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# Release of Tissue Taurine from the Oxygen-Deficient Perfused Rat Heart (40082)

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Taurine, 2-aminoethanesulfonic acid, is present in high concentrations in cardiac muscle (1). The function of this naturally-occurring substance has not been defined, although it has been implicated in maintenance of normal electrophysiological function of the heart. For example, it has been reported that taurine is capable of reversing electrocardiographic abnormalities induced by high concentrations of certain glycosides (i.e. digoxin and strophanthin K) (2, 3). In this context, taurine may be involved in regulating ion fluxes, specifically $K^+$ and $Ca^{2+}$ (4, 5).

We have recently reported both a regional heterogeneity in taurine levels within the dog myocardium and a generalized decrease in the taurine content in ischemic cardiac muscle (6). The ischemic left ventricle, induced by coronary artery ligation, demonstrated a loss in taurine content exceeding 45%. The loss of taurine in the myocardium could have occurred by at least two mechanisms: (a) metabolism or (b) release into the general circulation. While it has been demonstrated in tracer studies that taurine is indeed metabolized to isethionic acid, the rate is extremely slow (7, 8). Therefore, in quantitative terms the loss of taurine during short periods of ischemia (4 hr) due to metabolism could be eliminated as a possible mechanism. Since taurine is normally present in blood, albeit in low concentration, and since ischemic damage is principally a manifestation of cellular hypoxia, we have attempted to reproduce taurine loss in an isolated heart perfused with a taurine-free and oxygen-deficient buffer.

The present report shows, indeed, that myocardial cellular hypoxia resulted in a loss of tissue taurine *per se*. This taurine was recovered quantitatively in the buffered perfusate.

*Materials and methods. Animals.* Male Sprague–Dawley rats weighing 250–300 g were given free access to Purina rat chow and water.

*Perfusion system.* The animals were anesthetized with ether, the chest opened, heart excised and mounted on the perfusion apparatus as described elsewhere (9–11). Coronary perfusion pressure was constant at 82 mmHg. One group of hearts was perfusion-washed in a nonrecirculated system for 5 min with Krebs–Henseleit bicarbonate buffer containing 5.5 m*M* glucose. The ventricles were freeze-clamped with Wollenberger tongs cooled in liquid nitrogen and the frozen tissue analyzed for "initial" taurine content. A second group of hearts was perfused for an additional 30 min ("residual") before freeze-clamping and analysis. Each of the groups mentioned above was further divided into two groups; in one group, the buffered perfusate was equilibrated with 95% $O_2$–5% $CO_2$ (oxygenated controls) and in the other, the medium was gassed with 95% $N_2$–5% $CO_2$ (oxygen-deficient). The perfusion medium was sampled at 10-, 20- and 30-min intervals and aliquots taken for taurine analyses. Mean coronary flow rates (ml/min/g wet wt ± SE) were 10.9 ± 0.8 and 16.8 ± 1.0 for oxygenated controls and oxygen-deficient hearts, respectively. Mean heart rates (beats/min ± SE) were 286.6 ± 24.0 for oxygenated hearts and 130.0 ± 38.7 for oxygen-deficient hearts.

*Estimation of tissue and perfusate taurine.* The cardiac tissue was weighed in the frozen state and then homogenized in 3 vol of cold 2% perchloric acid with a Virtis Model 45 homogenizer for 20 sec at half speed. The homogenate was centrifuged for 10 min at 12,000*g*. Aliquots of the supernatant were analyzed for taurine content by an amino analyzer procedure as described previously (12).

A portion of ventricle from each heart was dried to constant weight and the dry: wet ratio determined. All data are expressed on a dry weight basis. A programmed statistical analysis of the data was performed on an IBM 370/145 computer.

*Results.* The effects of oxygen deficiency



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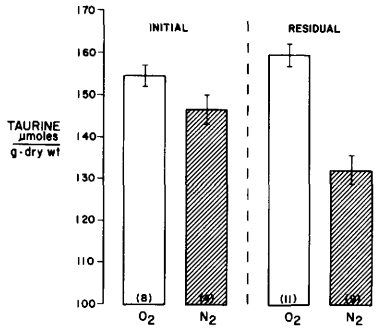


FIG. 1. Taurine content in rat heart after perfusion with or without oxygen. Hearts were perfused with Krebs–Henseleit bicarbonate buffer containing 5.5 m*M* glucose for 5 min ("initial") or for an additional 30 min ("residual"). Clear bars and hatched bars show values for tissue taurine in hearts perfused with buffer equilibrated with 95% $O_2$–5% $CO_2$ and 95% $N_2$–5% $CO_2$, respectively. Each value represents the mean ± SE of the number of hearts indicated in parenthesis.

on tissue taurine content are shown in Fig. 1. Values shown on the left of the figure represent taurine content after a nonrecirculated short-term washout of the coronary vascular bed and thus reflect a more accurate estimation of initial tissue taurine. The apparent difference between values from normoxic and anoxic perfused hearts was not statistically significant ($P > 0.10$). Comparison of "initial" and "residual" values showed that tissue taurine levels remained unchanged after 30 min of well-oxygenated perfusion ($P > 0.10$). However, a similar period of perfusion under anoxic conditions resulted in a decrease in content of taurine ($P < 0.02$).

Since taurine was not present in the perfusion medium initially, samples of perfusates were collected at various time intervals during recirculated perfusion. The results are shown in Fig. 2. In well-oxygenated control hearts, a small quantity of taurine was detected after 10 minutes of perfusion. In this group of hearts, perfusate taurine amounted to less than 3% of initial tissue values which was within methodological error. No further release of taurine was observed after the initial 10-min sampling. In hearts perfused with oxygen-free medium, taurine release increased three- to fourfold after 10 min of recirculated perfusion and continued to increase through 30 min. Comparison of values for tissue taurine loss and those for appearance of taurine in the perfusion medium of oxygen-deficient hearts after 30 minutes indicated approximately 100% recovery.

*Discussion.* The role of taurine in tissue function is for the most part uncertain; the exception being its known conjugation with specific bile acids (13). In recent years, however, evidence has accumulated to suggest a functional role of taurine in heart; a tissue which contains a relatively high content of this sulfonic amino acid. Observations by Kocsis *et al.* (14) and Crass and Lombardini (6) have shown that dog myocardial tissue displays a regional heterogeneity in taurine content, including an increasing outer to inner transmural gradient across the left ventricular wall.

A potentially important function of taurine in heart was suggested in the experiments of Read and Welty (2) and others (3). These workers noted that infusion of taurine into animals demonstrating glycoside-induced electrocardiographic abnormalities resulted in the disappearance of the arrhythmias. These and other more recent observations which have recently been reviewed (15) have implicated taurine, or its principal metabolite, isethionic acid, in regulation of intracellular $K^+$ and $Ca^{2+}$ fluxes. In this context, we recently postulated (6) that a contributing factor to the arrhythmias characteristic of acute myocardial ischemia could be the loss of intracellular taurine. The cardiac muscle cell maintains a large taurine gradient relative to the extracellular fluid (3). A decrease in cellular phosphate potential secondary to severe

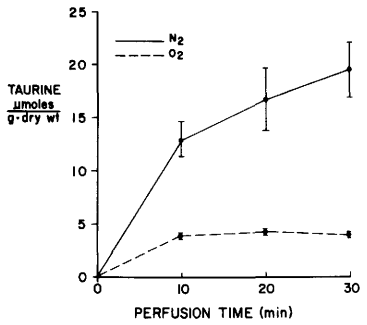


FIG. 2. Release of tissue taurine into perfusate during anoxic perfusion of the isolated rat heart. Hearts were perfused with Krebs–Henseleit bicarbonate buffer containing 5.5 m*M* glucose equilibrated with either 95% $N_2$–5% $CO_2$ (solid line) or 95% $O_2$–5% $CO_2$ (broken line). Each value represents the mean cumulative taurine value in the recirculated perfusate of six hearts at the times indicated ± SE.

hypoxia may, as in the case of $K^+$ loss (16), be the basis of taurine loss observed in ischemic heart muscle (6). Taurine loss is particularly large in the subendocardial region where density of conduction tissue is greatest (14, 17).

In the present studies, isolated rat hearts, deprived of oxygen to simulate ischemia-induced cellular hypoxia, were perfused with a taurine-free medium to facilitate detection of possible tissue taurine leakage. In hearts perfused with oxygen for 30 min, the tissue content of taurine was not significantly changed (Fig. 1). However, within 10 minutes the perfusate contained 3.88 μmoles of taurine/g dry wt (Fig. 2). The recovery of taurine in the oxygenated perfusate was due, perhaps, to the short period of hypoxia (<1 min) when the heart was removed from the animal and mounted on the perfusion apparatus. Furthermore, the observation that the taurine release into the medium did not increase with time tended to support this conclusion.

In hearts perfused with the nitrogen mixture, a significant decrease in tissue taurine content was observed (Fig. 1). The "lost" taurine was recovered 100% in the perfusate (Fig. 2). Moreover, the release of taurine under conditions of oxygen deficiency increased with time (Fig. 2).

The data obtained in these experiments demonstrate that whole heart hypoxia, as in the case of acute ischemia *in vivo* (6), results in taurine loss. Furthermore, loss of taurine appears to involve leakage of taurine *per se* as opposed to hypoxia-induced metabolic conversion to a taurine metabolite. The decrement in the tissue pool(s) of taurine was recovered quantitatively in the perfusion fluid.

It would be of interest to determine whether a nonrecirculated perfusion system involving continuous removal of released taurine would potentiate tissue loss; and conversely, whether supplementation of the perfusion medium with exogenous taurine in anoxic or ischemic hearts could prevent tissue taurine leakage. Experiments to test these questions as well as efforts to correlate severity of electrophysiologic abnormalities with extent of taurine loss are currently in progress.

*Summary.* The effects of cellular hypoxia on taurine levels in rat hearts were determined. Hearts perfused with 95% $N_2$–5% $CO_2$ demonstrated a significant decrease in tissue taurine content when compared to control hearts perfused with 95% $O_2$–5% $CO_2$. The loss of taurine in oxygen-deficient hearts was time dependent over a period of 30 min. The perfusate when analyzed for taurine content contained 100% of the released taurine. Thus, metabolic conversion of taurine had no role in the disappearance of taurine from the rat heart.

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