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Possible mechanism of cardioprotective effect of angiotensin preconditioning in isolated rat heart

Ajay Sharma, Manjeet Singh *

Department of Pharmaceutical Sciences & Drug Research, Punjabi University, Patiala 147002, India

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Abstract

The present study is designed to investigate the mechanism of cardioprotective effect of angiotensin II preconditioning. Isolated perfused rat heart was subjected to global ischaemia for 30 min followed by reperfusion for 120 min. Coronary effluent was analysed for lactate dehydrogenase and creatine kinase enzyme release to assess the degree of cardiac injury. Myocardial infarct size was estimated macroscopically using triphenyltetrazolium chloride staining. Four episodes of angiotensin II preconditioning markedly reduced lactate dehydrogenase and creatine kinase release in the coronary effluent and decreased myocardial infarct size. Administration of prazosin (α_1-adrenoceptor antagonist) before global ischaemia reduced the extent of ischaemia–reperfusion-induced myocardial injury. Moreover, administration of prazosin during angiotensin II preconditioning or depletion of biogenic amines by reserpinisation (0.5 mg/kg i.p.) did not affect the cardioprotective effect of angiotensin II preconditioning. On the other hand, colchicine (5 mg/kg i.p.) or polymyxin B (50 μM) treatment markedly attenuated the cardioprotective effect of angiotensin II preconditioning. On the basis of these results, it may be concluded that the cardioprotective effects of angiotensin II preconditioning may be mediated through protein kinase C and may not involve release of norepinephrine or activation of α_1-adrenoceptor.

Keywords: Preconditioning; Angiotensin; Ischaemia; Prazosin; Colchicine or activation of α_1-adrenoreptors

1. Introduction

Angiotensin II preconditioning is reported to produce cardioprotective effect (Liu et al., 1995; Diaz and Wilson, 1997). We have recently reported that this cardioprotective effect may be mediated through the activation of angiotensin AT_1 receptors (Sharma and Singh, 1999). In cardiomyocytes, angiotensin AT_1 receptors are coupled with phospholipase C through G proteins. Stimulation of angiotensin AT_1 receptors leads to increased formation of inositol triphosphate, diacylglycerol and a subsequent increase in protein kinase C activity (Sadoshima and Izumo, 1993). Protein kinase C, which is implicated in the cardioprotective effect of ischaemic preconditioning in rat (Speechly-Dick et al., 1994; Mitchell et al., 1995; Tosaki et al., 1996), may also mediate the cardioprotective effect of angiotensin II preconditioning.

Angiotensin AT_1 receptors are demonstrated to be present on sympathetic nerve terminals of heart (Brasch et al., 1993). Stimulation of angiotensin AT_1 receptors facilitates norepinephrine release from sympathetic nerve terminals in rat heart (Musgrave et al., 1991). Moreover, angiotensin II is reported to increase the rate of norepinephrine synthesis and to inhibit its neuronal uptake (Peach et al., 1969; Starke, 1971). Since norepinephrine acts as an endogenous mediator of ischaemic preconditioning in rat (Banerjee et al., 1993; Hu and Nattel, 1995), there is a possibility that the cardioprotective effects of angiotensin II preconditioning may be mediated through release of norepinephrine. Therefore, the present study is designed to investigate the role of protein kinase C and norepinephrine in mediating the cardioprotective effect of angiotensin II preconditioning.

* Corresponding author. Tel.: +91-175-282461-66 ext. 6184; fax: +91-175-282881.

E-mail address: manjeet@pbi.ernet.in (M. Singh).

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2. Materials and methods

Wistar albino rats of either sex weighing 200–250 g were used in present study. The animal experiments were conducted in accordance with guidelines of the US National Institute of Health for care and use of laboratory animals. Angiotensin II (Hypertensin, Ciba-Geigy, Basel, Switzerland) and polymyxin B were directly added to Kreb's–Henseleit (K–H) solution. Prazosin hydrochloride (Sun Pharma, Baroda, India) was dissolved in minimum quantity of lactic acid and added to the K–H solution. Reserpine (Loba Chemie, Bombay, India) was dissolved in minimum quantity of glacial acetic acid and volume was made up with water. Solution of tyramine hydrochloride (Sigma, St. Louis, MO, USA) was prepared in normal saline . All other reagents used in the study were analytical grade of Loba Chemie.

2.1. Isolated rat heart preparation

Heparinized rats (500 U; i.p.) were sacrificed by stunning. Hearts were rapidly excised and immediately mounted on Langendorff's apparatus (Langendorff, 1895). The heart was enclosed by double-walled jacket, the temperature of which was maintained by circulating water heated to 37°C. The preparation was perfused at a coronary flow rate of 6–8 ml/min and a constant perfusion pressure of 70 mm Hg with K–H solution (NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; $MgSO_4 \cdot 7H_2O$, 1.2 mM; $NaHCO_3$, 25 mM; KH_2PO_4, 1.2 mM and $C_6H_{12}O_6$, 11 mM) maintained at 37°C and bubbled with 95% O_2 and 5% CO_2. Two thin silver electrodes fixed at the ventricular apex and origin of aorta were employed to record ECG (BPL MK 801, Banglore, India) for monitoring heart rate. Global ischaemia was produced for 30 min by closing the inflow of physiological solution and was followed by reperfusion for 120 min.

2.2. Assessment of myocardial injury

To determine the extent of myocardial injury, release of lactate dehydrogenase and creatine kinase was measured in the coronary effluent using 2,4-dinitrophenylhydrazine method (King, 1959) and Hughes method (Hughes, 1961), respectively. Values were expressed in international units (IU) per litre.

2.3. Myocardial infarct size

The heart was removed from the Langendorff's apparatus. Both the auricles and root of aorta were excised out and ventricles were kept overnight at −4°C. Frozen ventricles were sliced into uniform sections of about 2–3-mm thickness. The slices were incubated in 1% triphenyltetrazolium chloride at 37°C in 0.2 M Tris buffer (pH 7.4) for 30 min (Fishbein et al., 1981). The normal myocardium was stained brick red while the infarcted portion remained unstained. Infarct size was measured by volume and weight method as described earlier (Chopra et al., 1992).

2.4. Experimental protocol

A diagrammatic representation of experimental protocols is shown in Fig. 1. In all groups, isolated rat heart was perfused with K–H solution and allowed to stabilise for 10 min. Group I {Control; $n = 6$}, isolated rat heart was perfused for 45 min with K–H solution after stabilisation (period required to produce angiotensin II preconditioning) and then subjected to 30 min of global ischaemia followed by 120 min of reperfusion. In Group II {Angiotensin II (100 nM) preconditioned; $n = 6$}, isolated rat heart was perfused for 5 min with K–H solution containing angiotensin II (100 nM) followed by 5-min perfusion with K–H solution (containing no angiotensin II). This was repeated four times. Group III {Prazosin (100 nM) treated; $n = 6$}, the preparation was perfused with K–H solution containing prazosin (100 nM) for 45 min and then subjected to 30 min of global ischaemia followed by reperfusion with K–H solution (containing no prazosin) for 120 min. Group IV {Prazosin (100 nM) in angiotensin II preconditioning; $n = 6$}, isolated rat heart was perfused for 5 min with K–H solution containing prazosin before subjecting it to four episodes of angiotensin II preconditioning. Each episode comprised of 5-min perfusions with K–H solution containing angiotensin II (100 nM) followed by 5-min perfusion with K–H solution containing prazosin (100 nM). Group V {Prazosin (100 nM) treatment in angiotensin II preconditioning; $n = 5$}, the preparation was subjected to angiotensin II preconditioning as described in group II but prazosin was administered continuously before global ischaemia, even during the administration of angiotensin II. Group VI {Angiotensin II preconditioning in reserpinised (0.5 mg/kg i.p.) rats; $n = 6$}, rats were injected reserpine (0.5 mg/kg i.p.). After about 24 h, hearts obtained from these animals were retrogradely perfused on Langendorff's apparatus and subjected to angiotensin II preconditioning as described in group II. Group VII {Polymyxin B (50 μM) in angiotensin II preconditioning; $n = 6$}, isolated heart was perfused for 5 min with K–H solution containing polymyxin B (50 μM) before subjecting it to four episodes of angiotensin II preconditioning. Each episode comprised of 5-min perfusions with K–H solution containing angiotensin II (100 nM) followed by 5-min perfusions with K–H solution containing polymyxin B (50 μM). Group VIII {Colchicine (5 mg/kg i.p.) treated; $n = 6$}, rats were administered colchicine (5 mg/kg i.p.) and 30 min after the administration of colchicine, rats were sacrificed and hearts were removed from these animals. Isolated hearts were retrogradely perfused for 45 min and then subjected to 30 min of global

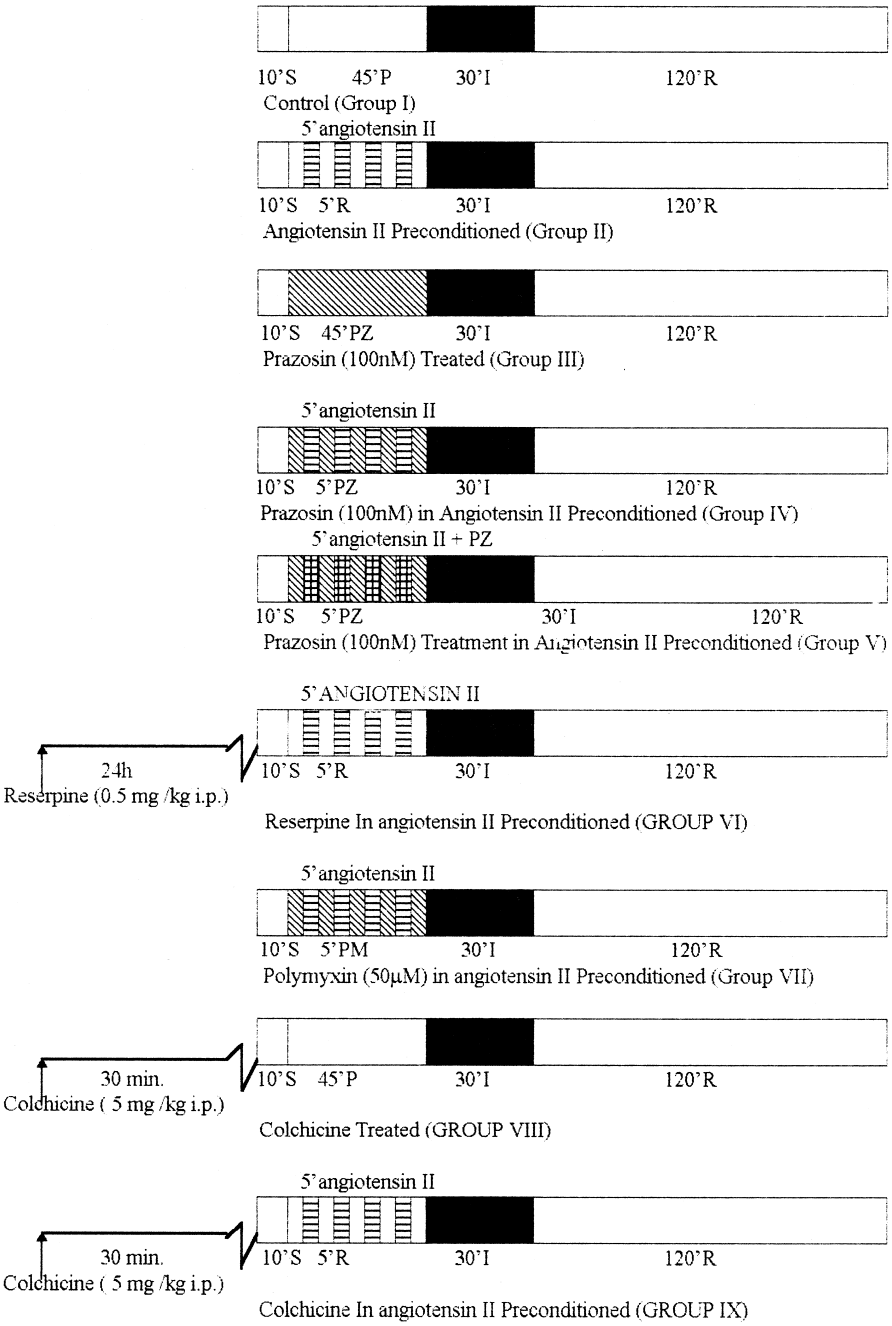

Fig. 1. Diagrammatic representation of experimental protocol. S = stabilization; P = perfusion with K–H solution; I = global ischaemia; R = reperfusion with K–H solution; angiotensin II, PZ or PM = perfusion with K–H solution containing angiotensin II (100 nM), prazosin (100 nM) or polymyxin (50 μM), respectively.

ischaemia followed by reperfusion with K–H solution for 120 min. Group IX {Colchicine (5 mg/kg i.p.) in angiotensin II preconditioning; $n = 6$}, rats were administered colchicine (5 mg/kg i.p.) and 30 min after administration of colchicine, animals were sacrificed and hearts were removed from these animals. Isolated hearts were retrogradely perfused for 5 min with K–H solution before subjecting them to four episodes of angiotensin II preconditioning as described in group II.

Coronary effluent was collected for 1 min immediately after stabilisation, before global ischaemia and immediately, 5, 15 and 30 min after reperfusion for lactate dehydrogenase and creatine kinase estimations.

2.5. Hemodynamic assessment of reserpinisation

To confirm depletion of catecholamines by reserpine, tyramine (2 mg/kg i.v.) induced change in mean arterial

blood pressure was noted in thiopental anaesthetised rats after 24 h of vehicle or reserpine (0.5 mg/kg i.p.) treatment. Left carotid artery and right juglar vein were cannulated to record mean arterial blood pressure using Condon's manometer and for tyramine administration, respectively.

2.6. Statistical analysis

Values for enzymatic data and infarct size are expressed as mean ± SEM. One-way analysis of variance was employed to calculate the statistical significance. Studentised range test and Dunnett's test were used as post hoc tests for multiple comparisons between groups and comparison with control group, respectively. $P < 0.05$ was considered to be statistically significant.

3. Results

Mean arterial blood pressure was significantly decreased in reserpinised rats (116 ± 3 vs. 100 ± 5 mm Hg). Moreover, reserpine pretreatment markedly attenuated tyramine (2 mg/kg i.v.) induced increase in mean arterial blood pressure (35 ± 0.34 vs. 12 ± 0.67 mm Hg; $P < 0.05$).

3.1. Effect of angiotensin II preconditioning on ischaemia–reperfusion-induced myocardial injury

Angiotensin II preconditioning markedly reduced the myocardial infarct size measured by volume and weight method (Fig. 2). Global ischaemia followed by reperfusion significantly increased the release of lactate dehydrogenase and creatine kinase in coronary effluent. Maximum release

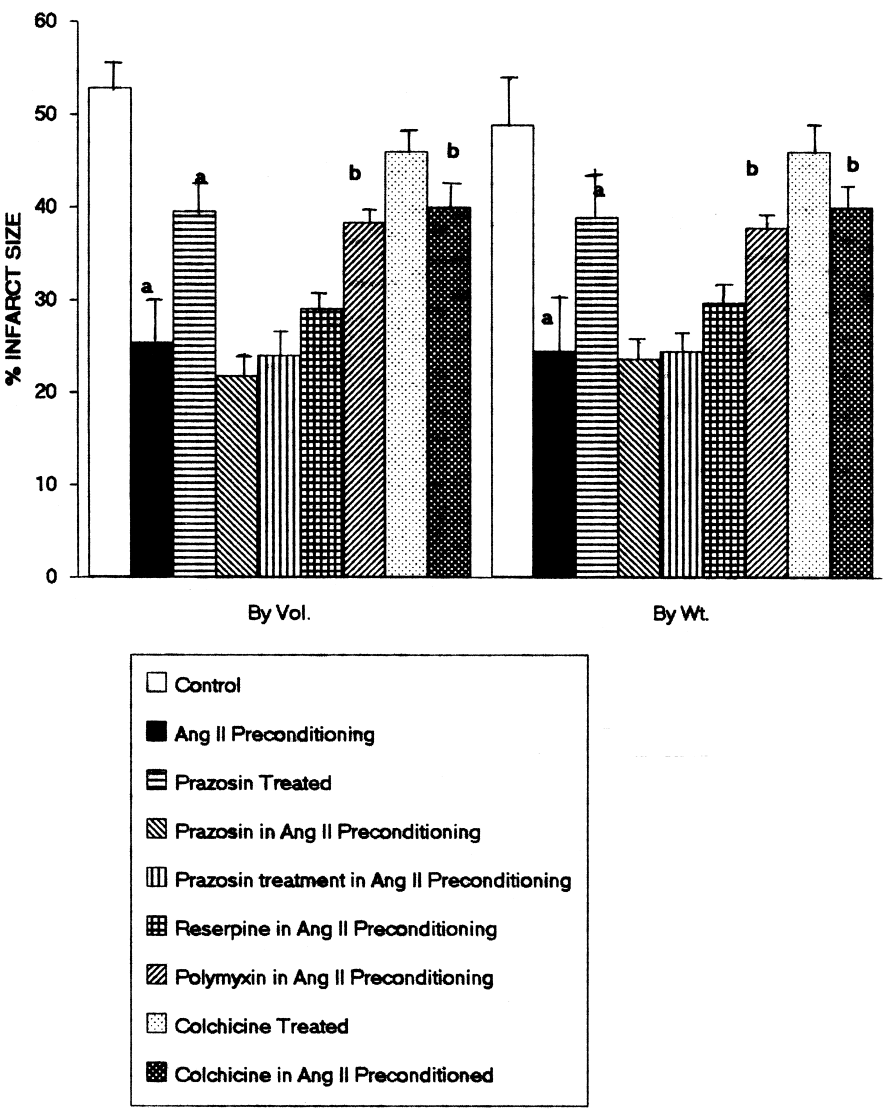

Fig. 2. Effect of pharmacological interventions on ischaemia–reperfusion-induced infarct size (measured by volume and weight method) in isolated rat heart. Values are expressed as mean ± SEM of six animals. a = $P < 0.05$ vs. control; b = $P < 0.05$ vs. angiotensin II preconditioning.

of lactate dehydrogenase was noted immediately and after 30 min of reperfusion (Fig. 3). On the other hand, peak creatine kinase release in coronary effluent was noted after 5 min of reperfusion (Fig. 4) and it declined subsequently with the ongoing reperfusion. Angiotensin II preconditioning significantly attenuated the increased release of lactate dehydrogenase and creatine kinase (Figs. 3 and 4).

3.2. Effect of prazosin and colchicine on ischaemia–reperfusion-induced myocardial injury

Administration of prazosin (α_1-adrenoceptor antagonist) significantly reduced myocardial infarct size, which occurred as consequence to sustained ischaemia and reperfusion (Fig. 2). Moreover, prazosin treatment before global ischaemia reduced ischaemia–reperfusion-induced release of lactate dehydrogenase and creatine kinase (Figs. 3 and 4). However, prazosin-induced reduction in infarct size and release of lactate dehydrogenase and creatine kinase was significantly less than that produced by angiotensin II preconditioning. Colchicine pre-treatment (microtubule disaggregator) produced no marked effect on myocardial infarct size, occurred as a result of global ischaemia and reperfusion (Fig. 2). Increased release of lactate dehydrogenase and creatine kinase observed as a consequence of ischaemia–reperfusion was also not altered with colchicine pre-treatment (Figs. 3 and 4).

3.3. Effect of various pharmacological interventions on cardioprotective effect of angiotensin II preconditioning

Prazosin administered during angiotensin II preconditioning or administered continuously before global is-

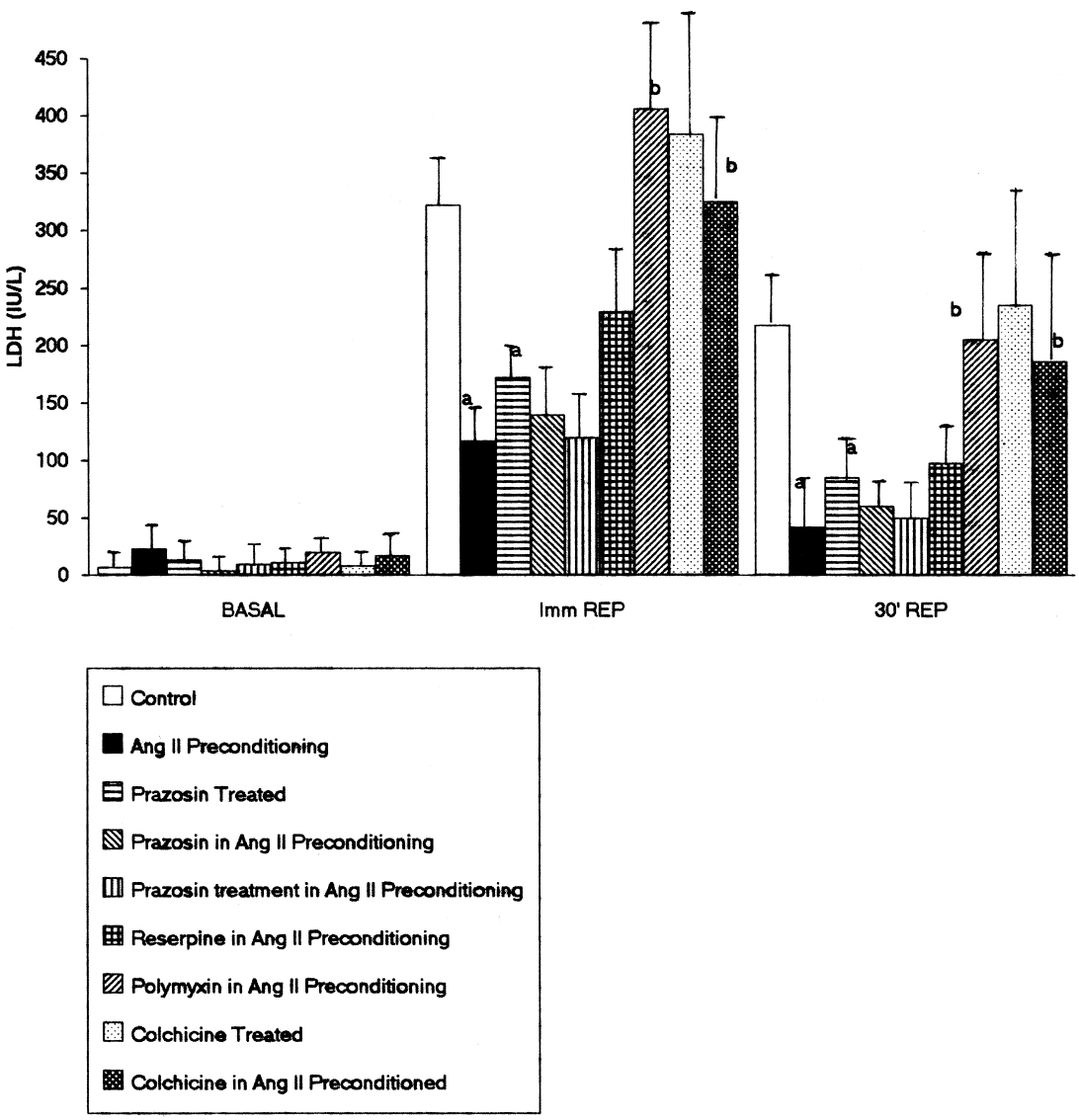

Fig. 3. Effect of pharmacological interventions on ischaemia–reperfusion-induced lactate dehydrogenase release in isolated rat heart. Values are expressed as mean ± SEM ($n = 6$). Lactate dehydrogenase was estimated in samples of coronary effluent collected before global ischaemia (Basal), immediately (Imm) and 30 min after reperfusion (REP). a = $P < 0.05$ vs. control; b = $P < 0.05$ vs. angiotensin II preconditioning.

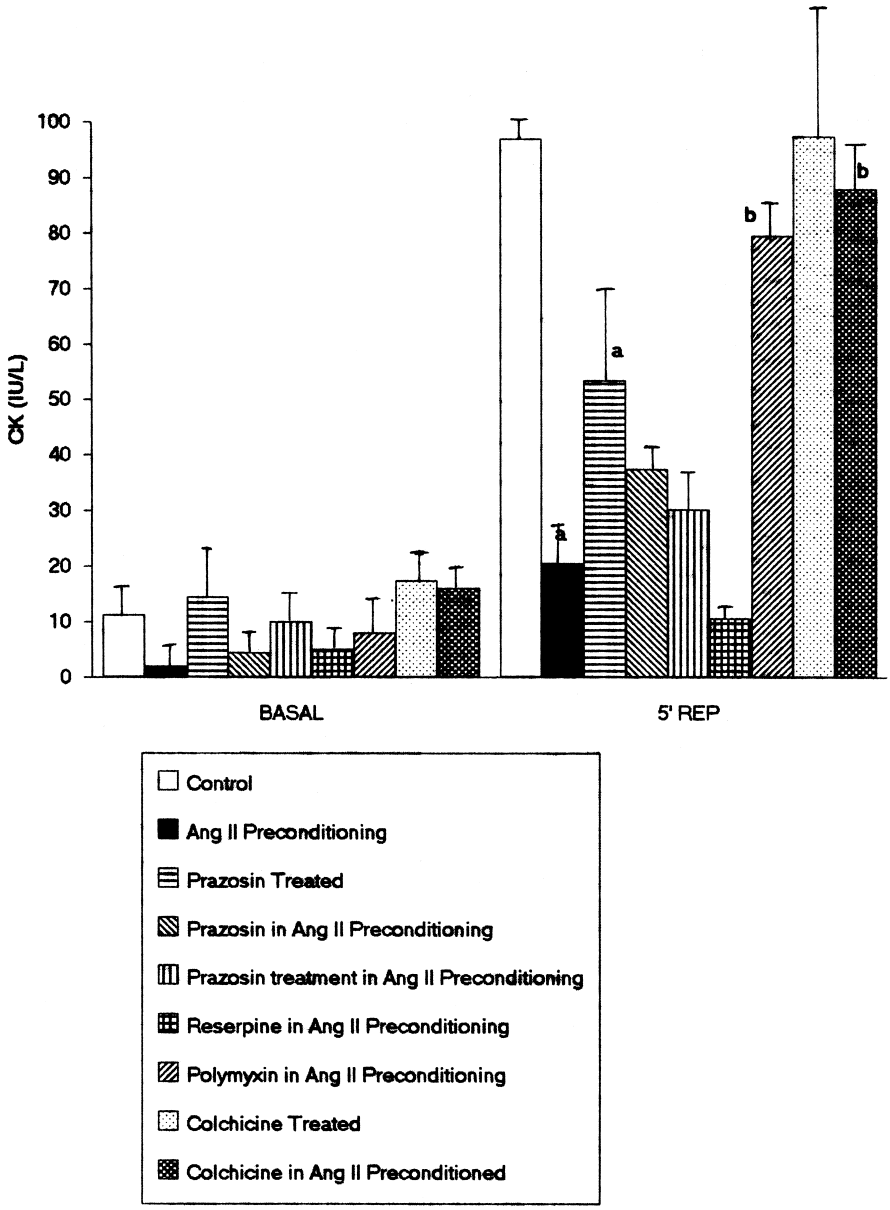

Fig. 4. Effect of pharmacological interventions on ischaemia–reperfusion-induced creatine kinase release in isolated rat heart. Values are expressed as mean ± SEM ($n = 6$). Creatine kinase was estimated in samples of coronary effluent collected before global ischaemia (Basal) and 5 min after reperfusion (REP). a = $P < 0.05$ vs. control; b = $P < 0.05$ vs. angiotensin II preconditioning.

chaemia, along with angiotensin II did not alter angiotensin II preconditioning-induced decrease in infarct size (Fig. 2) and release of lactate dehydrogenase and creatine kinase (Figs. 3 and 4). Moreover, reserpine treatment did not affect the cardioprotective effect of angiotensin II preconditioning measured in terms of decrease in infarct size and release of lactate dehydrogenase and creatine kinase. Colchicine treatment and administration of polymyxin B during angiotensin II preconditioning attenuated the cardioprotective effect {decrease in infarct size (Fig. 2) as well as decrease in lactate dehydrogenase and creatine kinase release (Figs. 3 and 4)} of angiotensin II preconditioning.

4. Discussion

Ischaemia is reported to activate the renin–angiotensin system (Juggi et al., 1993). Increased production of angiotensin II may jeopardize ischaemic myocardium by increasing cardiac contractility and constricting coronary vessels. It initiated to investigate the cardioprotective ef-

fect of angiotensin converting enzyme (ACE) inhibitors and angiotensin AT_1 receptor antagonists. The cardioprotective effect of ACE inhibitors is not reported to be mediated through angiotensin II but may be due to increased endogenous bradykinin production (Ertl et al., 1983), scavenging of free radicals (Martorana et al., 1990) and opening of ATP sensitive potassium channels (Sargent et al., 1993). Moreover, ACE inhibitors (Miki et al., 1993) and angiotensin AT_1 receptor antagonists (Richard et al., 1993) produced no limitation of infarct size. The above-mentioned findings do not implicate angiotensin II in ischaemia-induced cardiac injury. Therefore, the noted cardioprotective effect of angiotensin II preconditioning in the present study appears to provide a new clue to look into the issue of ischaemic myocardium. It may not be possible to use angiotensin II as a cardioprotective agent but study of cardioprotective effect of angiotensin II preconditioning may provide a new target site for therapeutic exploitation.

Angiotensin facilitates the release of norepinephrine from sympathetic nerve terminals (Musgrave et al., 1991). In buffer perfused isolated rat heart, sympathetic nerve terminals are the sole source of norepinephrine (Schomig et al., 1990), and norepinephrine is proposed to be an endogenous mediator of ischaemic preconditioning in rat (Banerjee et al., 1993; Hu and Nattel, 1995). Therefore, it may be possible that cardioprotective effect of angiotensin II preconditioning in rat may be mediated through the release of norepinephrine and activation of α_1-adrenoceptors. However, this contention is not supported by the results of the present study because administration of prazosin, an α_1-adrenoceptor antagonist, during angiotensin II preconditioning did not attenuate the cardioprotective effect of angiotensin II preconditioning. Since prazosin is a reversible α_1-adrenocepter antagonist, there is a possibility that α_1-adrenoceptors may become available for activation during angiotensin II perfusion. However, prazosin perfused along with angiotensin II as well as during intermittent reperfusion did not modulate the cardioprotective effect of angiotensin II preconditioning. These results suggest that cardioprotective effect of angiotensin II preconditioning may not be mediated through activation of α_1-adrenoceptors. In the present study, prazosin per se also produced cardioprotective effect as noted by Nayler et al. (1985). Therefore, it may be possible that per se cardioprotective effect of prazosin against ischaemia–reperfusion may have contributed to the noted cardioprotective effect of angiotensin II preconditioning in the presence of prazosin. However, this possibility appears to be remote because prazosin has not modulated the cardioprotective effect of angiotensin II preconditioning in the present study, while the same dose of prazosin in our earlier study has abolished the cardioprotective effect of ischaemic preconditioning (Sharma and Singh, 2000). Furthermore, depletion of biogenic amines by reserpinisation in study at hand also did not attenuate the cardioprotective effect of angiotensin II preconditioning. These observations suggest that norepinephrine release may not be involved in cardioprotective effect of angiotensin II preconditioning.

It is observed in our laboratory that angiotensin II preconditioning is mediated through activation of angiotensin AT_1 receptors (Sharma and Singh, 1999). Angiotensin AT_1 receptors are coupled through G proteins to phospholipase C in cardiomyocytes. Stimulation of angiotensin AT_1 receptors leads to increased formation of inositol triphosphate, diacylglycerol and a subsequent translocation of protein kinase C (Sadoshima and Izumo, 1993). Colchicine, in the dose employed in the present study, is reported to disrupt microtubules by binding to tubulin subunits (Limas and Limas, 1983; Sadoshima et al., 1992). Moreover, colchicine-induced microtubular disruption interferes with protein kinase C translocation in neuronal tissue (Bouron, 1997). Therefore, attenuation of cardioprotective effect of angiotensin II preconditioning with colchicine treatment in the study at hand may be explained on the basis of colchicine-induced microtubular disruption and consequent interference with protein kinase C translocation. This contention is partly supported by the observations of Liu et al (1994) with colchicine in ischaemic preconditioning in rabbit heart. Polymyxin B selectively inhibits protein kinase C by competing with co-factor phosphatidylserine for binding at its allosteric site (Mazzei et al., 1982; Casnellie, 1991). In the present study, administration of polymyxin B completely attenuated cardioprotective effect of angiotensin II preconditioning and similar effect of polymyxin B is also noted on ischaemic preconditioning (Tsuchida et al., 1994; Ytrehus et al., 1994). It suggests that cardioprotective effect of angiotensin II preconditioning may be mediated through protein kinase C. It is perhaps the first report in literature in rat species.

On the basis of the above discussion, it may be concluded that the cardioprotective effects of angiotensin II preconditioning may be mediated through protein kinase C and may not involve the release of norepinephrine or activation of α_1-adrenoceptors.

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