ERYTHROPOIETIN STIMULATES NA+-INDEPENDENT TAURINE FLUX THROUGH A TYROSINE KINASE-DEPENDENT PATHWAY IN LITTLE SKATE (RAJA ERINACEA) ERYTHROCYTES

Mark W. Musch[1], Erin M. Davis-Amaral[2], and Leon Goldstein[2]
[1]Department of Medicine, University of Chicago, Chicago, IL 60637
[2]Department of Physiology, Brown University, Providence, RI 02912

Erythropoietin (EPO) is a major growth factor required for development of red blood cells. EPO binds to its receptor on relatively mature erythroid progenitors and stimulates tyrosine phosphorylation of a number of cellular proteins. In the present study, we showed that EPO (EPOGEN, AMGEN) stimulated Na+-independent taurine flux in the nucleated skate erythrocyte. EPO, in a rapid (<10 min.), and concentration-dependent fashion (1-25 units/ml), stimulated taurine flux (150% control) under isoosmotic (940 mOsMoles/l) conditions. EPO-stimulated taurine flux was inhibited by the band 3 inhibitor, pyridoxal-5-phosphate (2mM) and the tyrosine kinase inhibitor, genistein (100μM).

To determine the mechanism of EPO action, cell lipids were analyzed for diacylglycerol (DAG) mass and phospholipid turnover. EPO stimulated a rapid (2 min.) increase (30%) in DAG. Since DAG can be derived by phospholipase C or D dependent pathways, several approaches were taken to differentiate between the pathways. There was an early (2 min.) increase in cellular phosphatidic acid that was blocked by the DAG kinase inhibitor R59022, suggesting a rapid activation of phospholipase C. A slower, more prolonged increase in DAG at longer times (5-30 min.) was not inhibited by R59022, suggesting a delayed activation of phospholipase D. Consistent with this suggestion, when ethanol was included in the incubation to promote the phospholipase D-dependent formation of phosphatidyl ethanol, this product appeared at the later time points (5 min.).

To test for tyrosine kinase activation, tyrosine phosphoproteins were immunoprecipitated using monoclonal antibody 4G10 and then separated on SDS-PAGE. Proteins were transferred to a PVDF membrane and probed with various antibodies. Using 4G10 to visualize the primary phosphoproteins, we found that EPO stimulated tyrosine phosphorylation of proteins with molecular masses of approximately 150, 120, 100, 75, and 35kDa. Using specific antibodies the 150kDa protein was identified as phospholipase C gamma-1 and the 100kDa protein as the skate homologue of band 3. PLC gamma-1 tyrosine phosphorylation increased rapidly (<2 min.) by 20-fold , and band 3 showed a 2 to 4-fold increase between 5 and 30 min. The nature of the other major phosphoproteins is unknown and one or more may be involved in the stimulated taurine flux.

In summary, EPO rapidly stimulates taurine flux in skate erythrocytes under isoosmotic conditions via a process involving tyrosine kinase. The roles of PLC gamma-1, DAG, and other signals remain to be clarified. Supported by NSF grant DOB 9102215 (LG) and NIH DK-38510 and DK-47722 (MWM).