VOLUME-ACTIVATED TAURINE EFFLUX FROM SQUALUS ACANTHIAS RECTAL GLAND CELLS

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Taurine efflux is one of the principal mechanisms responsible for regulatory volume decrease (RVD) in elasmobranch tissues (Boyd, T. et al., J. Exp. Zool. 199:435-442, 1977). Skate erythrocytes and liver cells as well as dogfish rectal gland cells have been the most frequently studied cell systems. While the mechanism of taurine efflux is relatively well-characterized in skate erythrocytes (Haynes, J. et al., Am. J. Physiol. 265:R173-R179, 1993) and liver cells (Ballatori, N. et al., Am. J. Physiol. 267:G285-G291, 1994), little is known about the transport mechanism in rectal gland cells (Ziyadeh, F. N. et al., Am. J. Physiol. 262:F468-F479, 1992). Regulation of taurine transport is poorly understood in all of the above systems.

The purpose of this investigation was to further clarify the mechanism of taurine transport during RVD in rectal gland cells and to determine if tyrosine phosphorylation plays a role in regulation of taurine transport. A primary monolayer cell culture system for shark rectal gland tubular epithelium provided an ideal system for further characterization of the taurine transport mechanism (Valentich, J. et al., Am. J. Physiol. 260:C813-C823, 1991).

One (1) to fourteen (14) day old rectal gland cultures from Squalus acanthias loaded with taurine by incubation with 1 μc/ml of ^{3}H-taurine (1mc/ml) in 1 ml of shark rectal gland tissue culture (SRGC) medium for 2 hours in 12 well plates at 15°C in a CO_2 incubator (5% CO_2). The cells in each well were then washed 3X with SRGC. The efflux of taurine was measured after a 15 min incubation at 15°C in isotonic (1000 mOsM) and hypotonic (600 mOsM) SRGC medium, in which Na^+ was replaced by Li^+. The amount of isotope in the cytosol of cells was determined at 0 time and 15 minutes after lysing cells in buffer containing 1% NP-40, 150 μM NaCl, 20 μM Tris base (pH 8), 5 μM EDTA, 10 μM NaF_2, 10 μM NaPPi, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μM Iodocetamide, 1 μM PMSF, and 1 μM NaVanadate. The lysate was subjected to centrifugation at 15,000 rpm in a microfuge for 30 minutes to generate a post-nuclear supernatant fraction. 0.2 ml of supernate (in duplicate) was added to 4.0 ml Optiflour for scintillation counting. Proteins in the supernatant fraction containing phosphorylated tyrosine residues were detected in the following way: The supernate was diluted 1:1 with sample buffer, containing 62.5 μM Tris HCl (pH 6.8), 2% SDS, 10% glycerol, 0.01% bromphenol blue, and β-mercaptoethanol. The sample was boiled for 7 minutes and subsequently subjected to SDS-PAGE (7.5% acrylamide). After electrophoresis, proteins were electrotransferred to Immobilon-P transfer membranes and subsequently probed with a monoclonal anti-phosphotyrosine antibody (4G10).

Rectal gland cells lost greater than 50% of intracellular taurine within 15 minutes when suspended in hypotonic medium, and the rate of efflux was substantially reduced when cells were shifted from hypotonic to isotonic medium after 5 minutes of incubation in the former medium (Fig. 1), suggesting that increased taurine efflux versus that observed in isotonic medium was an osmoregulatory response. While we have not yet determined whether taurine is metabolized during the loading period, previously published work indicates little or no metabolism of taurine during loading of a variety of elasmobranh tissues (King, P. et al. Mol. Physiol. 4:53-66, 1983). Thus, we interpret the loss of radioisotope from the cell as a measure of taurine efflux. In order to further characterize the transport mechanism, taurine efflux was measured in the presence and absence of the anion transport inhibitors: pyridoxal phosphate (2μM), DIDS (0.5μM) and NPPB (0.1μM). None of these inhibitors had a significant effect on taurine efflux, even when cells were preincubated for up to 15 minutes in the inhibitor, suggesting that the transport pathway does not involve an anion channel or Band 3 (Figs 2 and 3; data on NPPB not shown).

Five (5) prominent phosphorylated proteins and a number of minor bands were detected in the post-nuclear supernatant fraction. Only one of these proteins, a prominent band of approximately 42 kDa, exhibited an increased amount of phosphotyrosine when cells were incubated in hypotonic medium for 4 hrs and 10 min vs. isotonic controls which showed no change (Fig. 4). Hypotonic incubation in the presence of the tyrosine kinase inhibitors, genistein (100μM) and lavendustin B (50 μM), significantly inhibited taurine efflux and concomitantly reduced the phosphorylation of the 42 kDa protein, suggesting that this protein is involved in RVD.

These observations suggest that the membrane transporter involved in RVD in dogfish rectal gland cells differs from transporters previously described in skate erythrocytes and hepatocytes, since taurine transport is not inhibited by pyridoxal phosphate, DIDS or NPPB. We also provide evidence that a tyrosine phosphoprotein may be involved.

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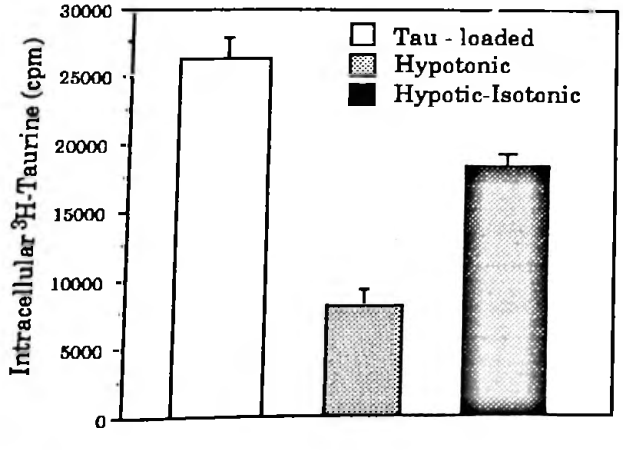

Fig. 1. Effect of medium osmolarity on taurine efflux from shark rectal gland cells (one day old cultures). Results are shown from a representative experiment. Counts per minute (cpm) of ^{3}H-taurine were measured in cells after preloading for 2h and after 15 min incubation in efflux medium. Tau-loaded, cpm after preloading; Hypotonic, cpm after efflux in hypotonic medium; and Hypotonic—Isotonic, cpm after efflux for 5 min in hypotonic medium followed by 10 min in isotonic medium. Values are means ±SE (n=3). All differences are significant (ANOVA, F = 134, $p<0.0001$ for every Scheffé comparison).

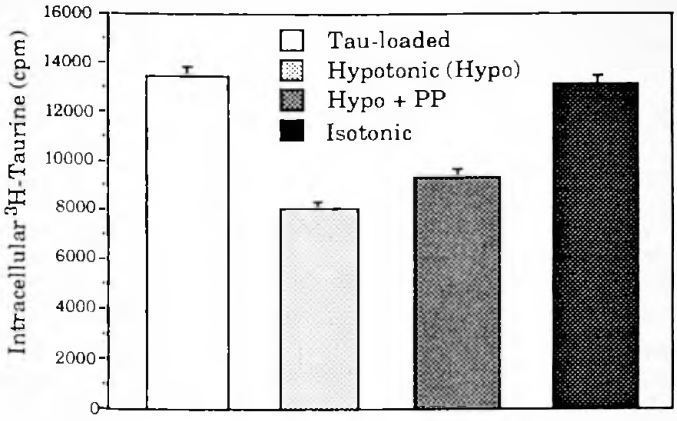

Fig. 2. Effect of pyridoxal phosphate(PP) on taurine efflux from shark rectal gland cells (2 day old cultures) in hypotonic medium (600mOsM). Results shown are from a representative experiment. The same experimental design was used as in Fig. 1. Tau-loaded, cpm after preloading; Hypo + PP, same as Hypo except efflux medium contained 2mM pyridoxal phosphate; Isotonic, cpm after efflux in hypotonic medium. Values are means ±SE (n=3). There is a significant difference between the mean values of Tau loaded cells and cells after efflux incubation in hypotonic medium (ANOVA, F = 85, $p<0.0001$ for Scheffé comparison). However, there is no significant difference between the mean values of cells after efflux in hypotonic medium and hypotonic medium plus pyridoxal phosphate (ANOVA, $p>0.05$ for Scheffé comparison).

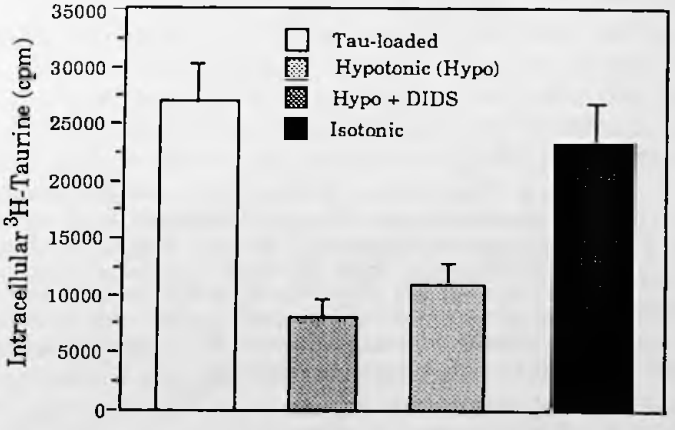

Fig.3. Effect of 4,4'-diisothiocyanostilbene-2,2'- disulfonic acid (DIDS) on shark rectal gland cells (2 day old cultures) in hypotonic medium (600mOsM). Results shown are from a representative experiment. Same experimental design as in Fig.1. Hypo + DIDS, hypotonic medium contained 0.5μM DIDS. Values are means ±SE(n=3). There are significant differences between the mean cpm of Tau-loaded cells and the cells incubated in hypotonic medium or cells incubated in hypotonic medium plus DIDS (ANOVA, $F=11.3$, $p<0.0001$). There is no significant difference between the mean cpm of cells incubated in hypotonic medium and cells incubated in hypotonic medium plus DIDS (ANOVA, $p>0.05$).

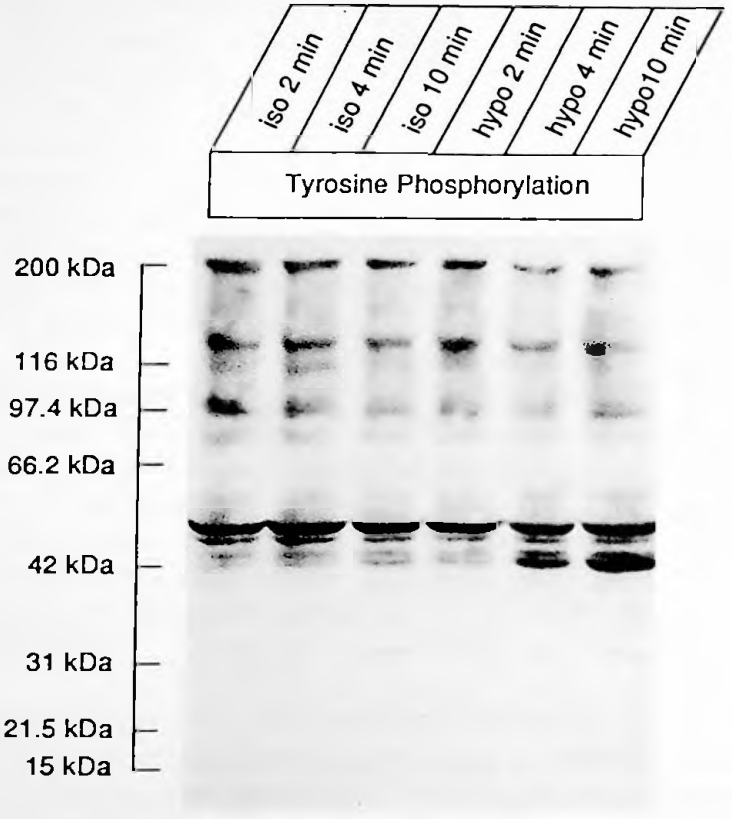

Fig.4. Western blot analysis of tyrosine-phosphorylated peptides in confluent primary cultures of SRG cells incubated in isotonic (control) and hypotonic media for 2-10 min. Postnuclear lysates were generated and subjected to SDS-polyacrylamide gel electrophoresis (7.5% acrylamide) and electrotransferred to membranes. Companion lanes probed with the anti-phosphotyrosine antibody 4G10 revealed multiple bands with a prominent new 42KDa band appearing at 4 minutes in hypotonic but not isotonic media.