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Myocardial taurine, development and vulnerability to ischemia

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Summary. Depleting intracellular taurine in heart cells improves their resistance to ischemia and reperfusion injury. The aim of this work was to see whether physiologically low levels of endogenous taurine also reflect a reduced vulnerability of the myocardium to cardiac insults. The myocardial concentration of taurine was measured during different stages of development and compared with vulnerability to ischemia and reperfusion injury in the rat and in pediatric patients undergoing cardiac surgery.

Rat hearts with relatively lower levels of taurine were significantly more resistant to an ischemic inult and there was a strong negative correlation between taurine content and recovery. Children's hearts had significantly lower taurine levels compared to infants' hearts which was consistent with their known increased resistance to an ischemic cardioplegic insult (Imura et al., 2001). This work shows that the changes in the concentration of myocardial taurine during development correlate with vulnerability to ischemia where low myocardial taurine is associated with improved recovery upon reperfusion.

Keywords: Taurine – Heart – Development – Ischemia

Introduction

Deprivation of the myocardium of oxygen and nutrients will induce an abnormal accumulation of ions and metabolites (See Suleiman et al., 2001 for a recent review). In particular, lactic acid accumulates, leading to a decrease in intracellular pH and an increase in intracellular Na^+ concentration. If coronary flow is restored quickly, then metabolic and ionic homeostasis are re-established and recovery occurs. However, reperfusion following prolonged ischemia can lead to irreversible damage caused by Ca^{2+} loading via the Na^+/Ca^{2+} exchanger and generation of reactive oxygen species. Myocardial protection strategies against ischemia, hypoxia and Ca^{2+}-depletion include attempts at reducing the accumulation of intracellular Na^+ concentration (Chapman et al., 1993a, b; Suleiman, 1994; Karmazyn et al., 2001). In addition to Ca^{2+} loading, it has been shown that Na^+ accumulation would contribute to osmotic-induced cell swelling, which has been implicated as a major cause of ischemic sarcolemmal damage (Steenburgen et al., 1985; Ruiz-Meana et al., 1995; Garcia-Dorado et al., 1993). Heart cells respond to cardiac insults/osmotic stress by releasing amino acids (Rasmusson et al., 1993; Kramer et al., 1981; Lombardini and Cooper 1981; Suleiman et al., 1992, 1993, 1997; Suleiman and Chapman, 1993; Schaffer et al., 2002a) including taurine, the primary organic osmolyte in the heart (Huxtable, 1992). The finding that taurine fluxes are dependent on the sodium gradient across the sarcolemma (Suleiman et al., 1993; Schaffer et al., 2002b) supports a close link between Na^+ (osmotic changes) and taurine.

Allo et al. (1997) have shown that drug-induced taurine-deficient myocardium is resistant to an ischemic injury, which supports a role for taurine in combating the osmotic imbalances during ischemia-reperfusion injury. Schaffer et al. (2002a) have further investigated the relationship between taurine depletion, osmotic load and protection against ischemia and reperfusion injury and suggested that the improvement in Na^+ handling could be a major cause for the cardioprotection seen in the taurine-deficient cardiomyocytes where these cells are likely to extrude Na^+ and retain taurine.

These conclusions were made under pathological conditions where cells were actively depleted of taurine. However, nothing is known of the relationship between physiological intracellular taurine and resistance to an ischemic insult. The concentration of endogenous taurine changes during development and therefore provides a convenient tool to test taurine and vulnerability to ischemia and reperfusion injury. In this work we show

developmental changes in the concentration of taurine in rat hearts that are paralleled by changes in vulnerability to ischemia and reperfusion injury. In addition and following our recent work (Imura et al., 2001) showing that children's hearts are more resistant to ischemic cardioplegic arrest than infants' hearts, we found that the latter hearts had significantly more taurine and interestingly only these lost taurine following ischemic cardioplegic arrest.

Methods

Isolated rat heart

Rats were anaesthetised and the hearts quickly excised and immersed in cold (4°C) Krebs-Henseleit buffer (KHB). For taurine measurements, the hearts were flushed with ice-cold buffer and the ventricular tissue immediately frozen in liquid nitrogen until processing for taurine extraction and determination. For functional measurements, hearts were canulated in the Langendorff mode as described previously (Awad et al., 1998). Aortic cannulation was performed with a blunted 23-gauge (4- and 7-day old hearts) or 21-gauge (14- and 21-day old hearts) needle and the hearts perfused at constant pressure (40, 45, 50 or 55 mmHg in the 4-, 7-, 14- and 21-day old hearts, respectively) and temperature (37°C). Left ventricular developed pressure (LVDP) was measured using an ultrathin intraventricular balloon which was introduced into the left ventricle through the left atrium and mitral valve after excising the left atrial appendage, and inflated slowly with water to give a left ventricular end-diastolic pressure of 3.5 to 6.5 mmHg. However, the success rate for measuring LVDP in 4-day old hearts was very low and therefore abandoned.

Pediatric patients

Forty-five pediatric patients (28 infants <12 months and 17 children ≥12 months) undergoing cardiac surgery for the same pathology (ventricular septal defect, VSD) at the Royal Hospital for Children, Bristol, between 2000 and 2002 were prospectively recruited. There were no emergency operations and no patients had preoperative respiratory or inotropic support. All operations were performed using cardiopulmonary bypass with ascending aortic and bicaval venous cannulation. Anesthetic technique was standardised for all patients (Imura et al., 2001). Myocardial biopsies (3–10 mg) were collected from the right ventricle (free wall of the trabecular portion) through the tricuspid valve by direct resection with surgical scissors. Control biopsies were taken from all patients ($n = 45$) immediately after crossclamping the aorta. In a subgroup of patients (13 infants and 5 children) a second biopsy was collected 20 min after reperfusion. In this group, hearts were arrested using cold crystalloid cardioplegia and were exposed to similar ischemic periods (cross-clamp time): 36 ± 5 min and 47 ± 9 min for infants and children respectively. All biopsies were immediately frozen in liquid nitrogen until processing for the analysis of taurine. Ethical approval from the local authority and informed consent was obtained for all patients.

Extraction and measurement of taurine in biopsy specimens

The procedure followed to extract taurine was similar to that described previously (Suleiman et al., 1997). In brief, frozen biopsy specimens were crushed under liquid nitrogen and the resultant powder was extracted with perchloric acid. An aliquot was taken for protein determination and the rest of the extracts were centrifuged at 1500 g for 10 minutes at 4°C. The supernatant was neutralised and processed for the determination of taurine. Protein determination was carried out using a protein determination kit from Sigma (Poole, Dorset, UK). Bovine plasma albumin (Sigma, UK) was used as a standard and the data were expressed per protein content. Taurine was separated from other free amino acids and its concentration determined using the Waters Pico-Tag method as previously reported (Suleiman et al., 1997). It is possible to compare concentrations of taurine expressed in different ways by dividing nmol/mg protein by 6.6 to give nmol/mg wet weight (assuming that 15% of wet weight is protein). The concentration per mg wet weight can then be converted into volume by assuming that 80% of the wet weight is water.

Statistical analysis

Data are expressed as mean ± SEM. The statistical significance of the data was determined using the Student's *t*-test and where appropriate analysis of variance (ANOVA) combined with Fisher's PLSD post hoc test. The significance of correlation was determined using Fisher's r to z. Values of $p < 0.05$ were considered statistically significant.

Results

Developmental changes in the concentration of taurine in rat hearts

Figure 1 shows the concentration of taurine in the ventricles of 4-, 7-, 14- and 21-day old and adult rats. It is evident that the concentration of taurine starts high but declines and reaches a nadir at 14-days old before climbing back to neonatal levels at 21 days. Adult hearts tended to have relatively more taurine compared to all developing ages, but this did not reach statistical significance.

Vulnerability of the developing heart to ischemia and reperfusion

Figure 2 shows the rate-pressure product (Heart Rate × LVDP) measured in hearts before 60 min ischemia and

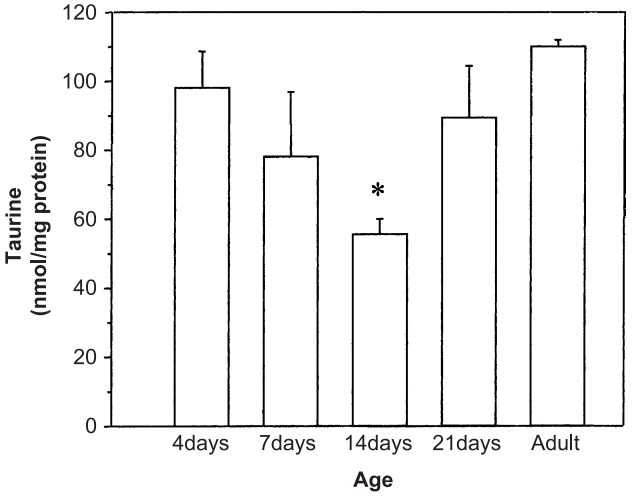

Fig. 1. The myocardial concentration of taurine in rat hearts during different stages of development. Mean ± SE ($n = 5$/group). $^{*}p < 0.05$ vs. all other concentrations

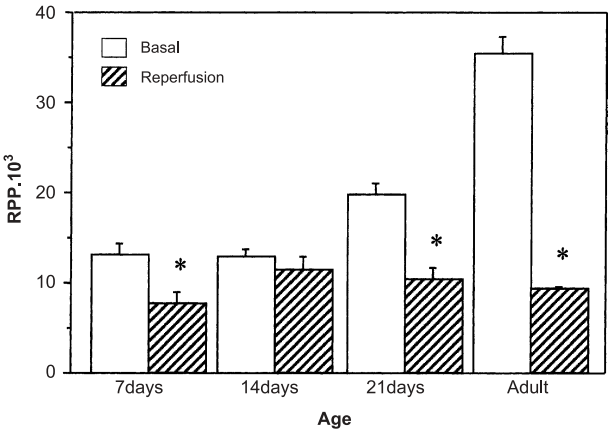

Fig. 2. The rate-pressure product (*RPP*) of isolated perfused hearts at different stages of development measured before (basal) and after 60 min of reperfusion following 60 min global normothermic ischemia. Mean ± SE (n = 3–6/group). *p < 0.05 vs. corresponding basal value

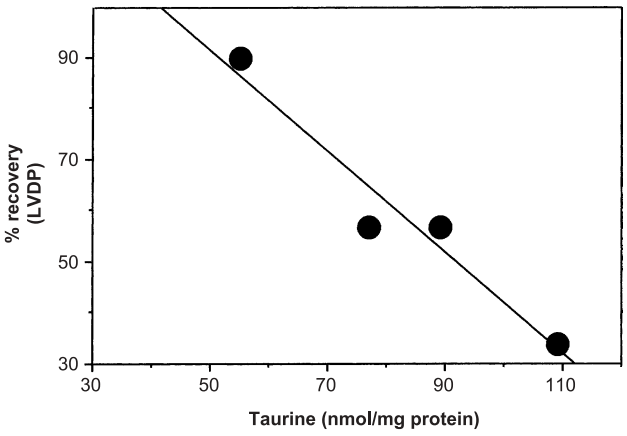

Fig. 4. Scattergram for the concentration of myocardial taurine in different age groups when plotted against percentage recovery. Values were taken from Figs. 1 and 3, which came from two different sets of hearts. There was a significant (p < 0.05) negative correlation between the two parameters

at the end of 60 min reperfusion. It was technically very difficult to insert a balloon into 4-day old hearts. However, early reports have shown 4-day old rat hearts to have the same vulnerability to ischemia and reperfusion as 7-day old hearts (Awad et al., 1998). It is evident that recovery was impaired in all age groups except 14-day old hearts (Fig. 2). Figure 3 shows the percentage recovery for all age groups with 14-day old hearts showing more than 90% recovery that was significantly more than all other ages. Figure 4 shows that there was a significant negative correlation (p < 0.05) between the concentration of taurine (Fig. 1) and percentage recovery in LVDP (Fig. 3).

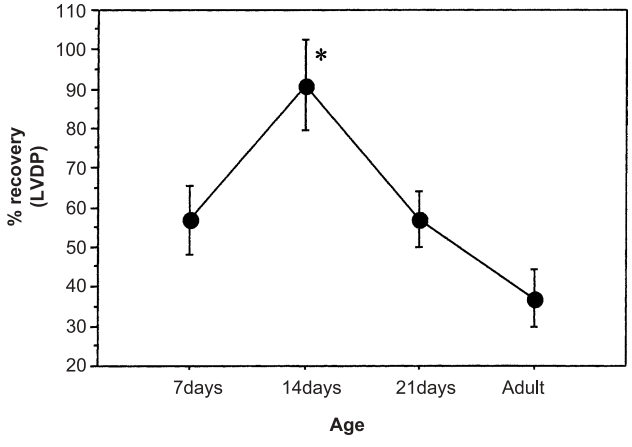

Fig. 3. Percentage recovery in left ventricular developed pressure in isolated perfused hearts at different stages of development and exposed to 60 min global normothermic ischemia followed by 60 min of reperfusion. Mean ± SE (n = 3–6/group). *p < 0.05 vs. all other values

Developmental changes in the concentration of taurine in patients' hearts

Patient characteristics are shown in Table 1. Figure 5A shows the basal concentration of taurine in hearts of children (≥12 months) and infants (<12 months) undergoing heart surgery. Children's hearts had significantly less taurine than infants' hearts. Figure 5B shows that these differences were also maintained in male and female patients. Figure 6 is a scattergram showing the concentration of taurine in individual patients.

Changes in the concentration of taurine during ischemic cardioplegic arrest

In a subgroup of patients (13 infants and 5 children) undergoing surgery using cold crystalloid cardioplegia, extra biopsies were collected 20 min after reperfusion. A significant fall (p < 0.05) in taurine was only seen in infant hearts (90 ± 8 to 63 ± 14 nmol/mg protein for infants compared to 54 ± 4 to 56 ± 6 nmol/mg protein for children).

Table 1. Patient characteristics

	Children	Infants
Sex (M/F)	9/8	15/13
Age (months)	78 ± 15	4.4 ± 0.4
Range (months)	(13–192)	(1–11)
Weight (kg)	25.4 ± 5.0	4.9 ± 0.2
O_2 saturation (%)	97.4 ± 0.4	97.5 ± 0.3

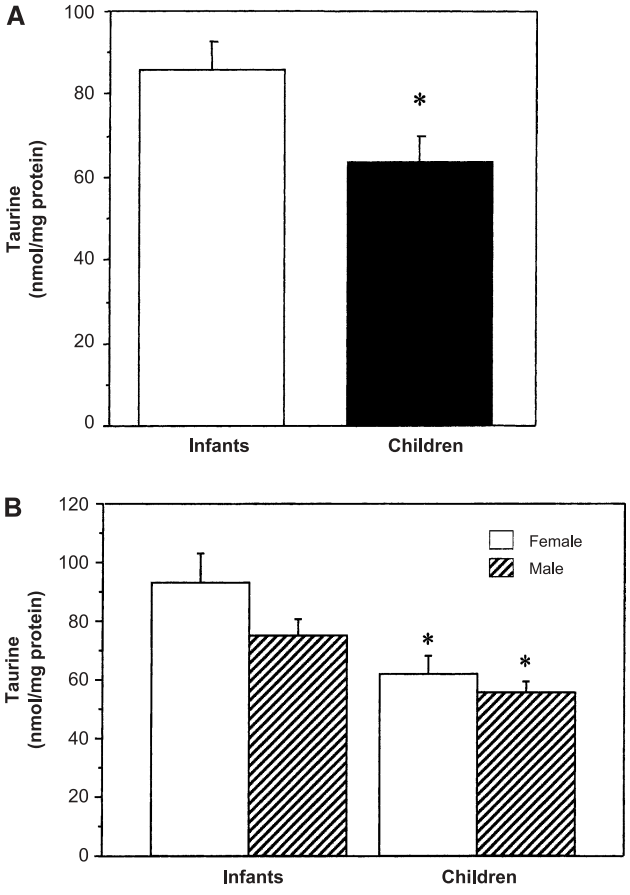

Fig. 5. The myocardial concentration of taurine in ventricular biopsies collected from hearts of children and infants undergoing open-heart surgery (**A**). Also shown are the concentrations of taurine in both groups separated into males and females (**B**). Mean ± SE. $^{*}p < 0.05$ vs. infants heart

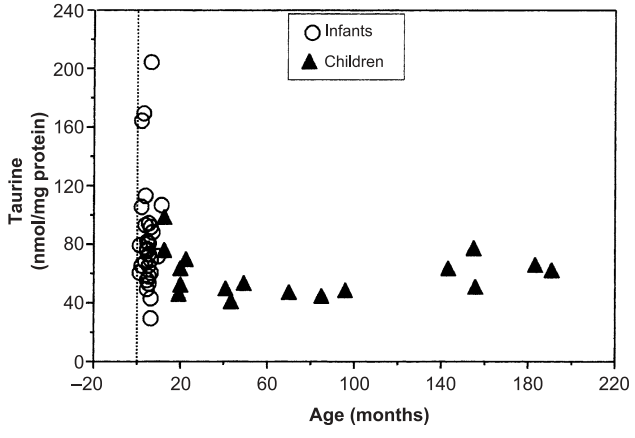

Fig. 6. The concentration of myocardial taurine in individual patients plotted against age and split into infant and child

Discussion

This work shows that the changes in the myocardial concentration of taurine during different stages of development are paralleled by changes in vulnerability to ischemia which is consistent with the view that low taurine renders the heart more resistant to an ischemic insult (Schaffer et al., 2002a). This association between low concentration of taurine and resistance to ischemia is evident in rat and human hearts. In contrast to most studies investigating the efficacy of taurine as a cardioprotective agent, this study did not involve modulation of myocardial taurine prior to an ischemic insult.

Age, taurine concentration and tolerance to ischemia in rat heart

In this study we found that the age-dependent changes in myocardial taurine concentrations are not simply an increase or decrease with age. The relationship follows an inverted bell-shaped curve with 14-day old rat hearts having significantly less taurine compared to younger or older ages (Fig. 1). Furthermore, adult hearts tended to have more taurine compared to all other age groups with nearly twice as much as the 14-day old hearts. These endogenous developmental (physiological) changes in taurine are not likely to be associated with disruption to the osmotic balance, in contrast to acute, pathological and sudden changes in taurine (e.g. drug-induced decrease in tissue taurine or during a cardiac insult). Presumably, heart cells will maintain the osmotic balance by mobilising other osmolytes (e.g. metabolic or ionic).

It is evident from this work that the age-dependent sensitivity to ischemia and reperfusion in rat hearts follows a bell-shaped relationship with 14-day old hearts showing a very high resistance to a severe ischemic insult. This is in agreement with an earlier study by Awad et al. (1998). The strong correlation between the taurine levels in the rat heart and resistance to an ischemic insult suggests a potentially important role for the amino acid in cardioprotection. This, however, may not be consistent with the view that taurine can be used to efflux Na^+ during ischemia and upon reperfusion by activating a Na^+/taurine symport (Suleiman et al., 1992; Suleiman, 1994; Chapman et al., 1993a, b). One would expect the higher outward gradient for taurine to be more effective at effluxing Na^+ and therefore more protective. However, it is possible that Na^+ accumulation in 14-day old hearts was not high enough to stimulate the symporter.

It has been proposed that low levels of taurine might benefit the cell by protecting against the development of a severe osmotic imbalance (Allo et al., 1997). The drug-induced taurine-deficient heart was found to be extremely resistant to ischemic injury (Allo et al., 1997) which was

attributed to taurine depletion reducing the osmotic load of the ischemic heart and could be related in part to the regulation of the intracellular Na^+ concentration, an effect thought to be linked to changes in tissue osmolality, intracellular pH, and/or the co-transport of taurine and Na^+. More interestingly, Schaffer et al. (2002a) demonstrated that a reduction in taurine levels before a chemical hypoxic insult resulted in a fall in the intracellular concentration of Na^+ and H^+. Under these conditions, heart cells will be better prepared to cope with the consequences of ischemia.

Developmental changes may include changes in a number of properties including transport systems (Ostadal et al., 1999). However, it is difficult to see why 14-day old hearts would experience sudden and opposite changes in activities of transport proteins. In an attempt to explain the age-related differences in the sensitivity to cardiac insults, researchers have proposed metabolism, calcium mobilisation, generation of oxygen free radicals and vascular resistance as possible mechanisms (Itoi and Lopaschuk, 1996; Jonas, 1998; Lopaschuk and Spafford, 1992; Matherne et al., 1996; Quantz and Chiu, 1993; Southworth et al., 1997). Finally, differences in taurine concentration would influence Ca^{2+} mobilisation differently. Taurine is known to modulate a variety of Ca^{2+}-linked activities (Schaffer et al., 1995).

Age, taurine concentration and tolerance to ischemia in pediatric hearts

In addition to the importance of age in determining vulnerability to cardiac insults, there are species-related influences. It is important therefore to find out whether the relationship between myocardial taurine concentration and resistance to an ischemic insult is also seen in other mammalian hearts. We have recently addressed the question of development and ischemia/reperfusion injury in pediatric patients undergoing cardiac surgery (Imura et al., 2001). We found children's hearts to be more resistant to ischemia compared to infants' hearts. In this study we found that infants had more myocardial taurine than children, again confirming the work on rat hearts showing that relatively lower levels of myocardial taurine are associated with an increased resistance to ischemia and reperfusion injury.

Our finding that children's hearts (with less taurine) did not lose significant amounts of taurine during ischemia and early reperfusion, suggests that these hearts were better prepared for an insult and that the stress was not sufficient to trigger a loss of taurine. It is possible, however, that a more severe ischemic insult would trigger a loss of tissue taurine from these hearts. It is also likely that these hearts had a reduced osmotic load as a result of having less taurine and therefore were able to withstand the insult better than those hearts with higher taurine (presumably with higher osmotic load). This reinforces the view that the reported contribution of the taurine/Na^+ symport towards cardioprotection is an oversimplification.

Finally, this work is in contrast to work described for cats and foxes (Pion et al., 1987; Moise et al., 1991) where the low concentration of taurine in the circulation appears to be responsible for the development of heart failure. This is likely to be due to species-related differences. Human and rat hearts do seem to have high concentrations of the amino acid. For example the relatively low levels of myocardial taurine in 14-day old rat hearts at nearly 60 nmol/mg protein (approximately 10 mmol/kg wet weight) is relatively high compared to other species (Huxtable, 1992). The same thing can be said for infants and children.

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