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Role of osmoregulation in the actions of taurine

Review Article

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Summary. Taurine regulates an unusual number of biological phenomena, including heart rhythm, contractile function, blood pressure, platelet aggregation, neuronal excitability, body temperature, learning, motor behavior, food consumption, eye sight, sperm motility, cell proliferation and viability, energy metabolism and bile acid synthesis. Many of these actions are associated with alterations in either ion transport or protein phosphorylation. Although the effects on ion transport have been attributed to changes in membrane structure, they could be equally affected by a change in the activity of the affected transporters. Three common ways of altering transporter activity is enhanced expression, changes in the phosphorylation status of the protein and cytoskeletal changes. Interestingly, all three events are altered by osmotic stress. Since taurine is a key organic osmolyte in most cells, the possibility that the effects of taurine on ion transport could be related to its osmoregulatory activity was considered. This was accomplished by comparing the effects of taurine, cell swelling and cell shrinkage on the activities of key ion channels and ion transporters. The review also compares the phosphorylation cascades initiated by osmotic stress with some of the phosphorylation events triggered by taurine depletion or treatment. The data reveal that certain actions of taurine are probably caused by the activation of osmotic-linked signaling pathways. Nonetheless, some of the actions of taurine are unique and appear to be correlated with its membrane modulating and phosphorylation regulating activities.

Keywords: Amino acids – Taurine – Osmotic stress – Signaling – Cell stretch – Ion transport – Phosphorylation

Taurine exerts a number of actions, but one of the most important is the modulation of ion transport. This important action has been implicated in many of taurine's regulatory effects, including its neuromodulatory, contrac-

tile, secretory and cytoprotective activity. Since taurine alters the activity of so many transporters and ion channels (Satoh and Sperelakos, 1998), it is logical to assume that it must modulate some central mechanism within the cell, such as membrane structure, protein phosphorylation status or the cellular content of key regulators (Huxtable, 1992). However, the possibility that some actions of taurine might be related to its osmoregulatory activity has not been entertained. Yet, overwhelming evidence tie the movement of taurine and ions during periods of osmotic stress. Following a hyposmotic insult, the cell rapidly extrudes both taurine and electrolytes while these same osmolytes rapidly accumulate in the hypertonic stressed cell, with the degree of osmolyte disruption dependent upon the severity of the osmotic imbalance (Pasantes-Morales et al., 1999; Hoffmann and Dunham, 1995). Since restoration of the osmotic balance depends upon the movement of both taurine and ions, the more taurine leaving or entering the cell, the less need to adjust the ion content of the cell. Taurine movement can also influence ion content through at least two other mechanisms. First, osmotic imbalances affect the size of the cytoplasmic compartment, which by inference determines the concentration of all cellular constituents. Since osmolytes, such as taurine, prevent dramatic alterations in the size of the cytoplasmic compartment, they affect the cellular concentration of all substances, including ions. Second, taurine movement minimizes other consequences of osmotic disruption, including the initiation of protein kinase-linked signaling pathways which alter the activity of key ion transporters (Hoffmann and Dunham, 1995; Sadashima et al., 1996). Interestingly, these same protein kinase signaling pathways are capable of inducing cell proliferation and promoting cell survival (Sadoshima et al., 1996; Häussinger and Schliess, 1999). Therefore, through its ability to regulate cell volume, taurine can have a major impact on ion homeostasis, as well as many other cellular events. The present review considers the involvement of osmoregulation in two important actions of taurine: (1) cation modulation and (2) protein phosphorylation and cell growth.

1 Modulation of ion movement by osmotic stress

a Regulatory volume processes

Mammalian cells have a limited ability to withstand severe osmotic imbalances. Therefore, in response to an osmotic imbalance, most cells rapidly adjust their cellular osmolyte content to minimize damage to the cell membrane. Following exposure to hypotonic medium, the stressed cell initially undergoes a transient volume increase followed by a process to restore original cell size known as the regulatory volume decrease. Similarly, the short term response to cell shrinkage involves a normalizing process known as the regulatory volume increase. Both taurine and ions play central roles in these regulatory volume changes.

b Regulatory volume decrease

In many cell types, the regulatory volume decrease is associated with both an activation of a Cl^- conductance and cellular efflux of K^+. While the K^+ efflux

event involves flux through both a K^+/Cl^- cotransporter and a Cl^- independent K^+ pathway (McManus et al., 1995; Hoffmann and Dunham, 1995), Cl^- efflux is mediated by several volume sensitive Cl^- channels (Nilius et al., 1996).

The physiological function of the K^+/Cl^- cotransporter has been examined in a number of cell types and appears to be cell dependent. In the red blood cell, the cotransporter plays a central role in regulatory volume decrease and participates in the volume changes occurring during sickle cell crisis (Cossins and Gibson, 1997). By comparison, in both secretory and reabsorptive epithelial cells, the cotransporter assumes two functions, one in promoting transcellular efflux and the other is volume regulation (Eveloff and Warnock, 1987; Sasaki et al., 1988). Interestingly, the K^+/Cl^- transporter only plays a secondary role in volume regulation in hepatocytes (Corasanti et al., 1990).

The K^+/Cl^- cotransporter usually exists in a nearly inactive state but is rapidly activated following an osmotic insult. The mechanism of activation is complex and involves several factors. According to the work of Dunham et al. using sheep red blood cells (1993), both a protein phosphatase and cytoskeletal changes are capable of shifting the transporter towards its active form. Countering these effects is a volume sensitive protein kinase which phosphorylates the transporter, converting it into a relatively inactive form. Cell swelling activates the transporter by inhibiting the protein kinase. While the activity of the K^+/Cl^- cotransporter is primarily determined by its phosphorylation state and the state of the cytoskeleton, it also appears to be sensitive to changes in pO_2 (Cossins and Gibson, 1997). Moreover, in the cardiomyocyte, a swelling dependent K^+ transporter, exhibiting some of the characteristics of the K^+/Cl^- cotransporter, is regulated by the membrane potential (Clemo and Baumgarten, 1997).

The nature of the Cl^- independent K^+ pathway varies in different cell types. While Ca^{2+} sensitive K^+ channels appear to play an important role in the regulatory volume decrease of many cell types (Sarkadi and Parker, 1991; Foskett, 1994), a nearly equal number of cell types extrude K^+ through Ca^{2+} insensitive, volume sensitive K^+ channels (Foskett, 1994; Jørgensen et al., 1997; Morales-Mulia et al., 1998; Vanoye and Reuss, 1999). In both red blood cells and epithelial cells, the Ca^{2+} dependency of the K^+ channel may involve calmodulin although that conclusion has been based on results using calmodulin inhibitors which are not altogether specific for calmodulin (Hoffmann and Dunham, 1995; Light et al., 1998). Irrespective of the mode of Ca^{2+} activation, the important feature in the activation process is the elevation in $[Ca^{2+}]_i$. Depending on the cell type, intracellular Ca^{2+} accumulation is tied to either lipoxygenase activation, enhanced phospholipase A_2 activity or phospholipase C stimulation (Hoffmann and Dunham, 1995; Thoroed et al., 1997; Light et al., 1998). Hoffmann and Dunham (1995) have concluded that osmotic stress *per se* does not directly activate either the Ca^{2+} dependent or Ca^{2+} insensitive K^+ channels. They argue that only after either sufficient cellular depolarization or an adequate elevation in $[Ca^{2+}]_i$ will the Ca^{2+} dependent or voltage dependent K^+ channels open, allowing the cell to adjust its volume.

The increase in Cl^- conductance during the regulatory volume decrease ($I_{Cl,vol}$) involves a specific group of Cl^- channels that are widely distributed in both excitable and non-excitable cells (Nilius et al., 1996). Although cell swelling is the primary initiator of $I_{Cl,vol}$, the Cl^- channels can also be activated in some cell types by cellular shape changes or shear forces (Basavappa and Ellory, 1996). The major function of the volume sensitive Cl^- channels is volume regulation, nonetheless, activation of the volume sensitive channels can also cause a relative depolarization of the osmotically stressed cell (Du and Sorota, 1997). In the case of the heart, this can shorten the action potential and under pathological conditions lead to arrhythmias (Vandenberg et al., 1997). Similar action potential shortening can trigger exocytosis in pancreatic β cells or chromaffin cells.

Several Cl^- channels have been identified which differ relative to conductance, voltage dependence and anion selectivity. One of the most widely distributed Cl^- channels is the anion organic osmolyte channel (VSOAC) which is an outwardly rectifying, intermediate conductance Cl^- channel capable of conducting both Cl^- and larger organic osmolytes, such as myoinositol and taurine (Strange and Jackson, 1995). In the eye, this channel may be intrinsically linked to the secretion of aqueous humor (Zhang and Jacob, 1997) while in the pancreas it is associated with the secretion of insulin (Best et al., 1996). However, Strange and Jackson (1995) maintain that the major function of the VSOAC channel is volume regulation. Another volume sensitive Cl^- channel, known as phospholemman, has been isolated from the heart and is capable of extruding both Cl^- and taurine, although it is more selective for taurine efflux (Moorman and Jones, 1998). The existence of taurine sensitive channels is not unique to the heart since many cells contain separate channels for Cl^- and taurine. As with other Cl^- channels, phospholemman exhibits voltage dependent inactivation (Chen et al., 1998) and modulation of Cl^- current by phosphorylation (Nilius et al., 1996; Mounsey et al., 1999). Also listed as putative Cl^- channels are Pglycoprotein and pI_{cln}, although several studies suggest that these proteins are regulators of the volume sensitive Cl^- channels rather than bona fide Cl^- channels (Krapivinsky et al., 1994; Hardy et al., 1995).

It is generally accepted that the extrusion of organic osmolytes, such as taurine, preserves the cellular content of the inorganic osmolytes. By sparing anions, taurine efflux minimizes the extent of Donnan imbalances that occur with substantial cellular losses of anions. Taurine loss also spares the cell from the loss of key cations, many which play a central role in the physiology of the cell.

c Regulatory volume increase

The regulatory volume increase occurs in response to a hyperosmotic stress and is characterized by an increase in cell volume. In some cell types, the cell volume is completely restored while in others the recovery is only partial. During the course of the regulatory volume increase, there is a net accumula-

tion of K^+, Na^+ and Cl^- by the cell. In lymphocytes, red blood cells and epithelial cells, the primary mechanism underlying the regulatory volume increase is the cellular accumulation of Na^+ and Cl^- through the combined actions of the Na^+/H^+ exchanger and the Cl^-/HCO_3^- exchanger (Hoffman and Simonsen, 1989). Interestingly, Ehrlich cells incubated with HCO_3^- free medium do not utilize the Na^+/H^+ exchanger to volume regulate, however, the Na^+/H^+ exchanger assumes an important role in volume regulation in the presence of 25 mM HCO_3^- (Pedersen et al., 1996). It has been proposed that extracellular HCO_3^- allows the Cl^-/HCO_3^- exchanger to maintain the pH_i within a range in which the Na^+/H^+ exchanger is active. In many cells, the pH buffering mechanism is extremely effective since the magnitude of Na^+/H^+ exchange activation increases as the hypertonic challenge intensifies (Pedersen et al., 1996). In these cells, the Na^+/H^+ exchanger acts as a nearly perfect osmometer to restore cell volume. However, in other cells, in which there is little buffering capacity and low Cl^-/HCO_3^- exchanger activity, activation of the Na^+/H^+ exchanger causes cellular alkalinization without an appreciable increase in cell volume.

Four isoforms of the Na^+/H^+ exchanger have been identified, but their response to hypertonic stress differs. While NHE-1 and NHE-2 are activated by acute hyperosmotic stress, NHE-3 exhibits the opposite response (Soleimani and Singh, 1995). This differential response may be important in epithelial cells which contain significant levels of NHE-3. It has been proposed that the inhibition of NHE-3 contributes to alterations in natriuresis and urinary acidification in conditions of high osmolality, such as advanced renal failure or glucosuria (Soleimani et al., 1998). Although ATP is required for osmotically-induced activation of NHE-1, phosphorylation of the exchanger *per se* is poorly correlated with its activation. There is some evidence supporting an ATP dependent, but phosphorylation independent, mechanism of activation (Demaurex and Grinstein, 1994). However, equally feasible is a phosphorylation step involving an accessory protein, whose phosphorylation status regulates the activity of the Na^+/H^+ exchanger (Soleimani et al., 1995; Pedersen et al., 1996). In this regard it is interesting that NHE-1 and NHE-2 are activated by protein kinase C while NHE-3 is inhibited by protein kinase C (Soleimani and Singh, 1995). Nonetheless, both protein kinase A (Sargeant et al., 1989) and calmodulin dependent protein kinase (Dascalu et al., 1992) have been implicated in osmotic-dependent activation of the Na^+/H^+ exchanger. The possibility that the regulation of the transporter may be cell dependent has been raised (Pedersen et al., 1996).

The involvement of the Na^+-K^+-$2Cl^-$ cotransporter in the regulatory volume increase of avian red blood cells was first described by Kregenow et al. (1981). Subsequent studies established the Na^+-K^+-$2Cl^-$ cotransporter as an important ubiquitous mechanism of cell volume regulation (Hoffmann and Dunham, 1995). Interestingly, the transporter is quite inactive in the unstressed cell. However, it responds rapidly to a hyperosmotic stress, with several factors contributing to its activation. In hypertonically stressed intestinal epithelial cells, an organized cytoskeleton is required for the activation of the Na^+-K^+-$2Cl^-$ cotransporter (Matthews et al., 1998). Other investigators

have implicated one or more protein kinases in the response to hypertonic stress (Mairbäurl and Herth, 1996; Wright and Rees, 1998). In Ehrlich cells, the regulatory volume increase is not inhibited by the calmodulin inhibitor, KN-62 (Hoffman and Dunham, 1995), raising questions about earlier conclusions supporting a role for the calmodulin dependent protein kinase in the activation of the Na^+-K^+-$2Cl^-$ cotransporter (Haas, 1989; Jensen et al., 1993). Similarly, the protein kinase C inhibitors, H-7 and chelerythrine, only attenuate shrinkage-induced activation of the Na^+-K^+-$2Cl^-$ cotransporter by 20–30% despite a dramatic reduction in shrinkage-induced activation of protein kinase C (Larsen et al., 1994). Clearly ruled out in Ehrlich cells is a role for cAMP dependent protein kinase in the hypertonic-induced activation of the transporter. Nonetheless, the protein phosphatase inhibitor, calyculin A, is a potent activator of the cotransporter (Hoffmann and Dunham, 1995). Thus, either activation of a volume sensitive protein kinase, inhibition of a protein phosphatase or a combination of both may contribute to shrinkage-induced activation of the Na^+-K^+-$2Cl^-$ cotransporter.

Although immediate benefits arise from the accumulation of inorganic osmolytes following a hypertonic insult, prolonged elevations in the intracellular concentration of the inorganic ions can interfere with important ionic interactions and perturb cellular macromolecules. Therefore, during the course of a chronic hyperosmotic insult, the cell shifts from accumulating inorganic osmolytes to the uptake of organic osmolytes. The most important organic osmolytes belong to one of three classes of compounds, the methylamines, the sugars and polyols or the amino acids and their derivatives. The accumulation of these organic osmolytes is enhanced as a result of hypertonic-induced upregulation of some key transporters, with those for betaine (methylamine), taurine (amino acid) and myoinositol (polyol) receiving the most attention (Burg et al., 1997). Also important is the increased expression of aldose reductase, an enzyme involved in the conversion of glucose to the polyol, sorbitol. As expected, the accumulation of sorbitol assumes an important pathological role in diabetes. Although the identity of the stimulus responsible for the acceleration of gene transcription remains to be determined, several studies have implicated $[Na^+]_i$ and $[K^+]_i$ in the process (Burg et al., 1997). Moreover, cis and trans osmotic response elements have been identified (Burg et al., 1997; Zhou and Cammarata, 1997). Yet, linking the osmotic signaling pathway to both the trans acting proteins and the tonicity responsive DNA elements is not trivial. One scenario proposed by Burg et al. (1997) suggests that the interaction of the trans proteins with the DNA elements might be facilitated by the phosphorylation of the trans proteins. This notion was supported by the observation that osmotic-mediated upregulation of the betaine transporter in osmotically stressed Madin-Darby kidney cells is blocked by the p38 kinase inhibitor, SB203580 (Sheikh-Hamad et al., 1998). However, in 3T3 adipocytes, inhibitors of ERK and p38 MAP kinase are incapable of blocking the effect of hypertonicity on myoinositol accumulation (Yorek et al., 1998). Moreover, neither the short term response (the regulatory volume increase) nor the longer term response (upregulation of the myoinositol transporter) to hypertonicity is affected in renal collecting

duct cells whose hypertonic sensitive isoform, jun kinase 2 (JNK2), has been mutated to an inactive form (Wojtaszek et al., 1998). Also ineffective in preventing the accumulation of myoinositol by hyperosmotically stressed 3T3 adipocytes are several inhibitors of protein kinase C, the tyrosine kinase inhibitor genistein, the p70 S6 kinase inhibitor rapamycin and the phosphatidylinositol 3-kinase inhibitor Wortmannin (Yorek et al., 1998). Thus, it remains unclear if specific protein kinases contribute to the enhanced expression of certain osmolyte transporters following a hypertonic insult. Also unresolved is the mechanism responsible for promoting the interaction of the trans proteins with the hypertonic sensitive DNA elements.

2 Involvement of osmotic stress in taurine-mediated alterations in ion movement

a Link between taurine and osmotic stress

Taurine plays an important role in the regulatory volume decrease, the long term response to hypertonicity and the maintenance of normal cellular osmolality (Hoffman and Dunham, 1995; Pasantes-Morales et al., 1999). Therefore, experiments designed to explore taurine function usually perturb the osmotic status of the cell. In many of these experiments, 20–30 mM taurine was added to the extracellular medium, with the aim of increasing the size of the intracellular taurine pool. However, the size of the intracellular taurine pool is very large while the rate of taurine uptake via the β amino acid transporter is slow (Atlas et al., 1984). Thus, changes in the size of the intracellular taurine pool only occur after chronic taurine exposure (Atlas et al., 1984). By inference, the effects seen seconds or minutes after taurine addition to the extracellular medium cannot be attributed to altered intracellular taurine levels. A more likely candidate for these acute effects is the rise in extracellular osmolality. Similarly, a significant decrease in the intracellular taurine pool leads to an osmotic imbalance, which in turn activates signaling pathways or sensing mechanisms designed to restore osmotic balance (Hoffmann and Dunham, 1995; Cazorla et al., 1998). Yet, the possibility that the osmotic-linked sensing mechanisms may contribute to the putative actions of taurine has not been previously considered. The aim of this section of the review is to examine various channels and transporters for possible relationships between the actions of taurine and those of osmotic stress.

b Effect of taurine and osmotic stress on $[Ca2^{+}]_i$

One of the first identified actions of taurine was the modulation of calcium movement. In 1973, Dolara et al. (1973a) showed that hearts exposed to calcium free medium containing 8 mM taurine exhibited a less marked decline in contractile force than control hearts perfused with calcium free medium lacking taurine. The difference in response between the two groups of hearts was traced to the retention of calcium by the taurine treated heart. Subse-

quent studies revealed that taurine's modulation of calcium movement was not restricted to one cellular organelle, but was detected using isolated sarcolemma (Schaffer et al., 1995), mitochondria (Dolara et al., 1973b; Palmi et al., 1999), nuclei (Bkaily et al., 1997), retinal P_1 synaptosomal membrane (Liebowitz et al., 1987), brain synaptosomes (Pasantes-Morales and Gamboa, 1980) and rod outer segments (Kuo and Miki, 1980). Based on these findings, it was assumed that the effects of taurine on calcium movement were mediated by one or more of these cellular organelles. In support of this notion, further studies revealed that taurine was capable of altering the composition and properties of the phospholipid bilayer and of modulating the phosphorylation status of membrane transporters (Schaffer et al., 1995). Since these latter effects also influence Ca^{2+} movement, it was proposed that modulation of membrane function may underlie the effects of taurine on Ca^{2+} movement (Schaffer et al., 1995). However, this hypothesis was based on the assumption that taurine addition to the extracellular medium altered the size of the intracellular taurine pool. Since taurine uptake via the β amino acid transporter is too slow to acutely change the size of the huge intracellular taurine pool, another mechanism now seems more appropriate. One likely scenario is that taurine affects $[Ca^{2+}]_i$ through an osmotic-linked mechanism. Elevations in extracellular taurine, as well as reductions in intracellular taurine, introduce osmotic imbalances which initially provoke cell shrinkage. This change in cell volume influences $[Ca^{2+}]_i$ through several mechanisms. First, cell shrinkage increases the concentration of all intracellular constituents. Not only will $[Ca^{2+}]_i$ be directly elevated because of the decrease in cell water, but will also increase secondary to a rise in $[Na^+]_i$. Second, cell shrinkage initiates a regulatory volume increase which is associated with the activation of several Ca^{2+} transporters and channels, such as the Na^+/Ca^{2+} exchanger (Wright et al., 1995). Third, cell shrinkage activates phospholipase C, leading to the production of IP_3 and the release of Ca^{2+} from intracellular stores (Hoffmann and Dunham, 1995). Yet, based on the response of individual transporters to taurine treatment and osmotic stress, it is obvious that only some of the transporters and channels are affected by the osmotic imbalances brought about by changes in taurine homeostasis.

One of the Ca^{2+} channels affected by both taurine and osmotic stress is the L-type Ca^{2+} channel. Satoh and Sperelakis (1993) found that the effects of taurine on L-type Ca^{2+} current in embryonic chick cardiomyocytes are extremely complex, depending not only on $[Ca^{2+}]_i$ and $[Ca^{2+}]_o$, but also on the site of taurine application (Table 1). When taurine was administered intracellularly at concentrations sufficient to induce a small degree of cell swelling, $I_{Ca(L)}$ was inhibited. On the other hand, extracellular administration of 20 mM taurine inhibited $I_{Ca(L)}$ when $[Ca^{2+}]_i$ was 10^{-7} M but enhanced $I_{Ca(L)}$ when $[Ca^{2+}]_i$ was reduced to 10^{-10} M. Using the cell attached voltage clamp technique, Satoh et al. (1996) observed a Ca^{2+} dependent effect of taurine on the open probability of the Ca^{2+} channel, with a preference towards the open state at 0.9 mM $[Ca^{2+}]_o$ but a shift towards the closed state when $[Ca^{2+}]_o$ was elevated to 5.4 mM. Since the response of the channel to taurine treatment is complex, it is likely that several factors contribute to taurine's actions, includ-

ing alterations in the lipid composition of the phospholipid bilayer, the osmotic status of the cell and the phosphorylation status of the channel. This view is borne out by evidence indicating that not only are the effects of taurine and osmotic stress not identical, but the effects of osmotic stress themselves are complex (Table 1). While cell shrinkage inhibits $I_{Ca(L)}$ (Ogura et al., 1997), cell swelling is without an effect in dog and guinea pig ventricle (Sasaki et al., 1992; Groh et al., 1996; Zhou et al., 1997) but in rabbit atria leads to a significant increase in $I_{Ca(L)}$ (Matsuda et al., 1996).

While most studies have focused on the effects of elevated extracellular taurine on $[Ca^{2+}]_i$, the effect of taurine depletion on Ca^{2+} movement has important pathological implications. Yet, the precise role that impaired Ca^{2+} handling plays in the development of the cardiomyopathy provoked by taurine depletion remains controversial. According to Eley et al. (1994), the Ca^{2+} transport abnormalities of papillary muscles excised from taurine depleted rats are not dramatic enough to explain the profound loss of force generation. Instead, the authors proposed that a reduction in myofibril activation secondary to a loss in contractile elements must be the primary force responsible for the decline in contractile function (Lake, 1993; Eley et al., 1994). In contrast to the conclusions of Eley et al. (1994), recent studies from our laboratory indicate that the major defect in the drug-induced taurine depleted myocyte is impaired relaxation (Schaffer et al., 1998). Our conclusions support the findings of Novotny et al. (1991), who showed that taurine depletion in the cat leads to an initial decline in diastolic function followed by the development of systolic defects. Since diastolic abnormalities are usually caused by impaired Ca^{2+} handling, it is not surprising that the relaxation phase of the calcium transient is prolonged in the taurine depleted cardiomyocyte (Fig. 1). A less anticipated result is the finding that cells incubated with medium containing 30 mM mannitol also exhibit a prolongation of the calcium transient (Fig. 1). These data imply that osmotic stress contributes to the relaxation defect that develops in the taurine depleted heart.

c Effect taurine and osmotic stress on $[Na^+]_i$

Evidence supporting an interaction between taurine and Na^+ movement was originally uncovered by Suleiman et al. (1992), who found that an increase in $[Na^+]_i$ was associated with enhanced taurine efflux from the cell. Conversely, taurine influx increased as $[Na^+]_o$ was elevated. This relationship has been largely attributed to the cotransport of taurine and Na^+ via the β amino acid transporter (Chapman et al., 1993). Yet, taurine also affects other Na^+ transport systems. Several investigators have shown that the addition of 20 mM taurine to the incubation medium both facilitates a slow inward Na^+ current (Sperelakis et al., 1996) and inhibits a rapid inward Na^+ current (Schanne and Dumaine, 1992; Satoh and Sperelakis, 1992; Sada et al., 1996), the latter attributed to Na^+ channel flux. Since both a rise in intracellular taurine and osmotic-induced cell swelling inhibit Na^+ channel flux in muscle (Cazorla et al., 1999), the two effects may be related. However, the effects of taurine on

Table 1. Summary of the effects of taurine and osmotic stress on ionic currents

Current	Modulator	Effect	Reference
L-type Ca^{2+}	Taurine (Extracellular)*,#		
	Low Ca^{2+}	Enhanced	Sawamura et al. (1990), Satoh & Horie (1997)
	High Ca^{2+}	Inhibited	Sawamura et al. (1990), Satoh & Horie (1997)
	Taurine (Intracellular)#		
	Low Ca^{2+}	Inhibited	Satoh and Sperelakis (1993)
	Swelling	Unchanged	Sasaki et al. (1992), Groh et al. (1996), Zhou et al. (1993)
		Increased	Matsuda et al. (1996)
Na^+-Ca^{2+} exchange	Taurine (Extracellular)•	Enhanced	Earm et al. (1993)
		Unchanged	Katsube & Sperelakis (1996)
	Swelling	Inhibited	Wright et al. (1995)
	Shrinkage	Enhanced	Wright et al. (1995)
Fast Na^+	Taurine (Extracellular)*,#	Inhibited	Schanne & Dumaine (1992), Satoh & Sperelakis (1992), Sada et al. (1996)
Taurine (Intracellular)*,#		Enhanced	Satoh & Sperelakis (1992)
	Swelling	Enhanced	Rehn et al. (1998)
		Inhibited	Cazorla et al. (1999)
Na^+/K^+ ATPase	Swelling	Enhanced	Whalley et al. (1993), Sasaki et al. (1994)
	Shrinkage	Inhibited	Whalley et al. (1993)
Delayed rectifier K^+ (Rapid)	Taurine		
	Low Ca^{2+}	Enhanced	Satoh (1995)
	High Ca^{2+}	Inhibited	Satoh (1995)
	Swelling	Inhibited	Sasaki et al. (1994), Rees et al. (1995), Wang et al. (1996), Lei & Kohl (1998)
		Unchanged	Groh et al. (1997)
Inward rectifier K^+	Taurine (Intracellular)*,#	Inhibited	Satoh (1999)
	Swelling	Unchanged	Sasaki et al. (1992), Rees et al. (1995)
ATP-sensitive K^+	Taurine (Intracellular)*,#	Inhibited	Han et al. (1996)
	Swelling	Enhanced	Van Wagoner (1993)
Cl^-	Taurine (Intracellular)	Enhanced	Taber et al. (1986)
	Swelling	Enhanced	Sorota (1992), Nilius et al. (1996), Vandenberg et al. (1997), Zhang & Jacob (1997)

*,# and • denote the administration of 10 mM, 20 mM and 30 mM taurine, respectively.

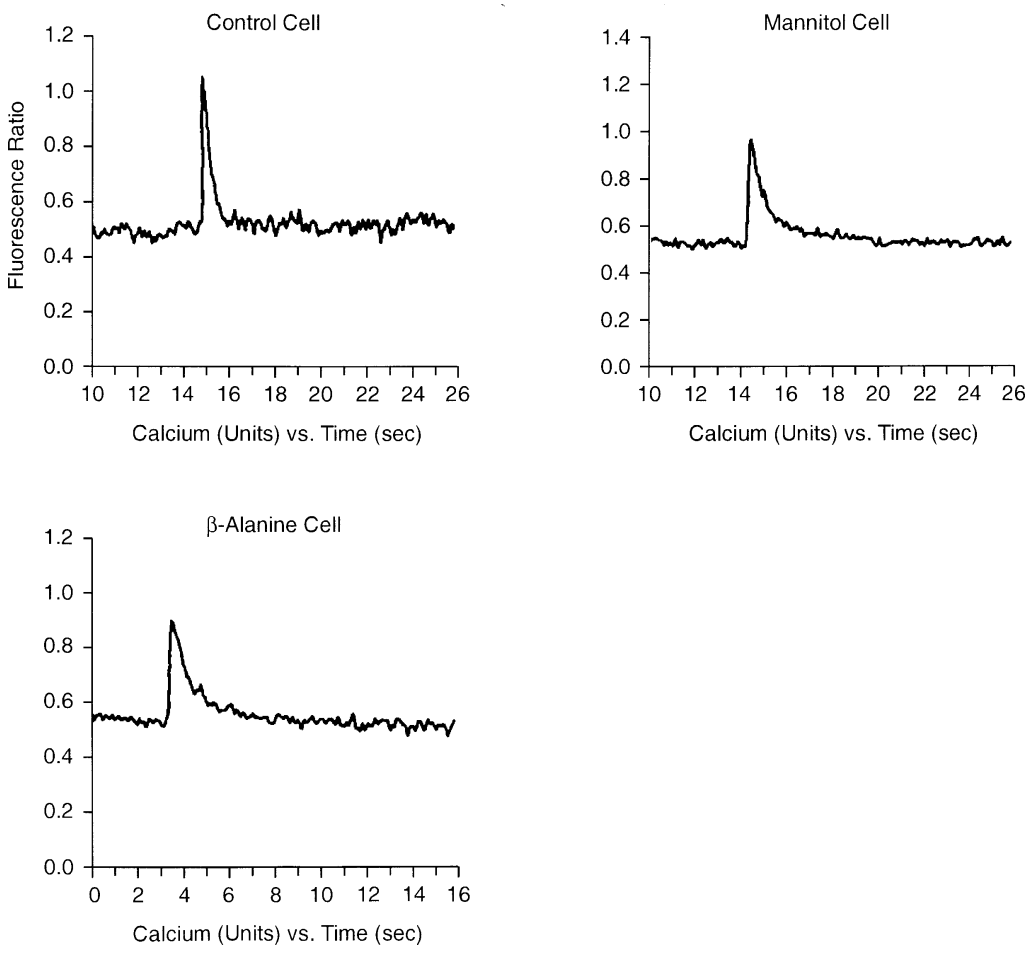

Fig. 1. Calcium transient profile of osmotically stressed cells. Isolated cardiomyocytes were incubated for 3 days in medium containing 5 mM glucose (control cell), 30 mM mannitol (Mannitol cell) or 5 mM β alanine (β alanine cell). The cells were then loaded with 2.0 μM Indo-1, a fluorescent calcium indicator. Calcium transients were monitored using a confocal microscope. The data are expressed as the fluorescence ratio, F_{410}/F_{490}, which is a measure of $[Ca^{2+}]_i$. Osmotically stressed cells exhibited significantly prolonged calcium transients

the Na^+ channel dwarf in comparison with the magnitude of taurine's effects on Na^+ flux via the Na^+-taurine cotransporter.

d Effect of taurine and osmotic stress on $[K^+]_i$

Taurine exhibits anti-arrhythmic activity. Since alterations in K^+ movement during phase 3 of the action potential are central to the activity of a number of anti-arrhythmic agents, it is not surprising that taurine is a regulator of K^+ transport. The K^+ current that dominates the repolarizing phase of the action potential (phase 3) involves K^+ movement through the delayed rectifier K^+

channel. The effect of taurine on these channels is largely assoicated with its effect on Ca^{2+} movement. While addition of taurine to the extracellular medium inhibits I_{kr} at high $[Ca^{2+}]_o$, it enhances the movement of K^+ at low $[Ca^{2+}]_o$ (Satoh, 1995). Because of the complexity of the taurine effect, it is unclear if the observed reduction in I_{Kr} following a hypotonic shock is at least partially mediated by taurine loss (Sasaki et al., 1994; Rees et al., 1995; Wang et al., 1996).

The ATP-sensitive K^+ channel assumes an important role in the cell during periods of energy deprivation, such as hypoxia. As ATP levels fall, the channel opens, allowing a premature repolarization of the cell. Since cell swelling also activates the channel, the increase in channel flux during hypoxia may be caused in part by the osmotic imbalances that develop during hypoxia. It has been proposed that some of the arrhythmias that occur during an ischemic event may be related to the activation of the ATP-sensitive K^+ channel (Wilde, 1994). Therefore, the observation that taurine inhibits the ATP sensitive K^+ channel is consistent with its anti-arrhythmic activity (Han et al., 1996). More significantly, the loss of taurine from the cell during hypoxia may render the myocyte more susceptibility to arrhythmias through its effects on the ATP sensitive K^+ channel. Because of the clinical significance of ischemia-induced arrhythmias, further studies examining the relationship between osmotic stress, taurine loss and the activation of the ATP sensitive K^+ channel are warranted.

Although taurine has no effect on Na^+/K^+ ATPase activity of isolated sarcolemmal membrane (Schaffer, unpublished observation), its effect on Na^+ pump activity of the intact cell has not been examined. This is unfortunate since osmotic stress has a unique mechanism of Na^+/K^+ ATPase regulation. Thus, the Na^+ pump could serve as an excellent tool to assess the contribution that osmotic stress makes towards the cell's response to taurine treatment.

The taurine depleted heart shows a characteristic prolongation in the QT interval and the time to either 75% or 95% repolarization (Lake et al., 1987). Since these effects can be reversed by taurine feeding, it has been assumed that these phase 3 action potential abnormalities are associated with taurine-induced alterations in either I_K or the Na^+/Ca^{2+} exchanger (Lake et al., 1987). Yet, osmotic swelling increases I_K and decreases $I_{Na/Ca}$ (Table 1). Therefore, it is possible that taurine depletion affects phase 3 K^+ and Ca^{2+} movement through an osmotically derived mechanism.

3 Osmotic stress and the phosphorylation cascade leading to cell hypertrophy

It is well recognized that cell distortion through axial stretching activates a series of protein kinase cascades which affect several cellular functions (Yamazaki et al., 1995). Among the protein kinases activated by cell stretching are raf-1 kinase, protein kinase C, the extracellular signal-regulated kinases (ERK), the stress activated protein kinases (SAPKs)/c-jun N terminal kinase and S6 kinase (Fig. 2). These same protein kinases are members of

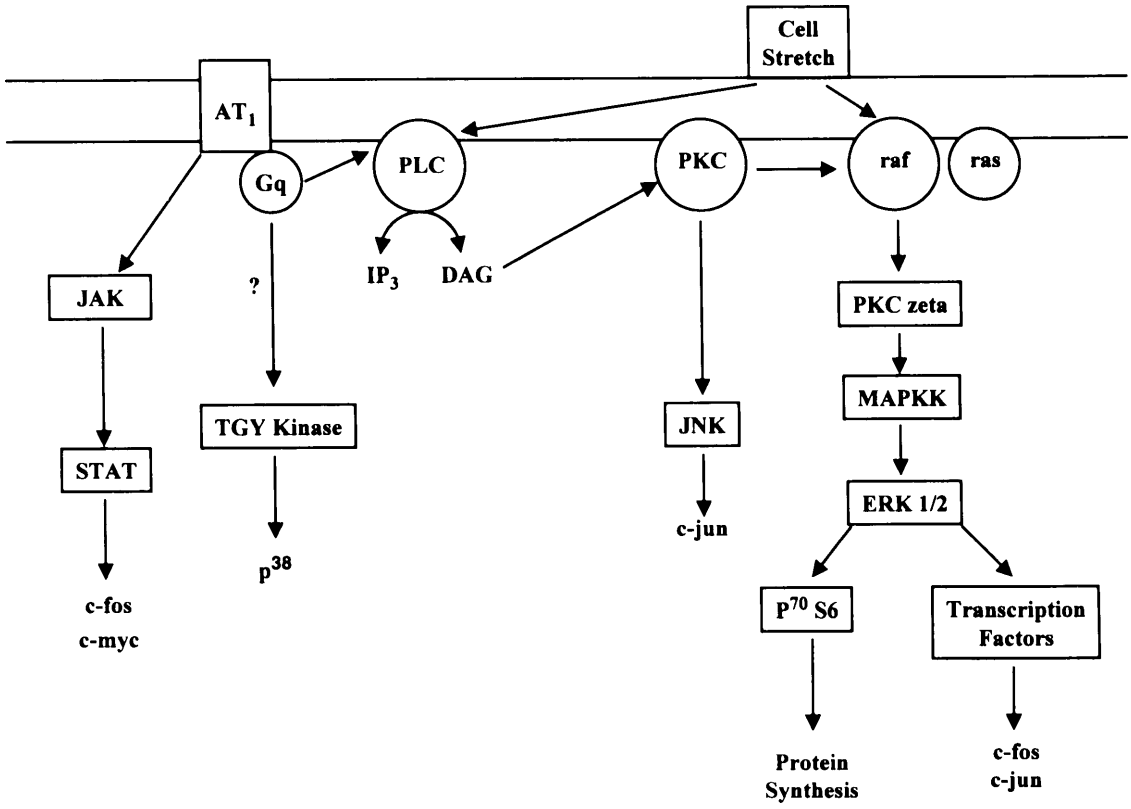

Fig. 2. Signaling pathways activated by angiotensin II and cell stretch. The abbreviations used are as follows: AT_1, angiotensin II type 1 receptor; G_q, G_q alpha subunit of heterotrimeric G-proteins; *JAK*, janus kinase; *STAT*, signal transducer and activator of transcription; *TGY kinase*, a kinase with a phosphorylation sequence of TGY; *PLC*, phospholipase C; IP_3, inositol triphosphate; *DAG*, diacylglycerol; *PKC*, protein kinase C, *JNK*, c-jun N-terminal kinase; PKC_{zeta}, protein kinase C-zeta isoform; *MAPKK*, mitogen-activated protein kinase kinase; *ERK*, extracellular regulated kinase; $p^{70}S6$, 70 or 85 kD form of S6 kinase

signaling cascades that are initiated by growth factors to mediate their hypertrophic responses (Dostal et al., 1997; Yamazaki et al., 1998). Significantly, hypotonic stress rapidly induces these same signaling pathways, although differences between osmotic swelling and mechanical stretching exist (Sadoshima et al., 1996). One of the characteristic features of mechanical cell stretching is the activation of p46 jun N-terminal kinase, but not the MAP kinase, p38 (Hamada et al., 1998). By comparison, hyperosmotic stress activates both p38 kinase and c-jun terminal kinase, as well as a third member of the MAP kinase group, the ERK kinases (Sadoshima et al., 1996). Since differences exist in the kinetics of protein kinase activation, as well as the degree of activation, the two types of osmotic stress appear to utilize separate mechanisms of action. Interestingly, both types of osmotic stress activate tyrosine kinases, although the substrates phosphorylated by these tyrosine kinases differ in the two types of osmotic stress (Sadoshima et al., 1996; Szaszi

et al., 1997). In the case of hypotonic stress, the activation of ERK 1 & 2 and the induction of the immediate early gene, c-fos, is blocked by the tyrosine kinase inhibitor, genistein. Thus, initiation of the hypertrophic cascade by hypotonic stress appears to require a tyrosine kinase step. It remains to be determined if the rapid activation of the tyrosine kinases also initiates similar hypertrophic responses to a hyperosmotic stress.

Although an increase in extracellular taurine (20 mM) causes cell shrinkage, the taurine challenge does not induce immediate early genes, stimulate protein synthesis and promote the re-expression of specific fetal genes (Takahashi et al., 1997; Azuma et al., 2001). Thus, the mild osmotic imbalance brought on by a chronic increase in extracellular taurine (20 mM) is insufficient to trigger the hypertrophic signaling cascades. However, taurine indirectly influences cellular hypertrophy and the growth-linked protein kinase cascades through its ability to block the actions of angiotensin II (Takahashi et al., 1997). Although the mechanism of taurine action has not been established, it is relevant that taurine attenuates the phosphorylation of a 29 kD protein (Figure 3). Since the downregulation of protein kinase C by phorbol myristate acetate (PMA) also blocks angiotensin II-induced phosphorylation of the 29 kD protein, the protein appears to be a substrate for one of the protein kinase C isoforms activated by angiotensin II. Therefore, taurine may inhibit the actions of angiotensin II by interfering with the activation of this protein kinase C isoform.

The effects of taurine treatment on protein phosphorylation are not restricted to the cardiomyocyte, but are also seen in the retina, where the phosphorylation status of several proteins is affected by taurine depletion (Lombardini, 1985). According to Lombardini et al. (1998) taurine depletion

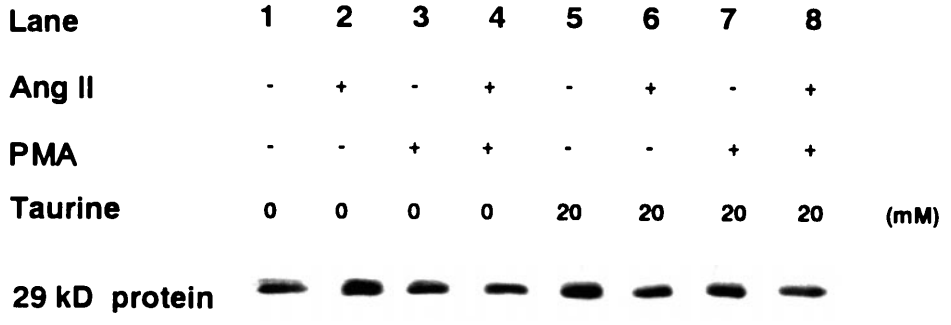

Fig. 3. Effect of taurine on the phosphorylation state of a 29 kD protein kinase C substrate. Isolated cardiomyocytes were incubated for 3 days with serum free medium containing either 0 or 20 mM taurine. On the last day, the two groups of cells were exposed to either 1 nM angiotensin II (Lanes 2 & 6), 100 nM phorbol myristate acetate (PMA, Lanes 3 & 7), the combination of 1 nM angiotensin II and 100 nM phorbol myristate acetate (Lanes 4 & 8) or no additions (Lanes 1 & 5). The cells were harvested and examined for the phosphorylation status of the 29 kD protein using Western blot analysis. Taurine enhanced the phosphorylation of the protein, but inhibited the phosphorylation of the protein by angiotensin II. Reproduced with permission from Azuma et al. (2001)

has its greatest effect on the phosphorylation of a 44 kD protein in the heart, which he identifies as pyruvate dehydrogenase, and a 20 kD protein in the retina, the latter being histone H2B. The effects of taurine on the phosphorylation state of histone H2B are interesting because they may explain some of the effects of taurine on cell proliferation described above. Similarly, the inhibition of pyruvate dehydrogenase by taurine depletion is consistent with the actions of taurine on cardiac metabolism (Schaffer et al., 1983). Although the effect of hyperosmotic stress on pyruvate dehydrogenase activity has not been investigated, it is significant that both taurine exposure and hyperosmotic stress elevate $[Ca^{2+}]_i$. Elevations in mitochondrial Ca^{2+} levels are known to activate pyruvate dehydrogenase through a decrease in the phosphorylation state of the dehydrogenase (Moreno-Sanchez, 1988).

Thus, taurine has a profound effect on cellular hypertrophy and energy metabolism. Although these effects of taurine are related, at least in part, to changes in the activities of key protein kinases, these protein kinases do not appear to be osmotic sensitive protein kinases.

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