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THE ROLE OF ANION CHANNELS IN OSMOTICALLY ACTIVATED TAURINE RELEASE FROM EMBRYONIC SKATE (*RAJA EGLANTERIA*) HEART

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Summary

Taurine, a major osmolyte of vertebrate hearts, is released from the skate heart at increased rates during hypotonic stress. We tested the hypothesis that this taurine release is mediated by chloride channels activated by swelling. Two inhibitors of the channels, NPPB and DIDS, inhibited the volume-activated release of taurine from embryonic skate hearts. These results support the hypothesis that swelling-activated chloride channels mediate the release of cardiac taurine.

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

Introduction

Elasmobranch hearts, like those of other vertebrates, contain high concentrations of the amino acid taurine (2-aminoethane sulfonate) which is used to maintain osmotic equilibrium between the cardiac cells and the extracellular fluid (Boyd *et al.* 1977). Taurine accumulation in the skate heart is mediated by a Na^+-dependent cotransport system that is capable of producing steep intracellular/extracellular concentration gradients (Forster and Hannafin, 1980*a*; Goldstein *et al.* 1993). When skate cardiac cells are exposed to a hypotonic environment, taurine is released down the concentration gradient into the extracellular fluid. This response, which is part of a regulatory volume decrease (RVD), has been observed both *in vivo* (Forster and Hannafin, 1980*b*) and *in vitro* (Forster and Hannafin, 1980*b*; Goldstein *et al.* 1993) in skate hearts as well as in cultured chick heart cells (Rasmusson *et al.* 1993). However, the mechanism of the response is not known. Recent studies carried out in cultured epithelial cells have yielded evidence to suggest that hypotonic stimulation of taurine (as well as other osmolytes) release from these cells might take place *via* swelling-activated Cl^- channels (Banderali and Roy, 1992; Jackson and Strange, 1993). Since swelling-activated Cl^- channels are known to be present in mammalian and avian cardiac cells (Vandenberg *et al.* 1994; Zhang and Lieberman, 1995; Zhang *et al.* 1993), we investigated the possibility that these channels might mediate osmotically induced taurine release from the skate heart. We used the embryonic heart of the clearnose skate (*Raja eglanteria* Bosc) to study this problem *in vitro*.

Materials and methods

Skate embryos used in this study were approximately one-third (25–29 days after oviposition) fully developed, with cardiac masses of 1.8±0.16 mg (mean ± S.E.M., N=18). Details of the capture, maintenance and breeding of the adult skates, the maintenance of the embryos and the surgical technique used to remove the hearts from the embryos have been described previously (Goldstein *et al.* 1993).

Cardiac taurine release was measured by preincubating embryonic hearts in 1.0 ml of isotonic elasmobranch incubation medium [EIM: isotonic medium is composed of (in $mmol\,l^{-1}$): 300 NaCl, 5.0 $CaCl_2$, 5.2 KCl, 2.7 $MgSO_4$, 15.0 Tris, 370 urea; 940 $mosmol\,l^{-1}$] (Forster and Hannafin, 1980*b*) for 3 h at 25 °C in porcelain depression slides to maximize the surface-area-to-volume ratio and the oxygenation of the medium. The incubation medium contained 0.1 $mmol\,l^{-1}$ taurine + [^{3}H]taurine (1.85×10^4 Bq). After preincubation, the hearts were removed from the medium, washed three times in separate beakers containing fresh isotonic medium and placed in separate depression wells. 1.0 ml of isotonic EIM was added to each of the wells. The incubation medium was withdrawn after 10 min and replaced with either hypotonic EIM [hypotonic medium is composed of (in $mmol\,l^{-1}$): 100 NaCl, 5.0 $CaCl_2$, 5.2 KCl, 2.7 $MgSO_4$, 15.0 Tris, 250 urea; 460 $mosmol\,l^{-1}$] or hypotonic EIM containing inhibitor. 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) was dissolved in water while 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) was dissolved in dimethylsulfoxide (DMSO); both were diluted 1:1000 in the incubation medium. Separate experiments showed that 0.1 % DMSO had no effect on taurine efflux rates. Tamoxifen was dissolved directly in the incubation medium. These media were removed after 10 min and replaced with similar fresh media for

another 10 min. All media were analyzed for ^3H by liquid scintillation spectrometry. Hearts were blotted lightly on tissue paper, digested in Soluene and assayed for [^3H]taurine as described previously. Statistical significance was tested by paired data analysis. Data points with inhibitor were matched against control data points without inhibitor for each time point.

NPPB was purchased from Biomol (Plymouth Meeting, PA, USA); DIDS and Tamoxifen {[*Z*]-2-[4-(1,2 diphenyl-1-butenyl)-phenoxy]-*N*,*N*-dimethylethanamine} were purchased from Sigma Chemical Company (St Louis, MO, USA). All other reagents were purchased from Sigma or Fisher Scientific (Springfield, NJ, USA).

Results and discussion

Fig. 1 shows the effects of two inhibitors of swelling-activated chloride channels on taurine release from embryonic skate hearts exposed to hypotonic EIM. In hearts incubated in isotonic media, taurine was released during the first 10 min of incubation. This release probably represents taurine trapped in the cardiac extracellular fluid and not removed during the washing procedure prior to incubation, since taurine release fell off rapidly after 10 min. In contrast, hearts incubated in hypotonic media continued to release taurine at elevated rates for at least another 20 min, indicating that both intracellular and extracellular taurine are released under hypotonic incubation conditions. Both NPPB and DIDS produced a significant inhibition of osmotically stimulated taurine release. NPPB at 100 μmol l^{-1} produced 55 % inhibition of osmotically activated taurine release at 30 min. In a separate experiment (not shown), we found that 10 μmol l^{-1} NPPB produced 33 % inhibition of osmotically activated taurine release. Fig. 1 also shows that 100 μmol l^{-1} DIDS, another inhibitor of swelling-activated chloride channels, produced a 30 % inhibition of osmotically activated taurine release at 30 min. Thus, although DIDS does inhibit taurine release, it is not as potent as NPPB in this regard.

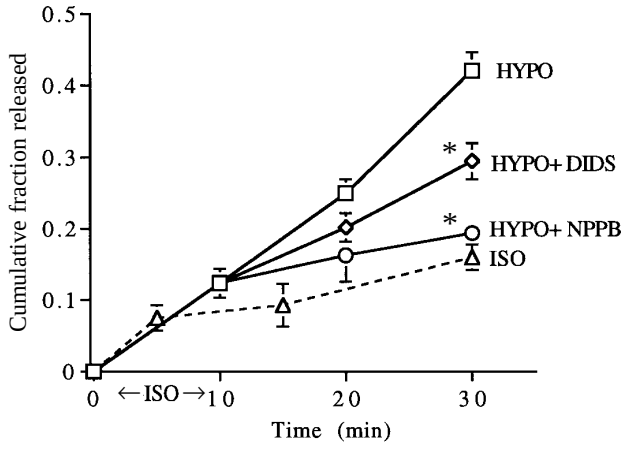

Fig. 1. Inhibition of osmotically activated taurine efflux by anion channel inhibitors. Values are means ± S.E.M. of 5–8 hearts per data point (data points without error bars have errors smaller than the size of the symbols). Results are expressed as the cumulative fraction of [^3H]taurine released: the fraction of [^3H]taurine originally present in the heart at time zero found in the medium at the times shown. * indicates $P<0.01$ compared with hypo-osmotic (HYPO) medium in the absence of inhibitors. HYPO, 460 mosmol l^{-1}. ISO, 940 mosmol l^{-1}. NPPB concentration, 100 μmol l^{-1}; DIDS concentration, 100 μmol l^{-1}. All hearts were incubated for 5 or 10 min in isotonic (ISO) medium and then transferred to one of the HYPO media shown, or to ISO medium, for an additional 20–25 min.

Since Tamoxifen has been shown to inhibit swelling-activated chloride channels in guinea pig cardiac myocytes (Vandenberg *et al.* 1994), as well as in other epithelial cells (Valverde *et al.* 1993), we tested the effect of this putative inhibitor on taurine release from skate heart. In two experiments, we found no effect of 10 μmol l^{-1} Tamoxifen (a concentration known to inhibit chloride channels in cardiac myocytes, Vandenberg *et al.* 1994) on taurine release. We did not test higher concentrations of Tamoxifen since Vandenberg *et al.* (1994) found that, even at 10 μmol l^{-1}, Tamoxifen could cause cell lysis in guinea pig cardiac myocytes.

The results presented in this study provide strong support for the idea that swelling-activated anion (Cl^-) channels are used by the embryonic skate heart to release taurine, and probably other osmolytes, during hypotonic stress. Our results are similar to those of Jackson and Strange (1993), who showed that NPPB and SITS (a DIDS analog) both inhibited swelling-induced taurine efflux in C6 glioma cells, a process thought to be mediated by volume-sensitive anion channels.

The presence of swelling-activated anion channels has been reported in cardiac cells of a number of mammalian and avian species (Vandenberg *et al.* 1994; Zhang and Lieberman, 1995; Zhang *et al.* 1993). Lieberman and his colleagues have provided evidence that these channels are involved in the release of taurine from chick heart. Smith *et al.* (1995) reported that reducing medium osmolarity from 290 to 180 mosmol l^{-1} stimulated taurine efflux by 42 % and chloride current by 72 % in cultured chick cardiac myocytes. NPPB inhibited the taurine response by 65 % and the swelling-activated chloride current by 54 %, demonstrating a close correlation of the effect of the inhibitor on the two responses. Zhang and Lieberman (1995) found that 10 μmol l^{-1} NPPB inhibited swelling-activated chloride current by 20 % and at 100 μmol l^{-1} NPPB this inhibition was increased to 60 %. Thus, NPPB inhibits swelling-activated chloride current in chick heart over the same concentration range as that shown to inhibit osmotically activated taurine release from skate heart. The results of Lieberman and his colleagues support our conclusion that swelling-activated chloride channels are directly involved in osmotically induced taurine release from skate heart.

The lack of effect of Tamoxifen on taurine release from skate heart is curious. Three explanations are possible. First, Tamoxifen may not inhibit chloride channels in skate heart as it does in the guinea pig heart. Second, inhibition of the chloride channel by Tamoxifen may not block the ability of the channel to mediate taurine release. Different inhibitors (NPPB *versus* Tamoxifen) may inhibit anion channels in different ways, not all of which render the channel inactive in taurine transport.

Third, the anion channel involved in taurine release from the skate heart might be different from that found in mammalian and avian hearts, despite the inhibition of taurine release from skate heart by agents such as DIDS and NPPB, which also inhibit anion channels in the hearts of higher vertebrates.

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