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EFFECT OF TAURINE ON CALCIUM LEVELS AND CONTRACTILITY IN GUINEA-PIG VENTRICULAR STRIPS

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Abstract—Taurine increases the calcium levels in guinea-pig ventricular strips at external calcium concentrations of 0.45, 0.9 and 1.8 mM. At 2.7 mM calcium, however, a decrease is observed. Analogous changes occur in contractile force. It is also seen that the superfusion of ventricular strips with taurine-free medium produces a decrease in taurine content at the end of 120 min superfusion. Taurine levels can be restored by superfusion with 10 mM taurine; a linear relationship exists between external taurine and internal taurine levels.

In acute *in vitro* experiments, taurine exerts a positive inotropic effect in guinea-pig hearts [1–5]. It has been proposed that the basic mechanism underlying this taurine action is by increasing calcium affinity for some cellular components [3, 6]. Taurine also seems to protect rat heart against calcium paradox* [7], while the effect of taurine on contractility in this animal is unclear; Dietrich and Diacono [2] in fact found a negative effect, and Schaffer *et al.* [8] a positive one. A taurine calcium interaction may be postulated from *in vitro* studies which show that: (1) the effect of taurine on contractility in guinea-pig hearts is more evident at low calcium concentrations [1, 4, 5]; (2) taurine increases calcium binding in heart [5] and calcium uptake in liver mitochondria [9]; (3) taurine prevents the 'negative inotropic effect' of verapamil [10] and of D600 [11] at cardiac level; (4) taurine increases calcium content in guinea-pig hearts [3, 12]; (5) there are conflicting results for the interaction of taurine with calcium binding sites on cardiac sarcolemma; Azari and Huxtable [13] reported that taurine decreases ^{45}Ca binding, while other authors [5, 6, 10] have found that taurine increases ^{45}Ca binding.

The possibility that taurine modulates heart calcium content, thereby influencing contractility, stimulated further *in vitro* research, the results of which are reported here.

MATERIALS AND METHODS

The experiments were performed on electrically-drive right ventricular strips from guinea-pig hearts. The animals (male, body wt 300–400 g) were killed by a blow on the head and bled. The hearts were quickly excised and two strips were dissected from the same right ventricle and washed in aerated bathing solution (for composition see below) at room temperature.

Each ventricular strip was attached to a platinum stimulating electrode and suspended individually in a glass organ chamber for recording isometric contraction.

The bathing solution containing (mM) NaCl, 115; KCl, 4.7; $CaCl_2$, 1.8; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; and glucose, 10, was continuously gassed with 95% O_2 and 5% CO_2 and maintained at 30°; the pH was 7.4. The solution flowed through the organ bath (volume 20 ml) at a constant rate of 6 ml/min. The force of contraction was measured with a force displacement transducer (Marb model 750) attached to a Marb recorder. The preparations were stimulated electrically with a constant current square wave pulse generator at 2 Hz and of 0.2 msec duration (Marb model 7/2/150); the intensity was twice the usual threshold current of 0.3–0.4 mA. Before experiments were started, the preparations were allowed to equilibrate for 60 min in the bathing medium. After equilibration the incubation fluid was changed; in fact one strip was exposed for 1 hr to a solution containing taurine and the other was exposed to a solution containing sucrose at equiosmotic concentrations with taurine. Variation in osmolarity obtained with sucrose, added to mimic the variation in osmolarity due to taurine, did not influence internal calcium, taurine loss or the contractility. Calcium concentrations were varied as described in the results. After the experiment, the strips were wiped and rapidly frozen by liquid nitrogen.

Calcium assay. The calcium levels in the heart were determined after digestion of tissue in concentrated HNO_3 with an atomic absorption spectrophotometer (Perkin Elmer model 303) according to Dolara *et al.* [3].

Taurine assay. Taurine was determined with a Perkin–Elmer S3B liquid chromatograph after perchloric acid homogenisation of the tissue following its reaction with dansyl-chloride according to Kruzs *et al.* [14].

Protein assay. This was performed according to Weichselbaum [15].

Materials. Taurine was obtained from Merck

* Calcium paradox is a phenomenon observed when the heart is perfused with zero calcium medium and reperfused with a medium containing calcium, leading to a massive calcium overload.

Table 1. Taurine levels (μmole/g protein) of guinea-pig ventricular strips and effect of different external taurine concentrations after 2 hr of superfusion

Ca^{2+} in medium (mM)	External taurine (mM)			
	0	4	10	20
0.45	21.6 ± 1.7	25.8 ± 3.9	53.5 ± 5.4†	109.9 ± 17.2†
0.9	21.0 ± 2.2	36.8 ± 3.0*	78.9 ± 11.8†	101.2 ± 19.0†
1.8	21.9 ± 2.4	27.3 ± 3.28	101.3 ± 6.6†	127.3 ± 13.6†
2.7	19.7 ± 2.7		88.0 ± 13.9†	
3.6	20.3 ± 1.5		67.9 ± 10.5†	

Taurine levels in right ventricular measured soon after dissection (control) were 72.16 ± 0.96 (n = 9). Results are means ± S.E. of 4–8 experiments.
P versus zero taurine in the external medium.
* $P \leq 0.05$; †$P \leq 0.001$.

(Darmstadt, F.R.G.). All other chemicals were of analytical or best commercial grade. Twice-distilled water was used throughout.

RESULTS

Guinea-pig ventricular strips superfused in the absence of taurine

Superfusion under these conditions results in a significant decrease in taurine content (about 70%) when measured at the end of the second hour. The residual taurine content is not influenced by varying the external calcium concentration (Table 1).

The internal calcium content is significantly influenced by external calcium at least between 0.45 and 2.7 mM (Table 2) a positive log–linear relationship exists between the log of the internal calcium levels and external calcium concentrations (Fig. 1). The variability between different hearts does not allow evaluation of the significance for the calcium concentration of 3.6 mM.

A linear relationship exists between contractility and external calcium concentrations (Fig. 2).

Taurine-treated guinea-pig ventricular strips

The levels of taurine in the taurine-superfused strips are higher in comparison with those found in the paired untreated strips (Table 1). In the presence of 20 M taurine the tissue level of this amino acid is increased to above control levels (Table 1). There is a positive linear relationship between internal and external taurine which is maintained at the various calcium concentrations (Fig. 3).

When the logarithm of internal calcium levels is plotted against external calcium concentrations in the presence of taurine, a bell-shaped curve is produced (Fig. 1).

The time-course of the effect of 20 mM taurine on the contraction is shown in Fig. 4; the other taurine

Table 2. Calcium content (μg/mg protein) in guinea-pig ventricular strips

External calcium concentration (mM)			
0.45	0.9	1.8	2.7
0.99 ± 0.17 (9)	1.06 ± 0.06 (20)	1.25 ± 0.08 (24)	1.48 ± 0.18 (23)

The calcium contents were measured after 1 hr superfusion with $CaCl_2$ (1.8 mM) and 1 hr incubation with the concentrations indicated above.
Values in parentheses indicate the number of experiments.
Results are means ± S.E.

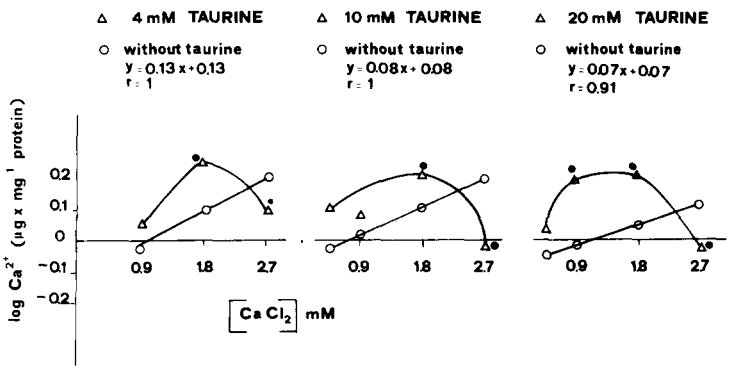

Fig. 1. Effect of external calcium on internal levels of this cation in the presence (△) or absence (○) of different concentrations of taurine. The experiments were performed in a parallel way as described in Materials and Methods. Results (means) are from 5–9 experiments. The statistical test used was the paired Student's t-test (●). $0.01 \leq P \leq 0.001$.

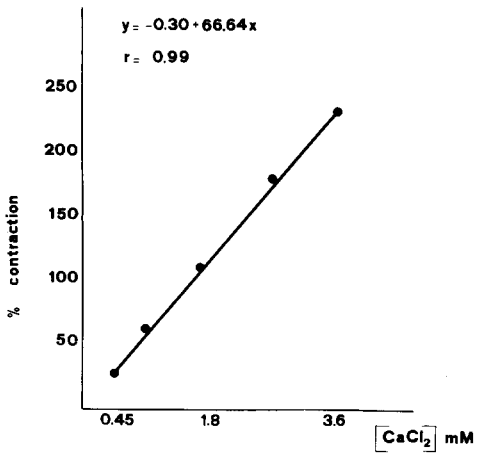

Fig. 2. Effect of different $CaCl_2$ concentrations on the contractile force of guinea-pig ventricular strips in the absence of taurine in the superfusion medium. Each point was the mean of all experiments performed at each calcium concentration.

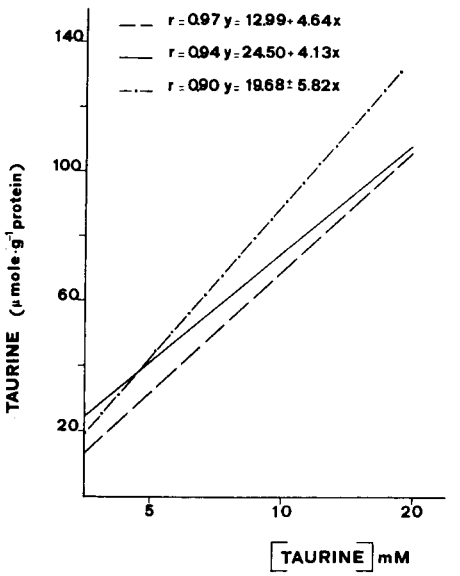

Fig. 3. The effect of different external taurine concentrations on internal levels. The straight lines were obtained by plotting data presented in Table 1 for each external calcium concentration. (–.–.–), 1.8 mM $CaCl_2$; (——), 0.9 mM $CaCl_2$; (– – –), 0.45 mM $CaCl_2$.

concentrations have the same kinetics. It may be seen that the time-course of taurine activity is dependent on external calcium concentrations. In fact at 0.45 mM taurine reaches a steady state after 10 min. At 0.9 mM it is possible to observe an initial decrease in contractile force followed by a complete recovery after 40 min. At 1.8 and 2.7 mM the taurine effect reaches a maximum after 30–40 min, respectively. In the presence of external taurine the contractility at each calcium concentration from 0.45 to 1.8 mM is higher than in the taurine non-treated strips. The positive inotropic effect is particularly evident at 0.9 mM calcium; in fact at this calcium concentration all taurine doses tested produce a significant dose-dependent increase in contraction (Fig. 5), while at 0.45 and 1.8 mM calcium only 20 mM taurine produces a significant increase in contractility. At calcium concentrations over 1.8 mM the taurine effect is reversed. The overall effect of each taurine concentration is therefore to produce a bell-shaped curve which crosses the zero line.

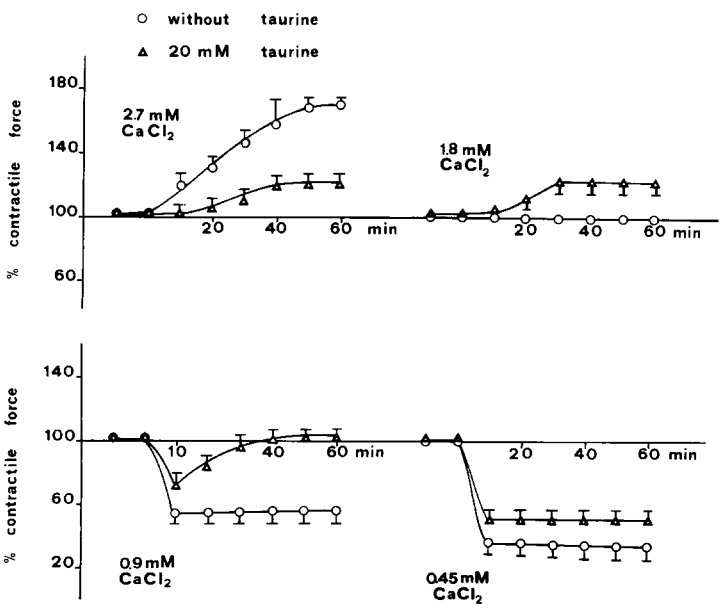

Fig. 4. Time-course of the effect of 20 mM taurine on cardiac contraction in guinea-pig ventricular strips in the presence of different calcium concentrations. The results are expressed as percentages of the initial contractile force during superfusion with normal medium. Each result is mean ± S.E. of 6–8 experiments.

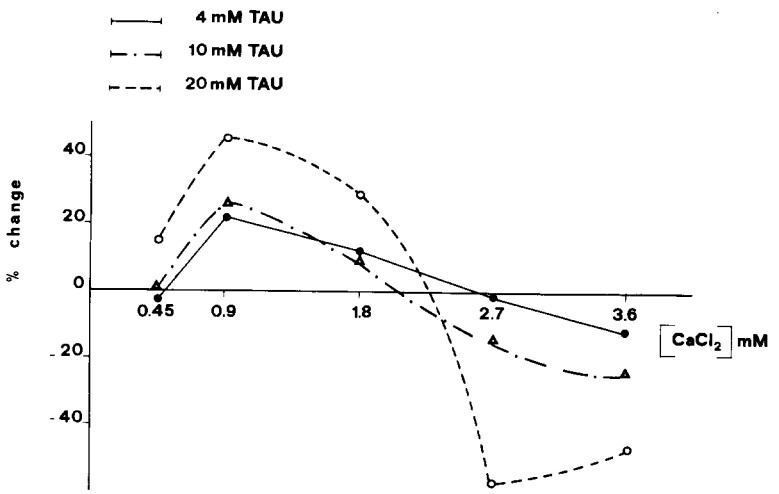

Fig. 5. Effect of different taurine concentrations in the presence of different calcium concentrations on the contractile force of guinea-pig ventricular strips. After a stabilization period of 1 hr with normal medium containing 1.8 mM $CaCl_2$, the strips were superfused with a media containing different calcium concentrations. The results are expressed as difference observed in the parallel strips. Using the paired Student's *t*-test the difference observed at 0.9 mM $CaCl_2$ with all taurine concentrations was statistically significant ($0.05 \leq P \leq 0.01$), while at 0.45 and 2.7 nM $CaCl_2$ only 20 mM taurine produced a statistical difference ($0.01 \leq P \leq 0.001$). At 3.6 mM $CaCl_2$, 10 and 20 mM taurine produced a statistically significant difference ($0.01 \leq P \leq 0.001$). Results are the means from 5–9 experiments.

DISCUSSION

That the positive inotropic effect of taurine in guinea-pig heart is a function of calcium in the medium or of cellular internal calcium has previously been demonstrated by Dolara *et al.* [3] and Dietrich and Diacono [2]. In fact Dolara *et al.* [3] found that calcium is taken up more extensively in the taurine-treated heart than in the control experiments; while Dietrich and Diacono [2] demonstrated that the positive inotropic effect was particularly striking in a low calcium medium.

The negative inotropic effect has been found in rat heart by Dietrich and Diacono [2], but it is known that the rat heart behaves differently in several respects (e.g. negative rate staircase, insensitivity to cardiac glycosides) [16] from other mammalian hearts. Moreover Bers *et al.* [17] suggested that the contractility in adult rat hearts depends on the greater amount of calcium release from sarcoplasmic reticulum, rather than on calcium binding to sarcolemma as in other species.

Our findings confirm the relationship between taurine's effect on contractility and calcium; they also provide the first demonstration that in a single species, the inotropic action of taurine may be positive or negative according to the calcium concentration in the external medium. The calcium levels found in the tissue after taurine offer an explanation of the variation of taurine effect on contractility relative to the external calcium concentration. In fact, in the presence of taurine, the log–linear relationship obtained by plotting log calcium content versus calcium concentration disappears, and a bell-shaped curve is obtained. In the taurine-treated strips maximum contractility and cellular calcium is reached around 1.8 mM, in the taurine-untreated strips around 3.6. Measurement of calcium levels at 3.6 mM are omitted because at this calcium concentration a big increase in variability is seen both in treated and untreated strips.

A biphasic effect of taurine has also been described on calcium binding. Chovan *et al.* [6, 10] and Khatter *et al.* [5] found that taurine increases calcium binding to cardiac sarcolemma when the calcium concentration in the medium is below 0.9 mM; Azari and Huxtable [13] found that taurine decreases calcium binding when the calcium concentration in the incubation medium is 2.5 mM. Chovan *et al.* [10] further demonstrated that taurine interacts with low-afffinity calcium binding-sites which are important for contractility [18].

The experiments presented here do not allow elucidation of the mechanism by which taurine exerts its effect. They do however show that restoration of the taurine content which is lost during superfusion is parallel to an increase in cellular calcium and contractility, almost as if the cellular taurine regulated the excitation–contraction coupling mechanism at calcium concentrations of or below 1.8 mM.

Taurine has been implicated in various pathological heart states. High myocardial taurine levels have been found both in patients who have suffered from congestive heart failure [19] and in experimental models of cardiac hypertrophy [20, 21]. During heart anoxia and ischemia a net efflux of taurine was observed which led to a net loss of tissue levels [22, 23]. Low taurine levels have also been found in a human syndrome characterized by mitral value prolapse and fibrosis of papillary muscle [24]. Besides their possible physiological relevance these results clearly illustrate the need to distinguish the pharmacological effects of taurine administered under conditions leading to restoration of the physiological

cellular content, from the ones obtained with a high dosage, which may interfere with the excitation–contraction coupling mechanism.

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