Effect of $G_{\alpha q/11}$ protein and ATP-sensitive potassium channels on prostaglandin E_1 preconditioning in rat hearts

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KEY WORDS G-proteins; potassium channels; prostaglandin E_1; ischemic preconditioning; signal transduction; ischemia-reperfusion injury

ABSTRACT

AIM: To investigate the effect of $G_{\alpha q/11}$ signaling pathway and ATP-sensitive potassium channels (K_{ATP} channels) on prostaglandin E_1 (PGE_1) induced early and delay-preconditioning protection in rat hearts. **METHODS:** Two series of experiments were performed in Wistar rat hearts. In the first series of experiment, all rats were pretreated with PGE_1 40 min or 23 h 20 min before the experiment. Ischemia-reperfusion injury was induced by 30 min coronary artery occlusion followed by 90 min reperfusion. Hemodynamics, infarct size, and scores of ventricular arrhythmias were measured. The expression of $G_{\alpha q/11}$ protein in the heart was measured by Western blot analysis in the second series. **RESULTS:** Preconditioning with PGE_1 (25 μg/kg) markedly reduced infarct size, left ventricular end-diastolic pressure, and scores of ventricular arrhythmia. The effect of PGE_1 was significantly attenuated by glibenclamide (1 mg/kg, ip), a nonselective K_{ATP} channel inhibitor. PGE_1 caused a significant increase in the expression of $G_{\alpha q/11}$ protein. **CONCLUSION:** Activations of $G_{\alpha q/11}$ signal pathway and K_{ATP} channel played significant roles in the cardioprotection of PGE_1 preconditioning in rat heart and might be an important mechanism of signal transduction pathway during the PGE_1 preconditioning.

INTRODUCTION

Ischemic preconditioning (IPC), a well-known phenomenon in which brief episodes of ischemia and reperfusion before a prolonged ischemic event limit myocardial cellular damage, has been shown to elicit both an acute and delayed phase of cardioprotection or a second window of protection[1]. The ATP-sensitive potassium channel has been suggested as an end-effector in the mechanism of ischemic preconditioning[2]. Recent studies show that the K_{ATP} channel mediates the myocardial protection induced by pharmacological agents such as adenosine agonist[3], opioids[4], flumazenil[5] and monophosphoryl lipid A (MLA)[6]. Hide *et al*[7] reported that PGE_1 preconditioning reduced myocardial infarct size in the rabbit by activation of K_{ATP} channels . $G_{\alpha q/11,}$ a member of G_α protein subunit plays an important role as a signal transduction pathway in protecting mechanism of ET-1 preconditioning and IPC[8]. The cardioprotective effects of PGE_1 have been attributed to systemic and coronary vasodilation, inhibition of platelet aggregation and in particular, inhibition of neutrophil activation. However, PGE_1 has been suggested a cardioprotection induced by pharmacological preconditioning in rabbit heart and rat heart[7,9].

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In this paper we investigate the effect of $G_{\alpha q/11}$ protein and K_{ATP} channel on PGE_1 preconditioning in rat hearts.

MATERIALS AND METHODS

Animals Male Wistar rats weighing 270-320 g (provided by Henan experimental animal center, Grade II, centificated No 2002LA-193) were anesthetized by intraperitoneal injection of pentobarbital sodium (45 mg/kg). Rats were intubated and ventilated with a respirator using a mixture of 100 % oxygen and room air (total volume of 1.2 mL per 100 g body weight; respiratory rate, 65-70 breaths/min).

Experimental protocol Two series of experiments were performed in the study. In the first series, all rats were subjected to 30 min ischemia and 90 min reperfusion (I/R). Myocardium ischemia/reperfusion (MI/R) group rats, injected with 2 mL saline 40 min before I/R (MI/R). PGE_1 early preconditioning protrection (EPP) group rats were injected 20 min PGE_1 (25 μg/kg) 40 min before I/R. PGE_1 delayed preconditioning protection (DPP) group rats were injected 20 min PGE_1 (25 μg/kg) 23 h 20 min before I/R. Glibenclamide (Gli) group rats were given glibenclamide (1 mg/kg) 30 min before I/R. Gli+EPP and Gli+DPP group rats were treated with glibenclamide 40 min or 23 h 20 min before I/R respectively, and glibenclamide 30 min before I/R. Hemodynamics, infarct size/area at risk (IS/AAR) of myocardium and scores of ventricular arrhythmia were measured. The expression of $G_{\alpha q/11}$ protein in sarcolemma was measured in the following groups by Western blot analysis in the second series: sham operated control (Control), MI/R, EPP and DPP.

Hemodynamics and scores of arrhythmia After rats were anesthetized, a catheter tip pressure transducer (PE_{50}) was inserted into the right carotid artery and advanced into the left ventricle for the determination of hemodynamics. Then, a midline thoracotomy was performed , the heart was exposed, and myocardial ischemia was produced by placing a 5-0 silk thread around the left anterior descending coronary artery (LAD), approximately 2-3 mm from its origin. Ischemia was maintained for 30 min. At the end of ischemia, the silk thread was released for 90 min reperfusion. The number of premature ventricular contractions (PVCs), episodes and duration of ventricular tachycardia (VT) and ventricular fibrillation (VF) in ischemia period and reperfusion period[8], left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), left ventricular maximum changes in positive pressure over time (+d*p*/d*t*) were recorded using ECG monitor and a 4-channel polygraph recorder in 15 min of ischemia period and 30 min of reperfusion period respectively.

Measurements of infarct size At the end of ischemia reperfusion, the LAD was reoccluded and Evans blue dye solution (1 mL of 2 % w/v) was injected into the left ventricle to distinguish perfused and non-perfused (area at risk) sections of the heart. The Evens blue solution stained the perfused myocardium, while the occluded vascular bed remains uncoloured. The rats were killed and the hearts were immediately excised, weighed, frozen, and stored in a freezer. After removal of the atria and right ventricle, the frozen heart was sliced into 1.5 mm thick 5-6 sections, and the slices were incubated in 1 % triphenyltetrazolium chloride (TTC) in pH 7.4 buffer for 20 min at 37 ºC. The slices were immersed in 10 % formalin overnight. Viable myocardium is stained in red color by TTC, whereas infarcted tissue is gray, nonischemic area is blue. The infarcted myocardium was dissected from the AAR under the illumination of a dissecting micro-scope. IS, AAR, and LV were determined by gravi-metric analysis. AAR was expressed as a percentage of the LV (AAR/LV), and IS was expressed as a percentage of the AAR (IS/AAR).

Western blot analysis For $G_{\alpha q/11}$ protein assay, heart tissue (100 mg) was homogenized in 2 mL ice-cold lysis buffer (50 mmol/L Tris-HCl, pH 7.2, 0.1 % deoxycholic acid , 0.1 % Triton X-100, 5 mmol/L ethylene diaminotetraacetic acid, 100 μmol/L phenylmethylsulfonyl fluoride). The lysates were sonicated on ice and centrifuged at 1000×*g* at 4 ºC for 10 min. The supernatant was further subjected to centrifugation for 20 000×*g* for 40 min at 4 ºC. The subsequent crude membrane pellet was resuspended in the homogenizing buffer (20 mmol/L Tris-HCl, pH 7.4, 1 mmol/L ethylene diaminotetraacetic acid, 1 mmol/L dithiothreitol, 100 μmol/L phenylmethylsulfonyl fluoride). Total protein concentration of membrane fractions was measured using the Lowry method. Prestained high molecular mass marker and 150 μg proteins from samples were separated on 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Separated proteins were transferred on to 0.45 μmol/L Nitrocellulose membrane. The membrane was blocked overnight at 4 ºC in 5 % skim milk and probed with primary antibody for

$G_{\alpha q/11}$. Primary antibody was diluted 1:200 in PBS. Horseradish peroxidase (HRP)-labeled anti-rabbit IgG was diluted in 1:5000 in PBS and used as secondary antibody. $G_{\alpha q/11}$ was visualized by enhanced chemiluminescence (ECL). Autoradiographs from Western blot analysis were quantified using Eagle eye II systerm.

Reagents PGE_1 was purchased from Shenyang Biochemical Co (No 010906). Glibenclamide, Evans blue, triphenyltetrazolium chloride (TTC) and BSA were purchased from Sigma Chemical Co. $G_{\alpha q/11}$ primary antibody, HRP-labeled IgG, ECL were the product of Santa Cruz Co.

Statistical analysis All values are expressed as mean±SD. One way analysis of variance (ANOVA) followed by Bonferroni's test was used for comparing the differences among multiple groups. Significant differences among groups were defined by $P<0.05$.

RESULTS

Hemodynamics Myocardical functional parameters, such as heart rate, LVSP, +d*p*/d*t*, and LVEDP were not significantly different among the six groups at baseline, the average body and heart weights were also similar among all the groups (data not shown). In the ischemic-reperfused heart, LVEDP was significantly lower in the PGE_1 pretreated groups as compared with the MI/R ($P<0.01$, Tab 1). However, the LVEDP was not significantly different between the EPP and DPP group. The PGE_1-induced improvement in LVEDP was abolished by glibenclamide in Gli+EPP and Gli+DPP (compared with MI/R, $P>0.05$). Glibenclamide itself had no significant effect on LVEDP. No significant changes in LVSP, +d*p*/d*t*, or heart rate were observed among the groups.

Infarct size During the early pretreat phase, preconditioning with PGE_1 resulted in significant decrease in the infarct size (% AAR) from 22.1 %±3.6 % in the MI/R group to 14.7 %±2.0 % in EPP group, a 33.4% reduction compared with the MI/R group. The infarct size increased significantly to 19.6 %±2.8 % ($P<0.01$) when glibenclamide was given 30 min before I/R in the PGE_1-pretreated rats. Glibenclamide itself had an infarct size of 23.2 %±2.7 %, which was not significantly different compared with the MI/R group ($P>0.05$). In the delayed preconditioning , the infarct size had a significant reduction (13.4 %±2.9 % in DPP group), compared with MI/R group, $P<0.01$. PGE_1-induced delayed protection was also abolished by glibenclamide as indicated by increased infarct size (21.4 %±3.1 %,

Tab 1. Hemodynamics of each group. *n*=10. $^bP<0.01$ *vs* MI/R. Pre: preischemia; I: ischemia 15 min; R: reperfusion 30 min. HR: heart rate; LVSP: left ventricular systolic pressure; LVEDP: left ventricular end diastolic pressure; +d*p*/d*t*: maximum positive change in pressure over time.

	MI/R	EPP	DPP	Gli	Gli+EPP	Gli+DPP
HR /beats·min^{-1}						
Pre	416±24	423±31	420±26	419±19	404±19	414±23
I	389±21	406±27	406±26	399±19	388±20	396±22
R	387±21	393±22	383±23	392±27	376±18	382±25
LVSP /mmHg						
Pre	128±17	119±18	123±15	132±20	108±19	127±22
I	117±15	124±14	119±20	126±16	98±18	120±18
R	112±17	120±18	120±19	129±17	104±16	123±17
LVEDP /mmHg						
Pre	25±5	25±4	26±4	25±5	26±4	24±5
I	31±4	23±3^b	22±3^b	31±4	34±5	32±5
R	42±4	22±4^b	21±4^b	39±6	40±5	39±5
+d*p*/d*t* /mmHg·s^{-1}						
Pre	435±665	4239±743	4407±772	4385±628	4280±692	4291±712
I	428±834	4372±783	4280±685	4328±732	4370±683	4186±677
R	403±657	4166±654	4310±613	4219±650	4173±598	4064±630

P<0.01). Compared with the early preconditioning, the infarct size was not significantly different in delayed preconditioning. The area at risk (% LV) was not different among the groups (Fig 1).

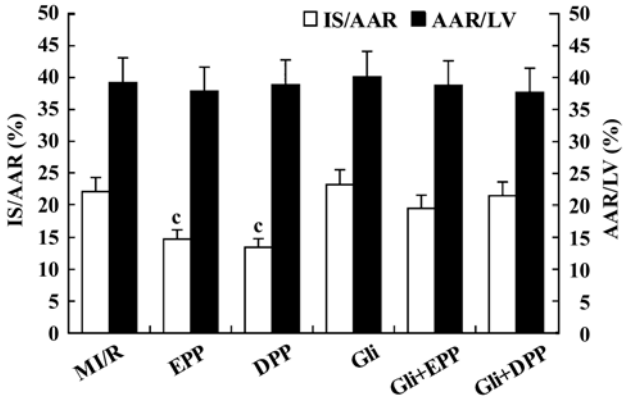

Fig 1. Effect of PGE_1 on myocardial infarct size. *n*=10. ^cP<0.01 *vs* MI/R. IS: infarct size; AAR: area at risk; LV: left ventricular.

Scores of arrhythmia Compared with MI/R group, the scores of ischemia phase (I) and reperfusion phage (R) in EPP and DPP groups significantly decreased (*P*<0.01). The protective effect was abolished by glibenclamide in Gli+EPP and Gli+DPP, but glibenclamide itself had no significant effect on arrhythmia. Scores of arrhythmia were not significantly different between the early and delayed preconditioning (Fig 2).

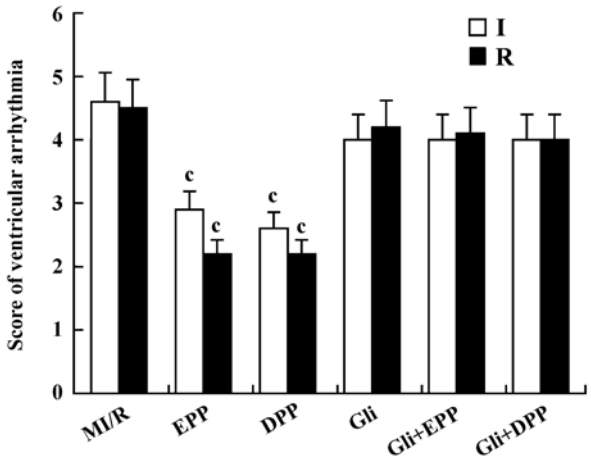

Fig 2. Effect of PGE_1 on the score of ventricular arrhythmia during 30-min occlusion and 90-min reperfusion. *n*=10. ^cP<0.01 *vs* MI/R. I: ischemia; R: reperfusion.

Expression of cardiac $G_{\alpha q/11}$ protein In comparison with Control group, $G_{\alpha q/11}$ protein expression was increased by 46.4 % (*P*<0.01) and 65.8 % (*P*<0.01) in EPP and DPP group respectively, while there was no significant difference in MI/R group. Interestingly, the expression of $G_{\alpha q/11}$ in delayed preconditioning was higher than early preconditioning (*P*<0.05) (Fig 3).

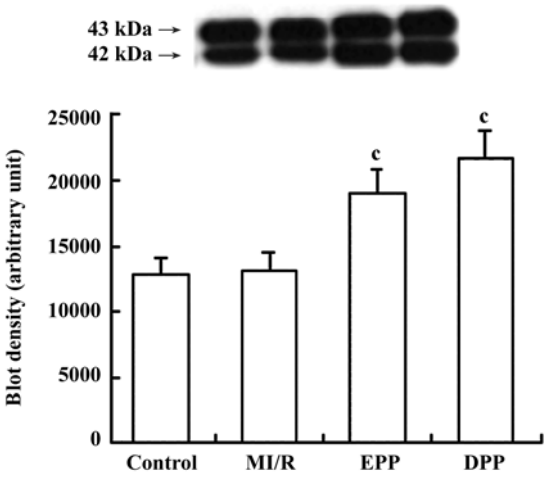

Fig 3. Immunoblotting analysis of $G_{\alpha q/11}$ in left ventricles of rats. Upper panel shows representative Western blots for $G_{\alpha q/11}$ and lower panel shows densitometric scores, *n*=8. ^cP<0.01 *vs* control.

DISCUSSION

Our results showed that PGE_1 induced an early and delayed cardioprotective effect in the heart as indicated by a significant decrease in the infarct size, scores of ventricular arrhythmias and LVEDP compared with the MI/R animals. The blocker of K_{ATP} channels glibencla-mide, when administered 30 min before ischemia-reperfusion, abolished the early as well as the delayed cardioprotection induced by PGE_1. No major differences in the heart rate, LVSP, +d*p*/d*t*, and AAR/LV were observed among the groups during the infarction protocol, suggesting that the changes in myocardial infarct size and scores of ventricular arrhythmias were independent of the systemic hemodynamics. PGE_1 pretreatment increased the expression of $G_{\alpha q/11}$ protein in EPP and DPP rat hearts. Taken together, our data suggested that pretreatment of rats with PGE_1 substantially reduced myocardial infarct size and ventricular arrhythmias, and the cardioprotective effects were mediated by K_{ATP} channels. Additionally, the $G_{\alpha q/11}$ protein signal-

ing was involved in the cardioprotective effect during PGE_1 preconditioning.

In our study, the protective effect in the heart was not significantly different between the early and delayed preconditioning induced by PGE_1. We did not perform time course of protection following PGE_1 treatment. Therefore, it was not clear whether this protection was sustained or was similar to the biphasic effect observed by ischemic preconditioning.

Recent studies have shown that vasodilatation and inhibition of platelet and neutrophil function are not a prerequisite for the cardioprotective effects of prostaglandins. Hide's study[7] demonstrated that pretreatment of rabbits with PGE_1 or PGE_0 caused reduction in myocardial infarct size, and the potent cardioprotective effects exerted by opening of K_{ATP} channels. Yamamoto's results[10] suggested that the PGE_1 protection of myocardium against ischemia was induced by inhibiting the myocardial L-type Ca^{2+} current. Our previous study[9] demonstrated that PGE_1 could protect ischemia-reperfusion myocardium from lipid peroxidation and enhence the activity of SOD in experimental rats, then it could modulate the balance of lipid peroxidation and anti-peroxidation effect *in vivo*.

It is now widely believed that K_{ATP} channels acts as the "end effector" of preconditioning induced by endogenous stresses[11-13] as well as pharmacological agents including adenosine agonist[3], flumazenil[5], opioid agonist and MLA[6] *etc*. Opening of the K_{ATP} channel has been shown to be protective due to the increase in the outward K^+ current resulting in the shortening of action potential, which in turn may spare ATP, thereby allowing less entry of Ca^{2+} into the myocyte. Decreased intracellular Ca^{2+} overload then results in less ischemic injury and better myocyte preservation[14]. Especially, opening of mitochondrial K_{ATP} channel leads to membrane depolarization, matrix swelling, slowing of ATP synthesis, and accelerated respiration[2,15,16], which due to myocardical protection by reducing infarct size and ventricular arrhythmias during preconditioning. Our present study also suggested that opening of K_{ATP} channels was the common mechanism which caused reduction of infarct size and ischemic arrhythmias in pretreatment of rats with PGE_1.

Recent study demonstrated that $G_{\alpha q/11}$ signal pathway was related to the protective mechanism of ET-1 pretreatment and ischemic preconditioning[8]. EP_1, EP_2, EP_3, and EP_4 are the four subtypes of prostaglandin E receptors. The EP_1 and EP_3 are coupled to $G_{\alpha q/11}$- phospholipase C (PLC) signal pathway. Interestingly, PGE_1 can act on EP_1 and EP_3 (subgroups A and D) receptors, and then activate PLC to release inositol 1,4,5,-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG)[7]. The latter compound in combination with intracellular calcium then causes the translocation and activation of protein kinase C (PKC). Activated PKC may phosphorylate secondary effectors. In our experiment, the expression of $G_{\alpha q/11}$ protein is significantly increased in PGE_1 pretreated (including EPP and DPP groups) animals. These suggested that the opening of K_{ATP} channels was based on activation of PKC, while the activation of the $G_{\alpha q/11}$ signal pathway (via activation of EP_1 and EP_3 receptors by coupled with PGE_1) is due to activate PLC, which enhences IP_3 /DAG signal pathway for the activation of PKC.

In summary, the present study demonstrated that pretreatment of rats with PGE_1 induced a significant decrease in myocardial infarct size and ventricular arrhythmias during regional ischemia and reperfusion. The cardioprotective effects of PGE_1 were due to activation of K_{ATP} channels, involved in activation of $G_{\alpha q/11}$-PLC signal transduction pathway via activation of EP_1 or more likely EP_3 receptors (coupled with PGE_1).

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