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Warm blood cardioplegia reduces the fall in the intracellular concentration of taurine in the ischaemic/reperfused heart of patients undergoing aortic valve surgery

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Summary. The effect of cold and warm intermittent antegrade blood cardioplegia, on the intracellular concentration of taurine in the ischaemic/reperfused heart of patients undergoing aortic valve surgery, was investigated. Intracellular taurine was measured in ventricular biopsies taken before institution of cardiopulmonary bypass, at the end of 30 min of ischaemic arrest and 20 min after reperfusion. There was no significant change in the intracellular concentration of taurine in ventricular biopsies taken after the period of myocardial ischaemia in the two groups of patients (from 10.1 ± 1.0 to 9.6 ± 0.9 μmol/g wet weight for cold and from 9.3 ± 1.3 to 10.0 ± 1.3 μmol/g wet weight for warm cardioplegia, respectively). Upon reperfusion however, there was a fall in taurine in both groups but was only significant ($P < 0.05$) in the group receiving cold blood cardioplegia (6.9 ± 0.8 μmol/g wet weight after cold blood cardioplegia versus 8.0 ± 0.8 μmol/g wet weight following warm blood cardioplegia). Like taurine, there were no significant changes in the intracellular concentration of ATP after ischaemia in the two groups of patients (from 3.2 ± 0.32 to 2.95 ± 0.43 μmol/g wet weight for cold and from 2.75 ± 0.17 to 2.62 ± 0.21 μmol/g wet weight for warm cardioplegia, respectively). However upon reperfusion there was a significant fall in ATP in both groups with the extent of the fall being less in the group receiving warm cardioplegia (1.79 ± 0.19 μmol/g wet weight for cold and 1.98 ± 0.27 μmol/g wet weight for warm cardioplegia, respectively). This work shows that reperfusion following ischaemic arrest with warm cardioplegia reduces the fall in tissue taurine seen after arrest with cold cardioplegia. Accumulation of intracellular sodium provoked by hypothermia and a fall in ATP, may be responsible for the fall in taurine by way of activating the sodium/taurine symport to efflux taurine.

Keywords: Amino acids – Taurine – Aortic valve surgery – Cold and warm cardioplegia

Introduction

The slowly metabolised non-protein β-amino acid taurine is present at high concentration in mammalian heart cells (ranging between 3–40 mM) but at a much lower concentration in the plasma (0.05–0.2 mM) thus creating a large concentration gradient across the sarcolemma (see Chapman et al., 1993a,b). This gradient is maintained by a Na^+-taurine symport using the Na^+ electrochemical gradient (Suleiman et al., 1992; Chapman et al., 1993a,b). The concentration of taurine is raised in the blood of patients following acute myocardial infarction (Cooper and Lombardini, 1981), unstable angina (Bhatanger et al., 1990) and cardiac surgery (Cooper and Lombardini, 1981; Lombardini and Bricker, 1981). Work on animal experimental models has provided direct evidence for a fall in taurine in heart cells during cardiac insults (e.g. Kramer et al., 1981; Lombardini and Crass, 1981; Suleiman et al., 1992). A fall in tissue taurine will influence myocardial function as taurine affects cellular calcium homeostasis and taurine deficiency is associated with development of cardiomyopathy (Schaffer et al., 1987; Chapman et al., 1993; Puna et al., 1994; Suleiman, 1994).

In an earlier study, we measured the intracellular concentration of taurine in ventricular biopsies collected from patients with ischaemic heart disease undergoing coronary artery bypass surgery (Suleiman et al., 1993, 1997). We have now expanded on this study and investigated the effects of cold and warm blood cardioplegia on the intracellular concentration of taurine in biopsies collected from patients with a different cardiac pathology, hypertrophy and congestion associated with aortic stenosis.

Materials and methods

A total of 17 patients suffering from aortic stenosis with a mean transvalvar gradient of 69 ± 25 mmHg and myocardial hypertrophy were selected for this study. Valve replacement surgery was performed and the patients were randomised to one of two techniques of myocardial protection. In the cold blood group (n = 8) myocardial protection was induced using antegrade administration of hyperkalaemic (20 mM K^+) blood cardioplegic solution (blood and St Thomas' I cardioplegic solution 4:1 with extra K^+ added) (Suleiman et al., 1997). The cardioplegic solution was administered under pressure into the aortic root as a 1 litre bolus (4–6°C) at the start of the ischaemic period. Infusions of 300 ml were repeated at 30 min intervals or earlier if electrical activity returned. Warm blood cardioplegia was essentially the patients blood with added K^+ and Mg^{2+} to give a final concentration of approximately 20 mM K^+ and 5 mM Mg^{2+}. These levels were similar to the ones found in the cold blood cardioplegia. The warm blood cardioplegia was used for induction of myocardial protection in the same way as for the cold blood group, except for the temperature of cardioplegia which was around 34°C.

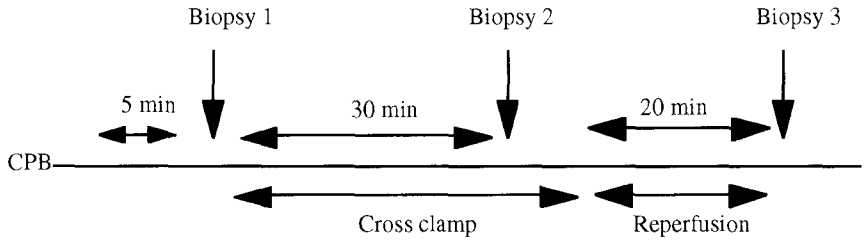
Operative procedure

Anaesthetic technique was standardised for all patients. Thiopentone (1–3 mg·kg^{-1}) was used for induction with 3–5 ug·kg^{-1} fentanyl, and volatile agents were delivered in 50% air-O_2 mixture for maintenance. Propofol (3 mg·kg^{-1}·h^{-1}) was given as an infusion during cardiopulmonary bypass and neuromuscular blockade was achieved by 0.1–0.15 mg/kg

Pancuronium Bromide. Alpha stat acid-base management was adopted. Initial anti-coagulation was accomplished with 3 mg·kg^{-1} body weight of heparin and was supplemented as required in order to maintain an active clotting time of 480 seconds or above. All operations were performed using cardiopulmonary bypass with ascending aortic cannulation and single-stage venous cannulation. Target systemic temperatures were between 32°C–34°C.

Collection of ventricular biopsy specimens

Myocardial biopsy specimens (4–12 mg wet weight) were taken from the apex of the left ventricle using a "Trucut" needle (Baxter Healthcare Corporation, IL 60015 USA). The protocol for biopsy collection is shown in Scheme 1. The first biopsy was taken 5 min after institution of cardiopulmonary bypass (CPB), the second after 30 min of ischaemia and the third after 20 minutes of reperfusion. Each specimen was immediately frozen in liquid nitrogen until processing analysis of amino acids. The analyses were performed by a research technician blind to the operative techniques used.



Scheme 1. Time points for collection of biopsy specimens during bypass surgery

Determination of taurine in biopsy specimens

The procedure followed to extract the amino acid was similar to that described previously (Suleiman et al., 1997). In brief, frozen biopsy specimens were crushed under liquid nitrogen and the resultant powder (taken as wet weight) was extracted with perchloric acid. The extracts were centrifuged at 1,500 g for 10 min at 4°C. The precipitate was weighed and the weight was used to express values per acid precipitate (see below). The supernatant was neutralised and taurine was determined according to the Waters Pico-Tag method as reported elsewhere (Cohen et al., 1989). Essentially, 100 μl of the extract was dried using vacuum centrifugation (Savant SV160, Farmingdale NY, USA). The free amino acid was derivatized using phenylisothiocyanate. The phenylisothiocarbamyl derivatized amino acids were separated by HPLC using a 30 cm Pico-Tag column (Millipore Corporation, Milford, MA, USA) with two Waters delivery pumps (A & B) at a constant flow of 1 ml/min with the following gradient: 100% A for 13.5 min, 97% A for 10.5 min, 94% A for 6 min, 91% A for 20 min, 66% A for 12.5 min and 0% A for 4 min. The solvents used were for A: 132 mM Na Acetate, 470 ml/l triethylamine, pH 6.4 and 6% acetonitrile. Solvent B was 60% acetonitrile. Derivatized amino acids were detected at 254 nm (46°C) using a Waters 486 detector. The acquired data was processed using the Millenium 2000 software supplied by Waters, Millipore (UK) Ltd., Watford, Herts. Chemicals needed to derivatize and separate taurine were obtained from Waters, Millipore (UK) Ltd., Watford, Herts.

In order to reduce the effect of variation in the biopsy specimen (Huxtable & Bressler, 1974), the concentration of taurine was reported in the following ways: μmol/g wet weight, μmol/g protein and μmol/g acid precipitate. Protein determination was carried out according to the Lowry method (1951) using protein determination kit from Sigma (Poole, Dorset, UK). Bovine plasma albumin (Sigma, UK) was used as a standard.

Taurine determination in plasma

Blood from patients undergoing open heart surgery was collected and processed before the operation. Blood plasma was deproteinised using Millipore 10K molecular weight cut-off limit ultrafiltration units for 15 min at 8,500 g. The procedure for taurine determination was the same as described above.

Determination of ATP in biopsy specimen

The intracellular ATP concentration was measured in tissue extracts used for the determination of taurine. A bioluminescent assay described elsewhere (Spielman et al., 1981) was used for ATP determination. ATP data was expressed as concentration per wet weight and per protein content only. Similar changes were also seen when the data was expressed per acid precipitate (data not shown).

Data collection and analysis

Data were expressed as mean value ± standard error (SE) unless otherwise stated. Differences within each group were analysed using ANOVA (Fisher's protected least significant difference) available on a Statview package provided on a Macintosh personal computer. The level of statistical significance was taken as 95%.

Results

The clinical information is presented in Table 1. The intraoperative management was similar. The postoperative course was uncomplicated, all patients being haemodynamically stable and needing no inotropic support. None of the patients showed any electrocardiographic signs of perioperative myocardial infarction.

Plasma and muscle taurine concentration

The intracellular concentration of taurine in control left ventricle biopsy was 9.9 ± 0.8 μmol/g wet weight, n = 17. However the plasma concentration was 86 ± 7 nmol/ml, thus giving a muscle to plasma ratio of approximately 136. The percentage protein content compared to the wet weight was 10.8 ± 0.3% (i.e. each g wet weight contains 0.11 g protein). The intracellular concentration of taurine in patients showing signs of clinical congestive heart failure (n = 8) was slightly but not significantly higher than the concentration found in patients without clinical congestive heart failure (Table 2). Furthermore there were no differences in the plasma concentration between the two groups (Table 2). Only two patients had ischaemic heart disease but also showed signs of congestive heart failure. These patients had relatively low myocardial taurine levels (7.7 ± 0.8 μmol/g wet weight, 14 ± 1.8 μmol/g acid precipitate and 77 ± 4.1 μmol/g protein).

Effect of ischaemia and reperfusion on intracellular taurine concentration

Following ischaemic arrest using cold blood cardioplegia, there was a small insignificant fall in the intracellular concentration of taurine (by 6%, 7% and

Table 1. Patient characteristics

	Warm blood cardioplegia (n = 9)	Cold blood cardioplegia (n = 8)
Age, yr	68.4 ± 6.6	60.4 ± 12.3
Sex, M/F	5/4	4/4
Diabetes	1	1
Hypertension	7	7
Ejection fraction (%)	45.6 ± 5.3	48.1 ± 6.5
Angina class I	2	1
II	4	4
III	3	3
LVF degree		
mild	4	4
moderate	5	4
Aortic gradient (systolic peak)		
<35	1	2
>35, <70	3	2
>70	5	4
Length of LVF		
Nil	4	3
(>6 months)	5	5
Bypass time, min	112 ± 18	109 ± 21
Ischaemic time, min	78 ± 12	83 ± 18

Preoperative and intraoperative variables of patients undergoing aortic valve surgery using cold blood or warm blood cardioplegia. Mean ± S.D. *LVF* left ventricular failure.

Table 2. Myocardial taurine concentration in patients with or without clinical signs of congestion

	Patients with no signs of clinical congestion (n = 9)	Patients with signs of clinical congestion (n = 8)	Level of significance P
Taurine (μmol/g wet weight)	9.24 ± 1.0	10.4 ± 1.1	0.46
Taurine (μmol/g acid precipitate)	20.9 ± 2.6	15.6 ± 1.5	0.13
Taurine (μmol/g protein)	97 ± 10	83 ± 9	0.32
Taurine in plasma (μM)	87 ± 9	85 ± 10	0.91

Resting taurine levels in myocardial tissue and in the plasma of patients with or without clinical signs of congestion. The concentration of taurine in control biopsies collected 5 min after institution of cardiopulmonary bypass and before ischaemic arrest, was used as the resting value. Values are mean ± SEM.

21% when the values were expressed per wet weight, protein content and acid precipitate respectively, Fig. 1). After 20 minutes reperfusion following ischaemic arrest with cold blood cardioplegia, there was fall in taurine which was significantly different from control and ischaemic levels (by 34%, 29%

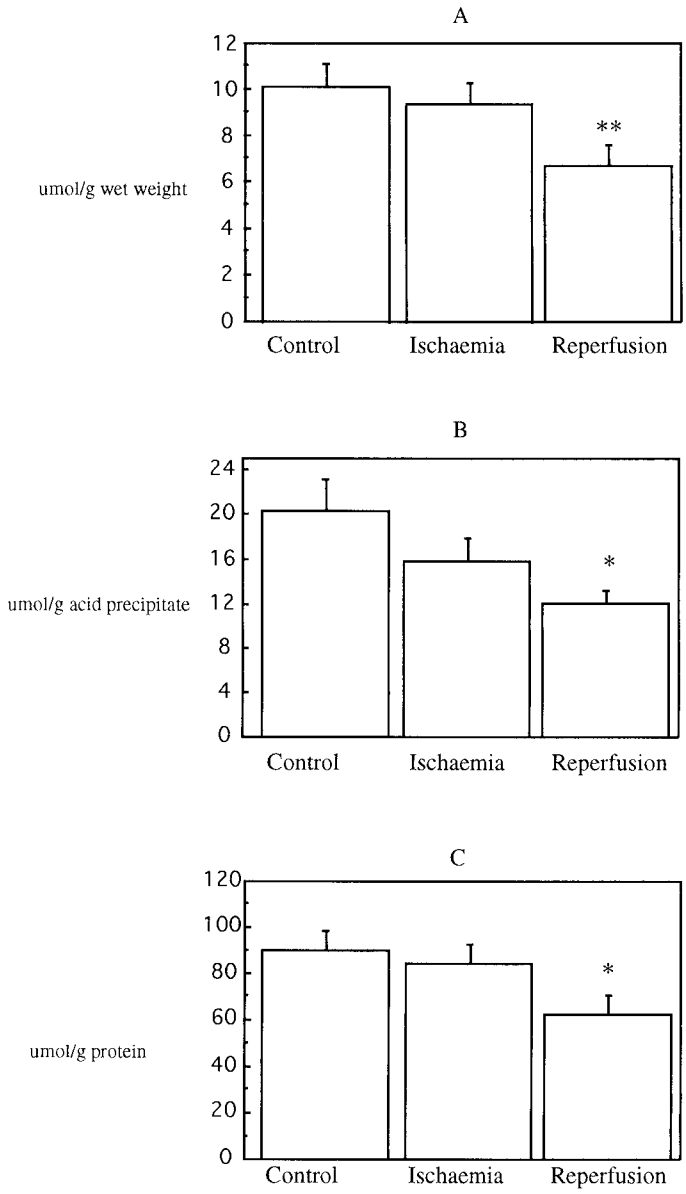


Fig. 1. Tissue taurine levels measured in ventricular biopsies taken from patients undergoing aortic valve replacement surgery using cold blood cardioplegia (n = 8). Control biopsy was collected 5 min after institution of cardiopulmonary bypass, ischaemic biopsy after 30 minutes ischaemic arrest and reperfusion biopsy after 20 min of reperfusion. Data was expressed per wet weight (**A**), per acid precipitate weight (**B**) and per protein content (**C**). **Significantly different for biopsy 3 vs. biopsies 1 and 2 ($P < 0.05$). *Significantly different for biopsy 3 vs. biopsy 1 ($P < 0.05$)

and 40% when values were compared to control and expressed per wet weight, protein content and acid precipitate, respectively) (Fig. 1).

Like cold cardioplegic arrest, warm cardioplegic arrest showed no significant change in the intracellular concentration of taurine following cardioplegic arrest (Fig. 2). However in contrast to the group receiving cold cardioplegia, reperfusion following warm cardioplegic arrest induced a small but insignificant fall (Fig. 2). The fall when compared to control was by 13%, 16%, 18% for values expressed per wet weight, protein content and acid precipitate, respectively.

Effect of ischaemia and reperfusion on intracellular ATP concentration

After 30 min of ischaemia, there was a small but insignificant fall in the intracellular concentration of ATP in the both groups of patients (Figs. 3 and 4). However upon reperfusion there was a significant fall in both groups which was more marked for the cold blood cardioplegia group (Figs. 3 and 4).

Discussion

In this work we present data showing that the intracellular concentration of taurine in the hypertrophic left ventricles with aortic stenosis at 9.9 $\pm$ 0.8 μmol/g wet weight is similar to the concentration found in hearts with ischaemic disease (earlier studies from the same laboratory reported values of 9.5 and 9.8 μmol/g wet weight (Suleiman et al., 1993; Suleiman et al., 1997). However the myocardial concentration of taurine in patients with clinical signs of congestive heart failure was slightly higher than hearts without clinical signs of congestive failure (Table 2). These findings are in contrast to an earlier suggestion that significantly higher taurine levels are present only in patients with congestive heart failure (Huxtable and Bressler, 1974). It is worthnoting however that this suggestion came from measurements of taurine in subjects who died from congestive heart failure, a situation that is different from the present study.

In an earlier study we have shown that ischaemic arrest using cold blood cardioplegic solution, did not induce a fall in taurine but on reperfusion a significant fall was seen (Suleiman et al., 1997). In the present study we show that similar changes also occur in hearts with different pathology. However this study shows for the first time that the fall in tissue taurine seen upon reperfusion following ischaemic arrest with cold blood cardioplegia, can be reduced with arrest using warm blood cardioplegic solution, indicating that the latter technique of myocardial protection reduces the extent of cellular changes which would eventually lead to loss of tissue taurine. It is possible however that longer periods of ischaemic arrest with warm blood cardioplegia would eventually lead to significant loss of tissue taurine.

A fall in taurine is likely to be due to transport because taurine is a non-protein amino acid and is very slowly metabolised (see Huxtable, 1992;

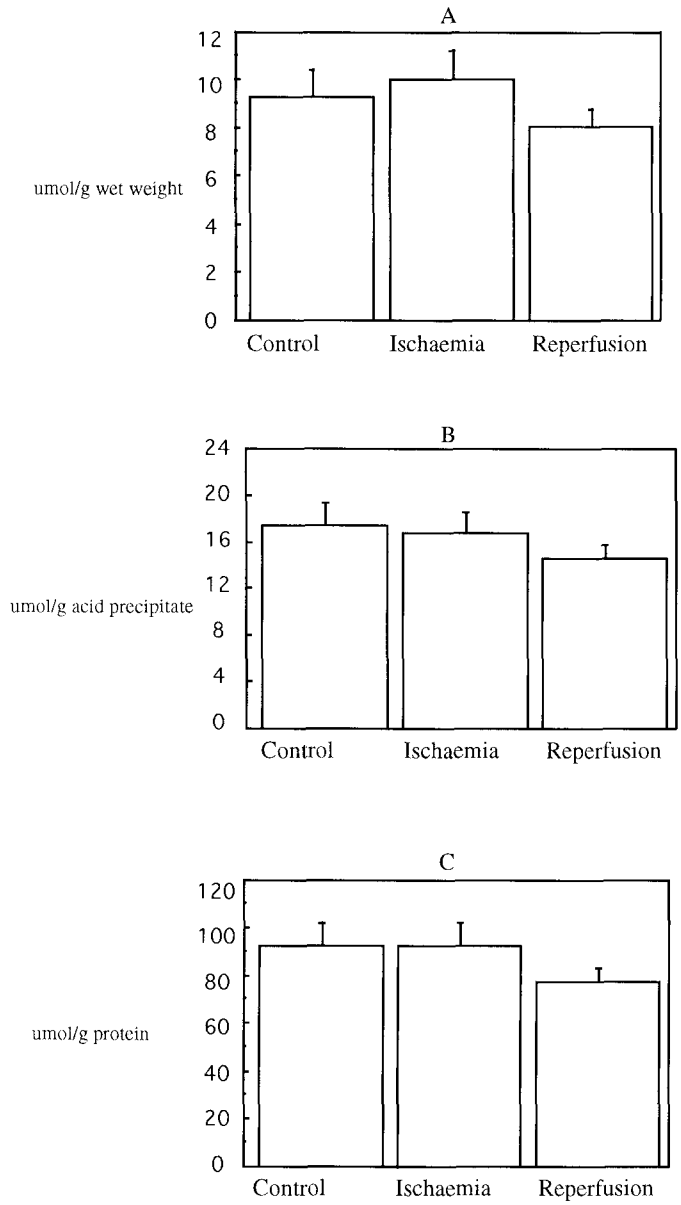


Fig. 2. Tissue taurine levels measured in ventricular biopsies taken from patients undergoing aortic valve replacement surgery using warm blood cardioplegia (n = 9). Control biopsy was collected 5 min after institution of cardiopulmonary bypass, ischaemic biopsy after 30 minutes ischaemic arrest and reperfusion biopsy after 20 min of reperfusion. Data was expressed per wet weight (**A**), per acid precipitate weight (**B**) and per protein content (**C**)

Suleiman, 1994). The transport of taurine is Na^+-dependent as shown by work on sarcolemmal vesicles, isolated heart cells and hearts (Chapman et al., 1993). Work on experimental models have shown that myocardial ischaemia is associated with a reduced Na-pump activity and an increased influx of Na^+

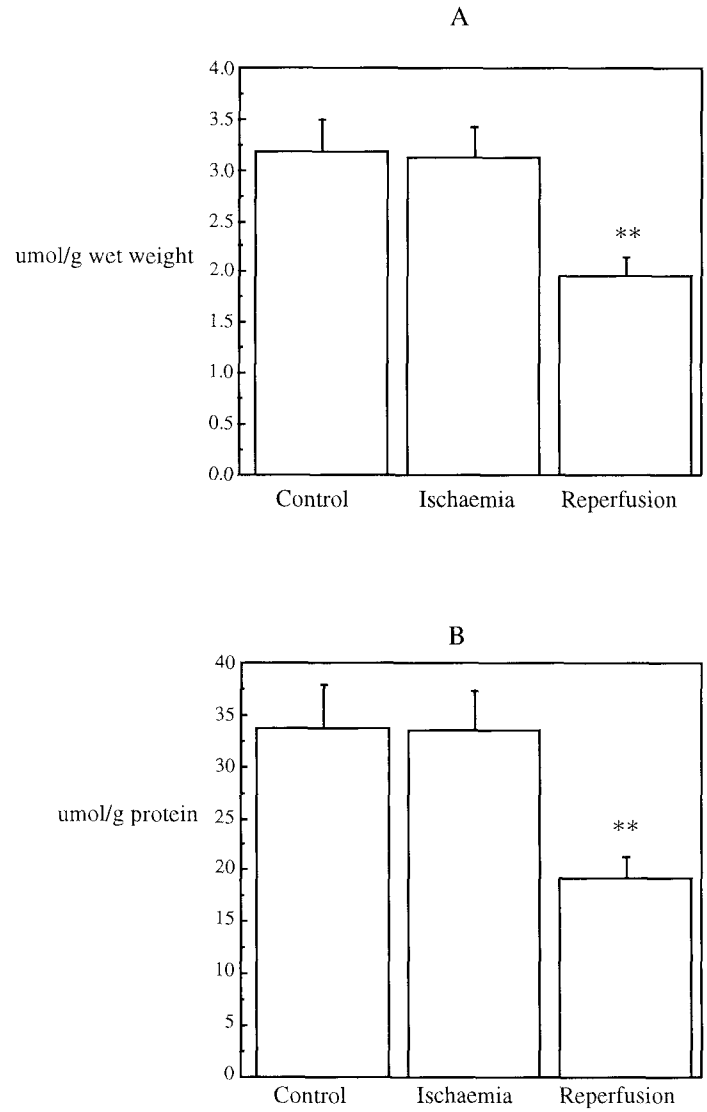


Fig. 3. Tissue ATP levels measured in ventricular biopsies taken from patients undergoing aortic valve replacement surgery using cold blood cardioplegia (n = 8). Control biopsy was collected 5 min after institution of cardiopulmonary bypass, ischaemic biopsy after 30 minutes ischaemic arrest and reperfusion biopsy after 20 min of reperfusion. Data was expressed per wet weight (**A**) and per protein content (**B**). **Significantly different for biopsy 3 vs. biopsies 1 and 2 ($P < 0.05$)

via the Na^+/H^+ result in an accumulation of $[Na^+]_i$ (Diederichs et al., 1990; Tani, 1990; Suleiman, 1994). As hypothermia inhibits the Na-pump (Suleiman and Chapman, 1990), a further rise in $[Na^+]_i$ is expected during hypothermic ischaemia. Therefore $[Na^+]_i$ accumulation during cold ischaemic arrest is likely to be higher than the rise resulting from ischaemia only. Therefore upon reperfusion, the higher levels of $[Na^+]_i$ in the cold cardioplegia group will lead to more Ca^{2+} loading resulting in an increased strain on cellular energy

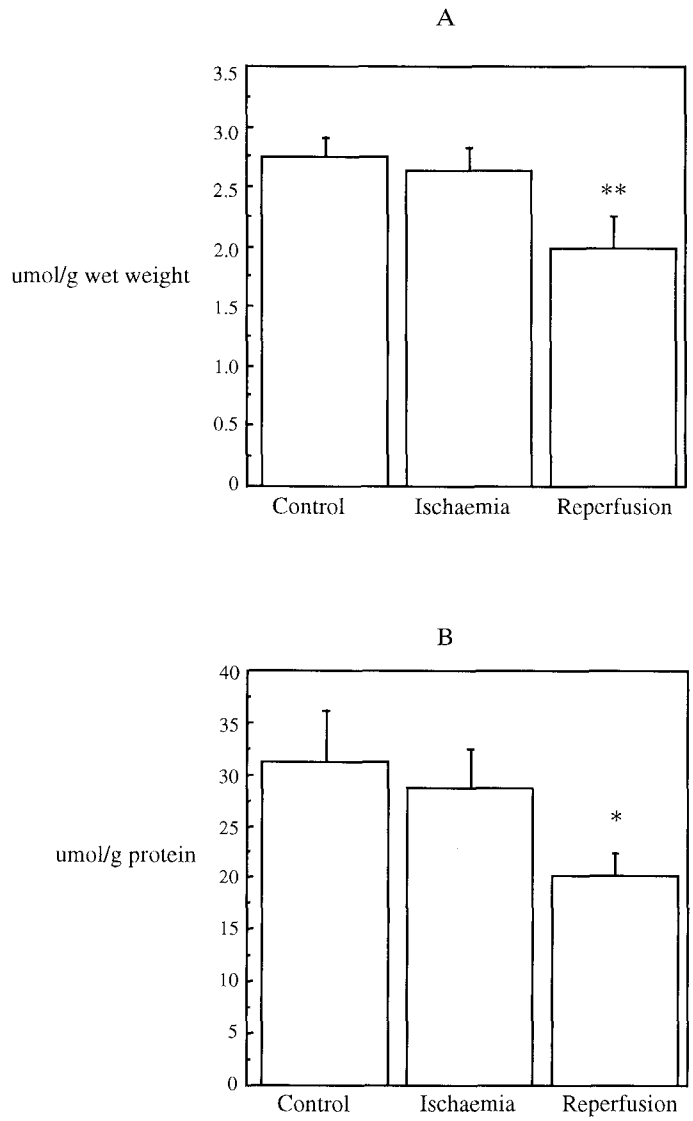


Fig. 4. Tissue ATP levels measured in ventricular biopsies taken from patients undergoing aortic valve replacement surgery using warm blood cardioplegia (n = 9). Control biopsy was collected 5 min after institution of cardiopulmonary bypass, ischaemic biopsy after 30 minutes ischaemic arrest and reperfusion biopsy after 20 min of reperfusion. Data was expressed per wet weight (**A**) and per protein content (**B**). **Significantly different for biopsy 3 vs. biopsies 1 and 2 ($P < 0.05$). *Significantly different for biopsy 3 vs. biopsy 1 ($P < 0.05$)

as shown by a further fall in ATP (Fig. 3) (Diederichs et al., 1990; Tani et al., 1990). On the other hand the extent of the rise in $[Na^+]_i$ in the warm cardioplegia group is expected to be less, resulting in a smaller fall in ATP (Fig. 4). The fall in ATP upon reperfusion in both groups is likely to be due to the resumption of mechanical activity and an attempt by the heart to reestablish ionic homeostasis, and not due to changes in the intracellular

concentration of taurine. A fall in taurine can be thought of as another tool used by heart cells to reduce the rise in $[Na^+]_i$ (via the Na^+-taurine symport) and therefore assist in recovery. That this mechanism was not activated during ischaemia when $[Na^+]_i$ is known to increase, could be because of low temperature influencing the transport mechanism. Furthermore and in contrast to reperfusion, the no flow situation during ischaemia may not facilitate the efflux of taurine and Na^+. The suggestion that Na^+ overload is responsible for the fall in the intracellular concentration of taurine is consistent with the proposed role for taurine as an osmoregulator (Pasantes-Morales and Schousboe, 1997).

Finally the loss of taurine in the cold cardioplegic group is likely to affect recovery following cardiac surgery as the amino acid has several important roles which include membrane stabilisation, Ca^{2+} mobilisation and is also implicated in the maintenance of normal cardiac cellular function as illustrated by the fact that its depletion is associated with the development of cardiomyopathy (see introduction).

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