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Hyperosmotic Pretreatment Reduces Infarct Size in the Rat Heart

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Summary

Preconditioning of the heart can be achieved by an ischemia/reperfusion stimulus, but also by stretching of the heart by an acute volume overload. Since manipulations of the extracellular osmolality affects cell size, we hypothesized that hyperosmotic pretreatment of the isolated perfused rat heart could reduce infarct size following regional ischemia (RI). Langendorff perfused rat hearts were subjected to 30 min RI by ligature of the main branch of the left coronary artery followed by 120 min reperfusion (control group). Ischemic preconditioning (IP-5') was achieved by 5 min total global ischemia and 5 min reperfusion prior to RI. Hyperosmotic pretreatment was accomplished by perfusion with a hyperosmotic buffer (600 mOsm/kg H_2O by adding mannitol) for 1 min, 2 min or 5 min. At the end of the experiments, the hearts were cut into 2 mm slices, incubated with triphenyltetrazoliumchloride before scanning and computerized for estimation of infarct size. The average infarct size (as percentage of area at risk) in the control group was 42 % and was significantly reduced to 16 % by ischemic preconditioning and to 17 % by 2 min hyperosmotic pretreatment. Neither 1 min nor 5 min hyperosmotic pretreatment reduced infarct size as compared to the controls. The infarct reducing effect of 2 min hyperosmotic pretreatment was not blunted by inhibition of protein kinase C (chelerytrine chloride), the Na^+/H^+-exchanger (HOE 694) or stretch-activated anion channels (gadolinium chloride). The results indicate that short-lasting hyperosmotic perturbations of the extracellular environment may precondition the heart to a subsequent ischemic insult.

Key words

Preconditioning • Osmolar concentration • Chelerytrine chloride • Gadolinium chloride • HOE 694

Introduction

A brief ischemic episode followed by a brief period of reperfusion enhances myocardial tolerance to subsequent, more prolonged ischemic episodes (preconditioning) (Murry *et al.* 1986) Preconditioning was initially demonstrated after short-lasting ischemia/reperfusion, but pharmacological and mechanical stimuli may also precondition the heart (Grover *et al.* 1990). It has long been known that ischemia dilates the left ventricle and previous studies have indicated that stretch *per se* may induce preconditioning (Przyklenk *et al.* 1993, Ovize *et al.* 1994). Stretch of myocardial cells can also be induced by

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manipulations in the extracellular compartment such as exposure to hyposmotic solutions or to isosmotic solutions after hyperosmotic exposure (relative hyposmosmolality). Most cells swell when they are exposed to hyposmotic solutions and shrink when exposed to hyperosmotic solutions. Isolated rabbit cardiomyocytes shrank by 29 % when exposed to 540 mOsm/kg H_2O and swelled by 41 % on exposure to 180 mOsm/kg H_2O (Drewnowska and Baumgarten 1991) Preliminary calculations from our laboratory indicate a rapid loss of 23 % of cell water when the isolated rat heart is perfused with 600 mOsm/kg H_2O. Thus, manipulations in cell size (stretch and shrinkage) can be achieved by changes in the surrounding osmolality.

Based upon the previous reports on mechanical preconditioning, we wanted to examine if stretch induced by pretreatment with isosmotic perfusion after a hyperosmotic stimulus could afford any immediate protection against ischemia. This was accomplished by infarct size measurements in an isolated rat heart model where hearts were perfused with a hyperosmotic solution (600 mOsm/kg H_2O) followed by isosmolal buffer and regional ischemia.

Material and Methods

Perfusion procedure

Seventy-five male rats (Sprague-Dawley, 200-250 g) were anesthetized with diethyl ether and heparinized (0.2 ml Heparin 1000 IU/ml i.v., Novo Nordisk, Copenhagen, Denmark). The experiments were performed according to the guidelines of the local ethical committee for animal experiments. Hearts were rapidly removed from the animals and after a brief (1 min) stay in ice-cold perfusion medium connected to an aortic cannula of a standard Langendorff retrograde perfusion system. Perfusion was in the global mode at 37 °C with a constant perfusion pressure of 100 cm H_2O.

The perfusion medium was glucose-containing Krebs-Henseleit bicarbonate buffer (gKHBB) containing (mM): NaCl 118.0, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.4 and glucose 11.1. The perfusate was equilibrated with 95 % O_2 and 5 % CO_2 to obtain a pH of 7.35-7.40 and was filtered (pore size 0.8 μm) before use. The hyperosmotic perfusate was made by adding mannitol to gKHBB.

Osmolality was measured by the freeze-point depression technique (Osmomat 030, Gonotec, Berlin, Germany) and was 290±4 mOsm/kg H_2O for gKHBB. Water for preparing the perfusates and dissolving drugs was tissue culture grade (18.2 MΩ resistivity) obtained by reverse osmosis and ultrafiltration (Elgastat Prima 1-3 and Maxima, Elga Ltd., High Wycombe, UK).

The perfusion system consisted of two parallel lines (one for gKHBB and one for the hyperosmotic perfusate) and a three-way valve which allowed changing between the perfusates. Pharmacological agents were introduced in a sideline at 1 % of main flow (Terufusion Syringe Pump, Terumo Corp., Tokyo, Japan) unless otherwise stated.

A fluid filled latex balloon was introduced into the left ventricle (LV) and connected to a pressure transducer for the recording of LV developed pressure (LVDP = LV systolic pressure – LV end-diastolic pressure). The balloon was filled with 0.05 ml water (unstretched) before insertion into the left ventricle. Pressure signals were amplified (Quadbridge, AD Instruments Ltd., Hastings, UK), digitally converted and processed by computer (MacLab unit and software, AD Instruments Ltd., Hastings, UK). The coronary flow rate (CFR) was measured by timed collections of the effluate.

Regional ischemia (RI) was accomplished with a snare around the main stem of the left coronary artery with a 3-0 silk suture. The ends of the suture were passed through a button (diameter 5 mm) and a vinyl tube. The thread ends were fastened to a device which allowed the two ends to be pulled gradually apart from each other perpendicularly to the vinyl tube. During this procedure, CFR and LVDP were followed continuously and the snare was secured with a clip immediately after a fall in both LVDP and CFR. Reperfusion was attained by removal of the clip and was followed by an immediate increase in CFR and LVDP. To maintain a constant temperature of 37 °C in all parts of the heart throughout the experiment, the hearts were submerged in gKHBB in a heated heart chamber.

Experimental protocol

After an initial stabilization period of 20 min, CFR and LVDP were measured. Five hearts with a CFR below 7 ml/min and/or LVDP below 70 mm Hg were excluded from further study. In seven hearts, an aortic root edema developed immediately during reperfusion with an apparently excessive coronary flow rate due to leakage from the aortic root. These hearts were also excluded from further study. In the control group, hearts were exposed to 30 min RI and 120 min reperfusion. In the preconditioning group (IP-5'), hearts were exposed to 5 min global ischemia (GI) followed by 5 min reperfusion, 30 min RI and 120 min reperfusion.

Hyperosmotic pretreatment was accomplished by a single period of hyperosmotic perfusion (600 mOsm/kg H_2O) for 1 min (600-1′), 2 min (600-2′) or 5 min (600-5′ followed by 5 min gKHBB, 30 min RI and 120 min reperfusion. A preconditioning effect of hyperosmotic pretreatment was postulated to be mediated through protein kinase C (PKC), the Na^+/H^+-exchanger and/or stretch-activated anion channels (SAC). To test this hypothesis, chelerytrine chloride (2 μM, selective inhibitor of PKC), and HOE 694 1 μM, selective inhibitor of the Na^+/H^+-exchanger) were introduced 10 min prior to and continued throughout the hyperosmotic pretreatment. In pilot experiments, infusion of 10 μM gadolinium chloride (inhibitor of SAC) gradually reduced LVDP and CFR to zero during the 10 min pretreatment. LVDP and CFR recovered slowly and only partly after cessation of infusion of gadolinium chloride. Because of these effects, gadolinium chloride was added to the hyperosmotic perfusate (10 μM) and delivered only during the hyperosmotic perfusion (2 min).

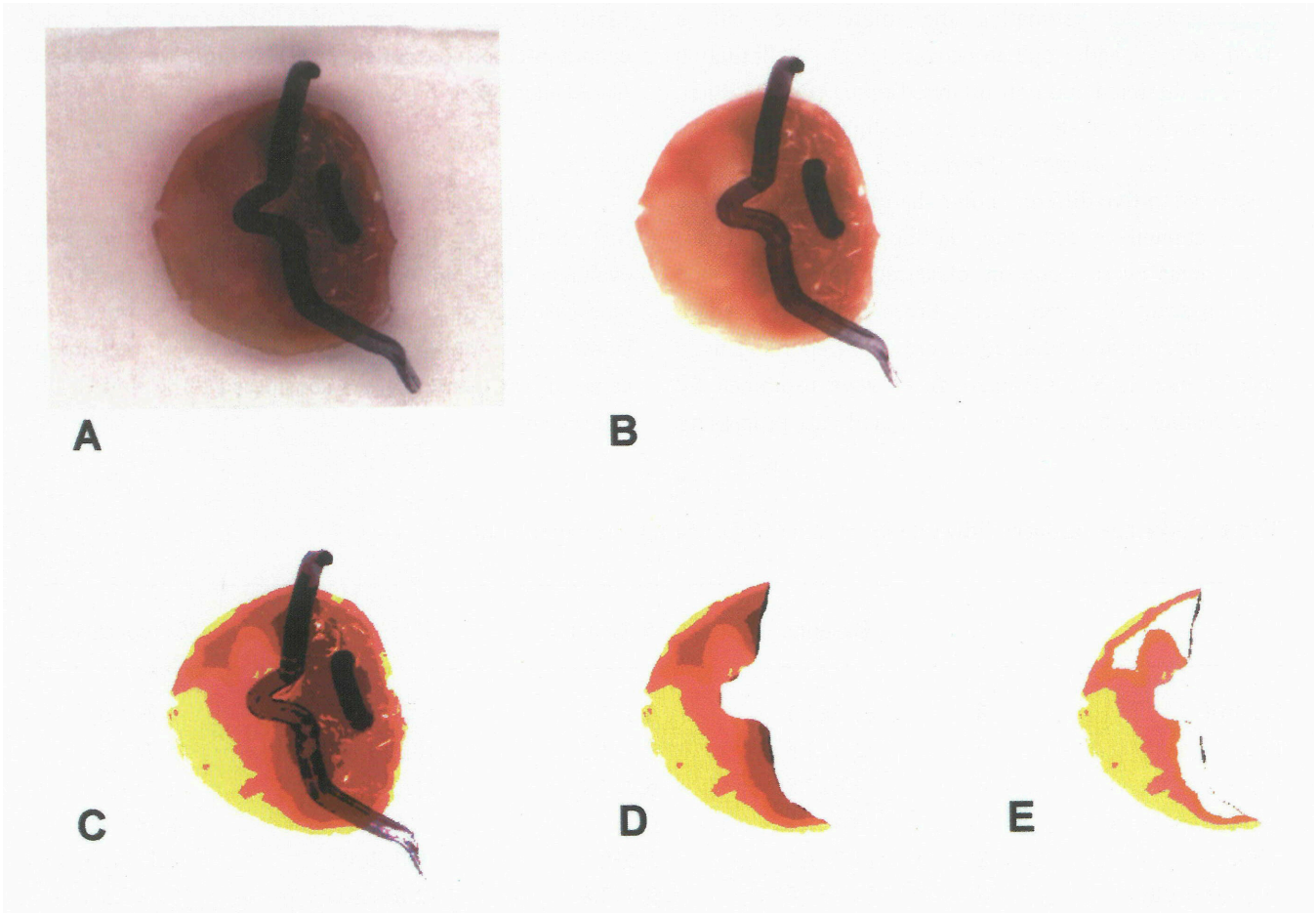

Fig. 1. *Infarct size measurement. After incubation of heart slices in TTC and formaldehyde, the slices were mounted between two cover glasses and illuminated with UV-light. Area at risk (AAR) was traced as the non-fluorescent zone. The cover glasses with the heart slices were then scanned. A: Primary scan of the heart slices between the cover-glasses. The black line separates AAR and the area not at risk, the latter on the upper part with a vertical black line. B: Adjustment of light and contrast. C: Posterizing of picture B and determination of pixel size of the whole heart slice. D: Removal of area not at risk and measurement of AAR. E: Removal of the non-infarcted area in AAR and measurement of the pixel size of the infarcted area in AAR.*

Measurement of infarct size

At the end of the experiment, the ligature was tightened and 2 ml of a 0.5 % suspension of zinc-cadmium fluorescent particles was slowly injected into the aortic root to mark the area at risk (AAR) as the non-fluorescent zone. The hearts were weighed and frozen overnight, thereafter cut perpendicular to the long axis in 2 mm slices, thawed and incubated in a 1 % solution of 2,3,5-triphenyltetrazoliumchloride (TTC) in phosphate buffer for 20 min at 37 °C. The slices were then immersed in 10 % formaldehyde for 72 h to enhance the contrast and then mounted between two cover-glasses. Area not at risk was marked as the fluorescent area under UV-light. The cover-glass with the heart slice and the

pen-marked border dividing the fluorescent and non-fluorescent area was thereafter scanned (Snap Scan, Agfa and Adobe Photoshop, version 4.0). In TTC-stained heart slices, the white areas are infarcted regions whereas the red areas are not infarcted.

The transition between infarcted and non-infarcted areas are in our opinion not clear-cut in the buffer-perfused rat heart, and a significant bias can thus be introduced by subjective assessment of the infarct area. We therefore developed a model in pilot experiments for estimating the infarct size with a standardized and operator-independent delimitation between infarcted and non-infarcted tissue (Fig. 1). First, the scanned heart slice picture was lighted up and the contrast was adjusted. Thereafter, the picture was posterized to five different color shades depending on the initial amount of red color in the heart slice. These adjustments were done in close agreement with the original scans of several heart slices and the procedure was compared and adjusted to previous reports (Bugge and Ytrehus 1996). All heart slices were examined by blind testing with the same scanning and data processing procedure. The pixel size of the whole heart slice, AAR and area not at risk was determined (pen-marked border was still visible). The pixel size of the infarcted area in AAR was determined and the ratio of infarct size vs AAR was finally calculated.

Chemicals

Chelerytrine chloride, gadolinium chloride and TTC were purchased from Sigma Chemical Co. (St. Louis, MO, USA). HOE 694 was a gift from Hoechst Marion Roussel (Frankfurt, Germany) and zinc-cadmium-chloride (particle size 1-10 μm) was purchased from Duke Scientific Corp. (Palo Alto, CA, USA).

Statistics

All values in the tables are expressed as means ± S.D. Statistical differences between the groups were evaluated by one-way analysis of variance unless otherwise stated. *Post hoc* analyses were performed with Bonferroni's multiple comparison test. A probability value of less than 0.05 was considered to be statistically significant.

Table 1. Average coronary flow rate in ml/min (±S.D.) during the experiment

	N	Baseline	5' ischemia	5' reperfusion	120' reperfusion
Control	8	11.5±1.1	6.3±1.4	11.8±2.4	7.8±2.6
IP 5'	9	11.3±1.5	5.8±1.3	10.2±1.5	6.7±1.9
600-1'	6	11.6±3.8	6.7±2.9	11.1±2.3	7.2±1.7
600-2'	7	13.4±1.1	6.5±0.7	11.6±1.4	8.7±1.9
600-5'	8	11.7±1.6	5.0±0.9	9.6±1.4	7.7±2.3
600-2'+ HOE	5	15.7±2.8*	9.0±3.8	12.8±4.6	9.7±2.8
600-2'+ Chel	5	17.2±2.5*	8.8±2.6	12.2±1.6	7.6±1.4
600-2'+ $GdCl_3$	8	12.5±1.9	4.6±1.3*	7.2±1.5*	6.3±1.2

Baseline = measurements immediately prior to regional ischemia (control and IP 5') and prior to hyperosmotic pretreatment, 5' ischemia = 5 min after onset of regional ischemia, 5' reperfusion = 5 min after onset of reperfusion of the ischemic area, 120' reperfusion = 120 min after onset of reperfusion.

IP 5` = After stabilization, 5 min total global ischemia, 5 min isosmotic reperfusion, 30 min regional ischemia and 120 min reperfusion.

600-1', 600-2' and 600-5': After stabilization, 1 (600-1'), 2 (600-2') or 5 (600-5') min of total global perfusion with hyperosmotic perfusate (600 mOsm/kg H_2O), 5 min isosmotic reperfusion, 30 min regional ischemia and 120 min reperfusion.

600-2'+ HOE and 600-2'+ Chel: Infusion of 1 μM HOE 694 (HOE) or 2 μM chelerytrine chloride (Chel) 10 min prior to and throughout hyperosmotic perfusion (2 min 600 mOsm/kg H_2O), thereafter as 600-2'.

600-2'+ $GdCl_3$: After stabilization, 2 min total global perfusion (600 mOsm/kg H_2O) containing 10 μM $GdCl_3$ followed by 5 min gKHBB, 30 min regional ischemia and 120 min reperfusion. *p<0.05 vs control.

Results

Functional variables

Values for CFR and LVDP are shown in Tables 1 and 2. Infusion of chelerytrine chloride and HOE 694 increased CFR significantly, whereas baseline values for the other groups did not differ significantly from the controls. CFR in the gadolinium chloride group was significantly lower than in the controls during RI and the early reperfusion period. LVDP was not significantly different between any of the groups throughout the experiment.

Table 2. Average left ventricular pressure in mm Hg (±S.D.) during the experiment

Group	Baseline	5' ischemia	5' reperfusion	120' reperfusion
Control	136±25.5	50±9.8	104±13.5	86±14.7
IP 5'	137±21.2	65±20.0	113±24.4	81±19.8
600-1'	104±36.7	26±12.0	95±21.4	70±16.7
600-2'	137±28.3	41±18.3	100±12.6	85±22.8
600-5'	110±15.5	25±12.7	95±13.9	76±10.0
600-2'+ HOE	102±27.9	38±16.3	84±18.9	83±23.4
600-2'+ Chel	96±12.2	35±14.1	103±11.0	72±19.8
600-2'+ $GdCl_3$	108±29.6	28±15.9	76±17.8	62±16.3

For legend see Table 1.

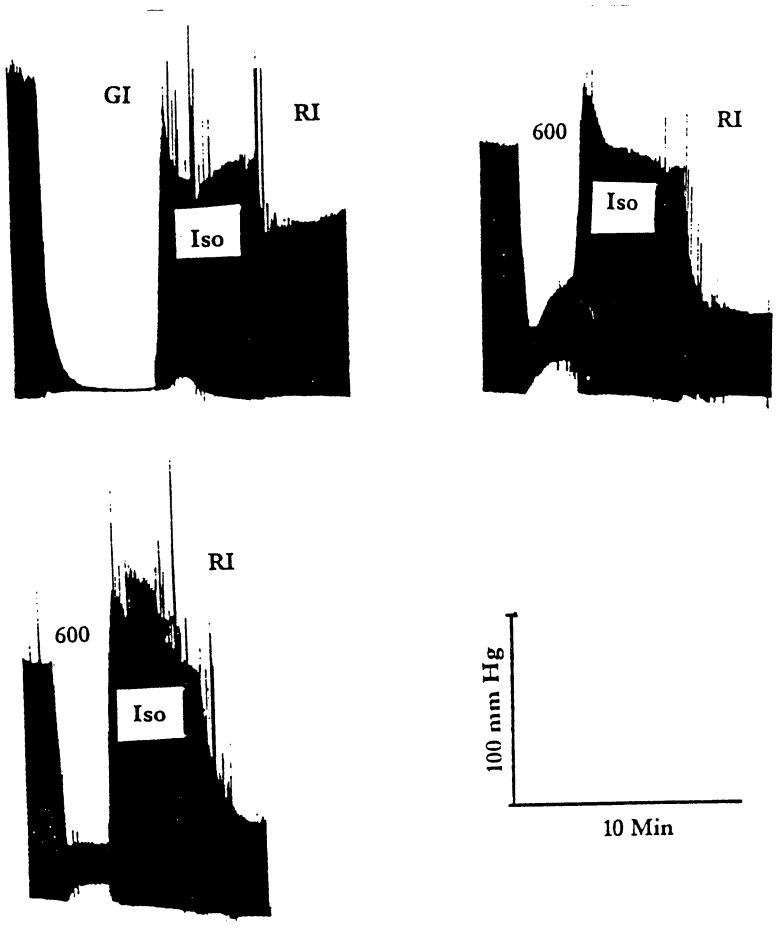

Fig. 2. *Representative tracings of the left ventricular pressure curve. A: Ischemic preconditioning group; perfusion with isosmolal buffer followed by 5 min global ischemia (GI), thereafter 5 min isosmotic reperfusion (Iso) before onset of regional ischemia (RI). B: Perfusion with isosmolal buffer followed by 2 min perfusion with 600 mOsm/kg H_2O buffer (600), thereafter 5 min isosmolal perfusion (Iso) before onset of RI. C: Same as B, but with HOE 694 (1 μM), an inhibitor of the Na^+/H^+-exchanger before and during the hyperosmotic perfusion.*

In the group subjected to ischemic preconditioning (5 min global ischemia), the developed pressure decreased immediately and was below 5 mm Hg for all the hearts after 5 min. When flow was re-established, LVDP increased rapidly and was 20 % below baseline values prior to RI (results not shown). In the groups subjected to hyperosmotic treatment, LVDP decreased to 25 % and partly recovered to 35 % of baseline values during hyperosmotic perfusion. Treatment with 1 μM HOE 694 abolished this contractile recovery. Re-exposure to gKHBB resulted in a rapid increase of LVDP above the baseline, but immediately prior to RI, LVDP returned to baseline values. Representative recordings are shown in Figure 2.

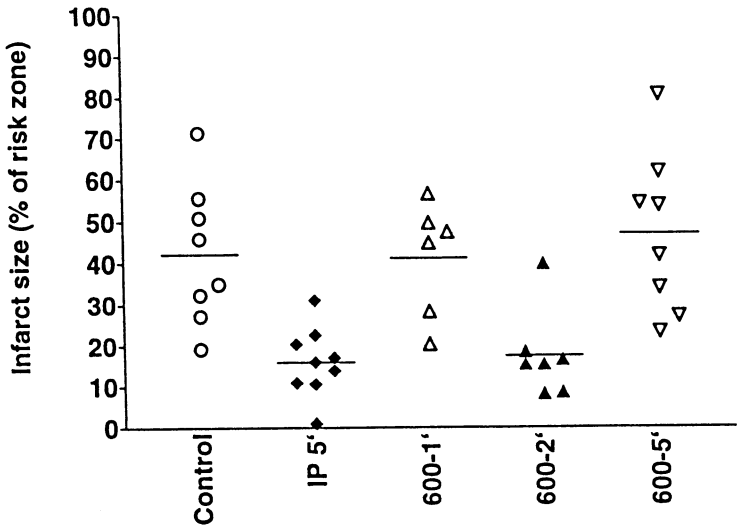

Fig. 3. *Infarct size as percentage of area at risk. Control: control group; IP-5': ischemic preconditioning; 600-1', 600-2'and 600-5': 1 min (600-1'), 2 min (600-2') or 5 min (600-5') of hyperosmotic perfusion. For further details see Materials and Methods.*

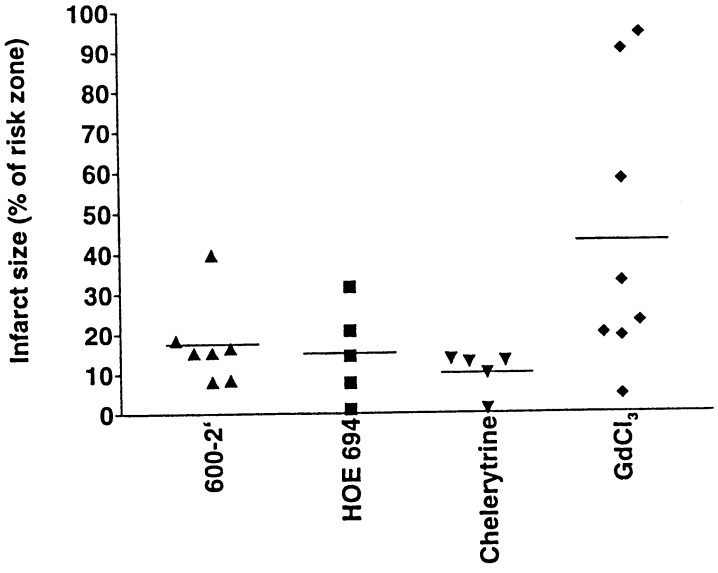

Fig. 4. *Infarct size after attempts to inhibit the infarct reducing effect of 2 min hyperosmotic pretreatment. 600-2'; 2 min hyperosmotic pretreatment; HOE 694; Infusion of 1 μM HOE 694 10 min prior to and throughout hyperosmotic perfusion (2 min 600 mOsm/kg H_2O); Chelerytrine; Infusion of 2 μM chelerytrine chloride 10 min prior to and throughout hyperosmotic perfusion (2 min 600 mOsm/kg H_2O); $GdCl_3$; 2 min total global perfusion (600 mOsm/kg H_2O) containing 10 μM $GdCl_3$. For further details see Materials and Methods.*

Infarct size

Infarct size in the different groups expressed as percentage of AAR is shown in Figure 3. In the control group, the average infarct size was 42 %. Ischemic pretreatment with 5 min GI reduced the average infarct size by 62 % (from 42 % to 16 %, $p<0.05$). The effect of hyperosmotic perfusion on infarct size was examined at three different times, i.e. 1 min, 2 min and 5 min. Neither 1 min nor 5 min exposure to 600 mOsm/kg H_2O reduced the infarct size compared to the control. Exposure to the

hyperosmotic buffer for 2 min prior to RI reduced the average infarct size by 58 % compared to the controls (from 42 % to 17 %, $p<0.05$).

To elucidate the possible mechanisms involved in the effect of hyperosmotic perfusion (2 min), HOE 694, chelerytrine chloride and gadolinium chloride were tested (Fig. 4). HOE 694 and chelerytrine chloride did not abolish the infarct-reducing effect of the 2 min hyperosmotic pretreatment (infarct sizes: HOE 694; 15 %, chelerytrine; 10 %, not significantly different from 600-2'). With gadolinium chloride, five of the eight hearts developed infarcts below 33 % whereas the remaining three hearts developed large infarcts despite a recovery of physiological variables which did not significantly differ from the other hearts. The median infarct size increased from 15 % (600-2') to 28 % ($GdCl_3$) with 10 μM gadolinium chloride ($p=0.054$, Mann-Whitney unpaired, two-tailed test). This indicates that the infarct-reducing effect of hyperosmotic pretreatment was abolished by gadolinium chloride in some, but not in all hearts.

Discussion

In this study, we have demonstrated that brief hyperosmotic pretreatment reduces infarct size following regional ischemia in the isolated rat heart. The duration of the hyperosmotic stimuli, however, seems to be of importance as a shorter (1 min) or longer (5 min) exposure did not afford any protection. Three lines of evidence made us to postulate why hyperosmotic pretreatment might be beneficial in preconditioning the heart to ischemia; the Ca^{2+}/protein kinase C axis, activation of the Na^+/H^+-exchanger and activation of stretch-activated anion channels (SAC). First, in experiments with sheep Purkinje fibers and ventricular strands exposed to hyperosmotic perfusates, a three- to four-fold increase in $[Ca^{2+}]_i$ was observed when solutions with twice isosmolal strength were applied (Lado *et al.* 1984). The same rapid and substantial elevation in $[Ca^{2+}]_i$ have been observed by others in ferret and guinea pig hearts exposed to hyperosmotic solutions (Allen and Smith 1987). Ca^{2+} has been suggested to play an important role in preconditioning through activation of PKC. Rat hearts exposed to an elevated extracellular $[Ca^{2+}]$ prior to total global ischemia (2.3 mM vs. 1.8 mM) achieved cardioprotection comparable to ischemic preconditioning, an effect which was lost when PKC was inhibited with chelerytrine chloride (Miyawaki and Ashraf 1997). On the other hand, a rapid Ca^{2+}-independent activation of PKC (74 % increase in one min) was found after hyperosmotic exposure of Ehrlich ascites tumor cells (Larsen *et al.* 1994). Recently, ischemic preconditioning was demonstrated to induce selective translocation and activation of the Ca^{2+}-insensitive PKCε and PKCη without any demonstrable changes in total myocardial PKC (Ping *et al.* 1997). Thus, hyperosmotic stimuli may activate PKC through Ca^{2+}-dependent and Ca^{2+}-independent mechanisms. Whatever the link between elevations of $[Ca^{2+}]_i$, activation of PKC and subsequent preconditioning, we were not able to inhibit the cardioprotective effect of 2 min hyperosmotic perfusion with chelerytrine chloride. The concentration of 2 μM was chosen on the basis of the reported IC_{50} for this drug (Herbert *et al.* 1990). In a previous report, this concentration abolished ischemic preconditioning, but had no effect on infarct size in the non-preconditioned rat heart (Bugge and Ytrehus 1995). Our results therefore indicate that the infarct reducing effect of hyperosmotic pretreatment is not mediated through activation of PKC.

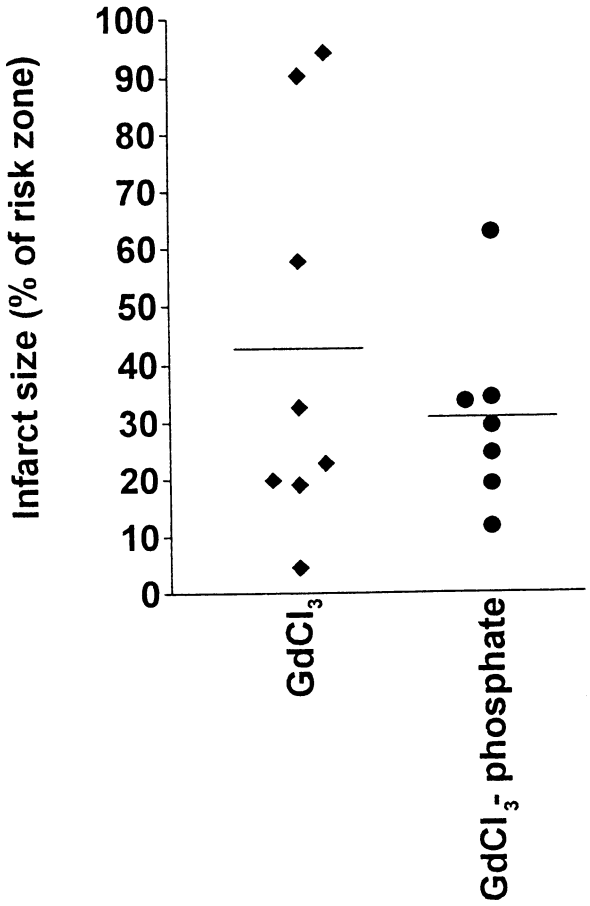

Fig. 5. *Infarct size after pretreatment with 2 min total global perfusion (600 mOsm/kg H_2O) with 10 μM $GdCl_3$ with buffer containing phosphate and without phosphate ($GdCl_3$ - phosphate).*

Secondly, activation of the Na^+/H^+-exchanger during hyperosmotic pretreatment could play a protective role. In a recent study it was observed that the Na^+/H^+-exchange activity was increased in the preconditioned myocardium as assessed by a faster recovery from acid loading (Ramasamy *et al.* 1995). The protective effect of ischemic preconditioning was attenuated by Na^+/H^+-exchanger inhibitors (EIPA) suggesting that an increased Na^+/H^+-exchange activity might be a prerequisite for the beneficial effect of ischemic preconditioning. The Na^+/H^+-exchanger can also be stimulated with hyperosmotic perfusion as in the present study. The contractile recovery, which was observed during hyperosmotic perfusion in the present study, was completely abolished by HOE 694 (Figs 2B and 2C). This suggests that a dose of 1 μM was sufficient to inhibit hyperosmotic activation of the Na^+/H^+-exchanger. Although speculative, activation of the Na^+/H^+-exchanger prior to ischemia might explain part of the lowered H^+ loading observed during the ischemic event in the preconditioned heart (Schjott *et al.* 1995). Since the hearts were exposed to isosmotic buffer without HOE 694 five minutes prior to RI, no persisting effect of the water-soluble drug would be expected. We could not, however, observe any effect of Na^+/H^+-exchange inhibition on the infarct-reducing effect of 2 min hyperosmotic pretreatment. It therefore seems unlikely that the effect is mediated through hyperosmotic activation of the Na^+/H^+-exchanger.

Finally, exposure of the heart to a hyperosmotic or to a relative hyposmotic environment (isosmolality after hyperosmolality) may lead to cell volume changes which could activate gadolinium-sensitive stretch-activated anion channels. Ovize *et al.* (1994) stretched canine hearts *in vivo* by an acute volume overload and found a reduced infarct size following 60 min of coronary artery occlusion. The effect was lost by pretreatment with gadolinium chloride, indicating that the effect was mediated through SAC activation. Intramyocardial injections of saline (4 x 0.15 ml) was recently demonstrated to reduce infarct size significantly in the rat heart *in vivo* (Whittaker *et al.* 1996). This effect was inhibited by addition of gadolinium chloride, indicating an effect of SAC on infarct size. The doses of gadolinium chloride used in the reported study was, however, about 40 times higher than in the present study and no hemodynamic effects of gadolinium chloride were reported. The reasons for the significant effect on infarct size without affecting contractile function remains unclear, but it could be due to differences between the *in vivo* and *in vitro* situation. Furthermore, the degree of cell swelling in a hyposmotic medium was reported to be significantly reduced by gadolinium chloride indicating that SAC play a role in modulating the cell volume of cardiac myocytes (Suleymanian *et al.* 1995). The use of gadolinium chloride as an inhibitor of SAC is, however, problematic since it may also act as an inhibitor of L-type calcium channels (Lacampagne *et al.* 1994). In pilot experiments, gadolinium chloride (10 μM) reduced LVDP and CFR to zero after 10 min isosmotic perfusion and thus had significant hemodynamic effects on the isolated hearts. To partly eliminate this problem, the hearts were exposed to 10 μM gadolinium chloride only during hyperosmotic perfusion (2 min). Nevertheless, both LVDP and CFR were below control values during ischemia and reperfusion, indicating a substantial effect on physiological variables (Tables 1 and 2). Another potential problem with the use of gadolinium chloride is the possible interaction with phosphate ions in the buffer. Rat ventricular myocytes exposed to 10 μM gadolinium chloride significantly reduced the fractional shortening and the calcium transient in a phosphate-free buffer indicating non-stretch dependent mechanisms of contractile development (Ward and White 1994). These effects were, however, not observed with phosphates in the buffer and it was therefore suggested that phosphate could potentially chelate the gadolinium chloride and neutralize its effects. To examine this possibility, we also investigated the effect of gadolinium chloride on infarct size using a phosphate-free buffer (Fig. 5). Neither the contractile function, nor the infarct size were significantly altered with the use of phosphate-free buffer, thereby excluding an effect of phosphate on gadolinium chloride in the present study.

The effect of gadolinium chloride on infarct size following pretreatment with 2 min hyperosmotic perfusion was highly variable (Fig. 4). In three hearts, the protective effect of 2 min hyperosmotic pretreatment was abolished, but in the remaining five hearts no effect could be demonstrated. We could not find any explanation for this striking variation in infarct size despite a close examination of physiological variables and experimental set-up. The slightly increased overall infarct size in the gadolinium chloride group could partly be due to its effects on coronary flow. A significantly lowered coronary flow was observed during reperfusion and this could have prevented sufficient reperfusion of the ischemic area. Thus, gadolinium chloride did not significantly attenuate the effect of hyperosmotic pretreatment on infarct size. The present results do not

allow any firm conclusions to be drawn about the role of SAC in hyperosmotic pretreatment.

Hyperosmotic exposure of the heart is frequently encountered in coronary angiography. Radiographic contrast media vary in composition, but osmolalities of 600-800 mOsm/kg H_2O, with increased viscosity and an apparently non-physiological electrolyte composition are routinely used. The heart is exposed to these substances for considerably shorter periods than in the present study (5-10 s), but the injections are repeated several times. We are not aware of any reports indicating a preconditioning effect of contrast media, but the results from the present study may, at least theoretically, indicate a hitherto undetected protective effect of contrast media prior to different cardiac interventions (surgery, PTCA, stenting).

In conclusion, we have demonstrated that short-lasting hyperosmotic exposure can induce preconditioning in the isolated perfused rat heart. The effect is independent of protein kinase C, activation of the Na^+/H^+-exchanger and of gadolinium-sensitive stretch-activated anion channels. The exact mechanism(s) of protection could not be determined in the present study. The potential clinical benefit of hyperosmotic preconditioning is not clear, but could involve presently used contrast media.

Abbreviations

RI = regional ischemia
GI = global ischemia
CFR = coronary flow rate
LVDP = left ventricular developed pressure
TTC = 2,3,5-triphenyltetrazoliumchloride
AAR = area at risk
PKC = protein kinase C
SAC = stretch-activated channels
μm = micrometer
μM = micromoles per liter
MΩ = megaohm

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