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Shape and size changes induced by taurine depletion in neonatal cardiomyocytes

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Summary. Taurine is a very important organic osmolyte in most adult cells. Because of this property it has been proposed that large changes in the intracellular content of taurine can osmotically stress the cell, causing changes in its size and shape. This hypothesis was examined by measuring cell dimensions of taurine deficient cardiomyocytes using confocal microscopy. Incubation of isolated neonatal rat myocytes with medium containing 5mM β-alanine led to a 55% decrease in intracellular taurine content. Associated with the loss of taurine was a reduction in cell size. Two factors contributed to the change in cell size. First, there was a shift in cell shape, favoring the smaller of the two cellular configurations commonly found in the myocyte cell culture. Second, the size of the polyhedral configuration was reduced after β-alanine treatment. These same two events also contributed to size reduction in cardiomyocytes incubated with medium containing 30mM mannitol. Nonetheless, some qualitative differences exist between cells osmotically stressed by increasing the osmolality of the incubation medium and decreasing intracellular osmolality. The results support a role for taurine in the regulation of osmotic balance in the neonatal cardiomyocyte.

Keywords: Amino acids – Taurine – Osmoregulation – Cell size – Cell shape

Introduction

Organic osmolytes play a very important role in the regulation of volume in most adult cells (Leem, 1996; McManus et al., 1995; Pasantes-Morales and Martin del Rio, 1990; Vandenberg et al., 1996). Typically, the osmotically responsive cell rapidly extrudes organic osmolytes following a hyposmotic insult, a process designed to limit the degree of cell swelling and trauma. By comparison, cell volume regulation in response to a hyperosmotic insult in-

volves the accumulation of organic osmolytes via a two step process, the slower one consisting of the activation and upregulation of proteins that either increase the biosynthesis or the transport of organic osmolytes.

An important organic osmolyte that contributes to volume regulation in most cells is the amino acid, taurine. Atlas et al. (1984) reported that taurine transport into mouse heart is enhanced by exposure to hyperosmolar medium. By contrast, large amounts of taurine efflux the myocyte subjected to a hyposmotic insult (Rasmusson et al., 1993; Vislie, 1983). The modulation of taurine levels in response to an osmotic challenge is particularly dramatic in the teleost, where taurine is the main osmolyte and generally accounts for 40–50% of environmentally-induced alterations in cellular osmolality (Vislie, 1983). Besides serving as an osmolyte, taurine has also been implicated in the regulation of calcium transport (Schaffer et al., 1994), phospholipid metabolism and membrane stability (Huxtable, 1992; Schaffer et al., 1995), free radical chemistry (Schuller-Levis et al., 1994), protein phosphorylation (Lombardini, 1992) and cellular development (Sturman, 1993).

Because of the importance of taurine's diverse actions, models of taurine deficiency have been developed to study the consequences of reduced cellular taurine content. One of the most widely accepted models of taurine deficiency is the nutritionally deprived cat. Since taurine is an essential nutrient in cats, an inadequate dietary source of taurine can dramatically lower intracellular taurine levels, producing retinal degeneration and a cardiomyopathy (Hayes and Carey, 1975; Pion et al., 1987). In contrast to the cat, most species are capable of synthesizing taurine, although intracellular levels of the amino acid can be dramatically reduced by feeding the animal a taurine transport inhibitor, such as β-alanine or guanidinoethane sulfonate (Mozaffari et al., 1986). Recently, we found that intracellular taurine levels of isolated cells can be depleted by incubating the cells with medium containing β-alanine. Thus, in this study the β-alanine procedure was used to decrease the size of the intracellular taurine pool of isolated neonatal rat cardiomyocytes. The potential osmoregulatory consequences of β-alanine-induced taurine depletion were then examined.

Methods

Rat neonatal myocytes were isolated according to the method of McDermott and Morgan (1989). The cells were suspended in minimal essential medium containing 10% newborn calf serum and 0.1 mM 5-bromo 2′-deoxyuridine and allowed to plate on dishes precoated with 0.1% gelatin. They were then placed in standard serum free medium containing 56 U/l insulin, 10μg/ml transferrin, 53 nM selenium and 0.25 mM ascorbate. Some of the cells were incubated in standard medium supplemented with either 5 mM β-alanine or 30 mM mannitol. Following a 5 day incubation with serum free medium, the cells were incubated with medium containing 5μM calcein/acetoxymethyl (AM) ester for 30 minutes. The calcein loaded cells were subsequently washed three times with calcein free medium and then placed in standard serum free medium containing either no additions, 5 mM β-alanine or 30 mM mannitol. The volume of the cell was evaluated by scanning the calcein fluoresence of the cell at various depths using a confocal microscope (Satoh et al., 1996). At each depth, the surface area and pixel thickness of the confocal cell image was determined. The volume of the cell was calculated from the surface areas of each cell

section, beginning at the bottom and proceeding systematically to the top of the cell. A computer interfacing with the confocal microscope was used to reconstruct the three dimensional image of the cell by summation of the individual cell cross-sections. The taurine content of the cell was determined as previously described (Mozaffari et al., 1986).

Results

Incubation of neonatal myocytes with medium containing 5 mM β-alanine led to a 55% decrease in the intracellular levels of taurine (Fig. 1). Since taurine is a major organic osmolyte in most cells, it seemed logical that the dramatic loss of taurine would cause an osmotic imbalance in the cell and thus lead to cell shrinkage. This was examined by loading cells with the fluorescent dye, calcein, and analyzing the distribution of calcein by confocal microscopy.

Using the confocal microscope, it was shown that normal neonatal rat myocytes cultured in serum free medium assumed either a rectangular or polyhedral configuration (Figs. 2 and 3). After a 5 day incubation in standard serum free medium, approximately 1/3 of the cells existed in the rectangular configuration. The dimensions of the largest cross-section of the rectangular cell were visualized by setting the observation angle of the confocal microscope at 90°. As seen in Figure 2A, the length (x dimension) of the largest section of a typical rectangular cell was approximately twice as large as the width (y dimension). From the image at a 10° angle, it is apparent that the depth (z dimension) of the rectangular cell was considerably smaller than either the width or length (Fig. 2B). Interestingly, the calculated depth of the

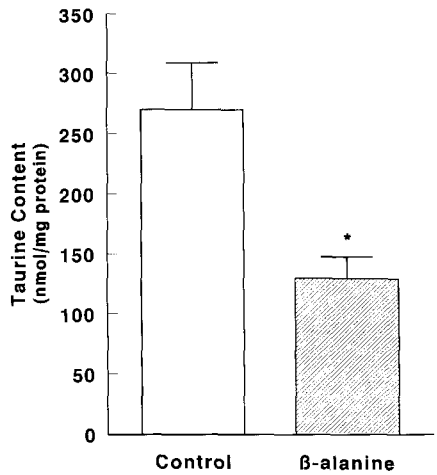

Fig. 1. Effect of β-alanine treatment on taurine content of isolated neonatal myocytes. Cells in culture were incubated with either 0 (*control*) or 5 mM β-alanine (*β-alanine*). After 5 days, the cells were digested with 2% perchloric acid and then run through an AG-50 WX8 column. The effluent was then assayed for taurine content. Data represent means ± S.E.M. of 5 different preparations. The asterisk denotes a significant difference from the control ($p < 0.05$)

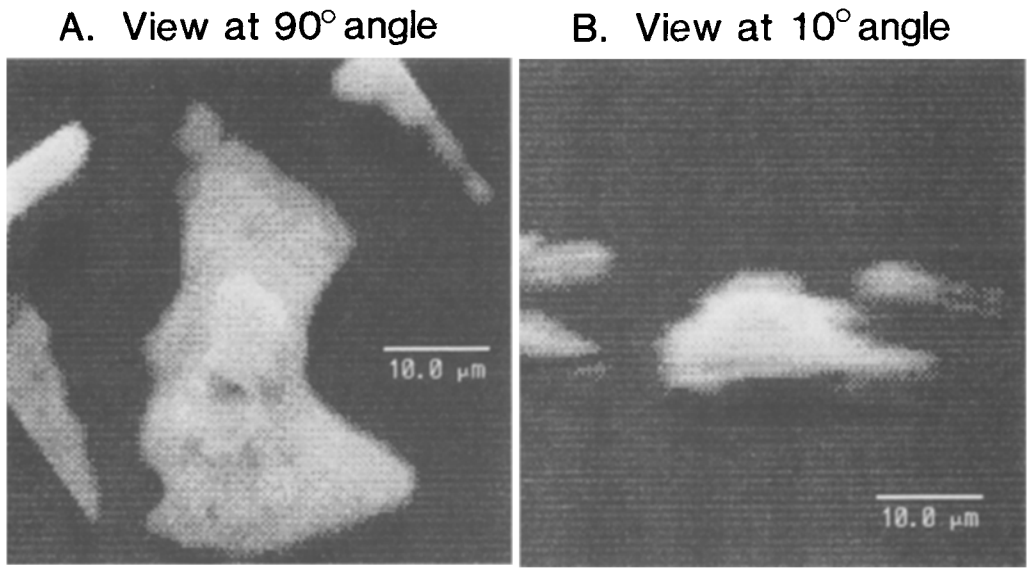

Fig. 2. Rectangular configuration of the neonatal rat myocyte. The cell was viewed from either a 90° (**A**) or a 10° angle (**B**). The three dimensional image was reconstructed from confocal microscope data

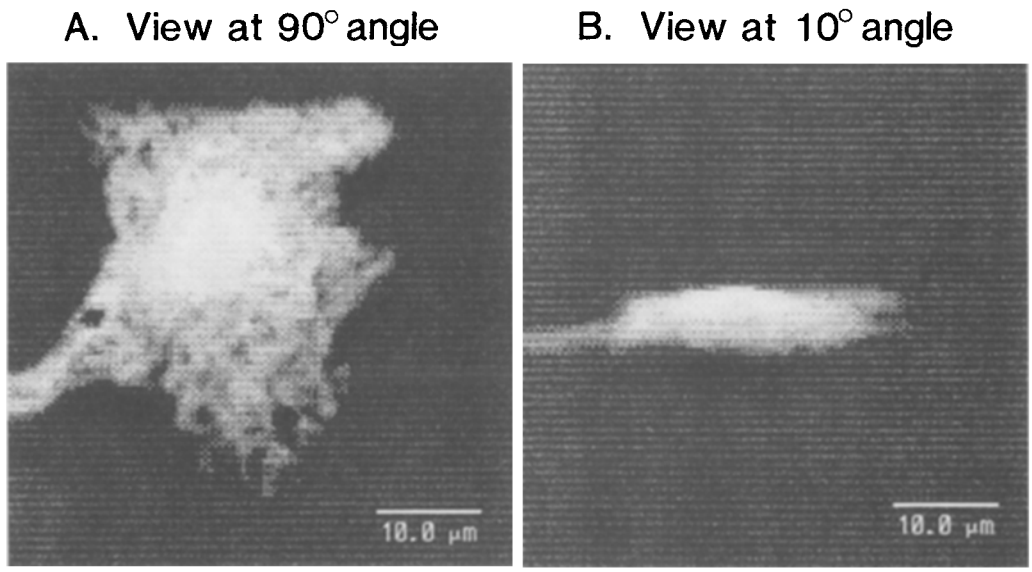

Fig. 3. Polyhedral configuration of the neonatal rat myocyte. The cell was viewed from either a 90° (**A**) or a 10° angle (**B**). The three dimensional image was reconstructed from confocal microscope data

neonatal myocyte ($z = 10.7\mu m$), was approximately the same as that of the adult rat myocyte (Satoh et al., 1996).

The polyhedral form of the neonatal myocyte was characterized by a rather large width in comparison to the rectangular form (Fig. 3A). As a

result, the surface area of the largest section of the polyhedral cell was slightly larger than that of the rectangular cell (Table 1). However, there was no significant difference between the depths of the polyhedral and rectangular cells (Figs. 2B and 3B, Table 1).

The effect of osmotic stress on the characteristics of each cell type was examined by placing neonatal rat myocytes in standard medium containing 30mM mannitol for 5 days. This led to a reduction in surface area of the largest cell section (Table 1). It also caused most of the cells to assume a rectangular shape (Table 1).

Table 1 summarizes the effects of β-alanine and mannitol on cell size and shape. Like mannitol, β-alanine also promoted a shift in favor of the rectangular cell form, although more cells retained the polyhedral shape after β-alanine treatment than following mannitol treatment. In addition to the shape change, β-alanine treatment also reduced the size of the polyhedral form (Table 1). Interestingly, the depth was not significantly altered by either β-alanine or mannitol treatment. Moreover, the surface area of the largest section of the rectangular form was not significantly reduced following β-alanine treatment. Thus, the decrease in cell volume in the β-alanine group was caused by a simultaneous reduction in cell size of the polyhedral form, as well as a shift towards the smaller cell type, namely, the rectangular form.

Discussion

The present study supports the notion that taurine serves as an important osmolyte in the cardiomyocyte. This cardiac function of taurine was first described by Vislie and Fugelli (1975), who examined the process of volume regulation in perfused flounder heart ventricles exposed to changes in

Table 1. Effect of mannitol and β-alanine treatment on size and shape of the neonatal cardiomyocyte

Cell type	Untreated	Mannitol treated	β-Alanine treated
Rectangular Form			
% found	33	90	62
depth (μm)	10.7 ± 0.5	9.8 ± 0.3	10.6 ± 0.3
area (μm²)	973 ± 51	836 ± 38*	904 ± 41
Polyhedral Form			
% found	67	10	38
depth (μm)	11.0 ± 0.4	10.2 ± 0.4	11.1 ± 0.4
area (μm²)	1140 ± 34#	965 ± 52*	986 ± 58*

Cardiomyocytes were incubated for 5 days in either normal medium (untreated), medium containing 30mM mannitol (mannitol treated) or 5mM β-alanine (β-alanine treated). The cells were then loaded with calcein and both their shape and configuration form established by confocal microscopy. Data shown represent means ± S.E.M. of 25–45 cells. Asterisks denote significant differences from the untreated control group ($p < 0.05$). The pound sign denotes a significant difference between the untreated rectangular and polyhedral forms ($p < 0.05$).

perfusate osmolality. They found that a major contributor to the regulatory volume increase that accompanied a hyperosmotic insult was a rise in cellular taurine content. Although teleosts were found to be very adept at volume regulation through alterations in taurine content, mammalian hearts also were capable of elevating taurine levels following a hyperosmotic insult (Atlas et al., 1984).

In contrast to hyperosmotic stress, hyposmotic stress is associated with a rapid loss of taurine. Rasmussen et al. (1993) calculated that taurine extrusion from chick heart cells accounted for 40% of total solute loss during a change in the osmolality of the extracellular medium from 280 mOsM to 150 mOsM. While taurine loss was found to contribute less to volume regulation following a more severe hyposmotic insult, their data suggest that taurine is the most important organic osmolyte of the cardiomyocyte.

The present study is unique because of the type of osmotic stress induced by β-alanine treatment. Rather than altering the osmolality of the extracellular medium, the experimental protocol resulted in a change in the osmolality of the cell. The amount of taurine in the neonatal myocyte is normally 270 $\pm$ 39 nmol/mg protein. Assuming a volume/protein ratio of 7.45 μl/mg protein (Rasmusson et al., 1993), the concentration of taurine in the cell is 36.2 mM. Thus, a drop in taurine content to 130 $\pm$ 18 nmol/mg protein results in at least a 19 mOsM decrease in intracellular osmolality, with the actual reduction dependent upon the amount of other ions that leaves the cell with taurine. Nonetheless, because 55% of intracellular taurine is lost during β-alanine treatment, it is not surprising that β-alanine treatment causes a reduction in cell volume and size.

However, the nature of the β-alanine-induced cell volume decrease is somewhat surprising. This may be related to the fact that the myocyte poses an interesting dilemma in regard to volume regulation. Koch-Weser (1963) showed that small elevations in the osmolality of the perfusion medium increase the force of contraction of isolated hearts while very large elevations in osmolality have an adverse effect on contactile function. These osmolarity-linked effects are largely mediated by changes in both $[Ca^{2+}]_i$ and the calcium sensitivity of the myofilaments (Allen and Smith, 1987; McDonald and Moss, 1995). Therefore, the contractile status of the heart is dependent not only on the degree of osmotic stress, but also the extent of volume regulation. Therefore, it is not surprising that severe taurine loss adversely affects the contractile state of the myocyte.

The cell adjusts to taurine loss through two mechanisms. First, there is a shift in favor of the rectangular form of the cell. Interestingly, the adult cardiomyocyte also exists in a rectangular configuration, although its y/x ratio (width/length) is considerably smaller than that of the neonatal myocyte. As seen in Figures 2A and 3A, the y/x ratio is approximately 0.75 and 0.55 for the polyhedral and rectangular neonatal cardiomyocytes, respectively. By comparison, the y/x ratio of the adult rat myocyte is typically about 0.25 (Satoh et al., 1996). Another major difference between adult and neonatal myocytes relates to size, with the adult being more than twice the size of the neonate (Satoh et al., 1996). Thus, the hyperosmotic induced

shift in cell shape does not represent merely a transition to the adult myocyte phenotype.

Second, taurine loss provokes a 15% decrease in the volume of the cell. This is caused primarily by a reduction in the size of each cross-section, with the surface area of the largest cross-section of the polyhedral cell being reduced from 1140μm^2 to 986μm^2 (Table 1). Taurine loss did not reduce cell depth, in agreement with the observation that the cell resists changes in cell depth (see Table 1).

Previous studies have shown that the pattern of cell shrinkage is dependent on cell type. While the length of the adult rat myocyte is reduced following an osmotic insult, it is extremely resistant to change in the osmotically stressed adult cat myocyte (Tanaka et al., 1996). The present study shows that while the surface area of the neonatal myocyte can shrink following an osmotic insult, the depth is very resistant to change.

In conclusion, a 55% reduction in the size of the cellular taurine pool leads to both cell shrinkage and a change in the shape of the cell. Although these effects resemble the changes induced by addition of 30mM mannitol to the incubation medium, some major differences are noted between the two types of osmotic stress.

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