GLUCOSE STIMULATION OF PHOSPHOLIPASE C IN THE BROCKMANN BODY OF THE LONG HORNED SCULPIN MYOXOCEPHALUS OCTODECIMSPINOSUS

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We have previously demonstrated that phospholipase C (PLC) plays an important role in glucose stimulated insulin secretion from the mammalian pancreatic islet (Kelley et al Endocrinology 134:1648-1654). Unlike receptor activated PLC which is well characterized in many tissues, the mechanism by which glucose activates PLC and the isozyme involved is unclear. The purpose of these studies was to determine if glucose activates PLC in the insulin secreting organ, Brockmann body, of the sculpin and to determine the isozymes of PLC present in this tissue. This model offers several advantages to its mammalian counterpart because the sculpin Brockmann body is separate from the exocrine pancreas and therefore much easier to isolate, and is many times larger than rat islets (5-10mg wet weight vs 5-10µg respectively) making it more suitable for biochemical studies. Furthermore, by comparing the signal transduction pathways in the sculpin to mammals, insights may be gained as to the role of PLC in glucose stimulated insulin secretion.

Brockmann bodies (two per fish) were isolated from sculpin that were anesthetized with MS222 (1/1000) and pithed. To determine inositol phosphate accumulation, groups of four Brockmann bodies were placed on filters and labeled with ^{3}H-inositol (40uCi/ml) for two hrs in a modified Ringer's solution that contained 150mM NaCl, 2.3mM NaH_2PO_4, 1mM CaCl, 0.6 mM $MgSO_4$, 5mM $NaHCO_3$, 2.6mM KCl, 2mM glucose, 10mM HEPES pH 7.5, and 0.1% BSA, and was gassed with 99%O_2/1%CO_2. The filters with the Brockmann bodies were then removed and washed with five ml of the modified Ringer's solution and incubated with 2mM glucose (basal) or 15 mM glucose (stimulatory) in the presence of 10mM lithium to prevent metabolism of inositol phosphates. After one hr the reaction was stopped with the addition of perchloric acid (final 10%) and total inositol phosphate content (IP1, IP2, and IP3) was determined by Dowex chromatography. PLC isozymes were determined by Western blot analysis of membranes using isozyme specific, mouse monoclonal PLC antibodies to PLC-β1, PLC-γ1, and PLC-δ1 (UBI, Lake Placid, NY). Freshly isolated Brockmann bodies (four) were obtained as described above and sonicated in 100 µl of solubilization buffer that contained 50mM Tris pH 7.6, 5mM EGTA, 5mM EDTA, 1% Triton X-100, 1mM dithiothreitol, 2mM phenylmethyl sulfonylfluoride, 0.1mM leupeptin, 5mM Benzamidine, 10µg/ml soybean trypsin inhibitor, 5µg/ml aprotinin, 2µM pepstatin A. The homogenate was centrifuged at 15,000 rpm in a tabletop microcentrifuge for 15 min and the supernant was then collected and subjected Western blot analysis using an ECL detection system.

To determine if glucose activates PLC in sculpin Brockmann bodies total inositol phosphate (IP) accumulation was measured in response to basal (2mM glucose) and 15 mM glucose. The addition of 15 mM glucose increased IP accumulation 1.83 fold above basal values from 375±18 to 686±168 cpm/Brockmann body/min (mean±SEM; n=3). While these studies are preliminary, they demonstrate that PLC activity is present in the sculpin Brockmann body and that glucose can stimulate this activity. Thus, activation of PLC may be an important pathway mediating glucose stimulated insulin secretion in the sculpin Brockmann body as it is in the mammalian islet.

Several distinct PLC enzymes have been identified from a variety of mammalian tissues, and three general families have been characterized, PLC-ß, PLC-γ, and PLC-δ. PLC-ß is activated by receptor-coupled G-proteins, PLC-γ is activated by tyrosine phosphorylation, and PLC-δ is likely activated by increased Ca^{2+} influx. We have identified the major PLC isozymes, PLC-ß1, PLC-γ1 and PLC-δ1, in rat islets by Western

blot analysis. In these studies, however, using the same mouse monoclonal PLC antibodies to PLC-β1, PLC-γ1 and PLC-δ1, we could not identify these isozymes in sculpin Brockmann bodies. In addition, Western blot analysis of the insulin secreting organ of the primitive Atlantic hagfish, Myxine glutinosa, also failed to show these PLC isozymes. The reason for this difference is unclear but may indicate that these isozymes are not present or are immunologically distinct from the mammalian isozymes. On the other hand, it is intriguing to speculate that glucose may activate a common isozyme of PLC in rat islets and sculpin Brockmann bodies that is distinct from the receptor activated isoforms previously identified in rat islets. Further studies may elucidate the isozyme and the mechanism of activation of PLC by glucose in insulin secreting tissues.

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