DIACYLGLYCEROL LEVELS IN HYPO-OSMOTICALLY TREATED ERYTHROCYTES FROM THE LITTLE SKATE Raja erinacea

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Cells placed in anisotonic media undergo volume changes, which are generally counteracted by volume regulatory responses in their solute transport systems. Like erythrocytes from other fish (Fincham et al., J. Membrane Biol. **96** : 45-56, 1987), red blood cells (RBC) from the little skate Raja erinacea, respond to changes in the tonicity of their medium by altering the rate of amino acid efflux across their membranes. Hypo-osmotic shock significantly increases taurine efflux, a response that can be mimicked by the application of phorbol esters or of calcium ionophore (Leite & Goldstein, J. Exp. Zool. **242** : 95-97, 1987). Phorbol esters stimulate protein kinase C from outside the cell; in vivo protein kinase C is activated by the membrane lipid diacylglycerol (DG). With the study reported here, we aimed to detect changes in the diacylglycerol content of skate RBC after treatment with a hypotonic medium.

Fresh blood was drawn as required from previously unused skates and centrifuged. The plasma was drawn off, the cells washed in 1 volume (wrt plasma volume) of Elasmobranch Incubation Medium (EIM, consisting of: 300 mM NaCl, 5.2 mM KCl, 2.7 mM $MgSO_4$, 5.0 mM $CaCl_2$, 370 mM Urea, and 15 mM Tris HCl, at pH 7.5 with an osmotic pressure (OP) of 930 mOsm) and resuspended to a hematocrit of 20% in 1:9 plasma:EIM.

Cells were incubated with 1 $uCiml^{-1}$ ^{14}C-arachidonic acid for 2 hours at 14°C, and after experimental treatment, the amount of radioactivity appearing as diacylglycerol was determined using thin layer chromatography. 1 ml of cells pre-incubated with ^{14}C-arachidonic was diluted with either EIM (control) or Diluting Solution (DS = EIM without NaCl and Urea; OP 75 mOsm). Suspensions diluted with DS had a final OP of 635 mOsm. Dilution treatment was terminated by commencement of the extraction procedure. The cells were extracted with 1:1 $CHCl_3$:MeOH, followed by 1N HCl and $CHCl_3$, and centrifuged to separate the phases. The lipid phase was washed with 1N HCl and the aqueous phases discarded. The lipid samples were dried under N_2, redissolved in hexane, and run beside known standards of dioleoylglycerol (Life Science Resources) on Silica Gel chromatographic plates in a hexane-based solvent bath. Plates were developed with molecular iodine, and DG identified by comparison with the standards. Areas from the gels corresponding to each lipid were scraped into vials for determination of radioactivity by liquid scintillation.

We found that dilution of the skate blood caused significant alteration of the diacylglycerol levels of the

blood cells. The DG level in hypo-osmotically shocked cells was elevated with respect to controls by 22 and 17% after 1 and 5 minutes respectively, but reduced to 20% below control levels at 2 minutes. All changes were significant, with $P < 0.05$. The rises in the level of radioactive DG in the RBC represent increases in the incorporation of arachidonic acid (the form of the introduced label) into DG. From this it can be inferred (a) that the turnover of DG has been enhanced, and (b) that there is likely to be concomitant enhancement of protein kinase C activity. The action of protein kinase C is likely to be phosphorylation of proteins, either in the membrane and directly involved with the volume-regulatory increase in amino acid flux, or cellular and influencing the flux indirectly.

A fall in DG level similar to that observed at 2 minutes has also been noted by Farese et al.(Science **236** : 586-589, 1987), studying myocytes. These authors also labelled cell lipids with ^{3}H-glycerol, and found the increase in labelled DG to be larger and better sustained. They attribute the difference to the different routes by which arachidonic acid and glycerol are directed to DG. Radio-labelling of skate erythrocytes may provide further information on the pathway of protein kinase C activation; the possibility is being investigated as part of our current research.

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