Pflügers Arch – Eur J Physiol (1999) 438:384–391

ORIGINAL ARTICLE

Claire Holloway · George Kotsanas · Igor Wendt

Acute effects of taurine on intracellular calcium in normal and diabetic cardiac myocytes

Received: 13 January 1999 / Received after revision: 12 March 1999 / Accepted: 14 April 1999

Abstract The present study investigated the acute effects of taurine on intracellular Ca^{2+} ($[Ca^{2+}]_i$) in normal and diabetic cardiac myocytes. $[Ca^{2+}]_i$ was monitored using fura-2 in single myocytes isolated from control or streptozotocin-treated rats and paced at frequencies between 0.33 Hz and 2.0 Hz in the absence or presence of 20 mM taurine. Increasing stimulus frequency resulted in significant increases in resting and peak $[Ca^{2+}]_i$, and amplitude of the Ca^{2+} transient in both control and diabetic myocytes. The amplitude of the Ca^{2+} transient and the extent of its increase with increasing frequency was, however, significantly lower in the diabetic myocytes. Taurine significantly increased resting $[Ca^{2+}]_i$, peak $[Ca^{2+}]_i$, and the amplitude of the Ca^{2+} transient in both control and diabetic myocytes at all stimulus frequencies examined. The degree of potentiation of the Ca^{2+} transient decreased with increasing stimulus frequency in control cells but not in diabetic cells. In the absence of taurine the decay of the Ca^{2+} transient was significantly slower in diabetic than control myocytes. Taurine was without significant effect on the time course of the Ca^{2+} transient decay in control cells, however, in diabetic cells it significantly accelerated the rate of decay. The data demonstrate directly that taurine is able to increase $[Ca^{2+}]_i$ and the amplitude of the Ca^{2+} transient in both normal and diabetic cardiac myocytes. In addition several of the effects of taurine appeared to be more pronounced in diabetic than control cells.

Key words Cardiomyocyte · Calcium · Insulin-dependent diabetes mellitus · Fura-2 · Taurine

C. Holloway (✉) · G. Kotsanas · I. Wendt
Department of Physiology, Monash University, Clayton, Victoria 3168, Australia
E-mail: Claire.Holloway@med.monash.edu.au
Fax: +61-3-99052547

Introduction

Taurine, a naturally occurring, sulphur-containing amino acid, is found in relatively high concentrations in many tissues. In particular, it is abundant in the mammalian heart where it constitutes approximately 50% of the total free amino acid pool [2, 31]. Although taurine is the major constituent of the free amino acid pool of mammalian myocardium its functional role remains unresolved. It is not a substrate for metabolism within the heart and does not participate in protein synthesis [19], however, taurine has been reported to influence contractility, suggesting that it may play an important role in modulating Ca^{2+} mobilisation. These effects of taurine appear to be paradoxical, in that it can either increase or decrease contractility, depending upon the pre-existing levels of contractility and the $[Ca^{2+}]$ within and around the cell [9, 10, 16, 27, 29]. Because of these actions it has been suggested that taurine may exert a "normalising" influence, such that when contractility is low it will increase it, while if contractility is high then taurine will act to reduce it [16, 27, 29].

Effects of taurine on a variety of Ca^{2+} and other ion transport pathways have been reported. For example, taurine has been shown to affect L-type Ca^{2+} channels [5, 6, 29], Ca^{2+} transport by the sarcoplasmic reticulum (SR) [28, 29, 31], K^+ and Na^+ currents [5, 26] and the Na^+/Ca^{2+} exchanger [29, 31]. The mechanisms by which taurine modulates the activity of these ion-handling pathways are not known, but may involve changes in membrane phospholipid microenvironments [15] or second messenger-mediated effects through binding to specific receptors [7].

The myocardial level of taurine is known to be altered in various pathophysiological conditions [1]. Significant increases in intracellular taurine content have been shown in both congestive heart failure and insulin-dependent diabetes mellitus, in which myocardial taurine levels are reported to be increased approximately two-fold [23, 27]. The diabetic heart is characterised by a depressed contractile reserve, slowed contraction and

slowed relaxation [8] and there is now compelling evidence that these impaired contractile properties stem predominantly from disturbances in Ca^{2+} handling [8, 33]. For example, the activities of the SR Ca^{2+}-ATPase, the sarcolemmal Ca^{2+}-adenosine 5'-triphosphatase (ATPase) and the Na^+/Ca^{2+} exchanger are all reportedly depressed in the diabetic heart [21, 22, 25, 30, 33].

Given that taurine appears to modulate contractility, possibly via actions on processes involved in Ca^{2+} handling, together with the fact that taurine levels are increased in the diabetic heart, the present study set out to examine the acute effects of taurine on intracellular $[Ca^{2+}]$ in normal and diabetic cardiac myocytes. The aims of the study were, firstly, to investigate whether the increase in contractility produced by taurine is, in fact, the result of increased Ca^{2+} mobilisation within the cell and secondly, to compare the acute effects of taurine on intracellular Ca^{2+} in normal and diabetic cardiac myocytes.

Methods

Induction of diabetes

Male Wistar rats weighing approximately 250 g were divided randomly into two groups (diabetic or non-diabetic). Rats assigned to the diabetic group received 60 mg/kg streptozotocin (STZ) by injection into the tail vein under halothane anaesthesia. STZ was dissolved in 0.1 M citrate buffer (pH 4.5). Each STZ-treated rat was subsequently housed with an age and weight-matched control rat. All animals were given free access to food and water for the following 8 weeks. Non-fasting blood glucose measurements were obtained for all rats at the beginning and conclusion of the experimental period. All experimental procedures and experiments complied with the guidelines of the National Health and Medical Research Council of Australia on the care and use of animals in research and had the approval of the Monash University Animal Ethics Committee.

Cell isolation procedure

After 8 weeks of untreated, insulin-dependent diabetes mellitus, animals were anaesthetised with chloroform and killed by decapitation. The heart was excised rapidly and placed in *N*-[2-hydroxyethyl]piperazine-*N*'-[2-ethane-sulphonic acid(HEPES)-buffered Krebs-Henseleit (K-H) solution with the following composition (mM): NaCl, 130; KCl, 4.8; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 1.5; HEPES, 10; glucose, 10 (pH 7.4). It was then cannulated via the aorta, mounted on a Langendorff perfusion apparatus and perfused at a constant pressure of 60 mmHg with oxygenated (100% O_2) HEPES-buffered K-H solution maintained at 37 °C. Once a steady heart rate had been attained, this solution was replaced with a nominally Ca^{2+}-free HEPES-buffered K-H solution to arrest mechanical activity. Collagenase (Worthington; Type II) and $CaCl_2$ were then added to the perfusate to final concentrations of 120–150 IU/ml and 50 µM respectively.

After 20 min of collagenase perfusion the atria and right ventricular free wall were removed and the remaining left ventricular tissue was teased apart and placed in a conical flask containing 10 ml collagenase solution. Myocytes were dispersed by gentle agitation at 37 °C for 5 min. The collagenase solution containing dispersed cells was decanted, filtered through thin nylon gauze (approximately 200 µm mesh) and then centrifuged for 2 min at about 60 *g*. The cell pellet was resuspended in 2–3 ml HEPES-buffered K-H solution containing 1 mM Ca^{2+} and 2.5% bovine serum albumin. This procedure was repeated several times and the viability of cells in the different fractions was determined microscopically. For all experimental protocols recordings were made only from cells that displayed normal morphology, had regular sarcomere spacing, and were quiescent in the absence of electrical stimulation at physiological bath $[Ca^{2+}]$ ($[Ca^{2+}]_o$). Although not systematically evaluated, the yield of cells from diabetic animals was generally lower than from control animals. Nevertheless in both groups a large proportion of cells (>40%) met the above criteria.

Measurement of intracellular $[Ca^{2+}]$

Intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) was monitored using the fluorescent indicator fura-2. Cells were loaded with fura-2 by incubation with 5 µM fura-2 acetoxymethyl ester (fura-2/AM) (Molecular Probes, Eugene, Ore., USA) for 15 min at room temperature. An aliquot of fura-2-loaded myocytes was placed in an experimental chamber (volume 2 ml) and allowed to settle and adhere to the base, which consisted of a poly-L-lysine-coated cover-slip. The chamber was mounted on the stage of a Nikon Diaphot 300 inverted fluorescence microscope and perfused with solution at 0.7–1.0 ml/min at 25 °C.

Fluorescence was recorded using a Cairn Spectrophotometer system (Cairn Research, Kent, UK) coupled to the microscope. Light originating from a 75 W xenon lamp was passed through a rotating filter wheel containing 340 nm and 380 nm filters to allow alternating excitation of the fura-2 loaded myocytes at these two wavelengths. An adjustable rectangular diaphragm restricted recording to one cell in the field of view. The emitted fluorescence was passed through a 510 nm emission filter and recorded by a photomultiplier tube. The output current of the photomultiplier was converted to a voltage and the signals were then digitised for subsequent storage and analysis using a personal computer. The ratio of the fluorescence at 340 nm excitation to that at 380 nm ($R_{340/380}$) was taken as an indication of the free $[Ca^{2+}]_i$. $R_{340/380}$ was sampled at a frequency of 100 Hz.

The fura-2 fluorescence signals were calibrated internally in each cell at the conclusion of the experiment as described by Kao [18]. The maximum fluorescence ratio (R_{max}) was determined by exposure of the cell to the Ca^{2+} ionophore ionomycin (7.5 µM) in the presence of solution containing 1.5 mM Ca^{2+}. Following determination of R_{max} an excess of the Ca^{2+} chelator ethyleneglycol-bis(β-aminoethylether)-*N*,*N*,*N*',*N*'-tetraacetic acid (EGTA) was added (final concentration 3 mM) giving the minimum fluorescence ratio (R_{min}). $[Ca^{2+}]_i$ was subsequently calculated from the experimental values of $R_{340/380}$, assuming the dissociation constant of fura-2 for Ca^{2+} to be 224 nM [12].

Experimental protocol

$[Ca^{2+}]_i$-frequency response

Cells were stimulated electrically using a pair of platinum electrodes that delivered square pulses of 10–20 V amplitude and 4 ms duration. For each cell, changes in $[Ca^{2+}]_i$ were recorded during steady-state stimulation at 0.33, 0.5, 1.0 and 2.0 Hz with the cell superfused with the standard K-H solution containing 1.5 mM Ca^{2+}. With each change in stimulus frequency, the cells were allowed to equilibrate to the new rate of pacing for 3 min before any recordings were made. Once a full set of recordings had been obtained under control conditions, 20 mM taurine was added to the superfusate. The cells were then left to equilibrate in the taurine-containing solution for 20 min, during which they were stimulated at 0.33 Hz. Following this equilibration period, recordings of $[Ca^{2+}]_i$ in the taurine-containing solution were obtained at the different stimulus frequencies as above.

Three parameters were determined in the quantitative analysis of the changes in $[Ca^{2+}]_i$ at each frequency. These were resting (diastolic) $[Ca^{2+}]_i$; peak (systolic) $[Ca^{2+}]_i$ and the amplitude of the

Ca^{2+} transient (peak–resting). For the recordings taken at 0.5 Hz the time course of decay of the Ca^{2+} transient was analysed also. This was done by fitting a single exponential function to the declining phase of the transient from 30–40 ms after the peak to 50 ms before the next stimulus. From this exponential fit a time constant for the decay of the Ca^{2+} transient was obtained for each cell under each condition.

Recirculation fraction

The fraction of Ca^{2+} recirculated within the cell (recirculation fraction), was estimated as described by Ravens et al. [24]. Cells were stimulated at 1.0 Hz until the Ca^{2+} transient amplitude had attained a steady state. Stimulation was then stopped for 30 s and recommenced at either 0.20, 0.25 or 0.33 Hz. The recirculation fraction was then determined from the linear relationship between the amplitude of post-rest Ca^{2+} transient *n* and *n*–1. A 5-min recovery period was allowed between experimental runs for cells to return to a steady-state at 1.0 Hz. Once a full set of recordings had been obtained under control conditions these were repeated in the presence of 20 mM taurine.

Statistics

All data are presented as means±SEM for *n* independent cell preparations. The effects of taurine on the $[Ca^{2+}]_i$-frequency response in control and diabetic cells were analysed using four-factor ANOVA. At each frequency, only those data sets in which complete, paired observations had been obtained (i.e. –taurine and +taurine) were included in the analysis, for both control and diabetic groups. The time course of decay of the Ca^{2+} transient data was analysed similarly.

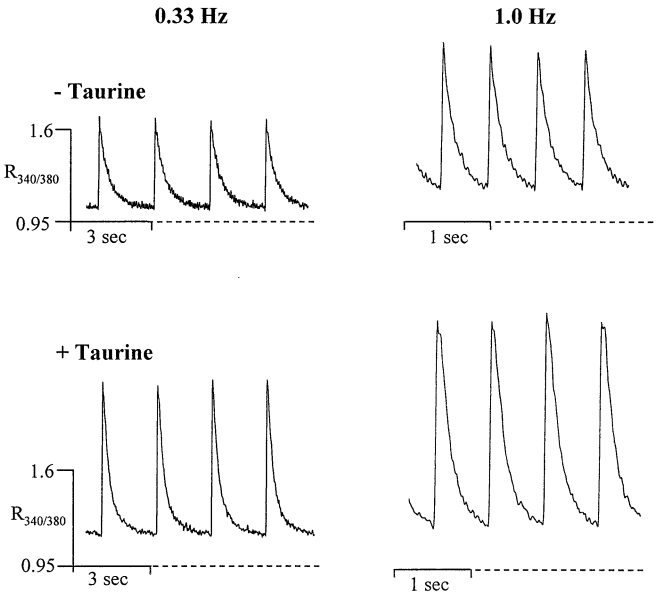

Fig. 1 Example of Ca^{2+} transients recorded from a control isolated rat cardiomyocyte in the presence (+*Taurine*) and absence (–*Taurine*) of taurine in response to two different stimulus frequencies. The *top panel* shows Ca^{2+} transients recorded during steady-state conditions at 0.33 Hz and 1.0 Hz in the absence of taurine, whilst the *bottom panel* shows transients from the same cell, at the same stimulus frequencies, but in the presence of 20 mM taurine

Results

Characteristics of experimental animals

Body weights and blood glucose levels of the experimental animals were measured prior to, and 8 weeks following, STZ treatment. Initial body weights of the animals in the control (*n*=11) and diabetic (*n*=8) groups were 282±6 g and 287±11 g respectively. Eight weeks after STZ treatment the diabetic animals had significantly lower body weights (304±12 g) than the controls (481±15 g). There was no significant change in blood glucose levels over the 8-week treatment period in the control animals, the initial and 8-week values being 6.6±0.5 and 6.2±0.7 mmol/l respectively. In contrast, a marked elevation in blood glucose was apparent within the diabetic group with mean values prior to STZ treatment of 7.8±0.4 mmol/l and after STZ treatment 29.3±2.1 mmol/l.

Effects of taurine on the frequency response in control myocytes

Figure 1 shows an example of Ca^{2+} transient recordings from a control cell at two stimulation frequencies in the absence and presence of taurine. Two features are evident. Firstly, there is an increase in the baseline, peak and amplitude of the Ca^{2+} transient with increasing stimulus frequency and, secondly, at each frequency the addition of taurine resulted in an increase in the baseline, peak and amplitude of the Ca^{2+} transient. Mean values of diastolic $[Ca^{2+}]_i$, peak $[Ca^{2+}]_i$, and the amplitude of the Ca^{2+} transient in control myocytes at different stimulus frequencies in the absence and presence of taurine are shown in Fig. 2. As stimulus frequency is increased, there are significant (P<0.02) increases in all three parameters of $[Ca^{2+}]_i$ both with and without taurine present. In addition, taurine itself increased diastolic and peak $[Ca^{2+}]_i$ significantly (P<0.01) at all frequencies (Fig. 2A, B). The increase in peak $[Ca^{2+}]_i$ was not just due to the increase in diastolic $[Ca^{2+}]_i$ since, as shown in Fig. 2C, the actual amplitude of the Ca^{2+} transient was also significantly increased by taurine at all frequencies.

Effects of taurine on the frequency response in diabetic myocytes

Figure 3 illustrates the mean effects of taurine on $[Ca^{2+}]_i$ in diabetic myocytes at different stimulus frequencies. As in the control myocytes, diastolic $[Ca^{2+}]_i$, peak $[Ca^{2+}]_i$, and the amplitude of the Ca^{2+} transient all increased significantly (P<0.02) with increasing stimulus frequency. Interestingly, the increases in peak $[Ca^{2+}]_i$ and amplitude of the Ca^{2+} transient with increasing frequency in the diabetic myocytes were significantly less than in control cells. The addition of taurine also significantly (P<0.01) increased diastolic $[Ca^{2+}]_i$, peak $[Ca^{2+}]_i$ and the

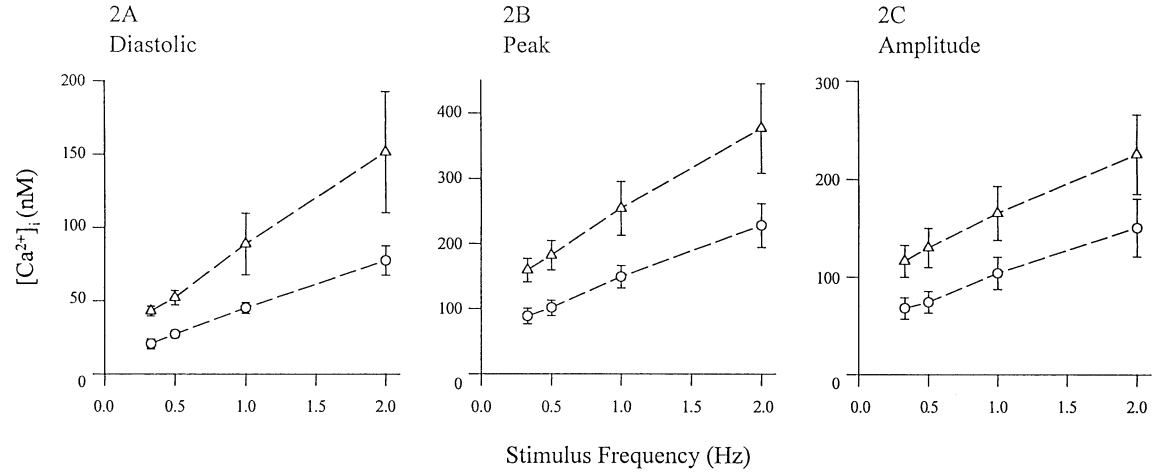

Fig. 2 Effects of 20 mM taurine on diastolic intracellular [Ca^{2+}] ([Ca^{2+}]$_i$), peak [Ca^{2+}]$_i$ and the amplitude of the Ca^{2+} transient in control cells at different stimulus frequencies. Means±SEM. At each different stimulus frequency paired comparisons only have been included, i.e. where data was obtained from the cell in both the absence (*open circles*) and presence (*open triangles*) of taurine at that frequency; $n \geq 8$ for each frequency. *$P<0.05$ vs. –Taurine

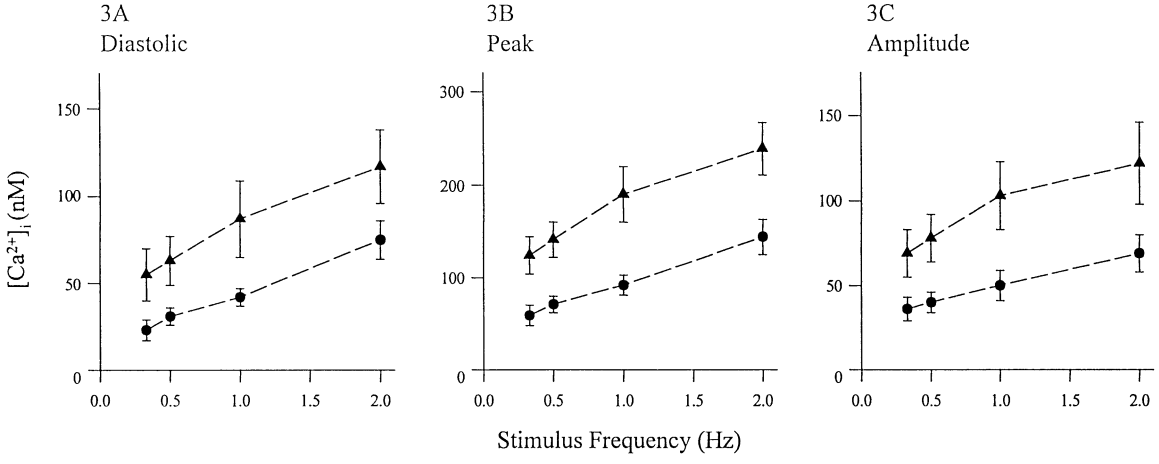

Fig. 3 Effects of 20 mM taurine on diastolic [Ca^{2+}]$_i$, peak [Ca^{2+}]$_i$ and the amplitude of the Ca^{2+} transient in response to changes in stimulus frequency in diabetic cells. Means±SEM. At each different stimulus frequency only paired comparisons for each cell have been included, i.e. where data was obtained from the cell in both the absence (*solid circles*) and presence (*solid triangles*) of taurine at that frequency; $n \geq 5$ for each frequency. *$P<0.05$ vs. –Taurine

amplitude of the Ca^{2+} transient in the diabetic myocytes at all stimulus frequencies.

Comparison of the effects of taurine in control and diabetic myocytes

To assess the relative effects of taurine on the [Ca^{2+}]$_i$ transient in control and diabetic cells, the extent of potentiation of the Ca^{2+} transient by taurine was determined for each cell. This was done by calculating the ratio of the amplitude of the Ca^{2+} transient in the presence of taurine to that in the absence of taurine. A value of 1 would thus indicate that taurine had no effect (i.e. no potentiation); a value of 2, for example, would indicate that taurine had doubled the amplitude. The results of this analysis are shown in Fig. 4. The trend within the control cells was for the degree of potentiation of the amplitude of the Ca^{2+} transient by taurine to decrease as the stimulus frequency increased. In the diabetic cells, however, taurine appeared to potentiate the Ca^{2+} transient similarly at all stimulus frequencies. Although not statistically significant, taurine does appear to potentiate the amplitude of the Ca^{2+} transient to a greater degree in diabetic cells at higher stimulus frequencies, whilst at lower frequencies the effect is similar in both groups.

Effects of taurine on the time course of the Ca^{2+} transient

Figure 5 illustrates the effects of taurine on the time-course of decay of the Ca^{2+} transient in a control (Fig.

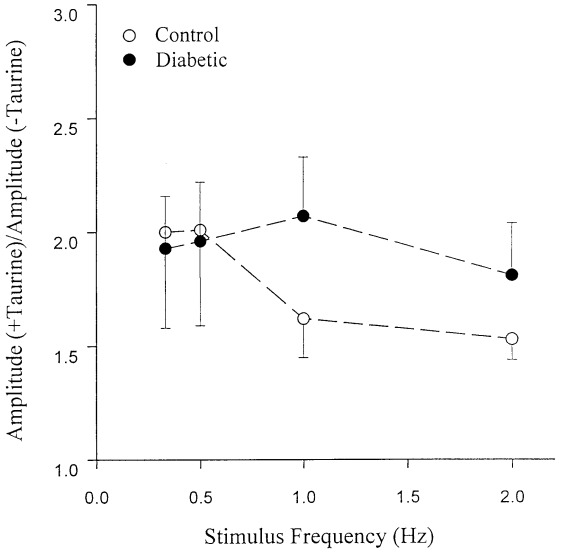

Fig. 4 Potentiation of the amplitude of the Ca^{2+} transient by 20 mM taurine in control and diabetic cells. Means±SEM. At each different stimulus frequency the amplitude of the Ca^{2+} transient in the presence of taurine has been expressed relative to the amplitude in the absence of taurine. Only paired comparisons for each cell within the control and diabetic groups have been included (i.e. where data was obtained from the cell in both the absence and presence of taurine at that frequency); n≥8 for each frequency in control group and n≥5 for each frequency in the diabetic group

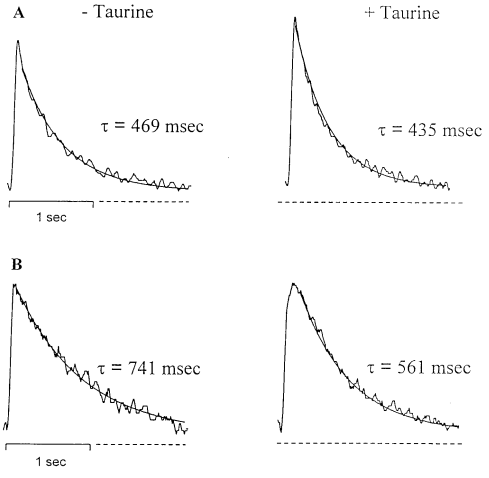

Fig. 5A, B Example of single Ca^{2+} transients from a control (**A**) and diabetic (**B**) cell in the presence and absence of 20 mM taurine. The transients were recorded during steady-state stimulation at 0.5 Hz and have been scaled to the same amplitude to facilitate comparison of their time course. The declining phase of each transient has been fitted with a single exponential function shown by the *smooth lines*. The time constant of each fitted function is shown to the *right* of each transient

5A) and a diabetic (Fig. 5B) myocyte stimulated at 0.5 Hz. In each case the declining phase of the transient was fitted with a single exponential function and from this a time constant was obtained. Taurine had no significant effect on the decay time constant in control cells, the mean values being 571±36 and 553±72 ms in the absence and presence of taurine respectively (n=11). In diabetic cells the addition of taurine significantly (P<0.02) increased the rate of decay of the Ca^{2+} transient with the corresponding values for the time constant being 723±36 ms in the absence of taurine and 570±50 ms in the presence of taurine (n=8). In the absence of taurine, the decay of the Ca^{2+} transient was significantly slower (P<0.02) in diabetic cells, but this difference was no longer apparent in the presence of taurine.

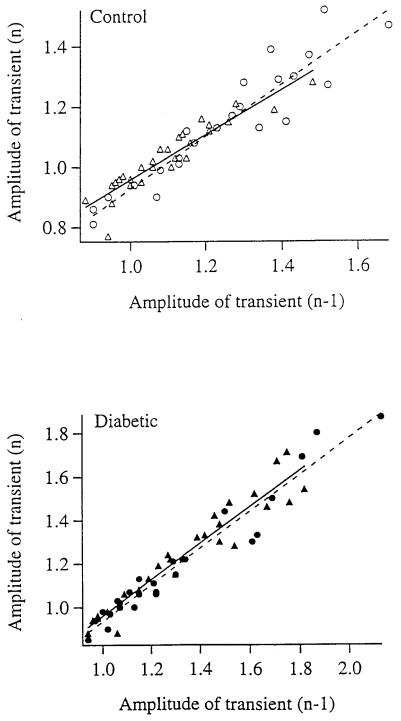

Fig. 6 The effects of 20 mM taurine on the recirculation fraction in control and diabetic myocytes. In both control and diabetic groups, a single linear function was fitted through the data both in the presence (*open triangles*) and absence (*open circles*) of taurine

Effects of taurine on the recirculation fraction

The fraction of mobilised Ca^{2+} recirculated within the cell (recirculation fraction) was estimated from the rate of decay of the potentiation of Ca^{2+} transients following a period of rest. During the decay of post-rest potentiation the relationship between the amplitude of post-rest contraction n, and the immediately preceding contraction n–1, is linear, and the slope of the relationship reflects the recirculation fraction [24]. This is shown in Fig. 6 in which the relationship between the amplitude of each post-rest transient (n) and its immediately preceding counterpart (n–1) during the decay of post-rest potentiation is shown for control and diabetic cells in the absence and presence of taurine. It is evident from Fig. 6 that taurine had no effect on the recirculation fraction in both experimental groups. The recirculation fraction in control cells was 0.87 (r^2=0.94) and 0.75 (r^2=0.93) in the

absence and presence of taurine respectively. Similarly in the diabetic cells the recirculation fractions were 0.85 (r^2=0.97) and 0.84 (r^2=0.96). It is also apparent that the recirculation fraction is no different in control or diabetic myocytes, either in the presence or absence of taurine.

Discussion

It has been well documented that taurine is capable of modulating the contractility of the heart and this has been linked to possible effects on Ca^{2+} movements [2, 28, 29]. The present study provides direct evidence that the addition of taurine to isolated cardiac myocytes increases resting $[Ca^{2+}]_i$, peak $[Ca^{2+}]_i$ and, most importantly, the amplitude of the Ca^{2+} transient. This increase in the amplitude of the Ca^{2+} transient suggests that taurine can enhance the mobilisation of Ca^{2+} within the cell upon stimulation, and reveals that the increase in peak $[Ca^{2+}]_i$ is not just secondary to an increased resting $[Ca^{2+}]_i$. These observations confirm that taurine can exert modulatory actions on the heart's contractility by influencing intracellular Ca^{2+} movements.

Increases in stimulus frequency resulted in increases in resting $[Ca^{2+}]_i$, peak $[Ca^{2+}]_i$ and the amplitude of the Ca^{2+} transient in both control and diabetic myocytes. The increase in the amplitude can be related to the "staircase" phenomenon seen in mammalian hearts where an increase in contractile strength accompanies increasing heart rate [6]. The frequency effect on peak $[Ca^{2+}]_i$ and amplitude of the Ca^{2+} transient appeared however, to be blunted in the diabetic cells compared with control cells and this may be attributable to depression of SR Ca^{2+}-ATPase activity or the number of SR Ca^{2+} release channels, both of which have been reported in diabetic cardiac muscle [25, 33]. The blunting of the increase in amplitude of the Ca^{2+} transient with increasing stimulus frequency could contribute to the depressed contractile reserve commonly seen in the diabetic heart by limiting the inotropic benefit of increases in heart rate [20, 25]. Indeed, in the present study, diabetic cells occasionally were unable to follow pacing at 2.0 Hz, a frequency that was never a problem for control cells.

Taurine had marked effects on $[Ca^{2+}]_i$ in both control and diabetic cells. At all frequencies in both groups taurine increased resting and peak $[Ca^{2+}]_i$ as well as the amplitude of the Ca^{2+} transient. An increase of approximately 30% in the amplitude of the Ca^{2+} transient by taurine has been reported previously in spontaneously contracting, cultured, mouse cardiac cells, however, this was associated with a decrease in the spontaneous rate of beating and resting level of Ca^{2+} [32]. The increase in the amplitude of the Ca^{2+} transient suggests that taurine may be increasing the flux of Ca^{2+} into the cell. Enhancement of both the L-type Ca^{2+} current and the fast component of the inward Na^+ current by taurine has been reported [5, 26]. A taurine-mediated increase in the L-type Ca^{2+} current would increase Ca^{2+} entry directly, while an increase in Na^+ current could have indirect effects via increased intracellular Na^+ levels and subsequent entry of Ca^{2+} via the Na^+/Ca^{2+} exchanger.

Taurine potentiated the Ca^{2+} transient to a similar degree in control and diabetic cells at the lower stimulus frequencies, however, at higher frequencies, taurine increased the amplitude of the Ca^{2+} transient in diabetic cells to a greater degree than in control cells. The lesser effect of taurine on control cells at the higher stimulus frequencies is consistent with an enantiostatic action of taurine as has been proposed by Huxtable [16]. This enantiostatic effect was suggested by several different studies in which taurine was found to exert effects on contraction and ion movements to produce changes in different directions depending upon the prevailing conditions of the cell [5, 10, 26]. In general, under conditions where $[Ca^{2+}]$ is low, taurine acts to increase contractility, whilst if contractility is already high, taurine tends to decrease it or produce little change. Similarly in the present study when the contractility of the control cells increased at higher stimulus frequencies the effects of taurine on the amplitude of the Ca^{2+} transient became less pronounced.

The reason for taurine's greater potentiation of the Ca^{2+} transient in the diabetic cells at the higher stimulus frequencies may be related to altered Ca^{2+} handling within the diabetic cells. It is generally believed that Ca^{2+} mobilisation and contractility are depressed in the diabetic heart [33]. Consequently, even at the higher stimulus frequencies used in the present study, the diabetic cells were in a condition of relatively low Ca^{2+} release and contractile activation. Under this circumstance taurine appeared, therefore, still to exert a substantial positive inotropic action.

It has been well documented that there is an increase in the level of taurine in the diabetic heart [23, 27]. Although the precise reasons for this increase have not been established, it may represent an attempt to offset the alterations in Ca^{2+} handling that occur in the diabetic state. In this way the depressed Ca^{2+} transporting activity in the diabetic myocytes may be overcome, at least partially, by the effects of the increased levels of taurine so that reasonable levels of contractility can still be maintained.

The rate of decay of the Ca^{2+} transient reflects the removal of Ca^{2+} from the cytosol. In the rat heart, SR Ca^{2+} uptake is believed to constitute the major Ca^{2+} removal mechanism [4]. In the absence of taurine, diabetic myocytes exhibited a significantly slower decay of the Ca^{2+} transient than control myocytes. This is consistent with the well-documented depression in the SR Ca^{2+}-ATPase activity in diabetes [21, 22]. In addition, depressed activities of the Na^+/Ca^{2+} exchanger and sarcolemmal Ca^{2+}-ATPase could also contribute to the slowing in the rate of decay of the Ca^{2+} transient in diabetic cells [33].

Taurine had no detectable effect on the rate of decay of the Ca^{2+} transient in control myocytes, however, in the diabetic myocytes taurine significantly increased the rate of decay. This indicates that in diabetic myocytes taurine is able to increase the rate of removal of Ca^{2+} from the cytosol presumably through actions on one of

the three main transport proteins responsible for the removal of Ca^{2+} from the cytosol during relaxation. Previous literature suggests that taurine has actions on both the SR Ca^{2+}-ATPase and Na^+/Ca^{2+} exchanger, which are considered to be the two primary cytosolic Ca^{2+} removal mechanisms contributing to relaxation [4]. An increase in the activity of both of these Ca^{2+} transporters by taurine has been reported [29, 31]. Interestingly, taurine has also been found to depress activity of the sarcolemmal Ca^{2+}-ATPase [28], however, the rate of Ca^{2+} extrusion by this transporter is generally considered too slow to contribute significantly to the decline of the Ca^{2+} transient on a contraction by contraction basis [3].

The recirculation fraction is the fraction of released Ca^{2+} that is recirculated within the cell between one contraction and the next, and has been shown to be a sensitive indicator of the inotropic state of the heart [17]. Interventions which exert a positive inotropic effect generally increase the recirculation fraction, whereas interventions which decrease the inotropic state of the heart may reduce the recirculation fraction. The recirculation fraction is also reduced under some pathophysiological circumstances in which cardiac function is impaired [14]. It was expected, therefore, that the increased mobilisation of intracellular Ca^{2+} observed with taurine would also be reflected in a higher value for the recirculation fraction. Taurine, however, had no effect on the recirculation fraction in either control or diabetic cells, indicating that, while it enhances intracellular Ca^{2+} mobilisation, the resulting changes do not alter the relative proportion of Ca^{2+} circulating within the cell. This may reflect effects of taurine on both the SR Ca^{2+}-ATPase and the Na^+/Ca^{2+} exchanger with any increase in SR Ca^{2+}-ATPase activity, which would normally increase the recirculation fraction, being offset by increased Ca^{2+} extrusion through the Na^+/Ca^{2+} exchanger. Interestingly, these experiments also revealed that there was no difference in recirculation fraction between control and diabetic myocytes. This indicates that the relative fraction of Ca^{2+} recirculated within diabetic myocytes is preserved despite the likelihood that the SR Ca^{2+}-ATPase activity is depressed.

From the present study it is clear that taurine affects the levels of Ca^{2+} within the myocardial cell, and must do this presumably by actions on some of the transport proteins and channels in the membranes of the myocyte. The mechanisms underlying such actions of taurine are as yet unknown, however, changes in membrane phospholipid distribution could be involved [27]. Taurine has been shown to inhibit phospholipid *N*-methyltransferase, which catalyses the conversion of phosphatidylethanolamine to phosphatidylcholine [13, 23]. The possibility exists, therefore, that taurine may alter the activity of transporters and proteins within the membranes of the cardiac myocyte through changes in membrane characteristics, although it is important to recognise that this remains speculative at the present time. Interestingly, a depression in sarcolemmal phospholipid *N*-methylation in diabetic myocardium has been observed [11] which may suggest a potential site of action for taurine within the diabetic myocardium.

This study has clearly shown that taurine is able to increase intracellular Ca^{2+} levels and Ca^{2+} mobilisation in isolated rat cardiac myocytes. There seems little doubt that this will account for the increase in contractility that has been shown previously to be produced by this amino acid. It is most likely that taurine exerts these actions through effects on one or more of the Ca^{2+} translocating mechanisms associated with either the sarcolemmal or SR membranes of the myocyte. Precisely which of these mechanisms is being modulated by taurine to produce the observed effects remains to be resolved, as does the means by which taurine achieves this modulation.

References

1. Azuma J, Hamaguchi T, Ohta H, Takihara K, Awata N, Sawamura A, Harada H, Tanaka T, Kishimoto S (1987) Calcium overload-induced myocardial damage caused by isoproterenol and by adriamycin: possible role of taurine in its prevention. In: Huxtable RJ, Franconi F, Giotti A (eds) The biology of taurine. Methods and mechanisms. Plenum, New York, pp 167–180
2. Azuma J, The Heart Failure Research with Taurine Group (1994) Long-term effect of taurine in congestive heart failure: preliminary report. In: Huxtable RJ, Michalk D (eds) Taurine in health and disease. Plenum, New York, pp 425–435
3. Barry WH, Bridge JHB (1993) Intracellular calcium homeostasis in cardiac myocytes. Circulation 87:1806–1815
4. Bassani JWM, Bassani RA, Bers DM (1994) Relaxation in rabbit and rat cardiac cells: species-dependent differences in cellular mechanisms. J Physiol (Lond) 476:279–293
5. Bkaily G, Haddad G, Jaalouk D, Gros-Louis N, Benchekroun M, Nalk R, Pothier P, D'Orleans-Juste P, Bui M, Wang, S, Sperelakis N (1996) Modulation of Ca^{2+} and Na^+ transport by taurine in heart and vascular smooth muscle. In: Huxtable RJ, Azuma J, Kuriyama K, Nakagawa M, Baba A (eds) Taurine. 2. Basic clinical aspects. Plenum, New York, pp 263–275
6. Chapman RA, Suleiman M, Earm YE (1993) Taurine and the heart. Cardiovasc Res 27:358–363
7. Chovan JP, Kulakowski E, Sheakowski S, Schaffer SW (1980) Ca^{2+} regulation by the low-affinity taurine binding sites of cardiac sarcolemma. Mol Pharmacol 17:295–300
8. Davidoff AJ, Ren J (1997) Low insulin and high glucose induce abnormal relaxation in cultured adult rat ventricular myocytes. Am J Physiol 272:H159–H167
9. Franconi F, Martini F, Stendardi I, Matucci R, Zilletti L, Giotti A (1982) Effect of taurine on calcium levels and contractility in guinea-pig ventricular strips. Biochem Pharmacol 31:3181–3185
10. Franconi F, Stendardi I, Matucci R, Failli P, Bennardini F, Antamini G, Giotti A (1984) Inotropic effect of taurine on guinea-pig ventricular strips. Eur J Pharmacol 102:511–514
11. Ganguly PK, Rice KM, Panagia V, Dhalla NS (1984) Sarcolemmal phosphatidylethanolamine N-methylation in diabetic cardiomyopathy. Circ Res 55:504–512
12. Grynkiewicz G, Poenie M, Tsien RY (1985) A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 260:3440–3450
13. Hamaguchi T, Azuma J, Schaffer SW (1991) Interaction of taurine with methionine: inhibition of myocardial phospholipid methyltransferase. J Cardiovasc Pharmacol 18:224–230
14. Hata Y, Shimizu J, Hosogi S, Matsubara H, Araki J, Ohe T, Takaki M, Takasago T, Taylor T, Suga H (1997) Ryanodine decreases internal Ca^{2+} recirculation fraction of the canine heart as studied by postextrasystolic transient alternans. Jpn J Physiol 47:521–530
15. Huxtable RJ (1987) From heart to hypothesis: a mechanism for the calcium modulatory actions of taurine. In: Huxtable RJ,

Franconi F, Giotti A (eds) The biology of taurine. Methods and mechanisms. Plenum, New York, pp 371–387
16. Huxtable RJ (1996) Taurine – past, present and future. In: Huxtable RJ, Azuma J, Kuriyama K, Nakagawa M, Baba A (eds) Taurine. 2. Basic and clinical aspects. Plenum, New York, pp 641–651
17. Juggi JS (1996) Recirculation fraction of the activator Ca^{2+}: index of the extent of Ca^{2+} loading of rat myocardium during ischemia-reperfusion. Can J Physiol Pharmacol 74:116–123
18. Kao JPY (1994) Practical aspects of measuring [Ca^{2+}] with fluorescent indicators. In: Nuccitelli R (ed) Methods in cell biology. Academic Press, San Diego, pp 155–181
19. Lake N (1994) Alterations of ventricular contractility and myofibril loss in taurine-deficient hearts. In: Huxtable RJ, Michalk D (eds) Taurine in health and disease. Plenum, New York, pp 335–343
20. Lopaschuk G, Tahiliani G, Vadlamudi R, Katz S, McNeill J (1983) Cardiac sarcoplasmic reticulum function in insulin- or carnitine-treated diabetic rats. Am J Physiol 245:H969–H976
21. Makino N, Dhalla K, Elimban V, Dhalla N (1987) Sarcolemmal Ca^{2+} transport in streptozotocin-induced diabetic cardiomyopathy in rats. Am J Physiol 253:E202–E207
22. Penpargkul S, Fein F, Sonnenblick E, Scheuer J (1981) Depressed cardiac sarcoplasmic reticulum function from diabetic rats. J Mol Cell Cardiol 13:303–309
23. Punna S, Ballard C, Hamaguchi T, Azuma J, Schaffer SW (1994) Effects of taurine and methionine on sarcoplasmic reticular Ca^{2+} transport and phospholipid methyltransferase activity. J Cardiovasc Pharmacol 24:286–292
24. Ravens U, Mahl C, Ohler A, Hardman SMC, Noble MIM (1996) Mechanical restitution and recirculation fraction in cardiac myocytes and left ventricular muscle of adult rats. Basic Res Cardiol 91:123–130
25. Ren J, Davidoff AJ (1997) Diabetes rapidly induces contractile dysfunctions in isolated ventricular myocytes. Am J Physiol 272:H148–H158
26. Satoh H (1996) Electrophysiological and electropharmacological actions of taurine on cardiac cells. In: Huxtable RJ, Azuma J, Kuriyama K, Nakagawa M, Baba A (eds) Taurine. 2. Basic and clinical aspects. Plenum, New York, pp 285–297
27. Schaffer SW, Azuma J (1992) Review: myocardial physiological effects of taurine and their significance. In: Lambardini JB, Schaffer SW, Azuma J (eds) Taurine: nutritional value and mechanisms of action. Plenum, New York, pp 105–121
28. Schaffer SW, Punna S, Duan J, Hamaguchi, T, Azuma J (1992) Mechanism underlying physiological modulation of myocardial contraction by taurine. In: Lambardini JB, Schaffer SW, Azuma J (eds) Taurine: nutritional value and mechanisms of action. Plenum, New York, pp 193–199
29. Schaffer SW, Ballard C, Azuma J (1994) Mechanisms underlying physiological and pharmacological actions of taurine on myocardial calcium transport. In: Huxtable RJ, Michalk D (eds) Taurine in health and disease. Plenum, New York, pp 105–121
30. Schaffer SW, Ballard-Croft C, Boerth S, Allo SN (1997) Mechanisms underlying depressed Na^{+}/Ca^{2+} exchanger activity in the diabetic heart. Cardiovasc Res 34:129–136
31. Steele DS, Smith GL (1992) Intracellular effects of taurine: studies on skinned cardiac preparations. In: Lambardini JB, Schaffer SW, Azuma J (eds) Taurine: nutritional value and mechanisms of action. Plenum, New York, pp 163–173
32. Takahashi K, Harado H, Schaffer SW, Azuma J (1992) Effect of taurine on intracellular calcium dynamics of cultured myocardial cells during the calcium paradox. In: Lambardini JB, Schaffer SW, Azuma J (eds) Taurine: nutritional value and mechanisms of action. Plenum, New York, pp 153–163
33. Tomlinson KC, Gardiner SM, Hebden A, Bennett T (1992) Functional consequences of streptozotocin-induced diabetes mellitus, with particular reference to the cardiovascular system. Pharmacol Rev 44:103–150