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Possible Involvement of Stretch-Activated Channels in the Cardioprotective Effect of Remote Aortic Preconditioning

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We sought to determine that whether acute and delayed cardioprotection offered by remote aortic preconditioning involves gadolinium sensitive-stretch activated channels. Hearts of sham operated rats, isolated 40 min and 24h after the isolation of abdominal aorta, were subjected to global ischaemia for 30 min followed by reperfusion for 120 min. Coronary effluent was analyzed for lactate dehydrogenase and creatine kinase release to assess the degree of cardiac injury. Myocardial infarct size was estimated macroscopically by using triphenyl tetrazolium chloride staining. Remote aortic preconditioning, immediately and 24 h before, subjecting the isolated heart to 30 min ischaemia and 120 min reperfusion markedly reduced lactate dehydrogenase and creatine kinase release in coronary effluent and myocardial infarct size. Intraperitoneal administration of gadolinium chloride (30 mg/kg) showed protection against sustained ischaemia and reperfusion whereas it did not seems to modulate the cardioprotective effect of remote aortic preconditioning. On this basis we concluded that the cardioprotective effect of remote aortic preconditioning did not involves stretch-activated channels.

Ischaemic preconditioning is a well-established phenomenon of ischaemia-induced protection against ischaemia either in the same tissue or in a tissue distant from the region in which preconditioning ischaemia is given. The latter phenomenon is termed as "remote preconditioning"[1]. Ischaemic preconditioning provides a dual phase of protection to the ischaemic myocardium. The first phase of protection appears immediately after the preconditioning episodes are given and wanes off gradually with time and is called 'classical preconditioning' or 'first window of protection (FWOP)[2]. The second phase of protection appears after 24 hours of the preconditioning trigger and is termed 'second window of protection (SWOP)[3]. Moreover, such protection has been shown to be mimicked by pharmacological stimulation of various plasma membrane receptors or ion channels[4-8]. Recently it has been observed that ischaemic episodes produced by aortic clamping induced a preconditioning-like effect which is due to volume overload since the effect is reported to be blocked by a stretch-activated ion channel blocker, gadolinium chloride ($GdCl_3$)[9]. Moreover, various studies have shown the presence of stretch-activated channels (SACs) in the rat heart[10]. On the basis of these observations we have generated a hypothesis that some stretch has been developed on the walls of the myocardium due to volume overload during renal remote preconditioning rather than due to vasoactive substances being released from the kidney. So the objective of the present study was to investigate the role of $GdCl_3$-sensitive SACs in ischaemia/reperfusion-induced myocardial injury with and without acute and delayed cardioprotective effects of remote aortic preconditioning.

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MATERIALS AND METHODS

Wistar rats of either sex weighing 200-300 g were used in this study. $GdCl_3$ (Sigma chemicals, St. Louis, MO) was

dissolved in distilled water immediately before use. All other reagents used in this study were of analytical grade (Loba Chemie, Mumbai).

Remote aortic preconditioning (RAPC):

Rats were anaesthetized with thiopental sodium (25 mg kg^{-1}, iv). A 2 cm long incision was given on the abdomen. Lower portion of the abdominal aorta was isolated and a suture (numbered 5/0) was passed beneath it away from the origin of renal arteries. Aorta was occluded by tying a shoe lace knot and was untied for reperfusion. Aorta was occluded for 5 min and was reperfused for 5 min. Four such episodes were used for preconditioning. In case animals were to be used after 24 h of aortic preconditioning, the abdomen was sutured in layers and animals were allowed to recover.

Isolated rat heart preparation:

Rats were anaesthetized by thiopental sodium (25 mg/kg i.v.) for performing surgical procedures and were heparinised 20 min prior to excision of the hearts (500 IU, i.p.). Hearts from heparinised rats were rapidly excised immediately or 24 h after four episodes of 5 min occlusion and 5 min reperfusion by occluding the abdominal aorta (in RAPC group) or after 40 min after isolation of abdominal aorta (sham group). The hearts were mounted on a Langendorff perfusion set-up[11]. The hearts were enclosed by a double walled jacket, the temperature of which was maintained by circulating water heated to 37°. The preparations were perfused at a rate of 6-8 ml/min and a constant perfusion pressure of 70 mmHg with Krebs Henseleit (K-H) solution (NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 25 mM; KH_2PO_4, 1.2 mM and D-glucose, 11 mM) maintained at 37° and bubbled with 95% O_2 and 5% CO_2. Two thin silver electrodes fixed at ventricular apex and origin of aorta were used to record electrocardiograms and heart rate. Global ischaemia was produced for 30 min by closing the inflow of physiologic solution, and was followed by reperfusion for 120 min.

First window of protection (FWOP):

Total thirteen groups of Wistar rats were employed to study the first and second window of protection immediately and after 24h, respectively, following RAPC. A diagramatic representation of experimental protocol is depicted in fig.1. In group 1 (sham group A; n=5), rats were subjected to surgical procedure for aortic isolation but aorta was not occluded. Hearts were excised 40 min after sham operation. Isolated hearts were perfused *ex vivo* and were subjected to global ischaemia for 30 min followed by reperfusion for 120 min. In groups II-VI ($GdCl_3$ treated control group A; n=5), rats were administered 0.5, 2, 3.5, 7.5

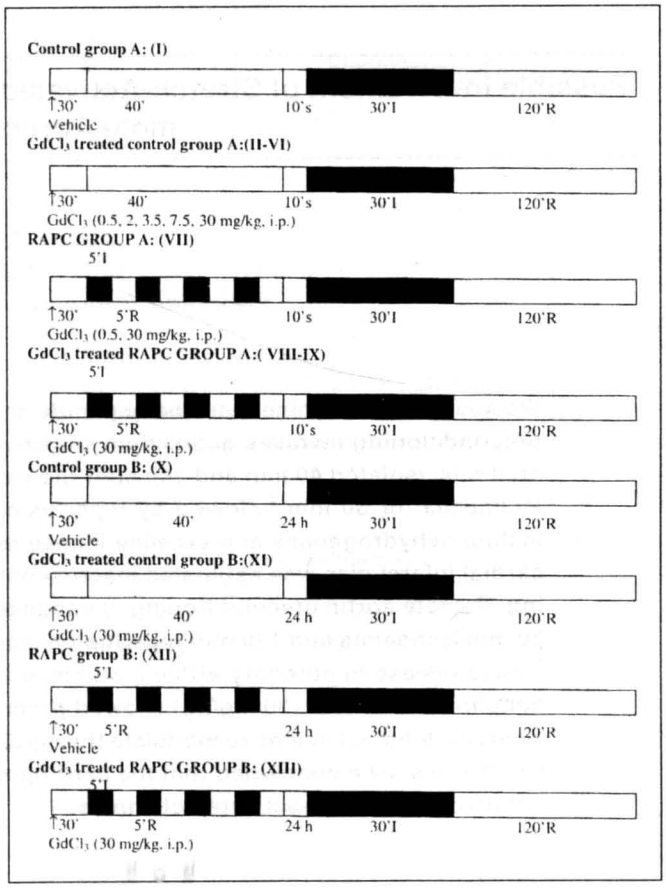

Fig. 1: Schematic representation of experimental protocol for acute and delayed remote aortic preconditioning.

I, R and S denotes global ischemia, reperfusion and stabilization respectively. Sham (□), RAPC (■), $GdCl_3$ (30 mg/kg) treated in sham (▥), $GdCl_3$ (30 mg/kg) during RAPC (▤).

and 30 mg/kg of $GdCl_3$ ip, respectively, 30 min before aortic isolation. Rest of the protocol was same as described in Group I. In group VII (remote aortic preconditioning group A; n=5), after aortic isolation, rats were subjected to RAPC as described earlier. Hearts were excised immediately after last episode of reperfusion, perfused *ex vivo* and were subjected to global ischaemia for 30 min followed by reperfusion for 120 min. In group VIII-IX ($GdCl_3$ treated remote aortic preconditioning group A; n=5), $GdCl_3$ (0.5 or 30 mg/kg, i.p.) was administered 30 min before subjecting the

rats to remote aortic preconditioning. Rest of the protocol was same as described in group VII.

Second window of protection (SWOP):

In group X (sham group B; n=5), rats were subjected to surgical procedure for aortic isolation but aorta was not occluded. Hearts were excised 24 h after sham operation. Rest of the protocol was same as in group I. In group XI ($GdCl_3$ treated control group B; n=5), $GdCl_3$ (30 mg/kg) was administered 30 min before aortic isolation. Rest of the protocol was same as described in group II. In group XII (RAPC group B; n=5), rats were subjected to remote aortic preconditioning and were excised 24 h after it. Rest of the protocol was same as in group VII. In group XIII ($GdCl_3$ treated RAPC group B, n=5), rats were administered $GdCl_3$ (30 mg/kg), 30 min before subjecting the rats to remote aortic preconditioning. Rest of the protocol was same as described in group VII.

Assessment of myocardial injury:

To determine the extent of myocardial injury following 30 min of global ischaemia and 120 min of reperfusion, release of creatine kinase (CK) and lactate dehydrogenase (LDH) was measured in the coronary effluent by using the method of Hughes[12] and the method of King[13], respectively. Coronary effluents were collected at immediate and 30 min of reperfusion for LDH estimation and at 5 min of reperfusion for CK estimation since their corresponding peaks was observed at the above mentioned time points during 120 min of reperfusion preceded by 30 min of global ischaemia. Values were expressed in International Units per litre (IU/l).

Myocardial infarct size:

Heart was removed from the Langendorff perfusion set-up. Both the auricles and the root of aorta were excised and ventricles were kept overnight at –4°. Frozen ventricles were sliced into uniform sections of 2-3 mm thickness. The slices were incubated in 1% triphenyl tetrazolium chloride (TTC) at 37° in 0.2 M TRIS buffer (pH 7.4) for 30 min. The normal myocardium was stained brick red, whereas the infarcted portion remained unstained. Infarct size was measured by the volume and weight method.

Statistical analysis:

Values for enzymatic data and infarct-size were expressed as mean±SEM. Statistical significance was calculated using one way ANOVA. Newman Keul's test was employed as post-hoc tests for comparison with control group and for multiple comparisons between groups, respectively.

RESULTS

Peak increase in release of LDH in coronary effluent of isolated rat heart subjected to global ischaemia and reperfusion was noted immediately and 30 min after reperfusion whereas peak increase in release of CK was noted after 5 min of reperfusion. $GdCl_3$ (2, 3.5, 7.5, 30 mg/kg, i.p.) administered 70 min before removing the heart for ischaemia/ reperfusion study *ex vivo*, markedly attenuated ischaemia/reperfusion-induced increase in release of LDH, CK and myocardial infarct size. On the other hand, low dose (0.5 mg/kg, i.p.) of $GdCl_3$ treatment produced no marked effect on ischaemia/reperfusion-induced increase in LDH and CK release and myocardial infarct size.

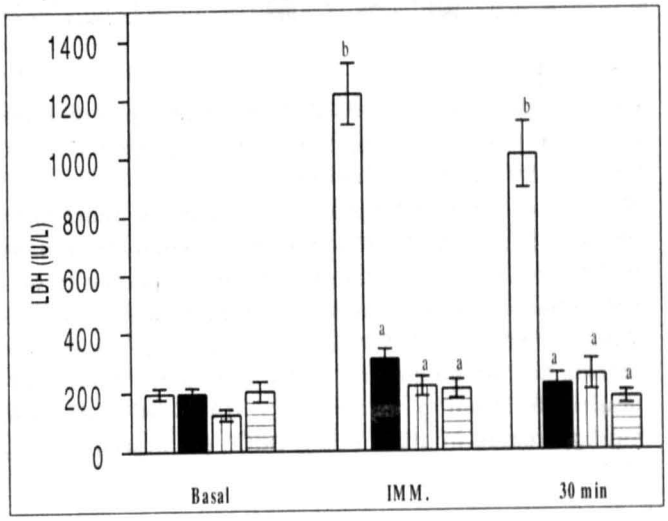

Fig. 2: Effect of acute RAPC and $GdCl_3$ pretreatment on LDH release from isolated rat hearts subjected to global ischemia and reperfusion

Effect of acute aortic preconditioning and gadolinium chloride ($GdCl_3$) pretreatment on lactate dehydrogenase (LDH) release from isolated rat heart subjected to global ischemia (30 min) followed by reperfusion (120 min). Values are mean±SEM of five experiments, P<0.05, Imm stands for immediate and RAPC indicates remote aortic preconditioning. Sham (□), RAPC (■), $GdCl_3$ (0.5 mg/kg) during RAPC (▥), $GdCl_3$ (30 mg/kg) during RAPC (▤).

Rat heart isolated immediately (acute) or 24 h (delayed) after four episodes of RAPC demonstrated a significant decrease in ischaemia/reperfusion-induced release of LDH (figs. 2 and 3), CK (figs. 4 and 5) and myocardial infarct size. Therefore, remote aortic preconditioning has produced acute and delayed cardioprotective effect. Administration of $GdCl_3$ 30 min before RAPC in low (0.5 mg/kg

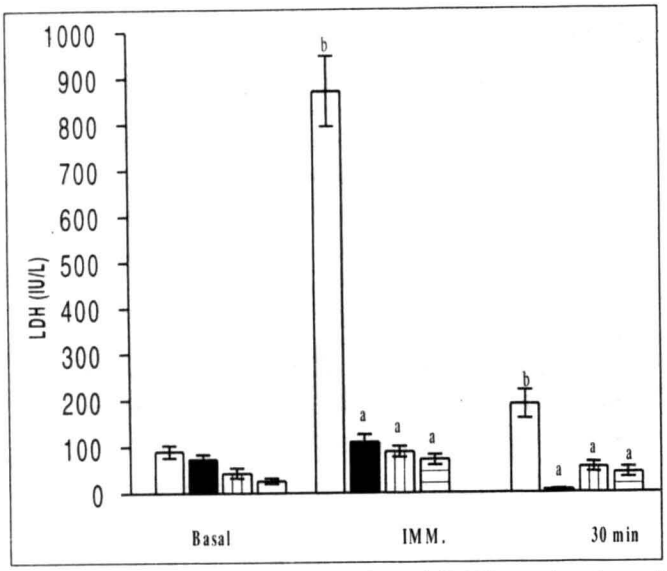

Fig. 3: Effect of delayed RAPC and $GdCl_3$ pretreatment on LDH release from isolated rat heart subjected to global ischemia and reperfusion

Effect of delayed aortic preconditioning and gadolinium chloride ($GdCl_3$) pretreatment on lactate dehydrogenase (LDH) release from isolated rat heart subjected to global ischemia (30 min) followed by reperfusion (120 min). Values are mean±SEM of five experiments, P<0.05, Imm stands for immediate and RAPC indicates remote aortic preconditioning. Sham (□), RAPC (■), $GdCl_3$ (30 mg/kg) treated in sham (▥), $GdCl_3$ (30 mg/kg) during RAPC (▤).

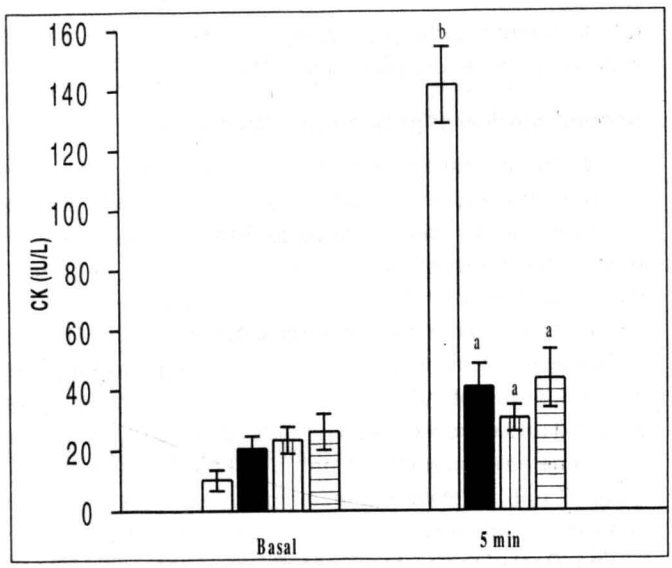

Fig. 4: Effect of acute RAPC and $GdCl_3$ pretreatment on CK release from isolated rat heart subjected to global ischemia and reperfusion.

Effect of acute remote aortic preconditioning and gadolinium chloride ($GdCl_3$) pretreatment on creatine kinase (CK) release from isolated rat heart subjected to global ischemia (30 min) followed by reperfusion (120 min). Values are mean±SEM of five experiments, P<0.05, RAPC indicates remote aortic preconditioning. Sham (□), RAPC (■), $GdCl_3$ (0.5 mg/kg) during RAPC (▥), $GdCl_3$ (30 mg/kg) during RAPC (▤).

i.p.) as well as in high (30 mg/kg i.p.) doses did not modulate significantly aortic preconditioning-induced decrease in LDH (figs. 2 and 3), CK (figs. 4 and 5) release and myocardial infarct size. $GdCl_3$ (0.5 and 30 mg/kg i.p.) pretreatment did not affect the acute and delayed cardioprotective effect of remote aortic preconditioning.

Global ischaemia for 30 min produced a marked decrease in coronary flow rate and heart rate (data not shown) and this decrease persisted during 120 min of reperfusion. $GdCl_3$ pretreatment and, acute and delayed RAPC produced no notable change in flow rate and heart rate.

DISCUSSION

Rat cardiomyocytes are reported to express mechanosensitive (MS) channels[14] and stretch-activated calcium channels (SACs), a subtype of MS channels, present abundantly in cardiomyocytes of rat[10]. Ventricular dyskinesia precipitated during ischaemia may lead to opening of MS channels, especially SACs. Therefore, the resultant influx of calcium may exacerbate ischaemic injury due to calcium overload. This contention is supported by the results of the present study because administration of $GdCl_3$, a selective blocker of MS channels[15] markedly attenuated ischaemia/reperfusion induced myocardial injury. These results are perhaps the first to implicate the MS channels in ischaemia/reperfusion injury. Moreover, it further supports an earlier observation that demonstrated an attenuation of ischaemia-induced ST-segment elevation by administration of $GdCl_3$ in dog[16].

After systemic administration $GdCl_3$ is reported to be precipitated in blood as carbonate and phosphate, which is subsequently taken up by tissue macrophages[17]. Resident tissue macrophages are reported to be present in the myocardium in the vicinity of the capillaries[18,19]. Therefore, it may be possible that the resident cardiac macrophages may have engulfed precipitated $GdCl_3$ that is released subsequently during myocardial ischaemia/reperfusion. It may

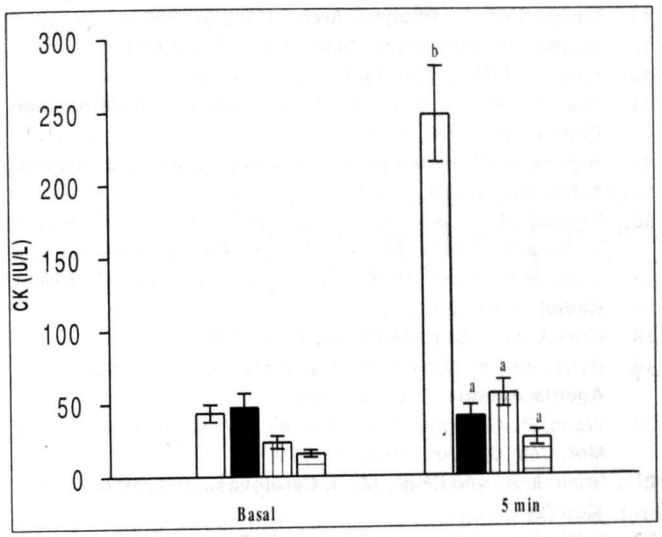

Fig. 5: Effect of delayed RAPC and ($GdCl_3$) pretreatment on CK release from isolated rat heart subjected to global ischemia and reperfusion

Effect of delayed remote aortic preconditioning and gadolinium chloride ($GdCl_3$) pretreatment on creatine kinase (CK) release from isolated rat heart subjected to global ischemia (30 min) followed by reperfusion (120 min). Values are mean±SEM of five experiments, P<0.05, RAPC indicates remote aortic preconditioning. Sham (□), RAPC (■), $GdCl_3$ (30 mg/kg) treated in sham (▤), $GdCl_3$ (30 mg/kg) during RAPC (▥).

account for the noted cardioprotective effect of $GdCl_3$ in isolated rat heart although it was administered *in vivo*. In the present study, an attempt was made to examine the dose-dependent cardioprotective effect of $GdCl_3$. It is interesting to note that $GdCl_3$ pretreatment in low (0.5 mg/kg, ip) as well as high (30 mg/kg, ip) doses did not alter the acute and delayed cardioprotective effect of RAPC. The selection of low and high dose is made on the basis that former produced no *per se* cardioprotective effect and the later is found to produce *per se* cardioprotection. However, all or none cardioprotective effect was noted with various doses of $GdCl_3$. It may suggest that cardiac macrophages from their large accumulation of $GdCl_3$ may release a small fraction of $GdCl_3$ during ischaemia and reperfusion, required to provide cardioprotection. However, data in hand is not sufficient to support this contention. Vasoactive substances have been implicated in the cardioprotective effect of ischaemic preconditioning[8, 20-21] but could not demonstrate the role of vasopressor agent like angiotensin in the renal preconditioning induced cardioprotection. Therefore, the present study was designed to investigate the role of MS channels in the observed cardioprotective effect of remote aortic preconditioning. Immediately and 24 h after four episodes of 5 min occlusion of aorta (distal to origin of renal arteries) followed by reperfusion for 5 min markedly protected the rat heart against ischaemia/reperfusion-induced myocardial injury. The observed acute and delayed cardioprotective effect of remote aortic preconditioning resembles earlier reported renal preconditioning induced cardioprotection. It is interesting to note that $GdCl_3$, a selective blocker of MS channels, pretreatment in low (0.5 mg/kg i.p.) as well as high (30 mg/kg i.p.) doses did not alter the acute and delayed cardioprotective effect of remote aortic preconditioning. The selection of low and high dose is made on the basis that a low dose (0.5 mg/kg) produced no *per se* direct cardioprotective effect and that a high dose (30 mg/kg) is documented in this study to produce *per se* cardioprotection against ischaemia/reperfusion injury. These observations suggest that $GdCl_3$ sensitive MS channels may not be involved in remote aortic preconditioning. These results are in contrast to some other reports, which have demonstrated attenuation of volume, overload induced cardioprotection by $GdCl_3$ in rabbit[9]. It suggests that $GdCl_3$ sensitive MS channels may be species specific. This contention is further supported by the observation of Falck *et al.* who demonstrated that $GdCl_3$ had no effect on the cardioprotective effect of hyperosmotic preconditioning in rat species[22]. Therefore, it may be suggested that $GdCl_3$ sensitive MS channel may not be involved in the cardioprotective effect of remote aortic preconditioning in rat species.

On the basis of results obtained in present study, it may be concluded that four episodes of 5 min occlusion of aorta followed by 5 min reperfusion significantly reduced ischaemia/reperfusion-induced myocardial injury recorded immediately and 24 h after remote aortic preconditioning. It suggests that remote aortic preconditioning produced an acute and delayed cardioprotective effect. $GdCl_3$ pretreatment markedly attenuated global ischaemia/reperfusion induced myocardial injury expressed in terms of infarct size and increased release of LDH and CK in coronary effluent. The noted cardioprotective effect may be due to blockade of $GdCl_3$ sensitive MS channels. Moreover, the resident cardiac macrophages may release a small fraction of $GdCl_3$ from its engulfed large store, to produce the reported all or none cardioprotection in isolated rat heart. Administration of $GdCl_3$ before remote aortic preconditioning did not alter the cardioprotective effect of remote aortic preconditioning. These result suggest that $GdCl_3$-sensitive SAC's or MS channel are not involved in acute and delayed cardioprotective

effect of remote aortic preconditioning.

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