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EFFECT OF DRUG-INDUCED TAURINE DEPLETION ON CARDIAC CONTRACTILITY AND METABOLISM

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Abstract—Myocardial taurine content was halved in rats maintained for 3 weeks on tap water containing either 1% guanidinoethylsulfonate (GES) or 3% β-alanine. The decrease in taurine content did not affect myocardial contraction; however, it significantly altered myocardial metabolism. The major effect of the treatment was a significant stimulation in the rate of glycolysis and lactate production. The rise in glycolytic flux was associated with activation of phosphofructokinase, presumably caused by a decrease in tissue citrate levels. GES-treated hearts also contained slightly lower creatine phosphate content, but since this effect was not observed in the β-alanine-treated hearts, it appeared to be independent of taurine depletion. The consequences of these metabolic changes are discussed.

Taurine (2-aminoethane sulfonic acid) is found in very high concentrations in excitable tissue, particularly in the heart where it comprises more than 50% of the total free amino acid pool [1]. Since the maintenance of the intracellular taurine pool requires an energy-dependent transport process and is regulated by hormones and adrenergic stimulation [2–4], it has been argued that taurine is physiologically important to the myocyte.

Insight into possible physiological roles for taurine comes from its well-established actions on the heart. The amino acid has been found to delay the washout of calcium from isolated heart preparations exposed to buffer lacking calcium [5, 6], reverse the negative inotropic effect of reduced perfusate calcium or exposure to calcium antagonists [7, 8], prevent myocardial necrotic lesions associated with calcium overload [9–11], improve clinical symptoms of congestive heart failure [12] and prevent cardiotoxicity caused by elevated concentrations of digoxin and epinephrine [13]. These observations have led to the hypothesis that one function of taurine is to modulate ion transport [14].

One other possible function of taurine is the regulation of energy metabolism. Perfusion of hearts with buffer containing taurine results in a stimulation of glucose utilization and glycogen synthesis [15]. Since these effects are greater when insulin also is added to the buffer, they support the recent contention that taurine functions as an agonist and/or regulator of insulin action [15, 16].

Other potential functions of taurine include osmoregulation [17], membrane stabilization [18] and detoxification of reactive metabolites [19].

The recent discovery of two drugs to reduce myocardial taurine content has introduced a powerful tool to test for possible physiological functions of the amino acid [20, 21]. In this report, we describe the effect of drug-induced taurine depletion on myocardial contractility and carbohydrate metabolism.

MATERIALS AND METHODS

Male Wistar rats (240–260 g) were fed Purina rat chow *ad lib.* and maintained for 3 weeks on either tap water (controls), tap water containing 1% (w/v) guanidinoethylsulfonate (GES) or tap water containing 3% β-alanine.

Hearts from control and taurine-depleted (GES- or β-alanine-treated) were perfused within 45 sec of decapitation on a standard working heart apparatus [15]. The perfusate consisted of Krebs–Henseleit buffer (pH 7.4) containing 5 mM glucose, 5 mM acetate, and the appropriate concentration of insulin. All hearts were paced at 300 beats/min and perfused for a 20-min stabilization period prior to initiation of the experiment. Most hearts were first exposed to buffer lacking insulin and then perfused with buffer containing insulin. Metabolic and hemodynamic parameters were measured before and after exposure to the hormone.

Cardiac work was calculated from coronary flow, aortic output and aortic pressure measurements as described by Lampson *et al.* [15]. Oxygen consumption was monitored continuously with a Clark oxygen electrode [15]. The rate of glucose utilization was determined by measuring the rate of tritium release from [3-^{3}H]glucose into water [15]. Coronary effluent samples were also assayed for rates of lactate and pyruvate production by standard spectrophotometric techniques [15].

Hearts used for determination of metabolic intermediates were rapidly frozen with a Wollenberger clamp precooled in liquid nitrogen. The frozen hearts were lyophilized, extracted and assayed for key metabolic intermediates using methods described by Kramer *et al.* [9]. Guanidinoethylsulfonate (GES) was assayed according to the method of Micklus and Stein [22] after being treated according to the procedure of Huxtable and Lippincott [23].

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RESULTS

Cardiac taurine content of rats maintained for 3 weeks on tap water containing 1% (w/v) guanidinoethylsulfonate (GES) fell from 77 ± 6 to 34 ± 1 μmoles/g dry wt. The 2-fold depletion in tissue taurine occurred in the absence of any detectable alteration in either body or heart weight. Moreover, the loss of taurine from the heart affected neither the mechanical function of the isolated heart perfused with buffer lacking insulin nor the response of the isolated heart to insulin (Table 1).

In contrast to the contraction and weight data, GES treatment dramatically altered myocardial metabolism. Glycolytic flux increased 80% in the GES-treated hearts (373 ± 13 in taurine-depleted vs 207 ± 5 μmoles/g dry wt/hr in control hearts). This stimulation in carbohydrate metabolism was caused in part by the 26% rise in glucose utilization (Fig. 1); however, the increase in glycogenolysis also contributed to enhance anaerobic metabolism. During the course of the first 15 min of perfusion, glycogen levels fell from 129.2 ± 7.9 to 101.0 ± 6.7 μmoles/g dry wt in the GES-treated hearts but remained constant at 101.8 ± 4.1 μmoles/g dry wt in control hearts. Thus, 113 μmole glucose equivalents/g dry wt/hr were mobilized for use by glycolysis in the GES-treated hearts, but no glycogen mobilization occurred in the control hearts.

The significant increase ($P < 0.05$) in glycolysis was also reflected in the rates of lactate and pyruvate production. In the absence of insulin, triose output was about 50% greater in the GES-treated hearts than in the controls (Fig. 2). Inclusion of insulin in the buffer stimulated both triose output and glucose utilization of both test groups. However, the effects of insulin on triose output and glucose utilization were considerably greater in the GES-treated hearts.

To clarify the effects of GES treatment on glycolysis, tissue content of key glycolytic intermediates was measured. The major effect of GES treatment was to decrease dramatically glucose-6-phosphate levels and increase fructose-1,6-bisphosphate content. When plotted in the classical crossover pattern (Fig. 3), these changes resulted in a crossover between glucose-6-phosphate and fructose-1,6-bisphosphate, suggesting that the rate-limiting enzyme phosphofructokinase is more active in the GES-treated hearts.

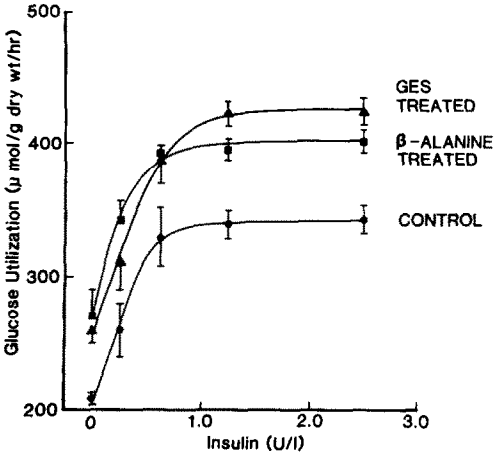

Fig. 1. Effect of taurine depletion on glucose utilization of the isolated rat heart. Hearts from GES-treated (▲—▲), β-alanine-treated (■—■) and untreated rats (●—●) were perfused with Krebs–Henseleit buffer supplemented with 5 mM glucose, 5 mM acetate and the indicated concentration of insulin. The rate of glycolysis was determined from the rate of tritium release from [3-^{3}H]glucose into water. Values shown represent means ± S.E.M. of six to eight hearts.

Stimulation of phosphofructokinase is usually associated with either a decrease in tissue ATP or citrate levels [25]. In the GES-treated hearts, the dominant factor controlling phosphofructokinase flux appears to be tissue citrate levels. While ATP was not affected significantly in hearts from the GES-treated rats, tissue citrate content fell 25% (Table 2).

The major concern with using GES to decrease tissue taurine content is that the drug accumulates within the cell (131 ± 3.7 μmoles/g dry wt in this study). Thus, the observed effects of treatment may be related to the accumulation of the drug rather than taurine depletion. To test the importance of this effect, β-alanine was used to deplete taurine within the heart. β-Alanine treatment was found to cause a similar 2-fold decrease in tissue taurine levels; however, unlike GES treatment, β-alanine does not accumulate within the heart [21].

Figures 1 and 2 show that β-alanine treament mediated changes in anaerobic metabolism similar

Table 1. Effect of taurine depletion on hemodynamics of the isolated heart

	Aortic output (ml/min)	Coronary flow (ml/min)	Cardiac output (ml/min)	Aortic pressure (cm H_2O)	Cardiac work (kg-m/g dry wt/min)
Control					
No insulin	34.0 ± 3.9	21.9 ± 0.9	55.9 ± 3.5	131 ± 4	0.33 ± 0.02
Insulin (2.5 units/l)	40.1 ± 3.3	23.9 ± 1.8	63.9 ± 4.1	138 ± 4	0.40 ± 0.01
Taurine depletion					
No insulin	32.2 ± 3.9	23.1 ± 1.6	55.3 ± 4.1	130 ± 5	0.33 ± 0.02
Insulin (2.5 units/l)	40.4 ± 4.5	23.1 ± 1.6	64.6 ± 5.9	137 ± 4	0.40 ± 0.02

Each data point represents mean ± S.E.M. of six to eight hearts.

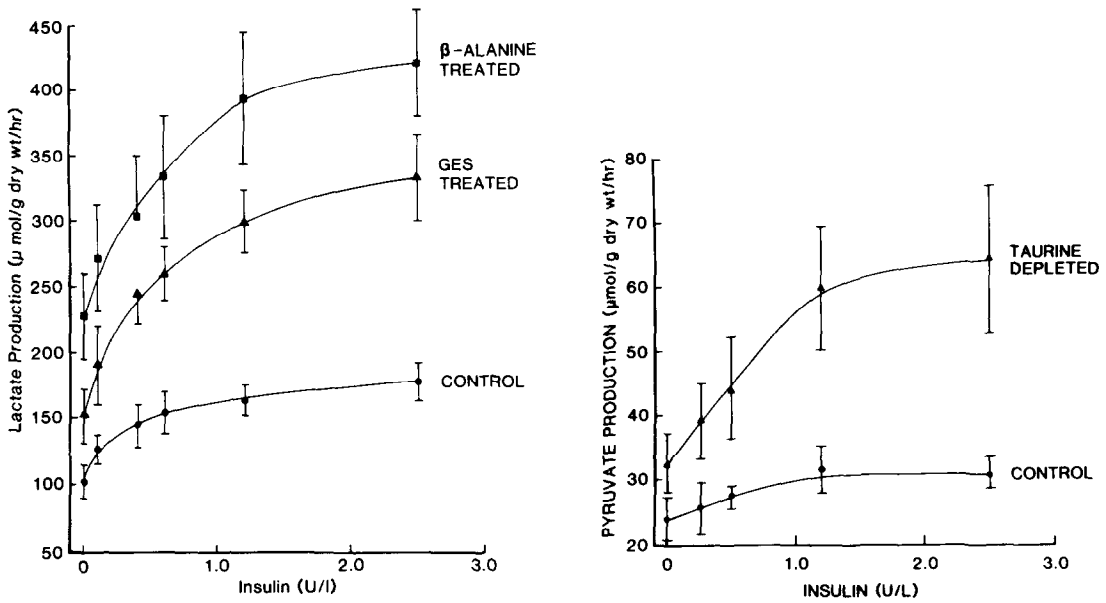

Fig. 2. Effect of taurine depletion on lactate and pyruvate production. Hearts from GES-treated (▲—▲), β-alanine-treated (■—■) and untreated rats (●—●) were perfused according to the method described in the legend of Fig. 1. Perfusate was collected and used to measure lactate (left panel) and pyruvate (right panel) production. Values shown represent means ± S.E.M. of six to eight hearts.

to those noted in the GES-treated hearts. Glucose utilization and triose output were both elevated to a similar extent in the two groups of taurine-depleted hearts. Moreover, a crossover between glucose-6-phosphate and fructose-1,6-bisphosphate was also observed in the β-alanine-treated hearts, indicating the importance of phosphofructokinase in β-alanine-mediated stimulation of anaerobic metabolism. The major difference between the two groups of taurine-depleted hearts was in high energy phosphate content. Whereas creatine phosphate levels were reduced from 28.3 ± 0.9 to 21.0 ± 0.7 μmoles/g dry wt in the GES-treated hearts, they were normal in the β-alanine treated hearts (28.9 ± 0.3 μmoles/g dry wt).

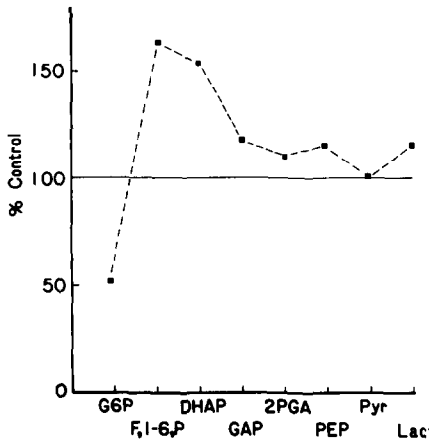

Fig. 3. Effect of taurine depletion on glycolytic intermediates. Hearts from GES-treated and untreated rats were perfused according to method described in the legend of Fig. 1. After 20 min of controlled perfusion, hearts from the two groups were freeze clamped with Wollenberger clamps. Tissue levels of key intermediates were measured using standard procedures. Data for taurine-depleted hearts are expressed as percent of the values for the control hearts. Control values are similar to those published previously [24]. Abbreviations: G-6-P, glucose-6-phosphate; F,1-6,P, fructose-1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde-3-phosphate; 2PGA, 2-phosphoglyceric acid; PEP, phosphoenolypyruvate; Pyr, pyruvate; and Lact, lactate.

DISCUSSION

Exposure of the isolated heart to buffer containing

Table 2. Effect of taurine depletion on key metabolic intermediates

Intermediate	Metabolic intermediates (μmoles/g dry wt) Control	GES-treated
ATP	21.99 ± 0.42	20.71 ± 0.48
ADP	5.26 ± 0.23	4.62 ± 0.21
AMP	2.97 ± 0.20	2.70 ± 0.18
Total adenine nucleotide	30.12 ± 0.56	28.30 ± 0.73
Phosphocreatine	28.32 ± 0.94	20.96 ± 0.73*
Citrate	1.88 ± 0.07	1.48 ± 0.05*

* Hearts from taurine-depleted and control rats were perfused as described in the legend of Fig. 1. Following freeze clamping of the tissue, samples were extracted and assayed. Values shown represent means ± S.E.M. of seven to twelve hearts.

* Denotes significant difference from control ($P < 0.01$).

millimolar concentrations of taurine leads to an increase in contractility [7, 8]. Chovan *et al.* [7] have suggested that, since the inotropic effects of taurine are only observed at nonphysiological serum taurine concentrations, they represent pharmacological actions of the amino acid. This view is in agreement with the present study in which a 2-fold decrease in taurine content was found to have no effect on mechanical performance of the isolated heart. However, it is in apparent contradiction to a recent study by Franconi *et al.* [26], who observed that taurine treatment restores both intracellular taurine content and contraction to their preparation, implying that elevations in intracellular taurine were responsible for restoration of myocardial contraction. The difference between the two studies may be related to the calcium concentration of the buffer. Franconi *et al.* [26] varied the calcium concentration, whereas only physiological calcium concentrations were used in this study. Since taurine effects are greater at lower calcium concentrations, the higher physiological levels may mask any abnormal response.

The most dramatic effect of taurine depletion is the stimulation of glycolysis and glycogenolysis. Crossover studies indicate that increased flux through glycolysis is caused by activation of the rate-limiting enzyme phosphofructokinase. Since taurine does not directly alter phosphofructokinase activity [15], the logical explanation for the data is that the decrease in citrate accounts for the increased activity of the enzyme in taurine-depleted hearts.

It is of interest that taurine depletion is associated with enhanced rates of glycogenolysis while increased plasma or perfusate taurine content results in the opposite response [15, 16]. Since both perturbations lead to a decrease in the taurine gradient across the cell, the change cannot be attributed to the gradient *per se*. Lampson *et al.* [15] have argued that the promotion of glycogen synthesis in isolated hearts perfused with buffer containing both insulin and taurine is related to the insulin-promoting activity of taurine. More recent studies reveal that taurine binds directly to the insulin receptor and, therefore, has insulin-like activity [16]. While the location of the taurine binding site on the receptor remains to be determined, it is possible that intracellular taurine content could influence the activity of the insulin receptor and play an important role in regulating glycogen metabolism.

The metabolic and mechanical properties of the β-alanine-treated and GES-treated hearts are virtually identical. The major difference is in the level of creatine phosphate, which was reduced significantly in the GES-treated hearts. Two reasons may account for the drop in creatine phosphate during GES treatment. First, GES may interfere with the transport of creatine into the cell [23]. Alternatively, GES may compete with creatine in the phosphocreatine kinase reaction. The latter possibility is intriguing in light of the work by Robin and van Thoai [27], who showed that phosphoguanidinoethane sulfonate can be formed in marine worm.

Decreases in retinal taurine content lead to very severe impairment of retinal function [28, 29]. The results of this study suggest that taurine depletion causes less dramatic changes in the heart. Nevertheless, the taurine-depleted hearts do exhibit abnormal energy metabolism and these changes may take on added importance in the stressed myocardium.

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