EFFECT OF CHLORIDE SECRETAGOGUES ON CYCLIC AMP FORMATION IN CULTURED SHARK (SQUALUS ACANTHIAS) RECTAL GLAND EPITHELIAL CELLS

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Chloride secretion in the shark rectal gland (SRG) is stimulated by several hormones and neurotransmitters involved in osmoregulation, including vasoactive intestinal peptide (VIP) and atrial natriuretic peptide (ANP). Previous work in our laboratory indicated that activation of SRG cells by VIP and ANP may involve multiple second messenger systems. In the native SRG, VIP stimulates the formation of cyclic AMP (Stoff et al., Am. J. Physiol. 237:F138, 1979) and in SRG slices VIP increases aequorin luminescence (Kelley et al., Bull. MDIBL 27:129, 1988). These results suggest that VIP activates both cyclic AMP and calcium-mediated signalling pathways. We have found that VIP stimulates the formation of inositol phosphates in ^{3}H-inositol-labeled SRG cultures (Ecay and Valentich, Bull. MDIBL 29:101, 1990), a result consistent with the coupling of VIP receptor occupancy to phospholipase A2 activation. The kinetics of the short-circuit current (I_{sc}) response to VIP also differ significantly from those observed in response to forskolin, dibutyryl cyclic AMP or 2-chloroadenosine. The latter secretagogues elicit a stable increase in I_{sc} which remains maximally activated for at least several hours. In contrast, VIP-stimulated I_{sc} is characterized by a transient peak followed by a decline over the course of 20-30 min to a steady state approximately 20-30% of the peak current. The principal objective of this study was to investigate the relationship between I_{sc} and intracellular cAMP kinetics in forskolin- and VIP-stimulated SRG cells.

Monolayer cultures were prepared as previously described (Valentich & Forrest, Bull. MDIBL 26:91, 1986). Briefly, collagenase-dissociated tubules were plated onto collagen gels supported with Nylon mesh to facilitate handling. Once cultures became confluent, they were used for experiments. Three to four replicate cultures were used for each data point. Intracellular cyclic AMP was measured using a commercial radioimmunoassay kit (New England Nuclear #NEK 033). Cultures were pre-incubated for 30 min HEPES-buffered, low bicarbonate shark Ringer with or without 10^{-3}M isobutyl methylxanthine (IBMX), a phosphodiesterase inhibitor. The pre-incubation Ringer was then replaced with fresh Ringer containing IBMX and 1mg/ml BSA with or without secretagogues and incubation continued for varying periods of time. Reactions were stopped and intracellular cyclic AMP extracted by rapidly aspirating the incubation Ringer and replacing it with ice cold 0.1N HCl. The extract was lyophilized and dissolved in a small volume of 50 mM sodium acetate buffer for RIA. Culture protein was determined using the Pierce BCA reagent after solubilizing cells in 0.1N HCl. Preliminary studies showed that the BCA reagent is insensitive to collagenous protein. Therefore, assay of total culture protein only detects cell-derived protein.

The first series of experiments were designed to compare the kinetics of change in intracellular cAMP levels in SRG cultures stimulated with forskolin or VIP. In the basal state, in the absence of IBMX, intracellular cyclic AMP

was approximately 3 pmols/mg protein. IBMX alone increased cAMP 3 fold. These values are essentially identical to those reported for the native unstimulated SRG (Stoff et al., ibid.). 10^{-6}M forskolin increased cAMP 10 fold in the presence or absence of IBMX, however the absolute amount of cAMP formed in the presence of IBMX was significantly greater. In the absence of IBMX, forskolin-stimulated cAMP remained maximally elevated for at least 40 min at a level of approximately 35 pmols/mg protein. In contrast, 10^{-7}M VIP elicited a 3 fold increase in cAMP which peaked at 12-15 pmols/mg protein 10-20 min following stimulation. However, by 40 min cAMP declined to a level near or only slightly above baseline. Peak cAMP levels were approximately 3 fold greater than those elicited by the same concentration of VIP in the native SRG (Stoff et al., ibid). These results demonstrate that the kinetics of the cAMP responses to both forskolin and VIP closely parallel the effects of these secretagogues on I_{sc}. The spontaneous decline in intracellular cAMP and I_{sc} in the continuous presence of VIP indicates that stimulus-response coupling undergoes desensitization. Since cultures which are desensitized to VIP remain fully responsive to forskolin or 2-chloroadenosine, it is likely that desensitization occurs in the mechanism which couples VIP receptor occupancy to adenylate cyclase activation. Alternatively, it is possible that the inositol phosphate signalling pathway, which is also stimulated by VIP, functions to terminate cAMP-mediated cell activation through negative feedback.

In previous studies we showed that I_{sc} is stimulated by ANP or high concentrations (10^{-3}M) of 8-bromo cGMP (Karnaky et al., Bull MDIBL 29:86, 1990). To determine if these responses result from secondary effects of ANP or 8-bromo cGMP on cAMP formation, we determined if these compounds modify intracellular cAMP levels in SRG cells. Experiments were carried out in the presence of IBMX since we have been unable to detect changes in intracellular cGMP in response to ANP in the absence of this phosphodiesterase inhibitor. 10^{-6}M ANP had no significant effect on intracellular cAMP, even though it increased cGMP nearly 60 fold (Karnaky et al. ibid). In addition, this concentration of ANP is at least 10-100 times greater than that required to activate I_{sc}. Therefore, we conclude that ANP effects of I_{sc} are not the result of an increase in intracellular cAMP. However, we found that 10^{-3}M 8-bromo cGMP elicited a 2 fold increase in cAMP. This increase may account for the small elevation in I_{sc} we have observed in response to high concentrations of this cGMP analog. However, additional experiments are necessary to determine if this apparent increase in cAMP is explained by cross reactivity of the cAMP antibody with 8-bromo cGMP. Exogenous cyclic nucleotide analogs have been reported to stimulate the synthesis of endogenous intracellular cyclic nucleotides by inhibiting cyclic nucleotide phosphodiesterase activity (Hall et al., Am. J. Physiol. 232:F368, 1977).

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