Amino Acids (2003) 27: 107–111
DOI 10.1007/s00726-003-0033-9

Amino Acids
Printed in Austria

# Validity of elevated interstitial levels of taurine as a predictor of myocardial ischemic injury

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Received March 10, 2003
Accepted April 25, 2003
Published online October 30, 2003; 

**Summary.** The microdialysis (MD) technique allows for continuous *in vivo* monitoring of dynamic changes in the interstitial levels of energy-related metabolites. The release of taurine from the myocyte has been suggested as a marker of ischemic injury. The relationship between (interstitial) taurine release and the degree of myocardial ischemic injury was evaluated following a 40 min long ischemia in a porcine heart-infarct-model. Different protocols of ischemia and reperfusion were used in order to achieve a graded level of myocardial injury. Both interstitial peak levels and the area under curve of taurine obtained during ischemia and reperfusion correlated with the degree of ischemic injury (assessed by developed infarct size estimation). The release of taurine in the myocardium measured by the MD-technique correlated with the degree of ischemic injury during ongoing ischemic insult. Hence, taurine determination in the MD-setting represents a powerful tool to follow the development of myocardial ischemic injury over time.

**Keywords:** Microdialysis – Ischemia – Taurine – Infarct size – Myocardium – Pig

## Introduction

The microdialysis technique has been demonstrated to be a powerful tool for studies of metabolic events in the myocardium (Jackson et al., 2000). The technique offers the advantage of continuous *in vivo* monitoring of dynamic changes in the interstitial levels of low-molecular metabolites, following different experimental interventions such as ischemia or drug administration. Although a number of energy-related metabolites can be analysed in the collected microdialysis samples, none of them has been shown to accurately reflect the extent of an ischemic injury. The release of the amino acid taurine from the myocyte has been suggested as a marker of ischemic injury (Crass and Lombardini, 1977; Suleiman et al., 1997). However, the relationship between taurine release and the degree of myocardial ischemic injury in a microdialysis setting has not been evaluated. To address this question, interstitial levels of taurine following a 40 min long ischemic insult were compared with the extent of ischemic injury (developed infarct size) estimated by triphenyl tetrazolium chloride- and fluorescein-staining. Using a porcine heart-infarct-model, different protocols of ischemia and reperfusion were used in order to achieve a graded level of myocardial injury.

## Material and methods

### *Animal preparation*

All experiments conformed to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe No 123, Strasbourg 1985), and were approved by the Ethical Committee for Animal Research of Uppsala University.

Twenty one domestic pigs of either sex were selected from a local stock regularly used for experimental surgery. The animals weighing between 30–35 kg were prepared as described earlier (Kavianipour et al., 2003). In brief, the animals were anaesthesized and sternotomy was performed. After exposing the heart, one or two diagonal arteries of the left anterior descending artery running over the left ventricle and adjacent myocardium were selected for ischemic studies. A patched (tetrafluoroethylene polymer pledgets $3 \times 6$ mm, Johnson-Johnson, Brussels, Belgium) suture (W.L. Gore and Associete', Evry Cedex, France) was then placed around the chosen artery. Pulling the free ends of the suture running through the patch while pressing the patch against the artery resulted in occlusion, whereas releasing the pressure resulted in reperfusion. This occlusion technique typically reduced the regional blood flow to less than 10% of normal blood flow during occlusion and back to normal or supra-normal blood flow upon reperfusion (Nilsson et al., 1995). Reperfusion was verified by regression of the changes induced by the occlusion (ST-segment elevation, visual regional cyanotic demarcation, systolic bulging and cessation of contraction). A more specific confirmation was achieved by detecting at least a doubling of interstitial lactate levels upon ischemia (data not shown).

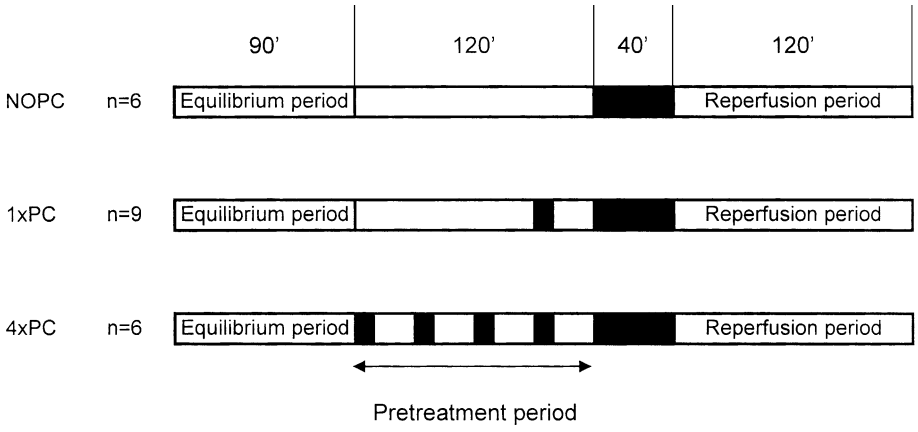


**Fig. 1.** Scheme of the experimental protocols. Abbreviations: (▮), regional myocardial ischemia

*Experimental protocol*

The experimental protocol is presented in Fig. 1. The animals (n = 21) were randomised in three groups and subjected to different protocols of ischemia and reperfusion. All animals were subjected to 120 min of pretreatment followed by 40 min of regional ischemia and 120 min of reperfusion. In one group (NOPC, n = 6), pretreatment consisted of 120 min of rest. In the other two groups the 40 min of regional ischemia was preceded by either one (1 × PC, n = 9) or four (4 × PC, n = 6) cycles of ischemia (10 min) and reperfusion (20 min) altogether comprising 120 min.

*Microdialysis technique*

The microdialysis technique has been described earlier (4). In brief, two microdialysis probes were placed in the left ventricular free wall, one in the anticipated ischemic tissue (ischemic probe) and another in a non-ischemic area (control probe). Starting with the equilibration period, the probes were perfused with a modified Krebs-Ringer phosphate buffer (sodium phosphate, 20 mM; magnesium chloride, 5 mM; sodium chloride, 110 mM, pH 7.4). Microdialysate samples were collected every 10 min. The flow rate was 2 $\mu$L/min and the dead space of the system was set to 10 $\mu$L, subsequently allowing 20 $\mu$L of microdialysate being collected with each 10 min sample. To ensure minimal degradation and evaporation bias the samples were immediately cooled upon collection in an ice bath and stored frozen at −18°C until analysis.

*Infarct size estimation*

The extent of ischemic injury (developed infarct size) was assessed by the ratio of necrotic area obtained by triphenyl tetrazolium chloride (TTC)-staining and the area of myocardium at risk (area at risk) obtained by fluorescein-staining as described earlier (Kavianipour et al., 2003).

*Biochemical analyses*

Components of the microdialysate were analysed with high performance liquid chromatography. Taurine was separated after precolumn derivatisation with orthophtaldialdehyde (Nucleosil 100 C 18 column, 60 × 4.0 mm) and measured by a fluorescence detector. All peaks were well separated from each other and the position of each metabolite in the chromatogram corresponded exactly with that of the standard.

*Statistical analyses*

Microdialysis data were estimated either as interstitial peak values or an estimate of area under the curve (AUC) of the microdialysis samples collected during the 40 min ischemia and the reperfusion period. Correlations were calculated with Pearson's rank test. A two-tailed p-value of $< 0.05$ was considered statistically significant.

## Results

Two dropouts were assignable to our studies, both belonging to the 1 × PC group. One was due to technical failure and the other due to ventricular fibrillation. There were no further episodes of ventricular fibrillation during the experimental procedures. Baseline levels of taurine were similar in the three groups. Interstitial levels of taurine increased following ischemia and declined upon reperfusion. Peak levels of taurine were reached during the second half of the 40 min long ischemia in all animals. Interstitial levels of taurine in non-ischemic tissue remained at baseline throughout the experiment. Both the interstitial peak levels of taurine and the AUC of taurine obtained during ischemia and reperfusion correlated with the developed infarct size as assessed by the ratio of necrotic area (TTC-staining) and the area at risk (fluorescein-staining) (Table 1, Fig. 2).

**Table 1.** Correlation coefficients (and equations) on reciprocal comparisons of interstitial levels of taurine and estimated infarct size in pig hearts

| Correlation vs. infarct size | r-value | p-value | Equation |
|---|---|---|---|
| Taurine, peak levels | 0.919 | <0.001 | y = 16,116Ln(x) − 57,659 |
| Taurine, AUC | 0.829 | <0.001 | y = 20,652Ln(x) − 165,99 |

*AUC*, Area under curve was calculated from the microdialysis samples collected during the 40 min ischemia and 120 min of reperfusion

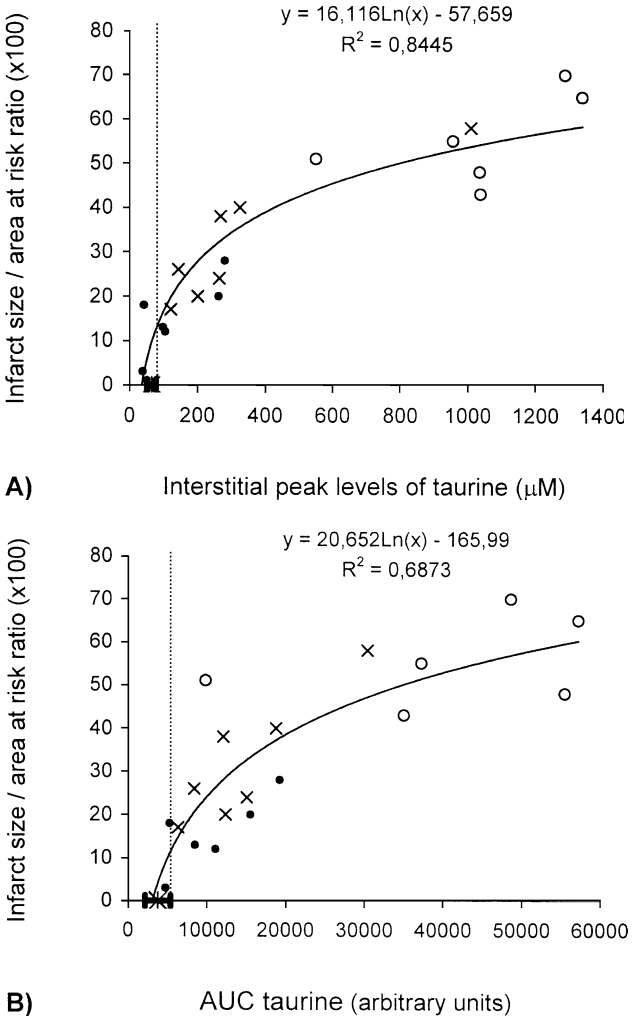


**Fig. 2.** Correlation of interstitial levels of taurine and the estimated infarct size/area at risk ratio in 19 pigs under various experimental conditions. A) Interstitial peak levels of taurine from ischemic probes. The mean and 95% confidence levels of data from non-ischemic probes (n = 19) is marked with a star and thick lines on the x-axis. B) AUC of taurine from all subjects calculated from the microdialysis samples collected during the 40 min ischemia and 120 min of reperfusion (ischemic probes). The mean and 95% confidence of data from non-ischemic probes (n = 19) is marked with a star and thick lines on the x-axis. Abbreviations: Open circles, the NOPC group; Cross, the 1 × PC group; filled circles, the 4 × PC group. Dotted vertical line, upper 95% confidence level of either interstitial peak level or AUC of taurine in the non-ischemic probes

## Discussion

In the current study it was desirable to attain different degrees of ischemic damage following a 40 min long ischemia. This was achieved by using two different protocols of ischemic preconditioning, an endogenous mechanism of heart protection (Murry et al., 1986).

In mammalian hearts taurine makes up about 50% of the free amino acid pool (Jacobsen and Smith, 1968). The physiological role of taurine in the myocardium is complex and not fully understood. It has been shown to sensitise contractile proteins to $Ca^{2+}$, and to increase the ability of the sacroplasmatic reticulum to store $Ca^{2+}$ (Sawamura et al., 1990; Steele et al., 1990). Furthermore, taurine possesses free radical scavenging properties and is believed to be involved indirectly in glutathione metabolism (Lin et al., 1988; Ohta et al., 1988). Taurine deficiency has been associated with dilated cardiomyopathy mediated via disturbances in calcium transport and angiotensin II response (reviewed in Schaffer et al., 2000). In addition, a role for taurine in development of diabetic cardiomyopathy has been described (reviewed in Militante et al., 2000). Addition of taurine has been shown to improve cardiac response to ischemic stress (Takahashi et al., 2000) and offer protection against the calcium paradox (Kramer et al., 1981). Taurine depletion was associated with improved resistance against hypoxia-induced necrosis and apoptosis in isolated myocytes (Schaffer et al., 2000).

Enhanced release of taurine was associated with acute and evolving myocardial injury (Crass and Lombardini, 1977; Lombardini and Cooper, 1981; Suleiman et al., 1997), suggesting its role as a marker of ischemic injury. However, recent works (Schaffer et al., 2002a, b) suggest that the taurine release may not be specific to ischemia. Taurine enters the myocyte via a $Na^+$/taurine symport mechanism with a presumed stochiometry of 1:1 (Suleiman et al., 1992). The activity the $Na^+$/taurine symport is responsible for a 200-fold gradient between cytosolic vs. plasma concentrations of taurine. Following events causing imbalance in intracellular $Na^+/Ca^{2+}$ concentrations (*i.e.* ischemia) the inward flow of taurine via the $Na^+$/taurine symport may be reversed (to an outward flow) in order to restore the intracellular $Na^+$ levels (Song et al., 1998). This in turn creates new opportunities for a myocytic $Na^+/Ca^{2+}$ antiport mechanism which is imperative for restoration of a disturbed intracellular $Ca^{2+}$ level due to energy crisis. Hence, the high intracellular levels of taurine may constitute an osmotic energy reserve which can be exploited to maintain ionic homeostasis and prevent $Ca^{2+}$ overload during osmotic stress. This line of reasoning is corroborated by others (Suleiman et al., 1992; Allo et al., 1997; Zemgulis et al., 2001). The extent/significance of such taurine-transport is not known. It is conceivable that taurine-transport according to above aids in maintaining ionic homeostasis (and subsequently limits the extent of taurine release/loss) at least during the initial phase of an ischemia. However, following sustained osmotic stress (*i.e.* prolonged ischemia) cell death and

membrane disruption occur causing a surge in taurine release. Support in this notion was offered by the hyperbolic shape of the correlation observed between the extent of ischemic injury (as assessed by infarct size development) and the interstitial levels of taurine (interstitial peak levels as well as AUC of repeated microdialysis measurements), in the present study. Hence, a low degree of ischemic damage (up to approx. 25% infarct size in Fig. 2A and 2B) was associated with a low pace of taurine release. While, following increased ischemic damage (beyond approx. 25% infarct size in Fig. 2A and 2B) the rate of taurine release was accelerated.

The taurine values obtained from the ischemic probe of two subjects overlapped with mean data from non-ischemic probes (see Fig. 2). Both subjects were from the $4 \times$ PC group. It is conceivable that the protective effect of the $4 \times$ PC preconditioning protocol modified the stress level in these subjects towards non-ischemic conditions, hence resulting in low infarct size and area at risk ratios. The cut off limit of such overlapping was approximately an infarct size/area at risk of 10%. Altogether this suggests that taurine release as assessed by the microdialysis technique represents an effective measure of the extent of ischemic injury. In addition, the use of the microdialysis technique in this respect allows *in situ* monitoring of the time sequence of the ischemic development in the pertinent anatomical region (data not shown). It should be kept in mind that data obtained by the microdialysis technique reflect local changes in interstitial level of metabolites. Through combining data of other metabolites obtained from the microdialysis samples the interpretation of taurine release can be refined.

One might argue that taurine release from the myocyte in response to the ischemic cycles of the pretreatment protocol in the preconditioned groups ($1 \times$ PC and $4 \times$ PC) resulted in successive depletion of the intracellular taurine levels, hence being explanatory to the lower levels of taurine observed in these groups during the 40 min long ischemia. However, an analysis of data obtained during the 120 min pretreatment period did not reveal any difference in taurine release between the groups. Moreover, the 40 min long ischemia was preceded by at least 20 min of reperfusion in all subjects, allowing for restoration of intracellular taurine levels.

The current study design would have benefited from additional acute markers of ischemia such as troponin T. However, there are no reliable methods for proper monitoring of troponins in pigs, and certainly not in the microdialysis setting (due to the high molecular weight of these compounds).

## Conclusion

The release of taurine in the myocardium (as measured by the MD-technique) correlated with the degree of ischemic injury (as evaluated by morphometric methods) during ongoing ischemic insult. Hence, taurine determination in the MD-setting would be a powerful tool to follow the development of ischemic injury over time.

## Acknowledgements

This study was supported by grants from The Swedish Heart and Lung Foundation, The Swedish Medical Research Council and The Norrland County Heart and Lung Foundation. The authors are indebted to Monica Hall and Åke Ericson, for their assistance.

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