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Regulation of calcium transport in drug-induced taurine-depleted hearts

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Drug-induced taurine depletion of rat heart led to the accumulation of free CoA, free carnitine and long-chain acylcarnitine, but a small decrease in long-chain fatty acyl-CoA. Although elevations in total tissue long-chain acylcarnitine levels have been linked to defective membrane function and the association of long-chain acylcarnitines with extramitochondrial membranes, these effects were absent in isolated sarcoplasmic reticulum prepared from taurine-depleted hearts. In contrast to the sarcoplasmic reticulum data, taurine depletion was associated with a significant decrease in ATP-dependent calcium uptake by isolated sarcolemmal vesicles. The major effect of taurine depletion on the sarcolemma was a 2-fold decrease in both the V_{max} of calcium transport and the activity of the Ca^{2+}-stimulated ATPase. Sarcolemmal vesicles prepared from taurine-depleted hearts also exhibited a decreased capacity to transport calcium in exchange for sodium, although the initial rate of the process was unaffected by taurine depletion. Since incubation of sarcolemma from taurine-depleted hearts with taurine could not overcome the effects of taurine depletion, it was concluded that the effects of taurine were not caused by a direct interaction of it with the calcium pump. Possible mechanisms of taurine action are discussed.

Introduction

Taurine (2-aminoethanesulfonic acid) is found in very high concentrations in the mammalian heart. Studies revealing that these high levels are maintained by an energy-dependent transport process led to the hypothesis that taurine plays an important physiological role in the heart [1]. This notion is supported by recent evidence that taurine-deficient animals exhibit abnormal myocardial function. The first report that taurine-depleted hearts were abnormal came from Mozaffari et al. [2], who focused on the myocardial metabolic defects of taurine depletion. Subsequently, it was found that rats, made taurine deficient using the taurine transport inhibitor β-alanine, were less able to resist stress related to either an ischemic insult or adriamycin toxicity [3,4]. Taurine-depleted hearts also were found to exhibit abnormalities in their contraction-relaxation cycle [3,5]. More recently, Pion et al. [6] found that cats fed on a taurine- deficient diet developed cardiomyopathy which could be reversed by treatment with taurine.

The basis for these multiple effects of taurine depletion is currently unknown. The hypotheses which have received the most attention have iden-

Abbreviation: Mops, 4-morpholinepropanesulfonic acid.

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tified taurine as a regulator of calcium movement [7], an osmotic agent [8], a modulator of energy metabolism [2] or a membrane stabilizer [9]. This report provides evidence that taurine depletion leads to defects in both energy metabolism and sarcolemmal calcium transport, which would be expected to alter calcium homeostasis of the myocyte.

Materials and Methods

Male Wistar rats were fed Purina rat chow ad lib and maintained for a period of 4 weeks on either tap water containing 3% β-alanine (taurine-depleted group) or tap water without the taurine transport inhibitor (control group). Myocardial taurine contents of the control and β-alanine-treated animals were 105 ± 6 and 45 ± 5 μmol/g dry wt, respectively.

The sarcolemmal preparation was isolated according to the method of Pitts [10]. Rat ventricles were washed, minced and then homogenized in 0.6 M sucrose, 10 mM imidazole (pH 7.0) (3.5 ml/g tissue) with five 20-s bursts of a Polytron (PT 10, setting 5). The resulting homogenate was centrifuged at $12000 \times g$ for 30 min and the pellet was discarded. After diluting (5 ml/g tissue) with buffer comprising 160 mM KCl/20 mM Mops (pH 7.4) (buffer A), the supernatant was centrifuged for 60 min at $95000 \times g$. The resulting pellet was suspended in buffer A and layered over a 30% sucrose solution containing 0.3 M KCl, 50 mM $Na_4P_2O_7$ and 0.1 M Tris (pH 8.3). After centrifugation at $95000 \times g$ for 90 min, the band at the interface was removed and diluted with 3 vol. of buffer A. Membrane sidedness was evaluated by the activity of ouabain-sensitive Na^+/K^+-ATPase [11] under basal conditions (32.5 ± 3.3 μmol P_i/mg per h) and in the presence of either 1.0 μM valinomycin (66.0 ± 4.0 μmol P_i/mg per h) or 1.0 mg alamethicin/mg protein (113.6 ± 9.3 μmol P_i/mg per h). According to the protocol of Doyle et al. [12], both the control and taurine-depleted membranes consisted of approx. 30% leaky, 30% inside-out and 40% right-side out vesicles.

In the sarcolemmal preparation, ouabain-sensitive Na^+/K^+-ATPase activity was 113.3 ± 12.9 and 114.3 ± 12.3 μmol P_i/mg per h in the control and taurine-depleted hearts, respectively. Since the sarcolemmal membrane contained little, if any, oxalate-facilitated calcium-uptake activity (values at 0.3 μM free calcium were 25.0 ± 12.5 and 23.8 ± 0.5 nmol/mg per min in the presence and absence of 2 mM oxalate, respectively) the preparation appeared to lack appreciable sarcoplasmic reticular contamination. The cytochrome-*c* oxidase activity of the membrane was unaffected by taurine depletion. While the purity factor relative to the homogenate for Na^+/K^+-ATPase was 12.7, it was 0.6 for cytochrome-*c* oxidase [13], indicating minimal mitochondrial contamination of the sarcolemmal preparation. The protein method used for all procedures was the Bradford assay.

The method used to assess Na^+-dependent Ca^{2+} uptake was described previously by Reeves and Sutko [14]. Sarcolemmal vesicles were loaded with Na^+ by incubation with 20 mM Mops containing 160 mM NaCl (buffer B). To begin the uptake process, 10 μg of membrane protein was added to 500 μl of 20 mM Mops (pH 7.4) containing 40 μM $^{45}CaCl_2$ and 160 mM KCl. After the desired period of incubation at 37°C, the reaction was arrested by the addition of 3 ml of ice-cold 20 mM Mops (pH 7.4) containing 160 mM KCl and 1 mM $LaCl_3$ and aliquots (100 μl) were withdrawn immediately, filtered through Millipore filters and then washed twice with the same solution. The filters were then dried and counted for radioactivity. The controls lacked a sodium gradient. This was achieved by first loading the vesicles with 20 mM buffer B and then incubating them with 20 mM of the same buffer (pH 7.4). Calcium binding was assessed after addition of 40 μM $^{45}CaCl_2$. The Na^+-dependent Ca^{2+}-uptake data were corrected for calcium binding and were expressed as nmol Ca^{2+} accumulated/mg protein.

The activity of the sarcolemmal calcium pump was assessed by measuring both Ca^{2+}-stimulated ATPase activity and ATP-dependent Ca^{2+} uptake. Ca^{2+}-stimulated ATPase activity was estimated by subtracting Mg^{2+}ATPase activity from total $(Ca^{2+} + Mg^{2+})$-ATPase activity. Measurement of Mg^{2+}ATPase and total $(Ca^{2+} + Mg^{2+})$-ATPase activity was carried out according to the method described by Makino et al. [15]. The data are expressed as μmol phosphate liberated/mg protein per h.

The extent of ATP-dependent Ca^{2+} uptake was etermined by preincubating sarcolemma (5 μg rotein) at 37°C for 5 min in 0.5 ml of 20 mM ris-Mops buffer (pH 7.0) containing 160 mM Cl and 5 mM $MgCl_2$ and the required amounts f $^{45}Ca^{2+}$ and EGTA to produce the desired free alcium concentration [15]. When desired, 20 mM aurine was included in the incubation medium. ccumulation of calcium was initiated by adding mM Tris-ATP (pH 7.0). After 1 min, the reaction was terminated by adding 3.0 ml ice-cold Cl-Mops containing 1.0 mM $LaCl_3$ and was hen immediately filtered. The filters were washed wice with 3.0 ml of buffer A and then counted. onspecific binding was measured in the absence f ATP and subtracted from the total accumulaion to yield uptake. The data were expressed as mol Ca^{2+} uptake/mg protein per min.

Microsomes enriched with sarcoplasmic reticuum were prepared from control and taurine-deleted rats using the method of Sumida et al. [16]. ssay conditions of the Ca^{2+}ATPase at a free-alcium concentration of 2.0 μM were described reviously by Tada et al. [17]. Oxalate-facilitated alcium uptake into the isolated vesicles was iniiated by addition of 5 mM Tris-ATP to a reaction nedium (37°C) consisting of 20–30 μg of microomal sarcoplasmic reticular protein, 40 mM istidine buffer (pH 6.8), 5 mM $MgCl_2$, 110 mM Cl, 2.5 mM Tris-oxalate, 5 mM sodium azide nd the appropriate concentrations of $^{45}CaCl_2$ nd EGTA to yield the desired free-calcium conentration [15,18]. The reaction was terminated fter 5 min by rapid filtration of the reaction olution onto 0.45 μm membrane filters. After vashing the filters three times with 3.0 ml of 40 nM Tris buffer (pH 7.2), the filters were dried nd placed in scintillation vials to be counted.

The carnitine and CoA derivatives were assayed in hearts after 20 min of perfusion on a tandard working heart apparatus. The perfusion nedium (37°C, pH 7.4) was Krebs-Henseleit uffer containing 5 mM glucose and 2.5 U/l nsulin. The hearts (paced at 300 beats/min) were rozen with Wollenberger clamps precooled in iquid nitrogen. After lyophilization, a known veight of freeze-dried tissue was homogenized in 3 ml of 6% (w/v) perchloric acid and then centriuged. For the CoA assay, the appropriate amount of dithiothreitol was added to yield a final concentration of 15 mM. The samples were first neutralized with 3 M K_2CO_3 and then assayed for free CoA using the α-ketoglutarate dehydrogenase reaction [19]. Long chain fatty acyl-CoA was determined as free CoA after hydrolyzing the ester for 15 min at 55°C in an alkaline medium (pH 11.5–12.0). The method of Idell-Wenger et al. [20] was used to correct long-chain acyl-CoA content for the presence of acid-soluble CoA. The levels of free carnitine in the supernatant were assayed by the carnitine acetyltransferase radioisotope procedure [21]. After alkaline hydrolysis of the pellet at pH 12.5–13.0 for 60 min at 70°C, the long-chain acylcarnitine content was assayed as free carnitine using the method of Idell-Wenger et al. [20]. Isolated sarcoplasmic reticulum was extracted with perchloric acid and then assayed according to the procedure described above.

Results

Table I reveals the effects of taurine depletion on myocardial levels of key CoA and carnitine intermediates. While the decrease in tissue taurine content mediated relatively small changes in free CoA, acetyl-CoA and long-chain fatty acyl-CoA content, it was associated with a 60% and 110% increase in free carnitine and long-chain acylcarnitine levels, respectively.

TABLE I

EFFECT OF TAURINE DEPLETION ON KEY CoA AND CARNITINE INTERMEDIATES

Hearts from taurine-depleted and control rats were perfused with Krebs-Henseleit buffer containing 5 mM glucose and 2.5 U/l insulin for a stabilizing period of 20 min before being frozen with Wollenberger clamps. Metabolic intermediates were extracted and assayed using standard procedures. Values represent means ± S.E. of 4–6 hearts.

Intermediate	(nmol/g dry wt)	
	control	taurine-depleted
Free CoA	421 ± 15	501 ± 13 *
Acetyl-CoA	19 ± 9	22 ± 4
Long-chain fatty acyl-CoA	87 ± 8	73 ± 6 *
Free carnitine	6304 ± 183	9997 ± 254 *
Long-chain acylcarnitine	184 ± 26	389 ± 27 *

* Significantly different from control ($P < 0.05$).

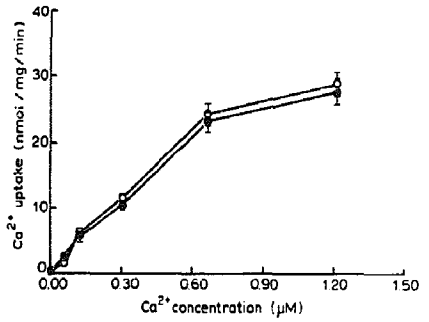

Fig. 1. Effect of taurine depletion on oxalate-facilitated Ca^{2+} uptake by sarcoplasmic reticular vesicles. Enriched sarcoplasmic reticulum were prepared from taurine-depleted (○——○) and control heart (●——●) using the method of Sumida et al. [16]. The rate of oxalate-facilitated calcium uptake by these sarcoplasmic reticular vesicles was evaluated according to standard procedures described in Materials and Methods. Taurine depletion had no significant effect on this transport process. Values shown represent the means±S.E. of four preparations.

TABLE II

LONG-CHAIN ACYLCARNITINE CONTENT AND (Ca^{2+} + Mg^{2+})-ATPase ACTIVITY OF SARCOPLASMIC RETICULUM PREPARED FROM CONTROL AND TAURINE-DEPLETED HEARTS

Sarcoplasmic reticulum was isolated from taurine-depleted and control hearts using the method of Sumida et al. [16]. (Ca^{2+} + Mg^{2+})-ATPase activity of these preparation was assayed at a calcium-free concentration of 2.0 μM. The acid-free and long-chain acylcarnitine contents of the isolated membranes were determined after extraction of the intermediates with perchloric acid. Values represent means±S.E. of four or five preparations.

Condition	(nmol/mg protein)		(Ca^{2+} + Mg^{2+})-ATPase (μmol P_i/mg per min)
	free carnitine	long-chain carnitine	
Control	2.18±0.19	1.63±0.37	3.95±0.11
Taurine-depleted	3.08±0.56	1.78±0.27	4.00±0.08

The significance of changes in long-chain acylcarnitine content usually lies in the ability of these lipid amphiphiles to modulate membrane processes such as sarcoplasmic reticular calcium pump activity [22,23]. Yet, in spite of the increase in total tissue long-chain acylcarnitine content in taurine-depleted hearts, the rate of calcium accumulation by sarcoplasmic reticulum isolated from these hearts was identical to the rate seen in sarcoplasmic reticulum from control hearts (Fig. 1). Similarly, the activity of sarcoplasmic reticular (Ca^{2+} + Mg^{2+})-ATPase was unaffected by taurine depletion (Table II). Since these results raised certain questions about the importance of the long-chain acylcarnitine changes, we examined long-chain acylcarnitine content of sarcoplasmic reticulum isolated from taurine depleted and control hearts. As seen in Table II, the long-chain acylcarnitine content of sarcoplasmic reticular membrane prepared from control and taurine-depleted hearts were identical. Thus, the failure to detect differences in sarcoplasmic reticular calcium transport between taurine-depleted and control membranes may be related to the removal of long-chain acylcarnitine from the membrane during the isolation procedure.

In contrast to the sarcoplasmic reticular calcium pump data, the sarcolemmal calcium pump was significantly affected by taurine depletion. As expected, the rate of ATP-dependent Ca^{2+} accumulation by isolated sarcolemma prepared from control hearts showed a typical hyperbolic curve with a K_m for Ca^{2+} of 0.21 μM and a V_{max} of 35.1 nmol/mg protein per min (Fig. 2). By comparison, calcium uptake was significantly depressed in sarcolemma prepared from taurine-depleted

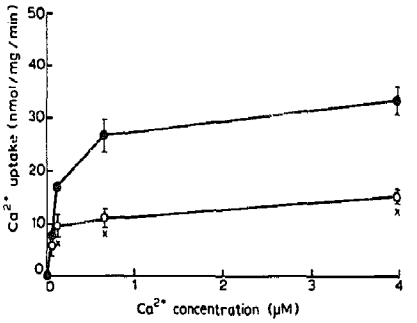

Fig. 2. Effect of taurine depletion on ATP dependent Ca^{2+} uptake by sarcolemmal vesicles. Sarcolemma were prepared from taurine-depleted (○——○) and control hearts (●——●). The rate of ATP-dependent Ca^{2+} uptake was determined over a free calcium concentration range of 0.06–4 μM. Taurine depletion significantly decreased the rate by reducing the V_{max}. Values shown represent the means±S.E. of four preparations. * Significantly different from the control ($P < 0.05$).

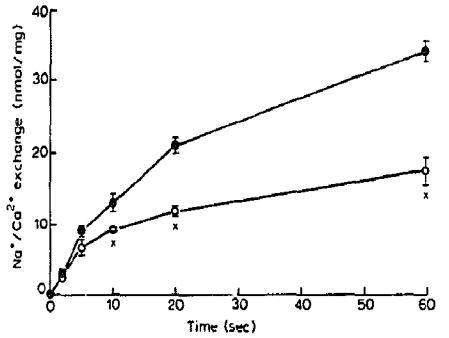

Fig. 3. Effect of taurine depletion on sarcolemmal Na^+–Ca^{2+} exchange. Sarcolemma were prepared from taurine-depleted (○——○) and control hearts (●——●). The extent of Na^+-dependent Ca^{2+} uptake by sodium-loaded vesicles was determined as a function of time after exposure of the vesicles to a buffer comprising 20 mM Mops (pH 7.4), 160 mM KCl and 40 μM $^{45}CaCl_2$. Taurine depletion had no effect on the initial rate of Na^+-dependent Ca^{2+} uptake but decreased the capacity of the uptake process. Values shown represent the means±S.E. of four preparations. * Significant different from the control ($P < 0.05$).

hearts. The decrease was caused by a 2-fold reduction in the maximal rate of calcium transport (V_{max} = 15.9 nmol/mg protein per min in taurine-depleted sarcolemma). In agreement, the activity of the enzyme thought responsible for ATP-dependent Ca^{2+} uptake (the Ca^{2+}-stimulated ATPase) was also 2-fold less in taurine-depleted membrane; ATPase activity was 17.0 ± 2.0 and 31.1 ± 2.3 μmol phosphate liberated/mg protein per h in taurine-depleted and control sarcolemma, respectively. Interestingly, the effects of taurine depletion on ATP-dependent Ca^{2+} uptake could not be reversed by addition of 20 mM taurine to the incubation medium.

One other transport process examined was the Na^+–Ca^{2+} exchange system. Under the conditions used in this study, the initial rate of Na^+-dependent Ca^{2+} uptake was unaffected by taurine depletion (Fig. 3). However, sarcolemma prepared from control hearts had a significantly greater capacity to transport calcium than did membrane isolated from taurine-depleted animals.

Discussion

The hypothesis that taurine influences calcium movement in the heart was originally based on studies by Dolara et al. [24], who reported that exposure of the isolated heart to high doses of taurine reduced the rate of calcium washout from the heart. Pharmacological doses of taurine were subsequently shown to decrease the severity of necrotic lesions in cardiomyopathic hamsters [25], reverse some of the effects of calcium overload in the calcium paradox model of heart failure [26], protect against the decline of calcium-induced slow action potentials during hypoxia [27] and improve myocardial contraction in hearts made hypodynamic by exposure to medium containing a calcium antagonist or low calcium levels [28].

The mechanism by which taurine regulates calcium transport has been an area of considerable interest. In 1978, Chovan et al. [29] reported that taurine stimulated the binding of calcium to isolated sarcolemma. This effect was not limited to sarcolemma, since calcium binding to phospholipid bilayers was also stimulated by taurine [30]. Nevertheless, the size of the calcium pool associated with the sarcolemma was considered particularly important because this pool is thought to play a role in myocardial contraction [7].

The results from this study raise the possibility that other mechanisms may contribute to the regulation of calcium movement by taurine. The most compelling evidence supports the notion that taurine alters the properties of the sarcolemmal calcium pump. The mechanism underlying the effects of taurine depletion on sarcolemmal Ca^{2+}-stimulated ATPase and ATP-dependent Ca^{2+} uptake is presently unknown. One problem faced in evaluating any change in calcium pump ATPase activity is the complexity of its regulation. Among the factors known to alter its activity are cAMP-dependent phosphorylation, calmodulin activation, phospholipid modulation, potassium stimulation and pH effects [31,32]. Taurine is thought to affect at least two of these factors – potassium movement and calmodulin action [33,34]. Moreover, the observation that the capacity for Na^+–Ca^{2+} exchange is altered by taurine depletion suggests that deficiency of taurine may alter membrane structure. The membrane stabilizing actions of taurine have been recognized for several years [9]. In this regard, it is important to remember that addition of taurine to the incubation medium did not alter the sarcolemmal calcium transport systems (Figs. 2 and 3).

The other means by which taurine can influence calcium movement is through the regulation of tissue long-chain acylcarnitine content. The importance of this intermediate lies in its association with extramitochondrial membranes. Under pathological conditions, such as diabetes or ischemia, the elevation of total tissue long-chain acylcarnitine content is believed to alter extramitochondrial membrane ion transport adversely and to contribute to the observed pathology [22,23,35]. In support of this view, Lopaschuk et al. [35] showed that the rise in total tissue long-chain acylcarnitine levels in insulin-dependent diabetes was associated with an increase in sarcoplasmic reticular long-chain acylcarnitine content and a reduction in sarcoplasmic reticular calcium pump activity. However, in this study a more complicated pattern emerged. Although total tissue long-chain acylcarnitine content of the taurine-depleted heart was elevated, there was neither an increase in the levels of long chain acylcarnitine associated with isolated sarcoplasmic reticulum nor a change in sarcoplasmic reticular calcium pump activity. One might speculate that the 'presumed' uncoupling of total tissue long-chain acylcarnitine levels and sarcoplasmic reticular content of this lipid amphiphile was caused by a washing out of the carnitine intermediate from the membrane during isolation. Alternatively, the results may reflect the absence of long-chain acylcarnitine regulation in taurine-depleted hearts. Without knowing the size of the in vivo sarcoplasmic reticular long-chain acylcarnitine pool, it is impossible to distinguish between these possibilities. Unfortunately, present-day technology does not enable one to obtain this information.

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