INTRACELLULAR CYCLIC GMP IN CULTURED SQUALUS ACANTHIAS RECTAL GLAND EPITHELIUM: EFFECTS OF ATRIAL NATRIURETIC PEPTIDE (ANP) AND ESCHERICHIA COLI HEAT STABLE ENTEROTOXIN (STa)

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Guanosine 3',5'-cyclic monophosphate (cGMP) acts as the major second messenger for both ANP and STa action. Both peptides increase intracellular cGMP through activation of a particulate isoform of guanylate cyclase (Tremblay et al., Advances in Second Messenger and Phosphoprotein Research 22 [eds. Greengard and Robison]: 319-383, Raven, New York, 1988). cGMP regulation of ion transport in both Cl-secreting and Na-absorbing epithelia has been previously reported, yet the underlying signalling mechanisms are poorly understood (Inagami, J. Biol. Chem. 264: 3043-3046, 1989; Needleman et al., Annu. Rev. Pharm. Tox. 29: 23-54). Our laboratory has recently demonstrated that ANP directly stimulates Cl secretion (measured as short-circuit current [I_{SC}]) and concomitantly elevates intracellular cGMP in cultured shark rectal gland (SRG) epithelium (Karnaky et al., Bull. Mt. Des. Isl. Biol. Lab. 29: 86-87, 1990). We have utilized cultured SRG epithelium as a model system to further investigate the role of cGMP in regulating Cl secretion following stimulation with ANP, and to determine the effects of STa on cGMP generation and Cl secretion.

Monolayer cultures of spiny dogfish (Squalus acanthias) SRG epithelium in 6 well culture plates were equilibrated with a low bicarbonate, HEPES buffered Ringer solution containing 1mM 3-isobutyl-1-methyl xanthine (IBMX) for 20 min. Cells were incubated for 10 min with varying concentrations of ANP (rANP, 5-28) or 10^{-7}M Escherichia coli STa. In addition, cells were incubated with 10^{-7}M ANP for time intervals ranging from 10 to 180s. Reactions were terminated with ice cold 0.1N HCl and assayed for cGMP by radioimmunoassay.

SRG monolayers incubated with ANP for 10 min exhibited dose-dependent elevations of intracellular cGMP levels beginning at a concentration of 10^{-8}M (data = mean $\pm$ S.E.; control = 336 $\pm$ 17, 10^{-8}M ANP = 1889$\pm$ 189 fmoles/mg protein; $p<0.001$; n=6), a finding which is similar to the dose-dependency of ANP stimulated Cl secretion reported by Karnaky et al. (*vide supra*, 1990). 10^{-6}M ANP increased intracellular cGMP nearly 50 fold (control = 336 $\pm$ 17, 10^{-6}M = 16686 $\pm$ 2047 fmoles/mg protein; $p<0.001$; n=6). Cl secretion elicited by 10^{-7}M ANP began within 2 min. and reached a maximum by 10 min (*vide supra*, Karnaky et al., 1990). Intracellular cGMP levels measured at 10, 30, 60, and 180s showed similar increases during this time interval (Fig. 1). These data suggest a close relationship between intracellular cGMP levels and Cl secretion in SRG epithelial cells.

Increases in intracellular cGMP and stimulation of Cl secretion by E. coli STa have been demonstrated in intestinal epithelia (Gianella and Drake, Immun. 24: 19-23, 1979) and the colonic cell line, T_{84} (Guarino et al. Am J. Physiol. 253: G775-G780, 1987). Forte et al. (Am J. Physiol. 255: F1040-F1046, 1988) have shown that E. coli STa elevates cGMP in slices of opossum kidney cortex and medulla, and in rat kangaroo kidney (PtK-2) cell lines. STa binding has also been localized in epithelial cells lining the small intestine, colon, gall bladder, cystic duct,

common bile duct, and trachea, as well as in epithelial cells forming the duodenal glands, liver, kidneys, and testis of opossum using ^{125}I-STa autoradiography (Krause et al., Cell Tissue Res. 260: 387-394, 1990). The occurrence of STa receptors in such a diverse list of epithelia suggests the presence of endogenous regulatory ligands involved in NaCl secretion. Incubating SRG monolayers with E. coli STa for 10 min at a concentration of 10^{-7}M increased intracellular cGMP levels 84%. This same concentration of ANP increased cGMP levels 40 fold (Table 1). 10^{-7}M STa, however, failed to stimulate Cl secretion (measured as Isc, data not shown). The modest, but significant increase in intracellular cGMP following E. coli STa exposure suggests the presence of an STa receptor coupled to cGMP formation in this tissue. An endogenous ligand for this receptor may exist in SRG.

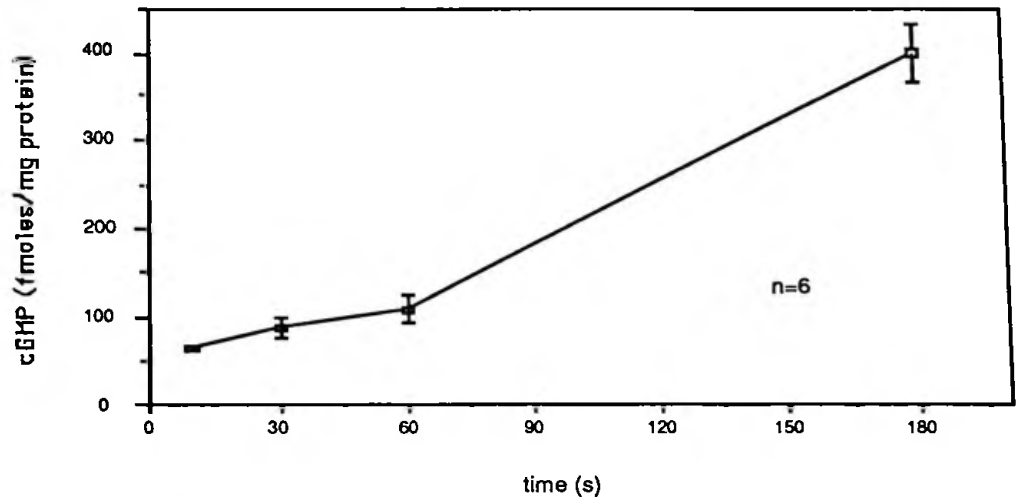

Fig. 1. Time course generation of intracellular cGMP. (Bars represent the S.E. of the mean. At 180s, cGMP levels were significantly different from basal levels; $p<0.001$).

Table 1. Intracellular cGMP levels following 10 min incubations with E. coli STa and ANP.

Control	10^{-7}M STa	10^{-7}M ANP
141±27 (7)	260±52* (7)	5857±1540** (7)

data = mean±SE fmoles cGMP/mg protein; *$p<0.05$; **$p<0.01$; (n).

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