PROTEIN KINASE C ACTIVATES CHLORIDE SECRETION IN CULTURED SHARK (SQUALUS ACANTHIAS) RECTAL GLAND EPITHELIAL CELLS

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Previous work in our laboratory demonstrated that chloride secretion by cultured shark rectal gland (SRG) epithelial cells is activated by elevated cytosolic calcium (Moran & Valentich Pediatr. Pulmonol. Suppl. 4, p. 116, 1989) and hormone and neurotransmitter inputs which stimulate the formation of cyclic AMP (Valentich & Forrest Bull. MDIBL 26:91, 1986), cyclic GMP (Karnaky et al. Bull. MDIBL 29:86, 1990), and inositol phosphate (Ecay & Valentich Bull. MDIBL 29:101, 1990) second messengers. In the present series of experiments we investigated the role of diacylglycerol and protein kinase C (PKC) in the regulation of SRG chloride secretion.

Monolayer cultures of SRG cells mounted in Ussing chambers (Valentich & Forrest, ibid.) were used to monitor short-circuit current (I_{sc}), a direct measure of chloride secretion in this preparation. PKC assays were performed on crude homogenates of native SRG using a commercial assay kit (Amersham #RPN 77). Minced rectal glands were homogenized using an ice cold Dounce homogenizer. The homogenization buffer contained: 50mM Tris-HCl, 5mM EDTA, 10mM EGTA, 0.3% beta mercaptoethanol, 10mM benzamidine, 50ug/ml phenylmethylsulfonyl fluoride, 10^{-2}M ouabain and 10^{-5}M vanadate. The homogenate was centrifuged for 7 min at 1000 rpm and 4°C in a refrigerated table top centrifuge and then diluted 10 to 40X with ice cold homogenization buffer before use. The PKC assay is based on the phosphorylation on a synthetic peptide which is a specific substrate for protein kinase C but a poor substrate for other protein kinases. Intracellular Cyclic AMP levels were measured using a commercial radioimmunoassay kit (New England Nuclear # NEK 033).

10^{-7}M phorbol 12,13-dibutyrate (PMA), a diacylglycerol analog and potent activator of PKC, rapidly stimulated I_{sc} when added to the apical bath (control = 12±3.2uA/cm^2; PMA = 52±2.9 uA/cm^2; mean±SD, n=3). In contrast, basolateral bath PMA had only a small effect on I_{sc} (control = 6.6±1 uA/cm^2; PMA = 12±3.2uA/cm^2; mean ±SD, n=3). The threshold concentration for PMA stimulation of I_{sc} from the apical side was 10^{-9}M. 10^{-6}M 4-alpha phorbol 12,13 didecanoate, a phorbol ester which does not activate PKC, had no effect on I_{sc}. Pre-treating SRG cultures with 10^{-6}M staurosporin, a relatively specific PKC inhibitor, prior to stimulation with PMA reduced the I_{sc} response by approximately 40%. This concentration of staurosporin had no significant effect on forskolin-stimulated I_{sc}, indicating that staurosporin pre-treatment does not perturb cyclic AMP-activated chloride secretion. 10^{-6}M PMA in the presence of 10^{-3}M isobutyl methylxanthine, a cyclic AMP phosphodiesterase inhibitor, did not modify intracellular cyclic AMP levels in SRG cells. This result shows that PMA activation of I_{sc} is not a secondary effect due to increased cytosolic cyclic AMP. We also determined whether PKC enzymatic activity could be detected in homogenates of native SRG. These experiments demonstrated the following: 1) An enzymatic activity which phosphorylates a specific PKC peptide substrate is present in the SRG, 2) Kinase activity was dependent on the presence of phosphatidyl-L-serine, phorbol 12-myristate 13-acetate and calcium in the reaction mixture, 3) PKC inhibitors are present in crude SRG homogenates since

serial dilution of the homogenate results in increased PKC activity. Partial purification of PKC from the crude homogenate using DEAE-cellulose chromatography (McArdle and Conn, Methods Enzymol. 168:287, 1989) would probably result in a significant increase in PKC specific activity, and 4) 0.7 X 10^{-6}M staurosporin inhibited kinase activity to the basal level observed in the absence of lipid, phorbol ester and calcium co-factors. To our knowledge, these are the first measurements of PKC activity in the SRG. In a previous study Schlondorff et al. (Bull MDIBL 18:46, 1978) reported cyclic AMP independent protein kinase activity associated with a membrane fraction of SRG which was insensitive to a specific inhibitor of cyclic AMP-dependent kinases. It is possible that at least a portion of this activity represented PKC.

Our results demonstrate that SRG cells contain PKC activity and that activation of this kinase with phorbol esters stimulates chloride secretion. The observation that PMA stimulates I_{sc} principally from the apical side of SRG cells suggests that PKC is preferentially localized in or near the apical plasma membrane. Coupled with our previous data demonstrating that calcium ionophores increase I_{sc} and apical plasma membrane chloride conductance when added to the apical but not the basolateral bath (Moran & Valentich, ibid.), the present data suggest the existence of a signalling mechanism in or near the apical plasma membrane which regulates the chloride conductance of this barrier. Our finding that receptors for atrial natriuretic peptide are present on the apical surface of SRG cells (Karnaky et al., ibid.) raises the possibility that this peptide may mediate the activation of a local apical signalling mechanism involving cyclic GMP, calcium and PKC-mediated protein phosphorylation.

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