VOLUME REGULATION IN SKATE (Raja erinacea) RED BLOOD CELLS: NO ROLE FOR EICOSANOIDS

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In the little skate Raja erinacea the taurine efflux response to hypoosmotic shock is an indicator of cell volume regulation. Efflux of taurine increased two-fold over 30 min. upon dilution of the medium (Leite and Goldstein. J. Exp. Zool. 242 : 95-97,1987). In skate red blood cells (RBC) there is an increase in the turnover of diacylglycerol (DG) when the cells are subjected to a hypoosmotic environment. The increased turnover of DG represents an increase in the incorporation of arachidonic acid (McConnell et al. M.D.I.B.L. Bulletin 27 : 114-115, 1987).

When arachidonic acid was added exogenously to Erlich ascites tumor cells, inhibition of cell shrinkage during regulatory volume decrease (RVD) was observed. These mammalian cells, when subjected to hypotonic medium showed a simultaneous increase in leukotriene (LT) production and decrease in prostaglandin (PG) production when measured by radioimmunoassay (Lambert et al. J. Membrane Biol. 98 : 247-256, 1987). PGE_2 and LTD_4 played contrasting roles, inhibiting and accelerating RVD respectively, in hypotonically swollen cells (Lambert J. Membrane Biol. 98 : 207-221, 1987).

Nothing is known about the role of prostaglandins and leukotrienes in cell volume regulation in non-mammalian vertebrates. Since increased levels of DG were observed in skate RBC in response to a hypoosmotic environment and the cleavage of arachidonic acid from DG could initiate the arachidonic acid cascade leading to the synthesis of prostaglandins and/or leukotrienes, the effects of prostaglandins and leukotrienes on skate RBC RVD (taurine efflux) were examined. In addition the synthesis of leukotrienes and prostaglandins were measured by radioimmunoassay to examine whether their production changed during hypoosmotic stress.

Blood was drawn from the caudal vessel of a previously unused skate using a heparinized 21 gauge needle and syringe. Blood was centrifuged for 5 min at 4,000 rpm and the plasma and buffy coat removed. Red blood cells were washed in 1 volume of elasmobranch incubation medium (EIM), 940 mOsM consisting of: 300 mM NaCl, 5.2 mM KCl, 2.7 mM $MgSO_4$, 5.0 mM $CaCl_2$, 15 mM Tris HCl, and 370 mM urea at pH 7.5. The RBCs were then resuspended at 20% hematocrit with EIM. This RBC suspension was preincubated with ^{3}H-taurine for 3 hr then washed once with EIM containing 10 mM taurine and once with EIM containing 1 mM taurine to remove excess ^{3}H-taurine. In the experimental incubations, 0.3 ml of the radioactive RBC suspension was added to 3.5 ml EIM which contained 0.1 mM taurine in a 25 ml erlenmeyer flask.

For experiments with prostaglandins or leukotrienes, concentrations of 10^{-6}M were added to flasks. For experiments with inhibitors, concentrations of 10^{-5}M were added to the flasks during the last half hr of preincubation and again for the experimental incubation. Incubations were carried out in a shaking water bath at 15 ± 1^{o}C. Cells tested in response to hypoosmotic stress were incubated in a hypotonic EIM (650 mOsM, prepared the same as the control EIM, except that NaCl and urea were decreased to 200 mM and 250 mM, respectively). 0.5 ml samples were taken at 0 and 30 min., centrifuged for 2 min at 4000 rpm and 0.3 ml of

supernatant was analyzed by liquid scintillation counting to determine amount of ^{3}H-taurine released. The remainder of the fluid was removed by aspiration, and the RBC pellet weight determined by weighing the tubes. Time zero pellets were resuspended in 1 ml 5% $HClO_4$, kept on ice for 10 min then centrifuged for 2 min. Supernatant (0.3 ml) was counted to determine how much ^{3}H-taurine remained in cell. Efflux was calculated from the amount of ^{3}H-taurine appearing in the medium and the radioactivity of the RBC pellet.

For the prostaglandin and leukotriene synthesis experiments the RBC suspension was prepared as above but not labeled with ^{3}H-taurine. For cells tested in hypotonic medium, the RBCs were resuspended in EIM (650 mOsm). RBC were incubated for two hr, centifuged at 4000 rpm for 5 min and supernatant analyzed by radioimmunoassay for the stable metabolites PGE_2, $PGF_{2\alpha}$, 6-keto $PGF_{1\alpha}$, and TXB_2 by a radioimmunoassay procedure which has been reported (Herman and Martinez. J. Exp. Zool. 248 : 101-108, 1988). PGE_2, TXB_2 and 6 keto $PGF_{1\alpha}$ antisera were obtained from Advanced Magnetics, Boston, MA. $PGF_{2\alpha}$ antiserum was a generous gift from Dr. F.W. Goetz, University of Notre Dame, Notre Dame, IN. The assay procedure for leukotrienes was identical with those used for the other eicosanoid metabolites, however, the antisera for LTC_4 (Advanced Magnetics, Boston, MA) had significant cross reactivity with LTD_4, LTE_4, and LTF_4. Therefore these values are reported as total sulfidopeptide leukotrienes (Table II). Prostaglandin and leukotriene standards (2 mg/ml in EtOH stored at -80°) were diluted to a final concentration of 10-10,000 pg/ml in 0.01 M potassium phosphate buffer. Incubations of tracer, antibody, and sample or standard in a total assay volume of 0.3 ml was carried out for 18-24 hrs. Following incubation, bound and free hormone were separated by addition of 0.7 ml dextran coated charcoal in potassium phosphate buffer, and centrifugation for 20 min. Supernatant (0.5 ml) was counted by liquid scintillation. Intraassay and interassay variations were less than 10%. Results represent the mean $\pm$ standard error of the mean (S.E.M.) for groups of 5-7 experiments. All data were analyzed for significance at $p < .05$ using the Student's t test for paired values.

Upon addition of leukotrienes or prostaglandins, the taurine efflux was not different from control cells. Under hypoosmotic conditions, taurine efflux was increased approximately three fold, and this response was not altered when cells were incubated with indomethacin a cyclooxygenase inhibitor or quinacrine, a phospholipase A_2 inhibitor (Table I). This suggests that neither prostaglandins nor leukotrienes play a role in skate RBC volume regulation.

The production of leukotrienes and prostaglandins did not increase significantly when skate RBC were subjected to hypoosmotic shock (Table II). In contrast, when Erlich ascites tumor cells were exposed to a hypoosmotic environment, there was an increase of LTC_4 and decrease of PGE_2 production. This decrease in PGE_2 production was attributed to insufficient release of arachidonic acid, which when added increased PGE_2 synthesis in the hypoosmotically stressed cells (Lambert et. al., 1987).

When skate RBC were subjected to a hypoosmotic environment in a previous study (McConnell et al., 1987), ^{14}C-DG levels increased as a result of an increase in the incorporation of ^{14}C-arachidonic acid. Arachidonic acid released from DG can be converted to prostaglandins, thromboxanes and prostacyclins via the action of cyclooxygenase (Hansen Wld. Rev. Nutr. Diet 42 : 102-134, 1983), or converted to leukotrienes via 5-lipoxygenase (Samuelsson Science 220 : 568-575, 1983).

TABLE I

Effects of Eicosanoids and Eicosanoid Inhibitors on the Efflux of Taurine from Skate Red Blood Cells.

Control Taurine Efflux	Addition	Experimental Taurine Efflux
0.67 ± 0.13	+ LTB_4	0.75 ± 0.08
0.72 ± 0.14	+ LTC_4	0.69 ± 0.10
0.81 ± 0.14	+ LTD_4	0.71 ± 0.10
0.76 ± 0.14	+ LTE_4	0.90 ± 0.18
0.68 ± 0.05	+ PGE_2	0.66 ± 0.30
0.68 ± 0.05	+ $PGF_{2\alpha}$	0.66 ± 0.17
control taurine efflux		0.29 ± 0.12
hypotonic EIM taurine efflux		2.69 ± 1.48
+ indomethacin in hypotonic EIM		2.23 ± 0.95
control taurine efflux		1.10 ± 0.50
hypotonic EIM taurine efflux		3.33 ± 0.73
+ quinacrine in hypotonic EIM		3.20 ± 0.92

Taurine efflux (μg/gm red blood cells/30 min) data are expressed as the mean ± S.E.M. Prostaglandin and leukotriene (n=3) concentrations were 10^{-6}, and inhibitor experiments (n=4) concentrations were 10^{-5}M. Control values were not different from experimental incubations in any group ($p < .05$).

TABLE II

Production of Prostaglandins and Leukotrienes from Skate Red Blood Cells Subjected to Hypoosmotic Stress.

	PGE_2	$PGF_{2\alpha}$	6-Keto $PGF_{1\alpha}$	TXB_2
control	57.3 ± 17.5	151.4 ± 52.5	36.0 ± 11.4	156.7 ± 23.7
hypotonic EIM	76.5 ± 31.2	213.1 ± 74.6	76.0 ± 33.9	222.2 ± 52.8

Total Peptide Leukotrienes	
control	230.5 ± 112.7
hypotonic EIM	149.0 ± 46.0

Values represent mean ± S.E.M. (n=7) in pg/ml containing 0.2 g RBC. Control values were not different ($p < .05$) from those in the hypotonic medium for any eicosanoid measured.

The reported increase in incorporation of arachidonic acid into the ^{14}C-DG of skate RBC in response to hypoosmotic stress implies that there was free arachidonic acid available. However, the arachidonic acid does not appear to be converted by cyclooxygenase or lipoxygenase to eicosanoids when the cells were hypoosmotically stressed. Therefore, in contrast to ascites tumor cells, skate RBC RVD does not appear to involve eicosanoids. This may reflect species differences or differences in volume regulation mechanisms between mammalian and elasmobranch cells.

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