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Augmented taurine release is not the mechanism of ischemic preconditioning's cardioprotection

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Summary. In ischemic preconditioning (IPC) a brief ischemic period protects the heart from a subsequent ischemic insult by an unknown mechanism. Osmotic swelling has been proposed to be a major cause of cell death when ischemic tissue is reperfused. The present study tests whether the preconditioned heart during reperfusion might release more taurine, an important osmolyte in the cardiac myocytes, to decrease cellular osmolarity, oppose swelling, and preserve viability. We collected the coronary effluent from isolated rabbit hearts for 10 min before and 10 min after preconditioning with 5 min of global ischemia. The heart then experienced 15 min of global ischemia and effluent was collected during reperfusion for 40 min. A control group was studied similarly but without the preconditioning ischemia. Fifteen min of ischemia was chosen to avoid any taurine release caused by ischemic cell death. Taurine was measured with HPLC. In the IPC group there was a postischemic release over baseline of $5.09 \pm 1.51\,\mu$mol (approx 3.3% of the total taurine pool), whereas in the control group the release was not significantly different, $5.72 \pm 1.67\,\mu$mol. The percent of the taurine pool lost from each heart during reperfusion was calculated based on an assumption of a total content of $20\,\mu$M taurine/gm wet weight. Since the amount of taurine released by the isolated rabbit heart following ischemia was not different in preconditioned and non-preconditioned hearts, we conclude that reduced swelling through taurine release is not the mechanism of the cardioprotective effects of IPC.

Keywords: Cell swelling – Ischemia – Preconditioning – Taurine

Introduction

The amino acid taurine is found in high concentrations in cardiac cells (about 50–60% of the total free amino acid pool) and serves as an important osmoregulator. The intracellular concentration of taurine, therefore, has a major influence on cell volume regulation, and cardiac cells can release taurine in response to a fall in extracellular osmotic pressure (Huxtable, 1992). In the non-ischemic cell extracellular sodium serves as the primary extracellular osmolyte to balance the osmotic effects of intracellular proteins and metabolites. However, ischemia dramatically alters intracellular osmolyte content. Breakdown of a single molecule of ATP to 1 molecule of AMP and 2 inorganic phosphate molecules triples the osmotic pull of the original single nucleotide molecule. Furthermore, accumulation of intracellular sodium following ion pump failure leads to a fall in the transmembrane sodium gradient. Both of these factors cause ischemic cardiomyocytes to swell. Cell swelling with subsequent membrane failure has been proposed to be the major mechanism of cell death during ischemia – reperfusion (Steenbergen et al., 1985). If cells are subjected to an osmotic insult following ischemia, hypoxia, calcium overload or acidosis, they respond with a substantial release of taurine (Song et al., 1996; Suleiman et al., 1997; Schaffer et al., 2002a). This movement of taurine out of the cell is an important process in the regulatory volume change that accompanies these insults. This acute taurine loss may be beneficial in ischemic myocytes to reduce the osmotic pressure inside the cell thereby reducing the likelihood of membrane rupture. Schaffer et al. (2002a) found that ischemia itself led to significant taurine release from cardiac myocytes with a peak at 30 min of ischemia, while reperfusion led to a second phase of taurine release.

Ischemic preconditioning (IPC) refers to a phenomenon whereby a brief sublethal episode of ischemia and reperfusion increases the resistance to myocardial infarction due to a subsequent prolonged ischemia. Since the first

description of IPC by Murry et al. (1986) numerous aspects of this powerful endogenous cardioprotection have been discovered, although the actual mechanism of the protection is still not understood. Our laboratory has repeatedly documented the protective effect of both ischemic and pharmacologic preconditioning. Thus in preconditioned hearts infarct size is consistently decreased by 60–75% (Cohen et al., 2000). To date we have learned a lot about the signaling pathway that eventually effects this protection, but the end-effector has proved to be quite elusive. In the present study we tested whether the release of taurine during ischemia – reperfusion might be greater in ischemically preconditioned hearts. If so, the greater release of taurine could reduce the osmotic pressure inside the cardiomyocytes and explain the mode of protection of IPC.

Methods

This study was performed in accordance with *The Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington, DC, 1996).

Isolated heart model

New Zealand White rabbits (1.5–2.7 kg) of either sex were anesthetized with sodium pentobarbital (30 mg/kg intravenously). A tracheotomy was performed and the rabbits were ventilated with 100% oxygen using a positive-pressure ventilator at a rate of 30–35 breaths per minute and a tidal volume of approximately 15 ml. A left thoracotomy was performed in the fourth intercostal space and the pericardium was opened to expose the heart. The heart was rapidly excised and mounted on a Langendorff apparatus by the aortic root. The heart was perfused with non-recirculating, taurine-free Krebs buffer consisting of (mM): NaCl 118.5; KCl 4.7; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 24.8; $CaCl_2$ 2.5; and glucose 10. The buffer was bubbled with 95% O_2/5% CO_2 to a pH of 7.35–7.45 and was maintained at a temperature of 37°C. Perfusion pressure was set at 75 mmHg by adjusting the height of the reservoir. All hearts were allowed to equilibrate for 20 min before the protocols were started.

Protocol

Figure 1 shows the experimental protocol. Two groups of hearts were studied. All hearts were subjected to 15 min of global ischemia and 40 min of reperfusion. During reperfusion the coronary effluent was collected for 4 periods of 10 min each. In preconditioned hearts one cycle of 5-min global ischemia/10-min reperfusion preceded the 15-min ischemic period. In these hearts 2 additional effluent samples were collected: 1 for 10 min prior to the preconditioning ischemia and the other during the 10-min reperfusion between the periods of ischemia. In control hearts that were not preconditioned only 1 10-min pre-ischemic baseline effluent sample was collected. In each sample taurine loss was calculated as the concentration in the effluent times the volume of the effluent collected.

Taurine measurement

The HPLC system consisted of a binary gradient pump (P2000, Spectra Physics, Mountain View, CA), an autosampler with a 10 μl sample loop (AS3000, Spectra Physics), and a single wavelength visual detector (UV1000, Spectra Physics). A Keystone Scientific (Bellefonte, PA) C_{18} reversed-phase column (250 × 4 mm, particle size 5 μm) was used.

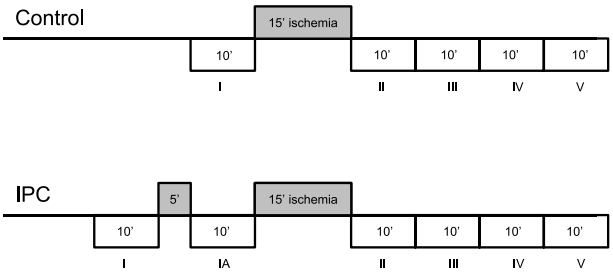

Fig. 1. Experimental protocol. Each box I to V represents a collection period of 10 minutes of coronary effluent. Both groups, control and ischemic preconditioning (IPC), were subjected to 15 minutes of global ischemia, while the IPC group had an additional 5 minutes of global ischemia prior to the long ischemia as a preconditioning stimulus

Determination of the taurine content of each sample was adapted from the method of Porter et al. (1988). The standard stock solution of taurine was prepared with 1:1 methanol – HPLC-grade water and diluted in Krebs buffer to concentrations of 2.5, 5, and 10 μM. The derivatizing reagent was formulated by adding 0.3% β-mercaptoethanol in *o*-phthalaldehyde (OPA) reagent solution (Jones and Gilligan, 1983; Piepponen and Skujins, 2001). The same volume of derivatizing reagent was added to 0.5 ml of either standard solution or coronary effluent in a screw-capped glass vial. After agitation for a total reaction time of 1 min samples were injected onto the HPLC machine. A linear gradient from 100% mobile phase A [0.05 M Na_2HPO_4 at pH 6.1 (adjusted with 85% phosphoric acid) with 0.5% acetonitrile and 1% tetrahydrofuran] to 100% mobile phase B [acetonitrile–tetrahydrofuran–water (70:1:29)] was run at a flow rate of 1.0 ml/min for 15 min. At the end 100% of mobile phase A was run for 5 min. The absorbance of the OPA adducts of taurine was monitored at 340 nm at a sensitivity of 0.01 absorbance unit full-scale. The taurine concentration of the samples was calculated from the peak area after comparison to the known standard curve.

Chemicals

All chemicals and reagents required for the isolated heart model and the HPLC measurements were purchased from Sigma Chemical (St. Louis, MO).

Data analysis

The statistical significance of the data was determined by using either one-way ANOVA with replication or the unpaired Student's t-test for comparisons between groups. Values of $p < 0.05$ were considered statistically significant.

Results and discussion

Ischemic preconditioned and control hearts were subjected to 15 min of global ischemia. In addition the IPC group had 5 minutes of global ischemia preceding the index ischemia. The duration of 15 minutes was chosen to cause as great an ischemic insult as possible without actually causing cell death. That would prevent any loss of intracellular taurine into the coronary effluent related to

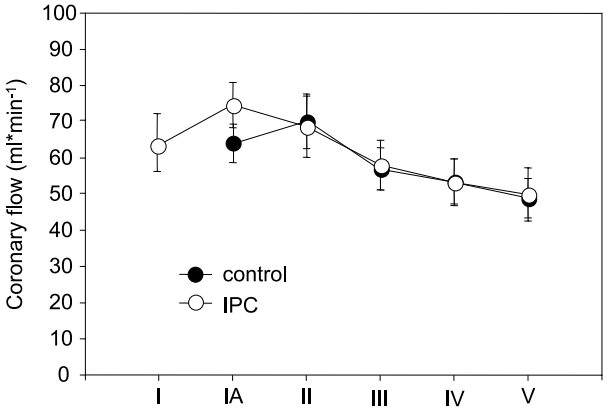

Fig. 2. Changes in coronary flow during the course of the experiment. There were no differences between the two groups. Abbreviations: see Fig. 1

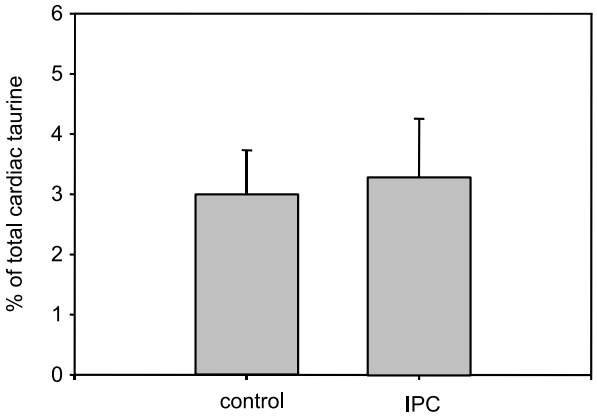

Fig. 4. Percentage of total taurine content of the heart released at reperfusion after 15 minutes of global ischemia. There was no difference between control and IPC groups

membrane failure. Thus any taurine loss from the heart in this study should represent active release. Because hearts were perfused with non-recirculating, taurine-free buffer, there was no possibility of any additional taurine uptake by the myocardium. Released taurine was continuously flushed out by the buffer perfusate. Therefore, the collected taurine represents the net change in cardiac taurine content. The coronary effluent was collected during equal 10-min periods and expressed as ml/min of coronary flow. Coronary flow did not differ between the groups at any time period (Fig. 2). Period IA in Fig. 2 serves as the baseline for the control group.

Taurine release at each time period is presented in Fig. 3. Note that a small amount of taurine was lost after the preconditioning ischemia. A large amount of taurine was released in the first 10 minutes of reperfusion, but there was no difference between IPC and control hearts. Figure 4 shows the total taurine released during the entire experiment for both groups of hearts. In the IPC group there was a postischemic release over baseline of $5.09 \pm 1.51\,\mu$mol (n = 4), whereas in the control group the release was not significantly different and totaled $5.72 \pm 1.67\,\mu$mol (n = 4). The percent of the taurine pool lost from each heart during reperfusion was actually quite small. Assuming a total content of $20\,\mu$M taurine/gm wet weight (Han et al., 1996), approx 3.3% of the total taurine pool was lost in IPC hearts (average heart weight 8.0 ± 0.5 g) and 3.0% was lost in control hearts (heart weight 9.5 ± 0.3 g). While IPC hearts tended to release more taurine, the difference seems too small to have much of a biological effect.

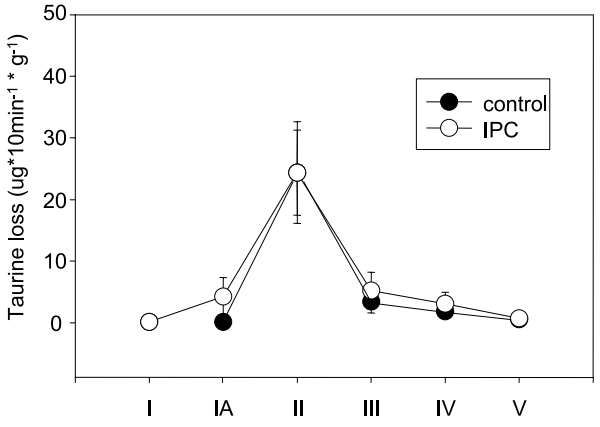

Fig. 3. Taurine loss over the time course of the experiment. Data are presented as the amount of taurine collected in the coronary effluent over any given 10-min period. Period II represents the sample collected in the first 10 min of reperfusion following 15 min of global ischemia. There was no difference between control and IPC hearts. Abbreviations: see Fig. 1

Although taurine does not presently have a well defined physiological role in the heart other than osmoregulation, it is clearly necessary for normal cardiac function as its depletion is associated with the development of cardiomyopathy (Kramer et al., 1981). Schaffer has presented evidence that taurine depletion can have a beneficial effect on the acutely ischemic heart (Allo et al., 1997; Schaffer et al., 2002b). On the other hand, it has been proposed that elevated taurine in the perfuate protects against reperfusion arrhythmias (Chahine and Feng, 1998), and, in conjunction with a low-calcium perfusate, reportedly preserves post-ischemic function (Oz et al., 1999). Previous studies in different models have shown release of large amounts of taurine from ischemic cardiac cells (Song et al., 1996, 1998a, b).

Schaffer et al. (2002a) noted that $1.6\,\mu$M/min of taurine was released into the coronary effluent from rat hearts

after an ischemic insult of 45 minutes. In the present study the peak rate of taurine loss after 15 min of ischemia was only about 24 μg/min/gram wet weight. The lower values in the present study are most likely related to the much shorter ischemic period. Unfortunately, taurine will also be washed out of dead cells making taurine release difficult to interpret when ischemic times are long. Under those conditions an increase in taurine release could represent active release indicating a protective response or it could simply reflect the consequence of increased cell death.

Every 1 mM of an osmotically active substance has an osmotic pressure of approximately 19 mmHg. Therefore, we calculate that the observed release of taurine in these experiments would result in a reduction of intracellular osmotic pressure of only 4.53 ± 1.34 mmHg in the control hearts and 4.99 ± 1.49 mmHg in the IPC group. This reduction in osmotic pressure will certainly contribute to an overall reduction of intracellular pressure during ischemia, but this level of pressure reduction seems far too small to be considered a significant element of cardioprotection. Furthermore, the difference between control and IPC groups was only 1 mmHg, suggesting that taurine release is not the mechanism by which preconditioning protects the heart against an ischemic insult. Taurine is the major component of the free amino acid pool; therefore, among all of the amino acids released it would most likely have the greatest impact on the reduction of osmotic pressure. We also measured aspartate and glutamate, other amino acids known to be abundant in the heart, but couldn't detect osmotically relevant release in these hearts (data not shown).

Thus, we found significant taurine release after a sublethal ischemic insult of 15 minutes in the isolated rabbit heart, but the amount released by the cells represented only a small fraction of the total taurine pool. In addition the amount of taurine released was too small to have much of an effect on the intracellular osmotic pressure of these hearts. Finally there was little difference in the amount of taurine released in ischemically preconditioned and non-preconditioned hearts. We, therefore, conclude that augmented release of taurine, a major osmolyte in the heart, is not the mechanism by which ischemic preconditioning protects against osmotic cell swelling and subsequent cell death following ischemia.

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