negative effect, we systemically injected iv glucose during the reperfusion period or at the time when glibenclamide was given. Therefore it seems unlikely that increased insulin or decreased blood glucose levels is responsible for effect of this compound to prevent Pc.

In summary, ischemic preconditioning on the rat small intestine is mediated by activation of K_{ATP} channels.

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K_{ATP}通道激活介导大鼠小肠的缺血预处理[1]

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关键词 小肠；格列苯脲；色满卡林；缺血预处理；钾通道

目的：研究缺血预处理对大鼠缺血再灌注损伤小肠的保护作用是否由K_{ATP}通道开放剂介导的. **方法**：用短暂反复夹闭肠系膜上动脉诱导预处理，观察其对长期缺血再灌注损伤小肠的保护作用，并用K_{ATP}通道开放剂色满卡林(Cro)和拮抗剂格列苯脲(Gli)进一步探讨其作用机制. **结果**：预处理对大鼠缺血再灌注损伤小肠具有保护作用，其作用可被Cro模拟，并被Gli取消. **结论**：预处理对大鼠缺血再灌注损伤小肠的保护作用是由K_{ATP}通道开放剂介导的.

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