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The Effect of Taurine on Contractility and Sarcolemmal Calcium Binding of Bullfrog Hearts (40919)[1]

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Abstract. The effect of taurine on myocardial contractility was studied utilizing the isolated perfused bullfrog heart. Within the range of 0.5 to 2.8 m*M* calcium in the perfusion fluid, taurine produced an increase in the developed force of the spontaneously beating isolated perfused bullfrog heart. The effect of taurine was calcium dependent having a large effect at low calcium concentrations but diminishing at high calcium concentrations. Taurine enhanced calcium binding to sarcolemma isolated from bullfrog hearts. The results suggest that taurine produces an inotropic response in the bullfrog heart by increasing calcium binding and calcium influx at the sarcolemma.

Taurine (2-aminoethanesulfonic acid) occurs universally in animals, both invertebrate and vertebrate (1). Prior to 1960 it was generally assumed that taurine was an end product of sulfur metabolism in the animal body, having no function other than conjugation with cholic acid in the liver and excretion in the bile (2). Since 1960 reports have appeared which suggest that taurine has other important physiological functions: that of modulating impulse flow across central neural synapses (3), and that of modulating myocardial excitability (4) and contractility (5).

While the effect of taurine on myocardial excitability may be mediated through an alteration of potassium flux (6), there is evidence that the effect of taurine on myocardial contractility is calcium related. Thus, it has been shown that taurine increases the calcium content of the isolated perfused guinea pig heart (7) and causes a positive inotropic response in such preparations (8). The substance antagonizes the negative inotropic effect of low calcium media (7) and enhances recovery of contractile force in calcium depleted guinea pig ventricle strips (8).

The site at which taurine acts in the heart is not clear. Dolara *et al.* (9) reported that taurine increased calcium binding and transport of sarcoplasmic reticulum (SR) isolated from guinea pig hearts. However, Entman *et al.* (10) and Jaqua (11) could find no effect of taurine on calcium binding or uptake of SR isolated from dog heart. The recent report of taurine receptors located on rat heart sarcolemma (12) drew attention to this structure as possibly important in the effect of taurine on myocardial contractility.

In view of the question on the exact site of taurine action, we utilized the bullfrog heart as a model. Frog ventricular myocardium lacks a transverse tubular system (13, 14) and possesses only a sparse sarcoplasmic reticulum (15). The frog heart relies on a direct influx across the sarcolemma for delivery of calcium to the contractile proteins (16, 17), rather than release from SR, as in the mammalian heart. The frog heart thus provides a model in which myocardial contractility can be related to events at the sarcolemma.

The present report demonstrates a correlation between the positive inotropic effect of taurine on the isolated perfused bullfrog heart and enhanced calcium binding of isolated myocardial sarcolemma.

Materials and methods. Myocardial contractility. Bullfrogs (*Rana catesbeiana*)

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from Mogul-Ed, Oshkosh, Wisconsin, were used. The animals were pithed, the chest opened, and a polyethylene cannula introduced into the ventricular chamber through the left truncus arteriosus. The entire heart, including the atria, was excised, flushed with perfusion fluid, and secured to the perfusion system. The perfusion system consisted of a series of reservoirs connected to a manifold which allowed switching from one solution to another without interrupting the flow to the heart. The solutions in the reservoirs were maintained 15 cm above the heart. The apex of the ventricle was joined by a nylon thread to a Statham force transducer (Model 397, 0.3 oz). Developed force was recorded on a Hewlett–Packard Model 7712 recorder. The hearts were allowed to beat spontaneously. Fluid from the reservoir entered the heart through the left truncus and exited from the right truncus.

The millimolar concentration in perfusion fluid was: NaCl, 111; KCl 2.7; K_2HPO_4, 1.8; Mg SO_4 1.1; $NaHCO_3$, 11.9; glucose, 5.5; $CaCl_2$, variable as stated. The solutions were constantly gassed with 95% O_2–5% CO_2. The pH of the gassed solutions was 7.05. The temperature was 20°.

When taurine was included in the perfusion fluid, the NaCl was decreased appropriately to maintain osmotic equality (0.5 mmole of NaCl omitted per mmole taurine added). The ventricular tissue of the bullfrog hearts used contained 0.87 ± 0.09 μmole of calcium per gram of tissue wet weight and the bullfrog plasma calcium was 1.4 ± 0.11 mmole per liter. Therefore, the calcium concentrations used in the present experiments varied from 0.5 to 2.8 m*M*, a range selected to include concentrations expected in the frog.

Isolation of sarcolemmal membranes. For isolation of sarcolemmal membrane fragments, the ventricles from 10 bullfrog hearts were pooled to give about 4 g of tissue. Sarcolemmal membrane fragments were isolated by a procedure developed in this laboratory (18). On the basis of the activities of putative marker enzymes, our sarcolemmal fraction was contamination with negligible, if any, amounts of sarcoplasmic reticulum and mitochondria.

Calcium binding. Calcium binding was measured by the Millipore filtration technique (19). Membrane preparations (100 μg of membrane protein) were incubated at 37° in a medium consisting of 50 m*M* Tris–HCl, pH 7.0, 5 m*M* $MgCl_2$, $CaCl_2$ as indicated containing 0.2 μCi $^{45}Ca^{2+}$, in the absence or presence of 2 m*M* ATP. The final volume was 2.0 ml. The reaction was started by the addition of calcium and terminated by filtering through Millipore filters (0.45 μm, 25 mm) with mild suction. The filters were washed with 8 ml of Tris–HCl buffer, pH 7.0, placed in scintillation vials, air-dried, and covered with 10 ml of Packard Insta-Gel scintillation fluid. Radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer. Calcium binding was calculated from the specific activity of the added $^{45}Ca^{2+}$ and the activity retained on the filter.

Protein was determined by the method of Lowry *et al.* (20); inorganic phosphate by the method of Fiske and Subbarow (21). Data were analyzed statistically by Student's *t* test. Significance was placed at the $P < 0.05$ level. Data are presented as the mean ± the standard error of the mean.

Results. Taurine and myocardial contractility. When taurine was added to the perfusion fluid, the sodium chloride was decreased (0.5 mmole of sodium chloride per mmole of taurine added) in order to accommodate the ionic contribution of taurine and maintain osmotic equality. As lowering the sodium concentrations is known to increase the contractile strength of frog ventricular (22) or atrial (23) strips, it was necessary to show that the effect of taurine was not due to simple reduction of the sodium concentration.

To this end, the contractile response of the bullfrog heart to increasing concentrations of taurine was compared to the contractile response obtained when the sodium was lowered and sucrose was substituted for taurine or when the sodium was reduced appropriately and no substitution made. The calcium concentration was held constant at 0.9 mmole per liter. The results are shown in Fig. 1. No significant effect on the contractility of the bullfrog heart was observed when the NaCl of the perfusion fluid

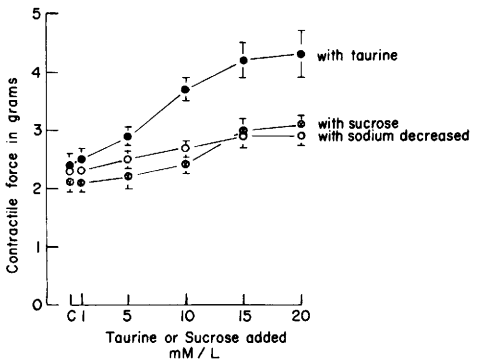

FIG. 1. The contractile response of the isolated perfused bullfrog heart to different concentrations of taurine. When taurine (or sucrose) was included in the perfusion fluid, NaCl was decreased appropriately. (●) The perfusion fluid contained taurine at the concentrations indicated and the NaCl was decreased (0.5 mmole per mmole of taurine added); (⊗) the perfusion fluid contained sucrose at the concentrations indicated and the NaCl was decreased (0.5 mmole per mmole of sucrose added); (○) the NaCl was decreased by one-half the amount indicated on the abscissa and no additions were made. The bars represent the standard error of the mean.

was decreased 0.5, 2.5 or 5 mmole per liter. When the NaCl was decreased 7.5 or 10 mmole per liter, a small (+20%), but significant, increase in contractile response was observed. As this increased contractility was not modified by addition of proper amounts of sucrose (or Tris buffer, not shown), the slight increase in contractility was due only to reducing the sodium concentration and not due to osmotic changes.

Taurine, at 1 m*M* concentration, had no effect on the contractility of the bullfrog heart, but at higher concentrations consistently increased contractility above that due to lowering the sodium concentration; +21% increase at 5 m*M*, +54% increase at 10 m*M*, +75% at 15 m*M*, and +79% at 20 m*M*. The difference in contractility between 15 m*M* taurine and 20 m*M* taurine was not significant.

As the effect of 10 m*M* taurine exhibited a response midway between the maximum (15 m*M*) and minimum (5 m*M*) we chose this concentration for further experiments. The ventricular tissue of the bullfrog hearts used contained 9.0 ± 0.03 μmole of taurine per gram of tissue wet weight, which corresponds roughly to 10 mmole of taurine per liter of tissue water.

Taurine, contractility, and calcium. Within the restricted range of calcium used in this study (0.5 to 2.8 m*M*), increasing the concentration of calcium in the perfusion fluid caused a linear increase in the maximum tension (P_{max}) developed by the ventricle of the bullfrog heart. Taurine (10 mmole per liter) consistently produced an increase in P_{max} above the control level at all levels of calcium studied, although the quantitative effect of taurine was calcium dependent. Figure 2 shows a typical recording of the response of the isolated perfused bullfrog heart to 10 m*M* taurine when the perfusion fluid contained 0.9 m*M* calcium. Taurine perfusion produced an immediate stepwise increase (+68%) in P_{max}. The rate of contraction did not change. When the taurine containing perfusion fluid was changed to control perfusion fluid, the P_{max} promptly decreased below the control level (−26%) but recovered to the control level within 30 sec. This suggests that taurine could be washed out of the heart easily.

The effect of taurine in increasing P_{max} was calcium dependent. Table I gives data on the effect of taurine at different calcium concentrations ranging from 0.5 to 2.8 m*M* in the perfusion fluid. At low concentrations of calcium (0.5 m*M*), taurine caused a large (+78%) increase in developed tension. At high concentrations of calcium (2.8 m*M*), the effect of taurine was small (+10%). At a calcium concentration of 1.4 m*M* in the perfusion fluid, which corresponds to the plasma level of calcium in the bullfrogs used, taurine would be expected to produce a 52% increase in P_{max}.

An interesting effect of taurine was observed at the higher calcium concentrations. Figure 3 shows typical recordings. Instead of the immediate taurine-induced increase in P_{max} exhibited at lower (0.5 and 0.9 m*M*) calcium concentrations, at higher calcium concentrations there appeared an immediate short-lived decrease in P_{max}. This was evident at 1.8 m*M* calcium and exaggerated at 2.8 m*M* calcium in the perfusion fluid. This reversal of taurine effect appears to occur at approximately 1.4 m*M*

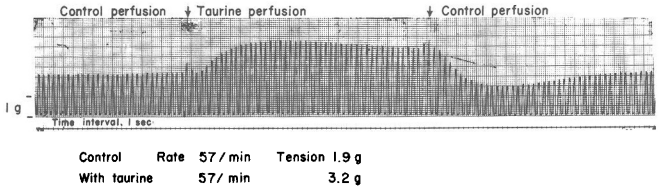

FIG. 2. The response of the isolated perfused bullfrog heart to taurine. The perfusion fluid contained 0.9 m*M* calcium; taurine was 10 m*M*.

calcium, the normal plasma calcium level of these bullfrogs. We are presently pursuing this problem. The data recorded in Table I refer to P_{max} after stability had been reached.

Taurine and sarcolemmal calcium binding. Purity studies. Table II shows the values obtained in the enzyme studies used to assess purity of our sarcolemmal preparation. The preparation exhibited Mg^{2+}-dependent, ouabain-inhibited, Na^+-K^+ ATPase activity characteristic of sarcolemmal membranes. Lack of sodium azide inhibition of the Na^+K^+ ATPase enzyme and lack (0 to trace) of succinate–cytochrome *c* reductase activity in this preparation suggests negligible mitochondrial contamination. We could demonstrate no Ca^{2+}-stimulated, Mg^{2+}-*dependent* ATPase activity in the preparation, which suggests no sarcoplasmic reticulum contamination (23, 25). Our sarcolemmal preparation exhibited high CA^{2+}-stimulated, Mg^{2+}-*independent* ATPase activity which suggests the presence of an effective mechanism for calcium efflux (25, 26).

Taurine and sarcolemmal calcium bind-

TABLE I. EFFECT OF TAURINE ON TENSION DEVELOPED BY BULLFROG VENTRICULAR MYOCARDIUM IN THE PRESENCE OF DIFFERING CONCENTRATIONS OF CALCIUM

Ca^{2+} concn	P_{max} (g)	Percentage change
0.5 m*M* Ca^{2+}		
control (7)[a]	1.8 ± 0.07[b]	
+ taurine (7)	3.2 ± 0.05	+78
0.9 m*M* Ca^{2+}		
control (10)	2.3 ± 0.08	
+ taurine (10)	3.8 ± 0.06	+65
1.8 m*M* Ca^{2+}		
control (9)	3.1 ± 0.07	
+ taurine (9)	4.3 ± 0.08	+39
2.8 m*M* Ca^{2+}		
control (7)	4.1 ± 0.10	
+ taurine (7)	4.5 ± 0.11	+10

Note. The perfusion fluid was a bicarbonate buffered Tyrode's solution, pH 7.05, continuously gassed with 95% O_2–5% CO_2 at 20°.

[a] Numbers in parentheses show the number of individual determinations.

[b] Mean ± SEM.

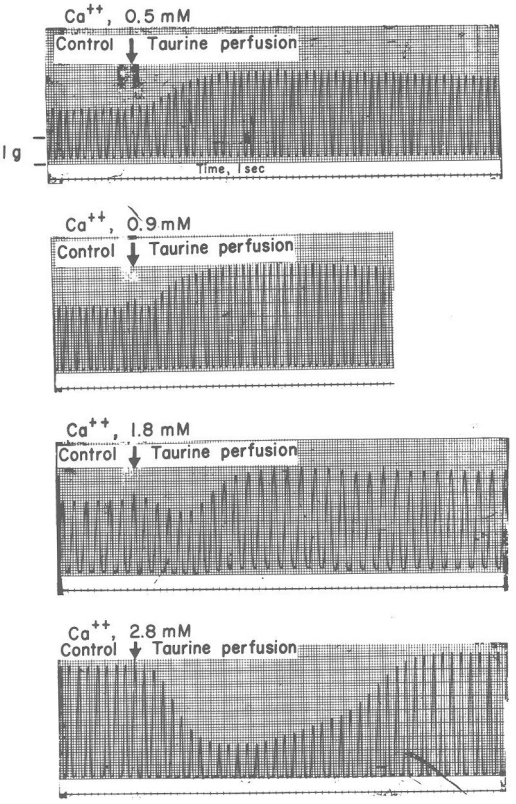

FIG. 3. The effect of taurine on the developed tension of hearts at different calcium concentrations in the perfusion fluid. The calcium concentration of the perfusion fluid is given at the top of each recording. At the arrow, the perfusion fluid was switched to one containing 10 m*M* taurine.

TABLE II. ENZYMATIC ACTIVITY OF ISOLATED BULLFROG MYOCARDIAL SARCOLEMMA

	ATPase activity (μmole P_i released/mg membrane protein/hr)						
	Na^+-K^+, Mg^{2+}-Dependent ATPase[a]			Ca^{2+}-Stimulated, Mg^{2+}-dependent ATPase[b]			Ca^{2+}-Stimulat
	Mg^{2+} ATPase	Mg^{2+}, + Na^+-K^+ ATPase	$Na^+ - K^+$ ATPase	Mg^{2+} ATPase	Mg^{2+} + Ca^{2+} ATPase	Ca^{2+} ATPase	Mg^{2+}-indepen ATPase[c]
ıtrol (5)[d]	2.8 ± 0.6[e]	9.0 ± 0.8	6.2 ± 0.7	3.1 ± 0.4	2.8 ± 0.3	0.0	54.0 ± 1.0
Ouabain (1 m*M*) (5)	2.6 ± 0.3	2.7 ± 0.8	0.0	2.9 ± 0.4	3.0 ± 0.5	0.0	53.6 ± 1.1
NaN_3 (1 m*M*) (5)	2.9 ± 0.6	8.9 ± 0.7	6.0 ± 0.4	3.0 ± 0.3	2.7 ± 0.4	0.0	52.9 ± 1.8

$Na^+ - K^+$ ATPase and Mg^{2+} ATPase were determined in a reaction mixture containing: Tris−HCl, 50 m*M*, pH 7.4; EGTA, 0.5 m*M*; $MgCl_2$, 5 m*M* m*M*; KCl 20 m*M*; Na_2 ATP, 2 m*M*; and 100 μg membrane protein in a final volume of 1.5 ml. The reaction (preincubated for 5 min at 37°) was sta addition of ATP and stopped after 10 min by the addition of 1 ml of 10% trichloroacetic acid (TCA). The Mg^{2+} ATPase was the activity measured in ce of NaCl and KCl. The $Na^+ - K^+$ ATPase activity was calculated as the difference in activity between the $Na^+ - K^+$, Mg^{2+} ATPase and th Pase.

The Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase activity was determined in a reaction mixture containing: Tris−HCl, 50 m*M*, pH 7.0; $MgCl_2$, 5 m*M* m*M*; EGTA, 0.2 m*M* or $CaCl_2$, 0.1 m*M*; and 100 μg membrane protein. The 5-min preincubated reaction was started by the addition of ATP and r 10 min by the addition of 1 ml of 10% TCA. The Ca^{2+}-stimulated ATPase activity was determined by subtracting the ATPase activity in the pres TA from that in the presence of Ca^{2+}.

The Ca^{2+}-stimulated, Mg^{2+}-independent ATPase activity was determined in a reaction mixture containing: Tris−HCl, 50 m*M*, pH 7.4; ATP, 4 m*M*; m*M* or Ca^{2+} 2 m*M*; and 100 μg membrane protein in a final volume of 1.5 ml. The Ca^{2+} ATPase was the difference between the basal EDTA vity and the ATPase activity in the presence of Ca^{2+}. Succinate−cytochrome *c* reductase activity, determined by the method of Tisdale (*in* "Met zymology, R. W. Estabrook and M. E. Pullman, eds.), Vol. 10, p. 213. Academic Press, New York, 1968), gave values that ranged from 0 to 0.00 ng membrane protein/hr which was 0 to 1.4% of the mitochondrial activity.

The numbers in parentheses represent the number of individual determinations.

The values represent the mean ± SEM.

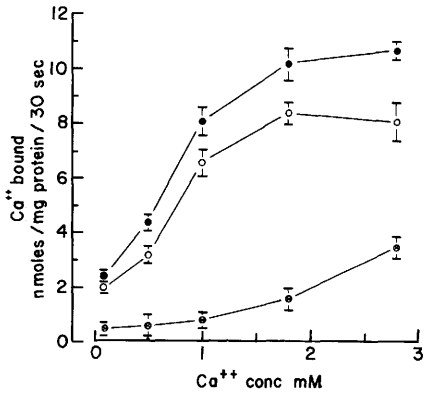

FIG. 4. The effect of taurine on calcium binding by sarcolemmal membranes isolated from bullfrog heart. (⊗) No ATP; (○) with ATP; (●) with ATP and taurine 10 m*M*.

ing. With bullfrog sarcolemma, calcium binding progressed rapidly, being complete in less than 30 sec. Calcium binding was increased in the presence of ATP (Fig. 4). In the control group, ATP-dependent calcium binding increased with increasing calcium concentrations up to a maximum at 1.8 m*M* calcium. Beyond 1.8 m*M* calcium there appeared to be a release of ATP-dependent bound calcium, while the passive calcium binding increased sharply. Except at 0.1 m*M* calcium concentration, taurine enhanced ATP-dependent calcium binding (22 to 38%).

Discussion. Although other interpretations are possible (27), we have assumed that tension development in the frog heart is directly related to a transsarcolemmal calcium influx entering the cell during depolarization (28, 29).

Calcium binding is a prerequisite for transport (30) and plays a role in the accelerated diffusion of calcium through the sarcolemma during the action potential (31). Our results show that taurine increased the maximal tension developed by the isolated perfused bullfrog heart and enhanced calcium binding of sarcolemma isolated from bullfrog hearts. We believe the two to be causally related and suggest that taurine enhances contractility in the bullfrog heart by enhancing sarcolemmal calcium binding.

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