J. exp. Biol. ***182****, 291–295 (1993)*
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SHORT COMMUNICATION

TAURINE TRANSPORT CHARACTERISTICS OF THE EMBRYONIC SKATE (*RAJA EGLANTERIA*) HEART

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Accepted 3 June 1993

The hearts of elasmobranchs, like those of other vertebrates, accumulate high concentrations of the amino acid taurine (Boyd *et al.* 1977), which is used to maintain osmotic equilibrium with the extracellular fluid. We recently demonstrated that embryonic hearts of the clearnose skate (*Raja eglanteria* Bosc) were able to accumulate taurine equally as well as hearts of adult skates and, as in adults, the accumulation was the result of transport from extracellular taurine across the cell membrane against a steep concentration gradient (Goldstein *et al.* 1990). In adult skate hearts, the uptake of taurine is Na^+-dependent and competitively inhibited by β-alanine (Forster and Hannafin, 1980*a*), indicating that transport occurs by the β-amino acid system found in many vertebrate hearts (Huxtable, 1992). During hypotonic stress, adult skate hearts release taurine and other amino acids. The Na^+/taurine cotransport system permits the skate heart to maintain high concentrations of intracellular taurine, while the hypotonicity-activated taurine release aids in cell volume regulation following dilution of the extracellular fluid. The aims of the present study were to determine whether taurine uptake in the embryonic skate heart is Na^+-dependent as in the adult and whether the skate heart has the capability of cell volume regulation (during hypotonic stress) before hatching.

Sexually mature clearnose skates (*Rajaeglanteria*) were captured, maintained and bred as described previously (Goldstein *et al.* 1990). Dates of laying for freshly deposited fertile egg-pairs were noted and all egg-pairs were incubated under the same conditions as for adult maintenance. Under these conditions, embryos develop at reproducible rates and complete their development and hatch after approximately 12 weeks (Luer and Gilbert, 1985). Skate embryos used in the present study had completed approximately one-third of their development (25–29 days after deposition), a stage determined previously to be useful for this type of experimentation (Goldstein *et al.* 1990). Access to embryos was achieved by carefully cutting a window through one of the flat sides of the leathery egg case. Embryos were removed from their egg cases, following the severing of yolk stalks, and pithed, and the developing hearts were excised using iridectomy scissors. Care was

Key words: skate, *Raja eglanteria*, heart, taurine transport, cell volume regulation.

taken to trim as much non-cardiac tissue as possible. Experiments were conducted in February in 1990–1992.

Taurine uptake was measured as before (Goldstein *et al.* 1990). Excised hearts were placed in 1.0ml of elasmobranch incubation medium (Forster and Hannafin, 1980*b*) in porcelain depression slides to maximize surface-to-volume ratio and oxygenation of the medium. The incubation medium contained 0.1mmol l^{-1} taurine + [^{3}H]taurine (1.85×10^4 Bq). In the Na^+ replacement studies, lithium salts were used to replace Na^+ in the incubation medium. Incubations were carried out at approximately 25°C. After incubation, the hearts were rinsed twice in fresh isotonic medium, blotted lightly and digested in 0.5ml of Soluene (Packard) at 50°C overnight. The solution was mixed with 10ml of Hyonicfluor (Packard) and assayed for ^{3}H by liquid scintillation counting. A sample of the incubation medium was also assayed for [^{3}H]taurine. The uptake of [^{3}H]taurine from the medium was calculated by dividing disintsmin^{-1} in the heart by the mass of the heart.

Taurine efflux was measured by preincubating hearts, as above, in 1.0ml of isotonic (940mosmol l^{-1}) elasmobranch incubation medium containing 0.1mmol l^{-1} [^{3}H]taurine (3.7×10^4 Bq) for 3h. After preincubation, the hearts were removed from the medium, washed three times in three separate beakers containing fresh isotonic medium and placed in separate depression wells. 1.0ml of 940mosmol l^{-1} isotonic Li^+ medium or hypotonic (460mosmol l^{-1}) Li^+ medium was added to each of the wells. The incubation medium was withdrawn after 5, 15 or 30min and assayed for [^{3}H]taurine as above. The hearts were blotted on tissue paper, digested in Soluene and assayed for [^{3}H]taurine as above.

DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid), probenecid [*p*-(dipropylsulphamoyl)benzoic acid], α-cyano-3-hydroxycinnamic acid and taurine were purchased from Sigma Chemical Company (St Louis). [^{3}H]taurine was purchased from N.E. Nuclear. All other chemicals were purchased from Sigma.

We have previously shown that the hearts of embryonic skates at 23–28 days after oviposition are able to accumulate taurine to the same degree as the hearts of adult skates (Goldstein *et al.* 1990). The uptake of taurine by the adult skate heart is sodium-dependent (Forster and Hannafin, 1980*a*). As shown in Fig. 1, taurine uptake is also sodium-dependent in the embryonic heart. Substitution of Li^+ for Na^+ in the incubation medium reduces taurine uptake by approximately 75%. Substitution of sodium by *N*-methyl-D-glucamine caused the same amount of inhibition. Forster and Hannafin (1980*a*) observed that about 80% of taurine uptake by atria of adult skates was Na^+-dependent. Thus, the Na^+-dependency is quite similar in embryonic and adult hearts.

In skate erythrocytes (Goldstein and Brill, 1991), Na^+-independent taurine uptake is activated by hypotonicity and inhibited by DIDS and other inhibitors of the anion exchanger. However, in the embryonic skate heart hypotonicity did not affect Na^+-independent taurine uptake. In addition, we found that DIDS (0.5mmol l^{-1}) did not inhibit the Na^+-independent taurine uptake by embryonic hearts incubated in hypotonic medium. Furthermore, two other anion transport inhibitors, 25mmol l^{-1}α-cyano-3-hydroxycinnamic acid and 1mmol l^{-1} probenecid, which are known to act on anion transport systems other than the anion exchanger (Aubert and Motais, 1975; Deuticke, 1982), did not inhibit Na^+-independent taurine uptake.

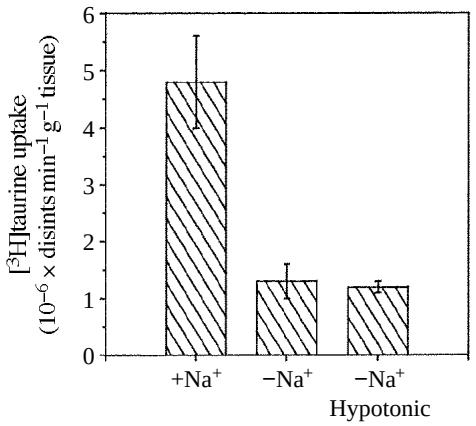

Fig. 1. Sodium-dependence of [^{3}H]taurine uptake by embryonic skate heart. Bars are means (± high and low values) of two hearts per treatment.

Forster and Hannafin (1980*b*) reported that both atrial and ventricular strips of adult skate (*R. erinacea*) hearts incubated in hypotonic media release increased amounts of ninhydrin-positive substances. Since a major fraction of these substances in skate heart is taurine, skate hearts, like other fish hearts (Vislie, 1983), show a volume-regulated taurine response. As shown in Fig. 2, embryonic hearts of the skate *R. eglanteria* also show this response. In iso-osmotic medium (940mosmol l^{-1}), taurine efflux is very low.

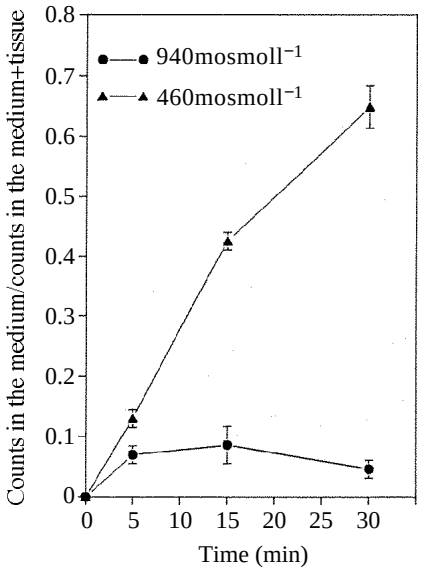

Fig. 2. Stimulation of [^{3}H]taurine efflux by hypotonic stress. Values are means (± high and low values) of two hearts per data point. Results are expressed as the fraction of [^{3}H]taurine originally present in the heart at time 0 found in the medium at the times shown.

In contrast, incubation in hypo-osmotic medium (460mosmol l^{-1}) produced a rapid efflux of taurine; after 30min of incubation, nearly 70% of the accumulated taurine was released into the hypotonic medium.

In skate erythrocytes, the hypotonicity-stimulated taurine efflux is markedly slowed by inhibitors of the anion exchanger band 3 (Goldstein and Brill, 1991). However, we found that 0.1mmol l^{-1} DIDS had no effect on taurine efflux from embryonic hearts incubated in hypotonic medium. Thus, in contrast to skate erythrocytes, embryonic skate hearts do not appear to use the anion exchanger in either their Na^+-independent taurine uptake or their volume-activated taurine efflux. The hypotonicity-activated taurine release is not due to damage to the cardiac myocyte cell membrane since, following 1h of exposure to hypotonic (460mosmol l^{-1}) incubation medium, embryonic skate hearts accumulated taurine to the same extent as hearts that had not been pre-exposed to dilute medium. The tissue/medium ratio at 1h was 2.4 in hearts pre-exposed to hypotonic medium, which compares favourably with the ratio of 5.4 reported for embryonic hearts incubated in isotonic medium for 3h (Goldstein *et al.* 1990). Thus, the embryonic heart shows a volume-activated taurine efflux but, unlike the situation in the erythrocyte, this efflux is not inhibited by DIDS. In fact, DIDS does not bind to the membrane fraction of embryonic heart (M. Musch and L. Goldstein, unpublished data), as it does to erythrocyte cell membranes, suggesting that the anion exchanger is not present in the cardiac cell membranes of embryonic skates. This raises the interesting question of the method of volume-activated taurine efflux in these cells, which must be *via* a heretofore unrecognized mechanism.

It is interesting that the volume-activated taurine release mechanism is present in the embryonic skate heart before hatching. This means that the system develops prior to the time when it will be needed to cope with changes in the osmolarity of the external environment. It will be important to discover the mechanisms and signals in the embryo that control the development of this transport system.

One of the authors (L.G.) was supported by NSF grant DCB-9102215. Partial support (C.A.L.) was provided by Walt Disney Imagineering grant 992BB-3022, in connection with the Living Seas. The authors wish to acknowledge Mr Jay Sprinkel for his assistance in preparing the graphic representations of the data.

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