Taurine depletion, a novel mechanism for cardioprotection from regional ischemia

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Allo, Simon N., Lillian Bagby, and Stephen W. Schaffer. Taurine depletion, a novel mechanism for cardioprotection from regional ischemia. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H1956–H1961, 1997.—Three processes that have been implicated in ischemic injury are impaired Ca2+ movement, altered osmoregulation, and membrane remodeling. Because the amino acid, taurine, affects all three processes, it seemed logical that changes in the myocardial content of taurine might affect ischemic injury. To test this hypothesis, infarct size and areas at risk were compared in isolated hearts from control and taurine-depleted rats after a 45-min ligation of the left anterior descending coronary artery and 2 h of reperfusion. Hearts of rats treated for 4 wk with the taurine inhibitor, β-alanine, exhibited a 57% reduction in the infarct size-to-risk area ratio. The degree of cardioprotection was found to correlate (r = 0.85) with the extent of taurine depletion, the latter dependent on the length of β-alanine feeding. When the taurine-depleted rats were fed taurine, myocardial taurine levels were restored and the cardioprotection was lost. However, addition of neither β-alanine (3%) nor taurine (20 mM) to the perfusion medium altered infarct size. We conclude that taurine depletion renders the heart resistant to injury caused by regional ischemia.

β-alanine; infarct size; osmoregulation

The ubiquitous sulfur-containing amino acid, taurine, is found in high concentrations in the heart, where it accounts for ~50% of the free amino acid pool (12). The exact role of taurine in cardiac function is not fully understood, although it has been purported to mediate a plethora of effects at the physiological, biochemical and molecular level (2, 12). Among its most important “physiological” actions in the heart are the modulation of Na+ and Ca2+ homeostasis (2, 9), the alteration in membrane structure and function (8), and the regulation of intracellular osmolarity (24). The importance of these actions is borne out by nutritional studies showing that cats fed a taurine-deficient diet develop cardiomyopathy (21).

Pharmacological doses of taurine also mediate several effects. One important action is its ability to exert a positive inotropic effect, which is independent of adenosine 3′,5′-cyclic monophosphate or Na+K+-adenosinetriphosphatase (2). Of equal importance is the finding that taurine treatment protects against injury in several models of heart failure, including the Ca2+ paradox, cardiomyopathic hamster, isoproterenol cardiotoxicity, and doxorubicin-induced cardiac damage (2, 16, 23). However, the most dramatic effect of taurine is observed in experimental heart failure, in which taurine treatment is reported to significantly reduce mortality (29). These animal studies have encouraged the use of taurine as a therapeutic agent. The most clinically relevant role of taurine to date has been its use for congestive heart failure in Japan, where clinical trials have revealed an improvement in New York Heart Association classification of patients, who have been treated with or without digoxin (1).

Although most studies to date have supported the notion that maintenance of high intracellular taurine levels in the heart is beneficial to normal myocardial function, recent studies by Chapman et al. (5) suggest that taurine efflux may help eliminate excess Na+ from the myocyte. Moreover, under pathological conditions in which the intracellular osmotic pressure rises, it is likely that the heart should benefit from a loss of the osmolyte, taurine. Thus, because both water and Na+ accumulate during ischemia and are thought to contribute to the severity of injury, it is logical to assume that changes in the size of the intracellular taurine pool should affect the outcome of an ischemic insult. To test this hypothesis, we examined the effect of drug-induced taurine depletion on cellular necrosis in a regional model of ischemia.

METHODS

Hearts from male Wistar rats weighing 250–300 g were taurine depleted by maintaining the rats on tap water containing 3% β-alanine for 4–28 days (9, 25). Some rats, referred to as the taurine-replete group, were maintained for 8 days on tap water containing 1.5% taurine after a 28-day treatment with 3% β-alanine. Control rats were age matched and maintained on normal tap water for the duration of the experiment. Neither taurine depletion nor repletion significantly altered rat body weight relative to the controls; body weight was 382 ± 28, 350 ± 21, and 365 ± 31 g for the control, taurine-depleted, and taurine-replete groups, respectively. Moreover, the dry weight of the heart was identical (0.29 ± 0.01 g) in all three groups. However, the protocols led to an alteration in the wet-to-dry weight ratio of the heart, which was 4.76 ± 0.06, 5.05 ± 0.10, and 4.73 ± 0.06 for the control, taurine-depleted, and taurine-replete groups, respectively.

After the appropriate period of taurine depletion and repletion, most hearts were perfused on a Langendorff apparatus with Krebs-Henseleit buffer containing 118 mM NaCl, 27.1 mM NaHCO3, 2.8 mM KCl, 1 mM KH2PO4, 1.2 mM MgSO4, 2.5 mM CaCl2, and 11 mM glucose, which was saturated with 95% O2-5% CO2 and maintained at 37°C. However, when the effect of exogenous taurine and β-alanine on ischemic injury of untreated, control hearts was examined, the Krebs-Henseleit buffer was supplemented with either 20 mM taurine or 3% β-alanine. For all experiments, the coronary perfusion pressure was fixed at 100 cmH2O.

To initiate the experiment, a 2-0 silk suture was loosely placed around the left main coronary artery and passed through a small vinyl tubing to form a snare. The hearts were then perfused under normal conditions for 20 min. After the stabilization period, coronary occlusion was effected in some...
of the hearts by pulling the suture through the snare and clamping the snare with a hemostat. The desired period of coronary artery occlusion (45 min) was followed by reperfusion for 2 h. The experiment was terminated by retightening the snare and infusing a 0.2% solution containing 1–10 µm zinc-cadmium fluorescent particles (Duke Scientific, Palo Alto, CA) into the aorta. The fluorescent particles were able to delineate the risk zone, which was defined as the region lacking fluorescence when observed with a 366-nm fluorescent lamp. After administration of the fluorescent particles, the hearts were removed from the perfusion apparatus and frozen for at least 2 h before being cut into 2-mm-thick slices. The slices were incubated for a period of 20 min at 37°C in a 1% solution of triphenyltetrazolium chloride dissolved in phosphate buffer (pH 7.4). The slices were then placed between glass plates, and the areas of the risk zones (delineated as nonfluorescent zones) and infarcted areas (lacking staining with tetrazolium) were determined by planimetry. The volumes of the risk and infarcted zones were determined by multiplying the area by the slice thickness. The infarct size was expressed as a percentage of the risk zone that was infarcted.

Determination of taurine levels. Cardiac taurine content was measured by the method of Shaffer and Kocsis (25). Hearts were perfused with Krebs-Henseleit buffer via aortic cannulation to remove the blood and then blotted, weighed, and frozen. After freeze drying, the samples were reweighed and homogenized with 2% perchloric acid. After neutralization with K$_2$CO$_3$, the supernatant was used for taurine determination.

An aliquot of the supernatant (20 µl) was diluted to 400 µl and then reacted with 0.1 ml of 2,4-dinitrofluoro-1-benzene (DNFB) in the presence of 0.1 ml of 1 M NaOH and 0.5 ml of dimethyl sulfoxide. The reaction was terminated after 30 s by the addition of 0.1 ml of 3 M HCl, which lowered the pH to 1.5–2.0. Deionized water was added to a final volume of 5 ml. Samples were then extracted with ethyl acetate (20 ml) for 10 min to remove the derivatized carboxylic amino acids and unreacted DNFB. The optical density at 355 nm of the aqueous fraction containing 2,4-dinitrophenyltaurine was determined. A taurine standard curve using 2–40 µg taurine was obtained for each assay.

Hemodynamic measurements. Hearts from control, taurine-depleted, and taurine-replete rats were perfused on a standard working heart apparatus with Krebs-Henseleit buffer supplemented with 11 mM glucose. After a 20-min stabilization period, several hemodynamic parameters were measured. Peak ventricular systolic pressure and heart rate were measured with a Statham P23 Gb pressure transducer by inserting a 22-gauge needle through the ventricular wall. Coronary flow was determined by collecting the coronary effluent.

Statistical analysis. All data involving multiple groups (control, taurine-depleted, and taurine-replete) were analyzed by analysis of variance, with the Newman-Keuls test used to determine the source of the significant difference. When only the taurine-depleted and control groups were compared, the Student’s t-test was used to determine significant differences.

RESULTS

Taurine depletion. Previous studies have shown that cardiac taurine pools can be significantly reduced by treating rats with the taurine transport inhibitor, β-alanine (9, 25). In accordance with those studies, we found that cardiac taurine levels were reduced from 105.0 ± 2.2 to 63.2 ± 5.3 µmol/g dry wt after 4 wk of β-alanine treatment (Table 1). This process is readily reversible, since the addition of taurine to the drinking water rapidly restored the myocardial taurine pool.

The degree of taurine depletion was dependent on the length of time the animals received β-alanine in their drinking water. Within the first few days after treatment with β-alanine, myocardial taurine levels fell abruptly. Thereafter, there was a slow decline in the size of the myocardial taurine pool, which reached a new steady state by ∼2 wk of treatment (Fig. 1).

Table 2 demonstrates that the baseline hemodynamic parameters were unaffected by either taurine depletion or repletion.

Effect of taurine depletion and repletion on myocardial infarct size. The size of the risk zone after ligation of the left anterior descending coronary artery was 0.4 cm$^2$ in all hearts examined (Table 3). After 45 min of ischemia and 2 h of reperfusion, control hearts exhibited an infarct size-to-risk area ratio of 55.68 ± 2.04%, which is similar to the value reported by other investigators for the same period of ischemia (4, 11, 17). Significantly, a 40% reduction in the size of the myocardial...
Table 2. Effect of taurine depletion on hemodynamic properties

<table>
<thead>
<tr>
<th>Hemodynamic Property</th>
<th>Control</th>
<th>Taurine Depleted</th>
<th>Taurine Replete</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>250 ± 22</td>
<td>250 ± 25</td>
<td>249 ± 27</td>
</tr>
<tr>
<td>Coronary flow, ml/min</td>
<td>21.9 ± 0.9</td>
<td>23.1 ± 1.6</td>
<td>26.3 ± 1.2</td>
</tr>
<tr>
<td>Peak systolic pressure, cmH2O</td>
<td>185 ± 11</td>
<td>179 ± 12</td>
<td>174 ± 6</td>
</tr>
<tr>
<td>Rate-pressure product x 1,000</td>
<td>46.1 ± 3</td>
<td>45.8 ± 10</td>
<td>43.2 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4–6 hearts. Hearts from control, taurine-depleted, and taurine-replete rats were perfused on a working heart apparatus with buffer containing 11 mM glucose. After 20 min of stabilization, heart rate, coronary flow, and peak systolic pressure were measured. No significant difference was noted between groups.

dial taurine pool resulted in a 57% decrease in the infarct size-to-risk area ratio (Table 3).

To further delineate the effect of taurine depletion on infarct size, we evaluated hearts whose taurine content was varied by treating rats with β-alanine for different intervals. Infarct size was maximally reduced after 2 wk of β-alanine feeding, coinciding with maximal depletion of the myocardial taurine pool (Figs. 1 and 2). Continuous feeding with β-alanine beyond 2 wk neither altered myocardial taurine levels nor affected the extent of cardioprotection from regional ischemia. However, exposure to β-alanine for shorter periods of time resulted in less taurine depletion and a corresponding smaller decline in infarct size.

Figure 3 reveals that a significant correlation ($r = 0.85$) exists between cardiac taurine levels and the infarct size-to-risk area ratio. Because β-alanine does not accumulate in the heart (data not shown), the observed reduction in the infarct size-to-risk area ratio appears to be caused by taurine depletion. This is supported by the observation that acute exposure to β-alanine (3%) in vitro had no effect on infarct size (Table 4). Similarly, addition of 20 mM taurine to the perfusion buffer throughout the experimental protocol did not alter infarct size. However, repletion of the cardiac taurine pool by maintaining taurine-depleted rats for 8 days on water containing 1.5% taurine completely eliminated the cardioprotective effects of taurine depletion (Table 3).

Table 3. Effect of taurine depletion and repletion on infarct size

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Taurine Depleted</th>
<th>Taurine Replete</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk area, cm$^3$</td>
<td>0.40 ± 0.03</td>
<td>0.39 ± 0.02</td>
<td>0.47 ± 0.04</td>
</tr>
<tr>
<td>Infarct size, cm$^3$</td>
<td>0.22 ± 0.02</td>
<td>0.10 ± 0.02*</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>Infarct size/risk area, %</td>
<td>35.7 ± 2.04</td>
<td>24.1 ± 5.2*</td>
<td>51.6 ± 5.1</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4–6 hearts. Hearts from control, taurine-depleted, and taurine-replete rats were perfused for a stabilizing period of 20 min. Hearts were then subjected to 45 min of regional ischemia and 2 h of reperfusion. Infarct size and risk area were determined as described in Methods. Percent of infarct size/risk area was calculated by dividing infarct zone volume by risk zone volume for each experiment. *Statistical significance from control and taurine-replete groups ($P < 0.05$).

DISCUSSION

The present study is the first investigation examining the effect of taurine in a myocardial regional ischemia model. The relevance of this study is that it uncovers a new and novel means of cardioprotection. It also introduces a new model to investigate mechanisms that contribute to cardioprotection against ischemic-induced cell necrosis.
Equally effective in reducing infarct size-to-risk between 63 and 73% after 45 min of regional ischemia. The maximal effect, a 57% reduction in taurine depletion results in cardioprotection from reperfusion-induced cell damage that occurs during coronary artery bypass surgery. However, they found that taurine prevented the decline in mechanical function induced by addition of neutrophils to the reperfusion medium. This protective effect of taurine was attributed to its ability to scavenge hypochlorous acid, thereby reducing the degree of oxidant damage mediated by the neutrophils. Similarly, the antioxidant effect of taurine has been implicated in taurine-mediated reductions in cell damage that occur during coronary artery bypass grafting (19). Interestingly, in the latter two studies, cardioprotection was only observed when extracellular taurine levels were significantly elevated, suggesting that the neutrophil neutralizing effect of taurine represents a pharmacological, rather than a physiological effect.

The present study contrasts with all previous ischemia studies because the experimental protocol focuses on changes in the intracellular taurine pool. A standard procedure was used to slowly lower the intracellular taurine pool. The value of this procedure was twofold. It permitted the regulation of myocardial taurine levels over a fairly wide concentration range and also allowed the reversal of the taurine defect merely by adding taurine to the animals’ water supply.

The most significant finding of this study is that taurine depletion results in cardioprotection from regional ischemia. The maximal effect, a 57% reduction in risk zone infarcted, is comparable in scope to other cardioprotective procedures. One of the most widely studied cardioprotective mechanisms, preconditioning, has been reported to reduce infarct size-to-risk area between 63 and 73% after 45 min of regional ischemia (4). Equally effective in reducing infarct size-to-risk area have been the Na+/H+ exchange inhibitors (4, 13). Two procedures that are slightly less potent in mediating cardioprotection are hyperthermia, a form of heat shock protein induction, and streptozotocin-induced diabetes, whose mechanism of cardioprotection is unknown; both conditions diminish the infarct size-to-risk area ratio ~33% (11, 17).

Although taurine depletion significantly reduces infarct size in the reperfused heart, it does not significantly improve recovery of contractile function (data not shown). Two explanations can be provided to account for this paradoxical observation. First, the area at risk is not fixed in the regional ischemia model, causing considerable variability in the recovery of mechanical function even among the control group. Second, taurine affects myocardial contractile function through alterations in Ca2+ movement and increased sensitivity of the myofibrils to Ca2+ (2, 9, 26). Although the degree of taurine depletion achieved in the β-alanine-treated rats does not induce a change in mechanical function (Table 2), more severe decreases in the intracellular taurine pool are associated with the development of a cardiomyopathy (21). This is relevant because massive amounts of taurine efflux the heart during an ischemia-reperfusion insult (16, 18). Thus, although the protected regions of the heart do not die, they exist in an unusually severe state of stunning. Consequently, the favorable effect of reduced infarct size may be balanced by the unfavorable effect of taurine depletion on contractile function.

Several factors support the conclusion that the β-alanine-mediated reduction in infarct size is directly related to taurine depletion. First, a negative correlation exists between taurine levels and the extent of cardioprotection (Fig. 3). Second, the cardioprotection is completely reversed by repleting the cardiac taurine pool. Third, the only known cardiovascular effects of β-alanine relate to the inhibition of taurine transport and the promotion of taurine efflux from the myocyte (9, 25). Fourth, the effects of β-alanine feeding cannot be duplicated by acute exposure of the isolated heart to either 3% β-alanine or 20 mM taurine (Table 4).

The mechanism by which taurine depletion is cardioprotective remains to be completely examined. One attractive hypothesis is that taurine, an effective osmolyte, plays a critical role in osmoregulation during ischemia-reperfusion (5). It has been established that taurine is rapidly lost from the heart after ligation of the circumflex branch of the left main artery (18) or after global ischemia followed by reperfusion (16). This ischemia-induced taurine loss may merely reflect a response of the ischemic heart to the accumulation of osmotically active agents, such as Na+, Pi, and lactate. Because taurine is an important osmolyte, its efflux from the cell effectively reduces the intracellular osmotic load, thereby diminishing the osmotic gradient across the cell membrane. According to Jennings and co-workers (14, 27), the intracellular osmotic load can lead to excessive cell swelling, which is thought to play a critical role in irreversible cell damage.

### Table 4. Effect of acute taurine and β-alanine exposure on infarct size

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Taurine (20 mM)</th>
<th>β-Alanine (3%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk area, cm³</td>
<td>0.40 ± 0.03</td>
<td>0.37 ± 0.04</td>
<td>0.51 ± 0.06</td>
</tr>
<tr>
<td>Infarct size, cm³</td>
<td>0.22 ± 0.02</td>
<td>0.18 ± 0.01</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td>Infarct size/risk area, %</td>
<td>55.7 ± 2.04</td>
<td>48.8 ± 5.0</td>
<td>48.8 ± 6.4</td>
</tr>
</tbody>
</table>

Values shown are means ± SE; n = 4 hearts. Hearts were perfused on a Langendorff apparatus with Krebs-Henseleit buffer containing no additions (control), 20 mM taurine, or 3% β-alanine. Perfusion protocol was identical to that described in Table 3.
Several investigators have attempted to reduce infarct size by decreasing the osmotic gradient across the cell membrane by raising the osmolality of the extracellular medium. This strategy has led to mixed results. Klener et al. (15) and Garcia-Dorado et al. (7) have reported a decline in infarct size in the hyperosmotically treated heart, whereas Harada et al. (10) found no influence of hyperosmolar mannitol on infarct size in the baboon heart. The present approach is seemingly related to that strategy; however, instead of raising the extracellular osmotic load, taurine depletion reduces the intracellular osmotic load. Nonetheless, two findings suggest that the cardioprotection noted in the taurine-depleted heart may not be related solely to the change in the osmotic pressure gradient. First, addition of hyperosmolar concentrations of β-alanine to the perfusate failed to reduce infarct size. Second, addition of 20 mM taurine to the perfusion medium with the aim of eliminating the taurine gradient across the myocyte membrane, did not influence infarct size.

Because the process of taurine depletion in the β-alanine-fed rat is complex, it is not surprising that multiple factors could contribute to the observed cardioprotection. An important consideration is that the taurine-depleted heart, to maintain an osmotic balance, presumably undergoes an adjustment involving modifications in the content of intracellular organic osmolytes as well as the activity of transporters involved in osmoregulation. A transporter whose activity is altered after an osmotic pressure insult is the Na\(^+/\)H\(^+\) exchanger (3). This transporter is of particular interest because inhibitors of the Na\(^+/\)H\(^+\) exchanger protect the heart against ischemic injury (13). Moreover, reduction in flux through the exchanger, either by manipulation of the cation composition of the myocyte or the intrinsic activity of the transporter, invariably leads to less cell damage during an ischemic-reperfusion or hypoxic-reoxygenation insult (13). Because taurine depletion appears to induce an osmotic stress, one would predict that the activity of the Na\(^+/\)H\(^+\) exchanger should be affected by β-alanine feeding.

Another important osmotic-sensitive transporter is the Na\(^+/\)Ca\(^2+\) exchanger (30). Previously, we demonstrated that the activity of the Na\(^+/\)Ca\(^2+\) exchanger is depressed in the taurine-depleted myocardium (9). Because this transporter is thought to play a pivotal role in Ca\(^2+\) overload-induced myocardial injury, it is a logical candidate for the cardioprotection of taurine depletion.

Taurine has another potential link to the regulation of intracellular cation homeostasis. The process of taurine uptake by the heart involves cotransport with Na\(^+\). According to Chapman et al. (5), taurine efflux utilizes this same Na\(^+\)-taurine cotransporter. This scenario would dramatically affect the ischemic heart because taurine efflux would be accompanied by a significant decrease in intracellular Na\(^+\) concentration. Thus damage to the heart would be minimized because both the osmotic and Na\(^+\) loads would be reduced. Although this scenario is attractive, in most noncardiac cells taurine efflux occurs via a Na\(^+\)-independent “volume sensitive organic osmolyte anion channel” rather than the taurine-Na\(^+\) cotransporter (28). Nonetheless, because the mode of taurine efflux during ischemia remains to be established, this interesting concept deserves further consideration.

The final possibility is that taurine depletion could influence the stability of the sarcolemmal membrane. Hamaguchi et al. (8) have reported that taurine serves as a potent inhibitor of phospholipid N-methyltransferase, the enzyme catalyzing the conversion of phosphatidylethanolamine to phosphatidylcholine. Because phosphatidylcholine is a bilayer former, whereas phosphatidylethanolamine is a nonbilayer former, taurine can cause local changes in the bilayer-to-nonbilayer content of the membrane. Recently, Post et al. (22) have argued that an elevation in the membrane content of bilayer formers protects the ischemic myocardium by stabilizing the membrane. Thus local changes in phospholipid content could occur in the taurine-depleted heart, which could affect the activity of a key enzyme or transporter and modulate the response to an ischemic-reperfusion insult.

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