EFFECTS OF TAURINE ON Ca²⁺-DEPENDENT FORCE DEVELOPMENT OF SKINNED MUSCLE FIBRE PREPARATIONS

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Summary

The effects of the naturally occurring amino acid taurine (2-aminoethanesulphonic acid) on isometric force development were investigated using skinned muscle fibre preparations. In atrial and ventricular pig heart muscles, as well as in fibres of slow abdominal extensor muscle of crayfish, an increase of submaximal isometric force was observed in Ca²⁺-activated skinned fibre preparations at physiological concentrations of taurine. The maximal isometric force remained unaffected in all preparations. It is assumed that taurine increases the Ca²⁺ sensitivity of the force-generating myofilaments in mammalian hearts and crustacean slow skeletal muscle fibres.

Introduction

Taurine occurs widely in animal tissues, often at concentrations exceeding those of most other free amino acids (Jacobsen and Smith, 1968). The highest concentrations have been detected in excitable tissues such as nerve and muscle (Huxtable, 1980), and a variety of physiological effects have been reported. The most established function is its contribution to osmoregulation in the muscle tissues of many marine animals, especially crustaceans (Shaw, 1958; Allen and Garrett, 1971; Dalla Via, 1989).

Taurine is also present in considerable amounts in heart tissues, constituting up to 50 % of the free amino acid pool in mammals (Awapara *et al.* 1950). Here, too, taurine has been proposed to maintain cardiac osmolarity (Thurston *et al.* 1981).

In addition, taurine modulates numerous Ca^{2+}-dependent processes in heart and other tissues (Chovan *et al.* 1980; Kramer *et al.* 1981; Huxtable, 1989; Dolara *et al.* 1973). Taurine influences cardiac contractility, inducing antiarrhythmic activity (Read and Welty, 1963) and exerting positive (in low-Ca^{2+} media) as well as negative (in high-Ca^{2+} media) inotropic effects on intact mammalian hearts (Dietrich and Diacono, 1971; Schaffer *et al.* 1978; Franconi *et al.* 1982). Furthermore, the positive inotropic effects of cardiac glycosides are potentiated by taurine (Guidotti *et al.* 1971).

Direct effects of taurine on force generation of the isolated contractile machinery have not yet been reported. We measured the isometric force production of Ca^{2+}-activated skinned muscle fibre preparations (see Stephenson, 1981) of pig heart and slow skeletal muscles of crayfish in the presence and absence of physiological concentrations of taurine. Some of our results have been published in abstract form (Galler and Hutzler, 1988).

Materials and methods
Biological materials and skinning procedures

Pig hearts were obtained from the local slaughterhouse. Crayfish (*Pacifastacus leniusculus*, Dana) originated from the University of Konstanz. Tissue concentrations of taurine were determined using a slightly modified HPLC technique described earlier (Haller and Lackner, 1987). For mechanical experiments, fibre bundles of pig heart muscle (trabeculae of the right atrium and musculi papillares in the right ventricle) were chemically skinned in a solution containing 50 % glycerol, 1 % Triton X-100, 2 mmol l^{-1} dithioerythritol (DTE), 10 mmol l^{-1} NaN_3, 5 mmol l^{-1} ATP, 5 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} EGTA and 20 mmol l^{-1} imidazole, pH 7.0 adjusted with KOH. Since preliminary mechanical experiments suggested that there are intracellular Ca^{2+}-sequestering vesicles, we extended the skinning procedure to 24 h at a temperature of about 4°C. Such treated preparations did not show any Ca^{2+}-translocating activity, but the velocity of force development was generally reduced. Maximum isometric tension was also diminished (about 1.5 N cm^{-2}). We have chosen these preparations to exclude any possible effects of Ca^{2+} translocation due to the activity of sarcoplasmic reticulum.

Skinned skeletal muscle fibres of crayfish were prepared by drying abdominal muscles (using silica gel, at −20°C) that had been frozen quickly in liquid nitrogen (see Stienen *et al.* 1983). For mechanical measurements, single fibres of superficial abdominal extensor muscles (Parnas and Atwood, 1966) were used.

For heart muscle preparations, the relaxation solution contained Mopso [3-(N-morpholino)-2-hydroxypropanesulphonic acid] (20 mmol l^{-1}), K_2H_2EGTA (10 mmol l^{-1}), Na_2H_2ATP (10 mmol l^{-1}), sodium phosphocreatine (10 mmol l^{-1}), magnesium propionate (13.4 mmol l^{-1}) and taurine (0 or 5 mmol l^{-1}). The maximal activating solution had the same composition, except that K_2H_2EGTA was substituted by CaH_2EGTA (10 mmol l^{-1}) and the magnesium propionate

concentration was $12.9\,mmol\,l^{-1}$. The ionic strength of the solutions was $0.11\,mol\,l^{-1}$. For the crayfish slow muscle preparation, relaxation and maximal activating solutions (ionic strength, $0.22\,mol\,l^{-1}$; solutions modified after Moisescu and Thieleczek, 1979) both contained Mopso ($60\,mmol\,l^{-1}$), Na_2H_2ATP ($8\,mmol\,l^{-1}$), sodium phosphocreatine ($10\,mmol\,l^{-1}$), caffeine ($15\,mmol\,l^{-1}$) and taurine (0 or $5\,mmol\,l^{-1}$). In addition, the relaxation solution contained K_2H_2EGTA ($50\,mmol\,l^{-1}$) and magnesium propionate ($8.3\,mmol\,l^{-1}$) and the maximal activating solution contained CaH_2EGTA ($50\,mmol\,l^{-1}$) and magnesium propionate ($7.4\,mmol\,l^{-1}$). In all cases, pH was adjusted to 6.70 at a temperature of $22\pm0.5°C$.

In preliminary experiments, different ion compositions of the bath solutions were tested to obtain optimal conditions for reproducible force values of the skinned muscle fibre preparations. A relatively low pH of 6.70 appeared most appropriate. In addition, the adjustment of the required free Ca^{2+} concentrations in the EGTA-buffered solutions is facilitated at this relatively low pH. For crayfish fast skeletal muscle fibre preparations no condition could be detected in which force transitions were reproducible enough to study effects on myofibrillar Ca^{2+} sensitivity. Thus, taurine effects could not be investigated in crayfish fast muscle fibres.

To obtain solutions with different Ca^{2+} concentrations, relaxing and maximal activating solutions were mixed in different ratios. Creatine kinase ($50\,i.u.\,ml^{-1}$) was added to all solutions immediately before the mechanical experiments. Precise concentrations of free calcium and magnesium ions in all bath solutions were achieved with an iterative computer program based on the equilibrium constants listed by Martell and Smith (1977). The free magnesium ion concentration in the bath solutions was $1\,mmol\,l^{-1}$ in the case of crayfish skeletal muscle fibres and $3\,mmol\,l^{-1}$ in the case of the pig heart preparations. Free Ca^{2+} concentrations of the activating solutions with and without taurine were measured with a Ca^{2+}-selective electrode (Schefer *et al.* 1986) calibrated with the solutions described by Tsien and Rink (1980) (ionic strength, $0.13\,mol\,l^{-1}$). Differences, due to different ionic strengths, of the activity coefficients of Ca^{2+} in the calibration and experimental solutions were corrected by applying the Debye Hückel formalism as described by Meier *et al.* (1980).

Isometric force measurements

The skinned fibres were mounted horizontally between a fixed glass needle and a force transducer (AE 801, SensoNor, Horton, Norway) with nitrocellulose dissolved in acetone. The preparations were 0.5–2 mm long with diameters of 70–$200\,\mu m$. After mounting, the fibres were immediately incubated in the relaxing solution. To improve the pattern of cross-striation, length changes of about 1% of total fibre length were induced for about 5 min by moving the glass needle sinusoidally with a vibrator (Ling V 101). Sarcomere length of the muscle fibres was measured with a laser beam (He–Ne laser from Spectra physics, model 102; 4 mW) according to the method described by Zite-Ferenczi and Rüdel (1978).

Sarcomere length of the heart preparations was set at 2.1 μm; that of crayfish slow skeletal muscle fibres was not changed after incubating the stiff dried fibres mounted at the apparatus for force measurements. It ranged from 7 to 10 μm.

The skinned fibre preparations were activated by incubation in solutions with different Ca^{2+} concentrations. An automatic cuvette transporting system provided a rapid change of bath solutions. Force signals were measured with a bridge amplifier connected to a chart recorder (Gould, Brush 220).

Results

Taurine concentrations in muscle tissues

Concentrations of taurine in different tissues and animals are listed in Table 1. The amount of taurine in atrial muscles of pig is smaller than in ventricular muscles ($P<0.05$). In crayfish, slow abdominal extensor muscles contain up to seven times more taurine than do fast abdominal extensor muscles. The ratio varies considerably in different animals.

Maximal isometric force

Maximal activating solutions with or without taurine were applied with or without intermittent relaxation to test the effects of taurine on maximum isometric force development. Up to concentrations of 20 mmol l^{-1}, no significant influence of taurine on maximum isometric force could be detected.

Submaximal isometric forces

Successive activations of crayfish slow skeletal muscle fibres by addition of Ca^{2+} were interrupted by total relaxation, whereas in pig heart preparations, Ca^{2+} concentration was raised gradually without intermittent relaxations. Activating solutions with and without taurine were applied alternately (Fig. 1).

In several experiments, the preparations were preincubated in a taurine-containing relaxation solution for different times (0–30 min) prior to the submaximal activations in the presence of taurine. In other experiments, crayfish slow muscle preparations were incubated continuously in taurine-containing media, but at the end of the experiments submaximal activations were applied in taurine-free

Table 1. *Taurine concentrations of different animal tissues*

Species	Tissue	Taurine content [μmol g^{-1} wet mass]	N	$\pm$S.D.
Pig	Right atrium*	3.79	4	0.25
	Right ventricle†	4.47	4	0.43
Crayfish	Abdominal extensor muscles			
	Fast muscle	2.49	5	0.52
	Slow muscle	16.1	5	3.75

*Trabeculae, † M. papillares.

media. Here, preincubation in taurine-free relaxation solution lasted 1 min. In all these cases of different incubation procedures, similar taurine effects were observed.

In most of the experiments on crayfish slow muscle, preparations were kept in taurine-free media for about 15 min during the first incubations in relaxation and activation solutions. Pig heart preparations were kept in taurine-free media for more than 24 h during the skinning procedure and the first mechanical experiments. When changing from taurine-free to taurine-containing media or *vice versa*, preincubations lasting from about 20 s (crayfish preparations) to 2 min (pig heart preparations) in the corresponding relaxation solutions were applied.
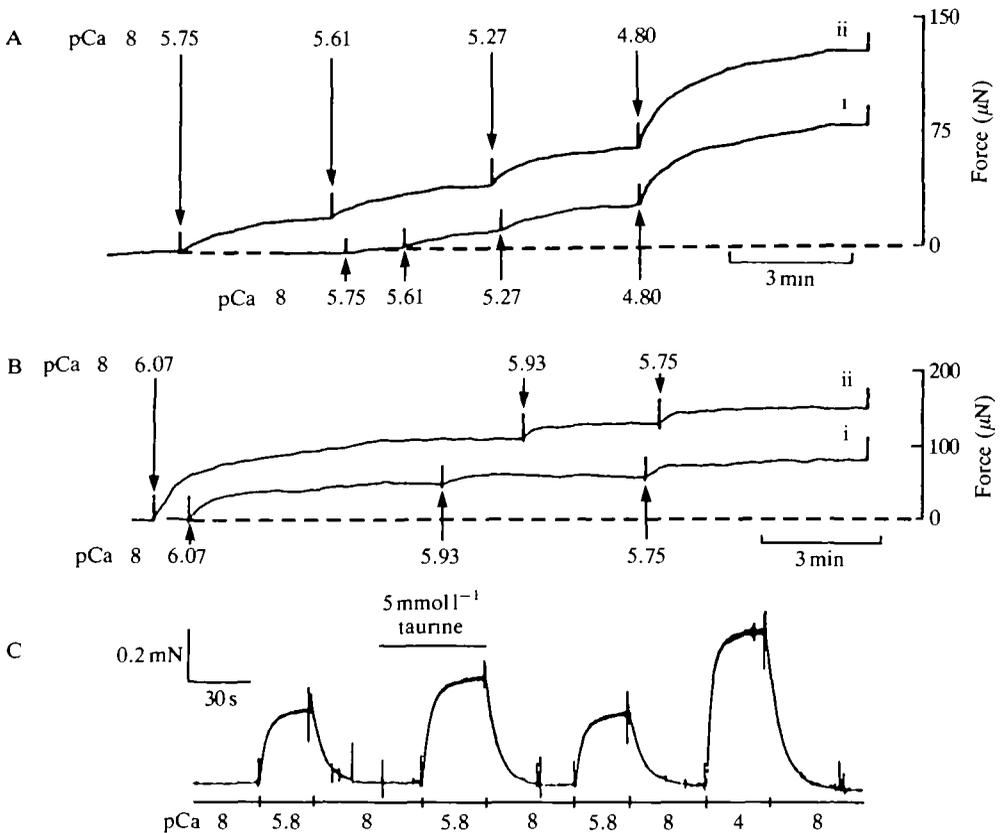

Fig. 1. Examples of mechanical experiments with an atrial (A) and a ventricular (B) skinned fibre preparation of pig heart and a slow skeletal muscle fibre of crayfish (C). Ai and Bi demonstrate increases of force after stepwise increase of Ca^{2+} concentration (expressed as $pCa = -\log[Ca^{2+}]$) in the absence of taurine. After maximal activation and relaxation at pCa 8 (not shown) the same stepwise Ca^{2+} increases were repeated in the presence of 5 mmol l^{-1} taurine (Aii, Bii). (C) A slow skeletal muscle fibre of crayfish is activated submaximally at pCa 5.8 in solutions with and without 5 mmol l^{-1} taurine. Single activations here are alternated with relaxations at pCa 8. Maximum isometric force is reached on the last activation at pCa 4.03.
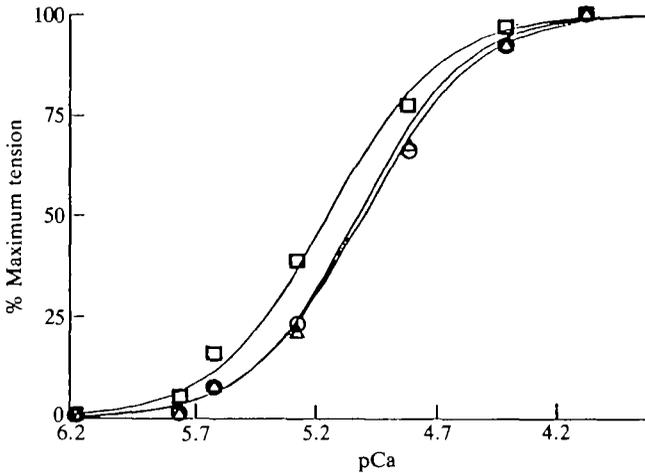
Fig. 2. Force values of a pig atrial muscle preparation relative to its maximal force are plotted against pCa. Curve-fitting resulted from the linear regression of a Hill plot. The shift of the pCa_{50} (pCa for half-maximal activation) towards lower Ca^{2+} concentration induced by $5 \, \text{mmol} \, l^{-1}$ taurine ($\square$) is 0.14 pCa units. One control curve ($\bigcirc$) represents Ca^{2+}-dependent force measurements before the addition of taurine, the other ($\triangle$) indicates the experiment after the removal of taurine.

Submaximal forces were enhanced by taurine in both cardiac and crayfish slow muscles. At about half-maximal activation the observed increase was $15.2\pm9.7\%$ (S.D.) of maximal force in atrial preparations ($N=8$), $17.9\pm13.0\%$ (S.D.) in ventricular preparations ($N=10$), and $13.2\pm6.5\%$ (S.D.) in crayfish slow skeletal muscle fibres ($N=12$) in the presence of $5 \, \text{mmol} \, l^{-1}$ taurine. Relative force plotted against pCa of the activating solutions and fitted with a Hill equation (Altringham and Johnston, 1982) revealed a shift of the curve towards lower Ca^{2+} values in the presence of taurine (Fig. 2). The increase of pCa_{50} (the negative logarithm of Ca^{2+} concentration for half-maximal activation) is significant (paired t-test, $P<0.001$) and similar for different preparations: pig atrial fibres, 0.13 ± 0.09 pCa units ($\pm$S.D., $N=7$); pig ventricular fibres, 0.15 ± 0.11 pCa units ($N=10$); crayfish slow muscle fibres, 0.10 ± 0.04 pCa units ($N=12$). Taurine did not significantly change the slope of the pCa–force curve in any preparation.

Discussion

Our measurements of taurine concentrations in different muscle tissues correspond with the findings in skeletal muscles of chicken (Airaksinen and Partanen, 1985) and fish (Haller and Lackner, 1987), where higher concentrations were found in the slow muscles than in the fast muscles. The higher taurine levels in pig ventricular muscles compared to those in atrial muscles also fit the above pattern, since ventricular muscle is considered to be slow and atrial muscle to be fast (Morano et al. 1988).

Physiological concentrations of taurine in pig heart and crayfish slow skeletal muscle fibres shift the pCa–force curve to lower Ca^{2+} concentrations without affecting the maximal force. These findings cannot be explained by a change of Ca^{2+} activity within the muscle fibre preparations as a result of Ca^{2+} movement through the sarcoplasmic reticulum. Pig heart preparations were treated with the non-ionic detergent Triton X-100, which removes membranes of sarcoplasmic reticulum (Meisheri and Rüegg, 1983). In the case of crayfish slow skeletal muscle fibres, all solutions for mechanical experiments contained $15 \, mmol \, l^{-1}$ caffeine, which prevents accumulation of Ca^{2+} in the sarcoplasmic reticulum (Nagasaki and Kasai, 1983). In addition, a constant Ca^{2+} level was maintained using high concentrations of the Ca^{2+} buffer EGTA ($50 \, mmol \, l^{-1}$). The possibility, therefore, that taurine acts *via* a change of Ca^{2+} concentration mediated through the sarcoplasmic reticulum can be excluded. We assume instead that taurine increases the Ca^{2+} sensitivity of the force-generating structures in pig heart and crayfish slow skeletal muscles.

As to the mechanism of action of taurine, its dipolar character may be responsible for the effects mentioned above, since taurine weakens charge-dependent protein–protein interactions. The troponin-I–actin binding which inhibits the force-generating myosin–actin interaction (El-Saleh *et al.* 1986) could theoretically be weakened by taurine. This would lead to the observed calcium-sensitizing effect (Rüegg, 1987).

The positive inotropic effect of taurine on living mammalian hearts (e.g. Dietrich and Diacono, 1971; Dolara *et al.* 1973) is usually thought to be caused by an increase in intracellular Ca^{2+} concentration mediated by taurine. Franconi *et al.* (1982) measured the force and tissue Ca^{2+} concentrations of superfused guinea pig ventricular strips at different external $CaCl_2$ concentrations in the presence and absence of taurine. They found only a coarse positive correlation between the taurine-mediated change in contractility and the taurine-induced change in the Ca^{2+} concentration of the heart tissue at different external $CaCl_2$ concentrations. The maxima of the two parameters appeared at different external $CaCl_2$ concentrations. The maximal taurine-mediated increase of internal Ca^{2+} concentration occurred at an external $CaCl_2$ concentration of $1.8 \, mmol \, l^{-1}$, whereas the maximal taurine-induced positive inotropic effect appeared at an external $CaCl_2$ concentration of $0.9 \, mmol \, l^{-1}$. Thus, in the presence of taurine, the increase of force is not always strictly correlated with an increase of internal Ca^{2+} concentration. Therefore, the positive inotropic effect of taurine may not be an exclusive effect of increased intracellular Ca^{2+} in intact heart muscle fibres; our study suggests that it may be partially due to enhanced Ca^{2+} sensitivity of force-generating myofilaments.

The potentiation of the positive inotropic effect of cardiac glycosides on intact mammalian hearts by taurine (Guidotti *et al.* 1971) may also be explained by the Ca^{2+}-sensitizing action. For these reasons, further attention should be paid to taurine as a potential moderate cardiotonic drug.

Since changes in taurine concentration appear in mammalian hearts and in

crustacean skeletal muscles in different physiological states, the Ca^{2+}-sensitizing effect of taurine could have physiologically important implications. Congestive heart failure in humans (Huxtable and Bressler, 1974) and dogs (Peterson *et al.* 1973) is accompanied by an increased taurine concentration in the heart tissues affected. Probably taurine is increased because of its Ca^{2+}-sensitizing effects on myofibrils.

In some crustacean species (e.g. *Carcinus, Eriocheir* and *Palaemon*; see Shaw, 1958; Allen and Garrett, 1971; Dalla Via, 1989) changes in sarcoplasmic taurine concentration were found to be dependent on extracellular osmolarity. Implications for muscle contraction are still unknown.

Apart from the naturally occurring sarcoplasmic imidazoles carnosine and *N*-acetyl histidine (Harrison *et al.* 1986), no endogenous Ca^{2+} sensitizers have yet been found. Non-endogenous Ca^{2+}-sensitizing drugs (submazole, Herzig *et al.* 1981; caffeine, Wendt and Stephenson, 1983) are imidazole derivatives. With taurine, a Ca^{2+} sensitizer with different chemical properties has been found. It is possible that other sarcoplasmic amino acids and peptides (e.g. glutamine and glutathione) may also have modulatory effects on the Ca^{2+} sensitivity of myofibrils.

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