EVIDENCE FOR A GUANYLIN/GUANYLATE CYCLASE SIGNALING SYSTEM IN THE INTESTINE, BUT NOT IN RECTAL GLANDS OF THE DOGFISH SHARK (*SQUALUS ACANTHIAS*)

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Guanylin (G) peptides activate membrane receptor-guanylate cyclase (R-GC) and regulate the intestinal secretion of chloride (Cl^-), bicarbonate and fluid in mammals via cGMP. G-like peptides and/or R-GC-receptors have been identified in all vertebrate classes including freshwater fish (Forte, *Reg. Peptides.* 81:25-39, 1999). Conflicting reports exist regarding the existence of a G-cGMP regulatory pathway in rectal glands of the dogfish shark. Silva et al. reported that guanylin or E. coli heat-stable enterotoxin (ST) stimulate Cl- secretion in perfused rectal glands, and this response was not inhibited by procaine (Silva et al., *Bull. MDIBL* 36:53-54, 1997; Silva et al., *Bull. MDIBL* 37:73, 1998), suggesting that guanylin and ST are not acting on nerves. In contrast, guanylin does not stimulate Cl^- secretion in perfused tubules isolated from rectal glands (Greger et al., *Pflugers Arch* 438:15-22, 1999). Treatment of primary cultures of rectal gland epithelial cells with ST did elicit small increases in cGMP levels, whereas atrial natriuretic peptide stimulated 40-fold increases in cGMP levels (Kennedy et al., *Bull. MDIBL* 30,102-103, 1991). In addition, ST, and either rat or human guanylin did not stimulate Cl^- secretion in cultured rectal gland epithelial cells (Kennedy et al., op. cit.; Karnaky et al., *Bull. MDIBL* 38, 70-71, 1999). We also found that 1 μM ST does not stimulate cGMP production in rectal gland slices in vitro (Karnaky et al., *Bull. MDIBL* 38, 70-71, 1999). In this report, we further investigated the effects of G family peptides on Cl^- secretion in the perfused rectal gland and on cGMP responses in the intestine of *S. acanthias.*

Rectal glands were obtained from male dogfish sharks, *S. acanthias*, weighing 2-4 kg, which were caught by gill nets in Frenchman's Bay, ME, and kept in marine live cars until use, usually within 3 days of capture. Sharks were killed by pithing the spinal cord. Rectal glands were excised, and cannulae were placed in the artery, vein, and duct as described previously (Kelley et al., *J. Clin. Invest.* 85:1629-1636, 1990). Monolayer cultures of dogfish SRG epithelium maintained on collagen-coated nylon mesh were used for measuring the Isc in Ussing chambers (Valentich, *Bull. MDIBL* 26:91-94, 1986). Mucosa from proximal intestine was isolated and ~0.5 g of tissue was incubated in 1 ml of shark-Ringers solution containing 1 mM zaprinast, an inhibitor of cyclic GMP metabolism, at 18 ^{0}C. Six samples were used for control and 6 samples were treated with 1μM E. coli ST for 60 minutes. Tissue cGMP was measured by RIA and values are expressed per mg of tissue protein. Treatment of isolated glands with 1μM human uroG, opossum G or E. coli ST by continuous perfusion using a paired and blinded method did not significantly influence Cl^- secretion, whereas infusion of 10 μM forskolin elicited substantial increases in Cl^- secretion. Confluent cultures of rectal gland cells raised on collagen-coated filters had no Cl^- (short-circuit current, Isc) responses to these peptides when delivered to either the basolateral or apical surfaces of the model epithelium. The radioligand, ^{125}I-ST, specifically labels G receptor-GCs in the intestines of vertebrates, including freshwater fish, but not in rectal glands. However, treatment with 1 μM *E. coli* ST of mucosa isolated from dogfish proximal intestine elicited significant increases in tissue cGMP from a basal level of 229 ± 10 to 300 ± 19 pmol of cGMP per mg protein ($p<0.05$). Thus, G peptides do activate a R-GC signaling protein located in the proximal intestine of *S. acanthias.*

We conclude that the G-cGMP signaling pathway is present in the intestine of dogfish sharks, which evolved more than 300 million years ago. However, we found no evidence for a possible regulatory action of G peptides to influence Cl^- secretion in the isolated perfused rectal gland. Supported by MDIBL New Investigator Awards, NIH grant DK 34208 and NIEHS grant P30-ES3828. Publication No. 186 of the Grice Marine Biological Laboratory, University of Charleston. JB was supported by a Hancock County Young Scholars Award. EB was a Burroughs-Wellcome Fellow from Hillside H.S. in Durham, NC. SD was a recipient of a Research Experience for Undergraduates from the NSF. AP was supported by a Hancock County Young Scholars Award.