# HUMAN UROGUANYLIN, OPOSSUM GUANYLIN AND HEAT STABLE *E. COLI* ENTEROTOXIN DO NOT STIMULATE CHLORIDE SECRETION IN THE PERFUSED RECTAL GLAND OF THE DOGFISH SHARK *(SQUALUS ACANTHIAS)*

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The guanylin family of cGMP-regulating peptides consists of three types of peptides: bacterial heat-stable enterotoxins (STa), guanylin and uroguanylin. These small heat-stable peptides activate cell-surface receptors that have intrinsic guanylate cyclase (GC) activity. Two receptor GC signaling molecules have been identified that are highly expressed in the mammalian intestine (GC-C) and kidney (OK-GC); both are selectively activated by the guanylin peptides (Forte et al., *Am. J. Physiol.* 278: F180–F191, 2000). In the shark rectal gland, there are conflicting data on the effects of these peptides. Bolus injections of guanylin and STa peptides into the rectal gland artery modestly increased chloride secretion in the perfused rectal gland (Silva et al., *Bull MDIBL*, 36: 53-54, 1997). However, guanylin did not stimulate chloride secretion in isolated perfused tubules of the gland that subsequently responded to C-type natriuretic peptide, a known activator of rectal gland guanylate cyclase (R. Greger, personal communication). Additionally, STa and rat and human guanylin do not increase chloride secretion in cultured monolayers of rectal gland tubular cells. STa also does not stimulate cGMP generation in fresh tissue slices of the gland (Karnaky et al., *Bull MDIBL,* 38: 70-71, 1999). We therefore carried out a detailed study of guanylin peptides using constant infusions of specific peptide preparations that were highly active in mammalian systems, as determined in the Forte laboratory.

Three guanylin peptides, human uroguanylin, opossum guanylin, and *E. coli* heat stable enterotoxin (STa) were examined. (Forte et al., *Ann. Rev. Physiol.,* 62:673-695, 2000). Rectal glands were perfused as described previously (Lehrich et al., *J. Clin. Invest.* 101:737-745, 1998). After thirty minutes of basal perfusion with shark Ringer's, a guanylin peptide was infused at a constant concentration for twenty minutes and then forskolin was added to the perfuate to test the responsiveness of the gland. Chloride secretion in the dogfish shark rectal gland was not stimulated by human uroguanylin (up to 1$\mu$M), opossum guanylin (up to 1$\mu$M), or *E. coli* heat stable enterotoxin (up to 400nM). (Figures 1-3).

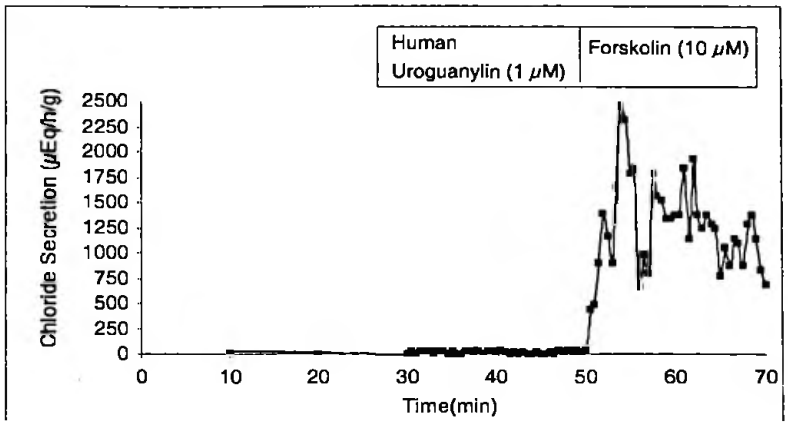


Figure 1. Human uroguanylin (1μM) added at t=30 for a duration of twenty minutes did not increase $Cl^-$ secretion of the rectal gland above basal values. Forskolin (10μM) added at t=50 resulted in a prompt stimulation in $Cl^-$ secretion, indicating that the gland was responsive to a secretagogue. The figure is representative of three experiments.

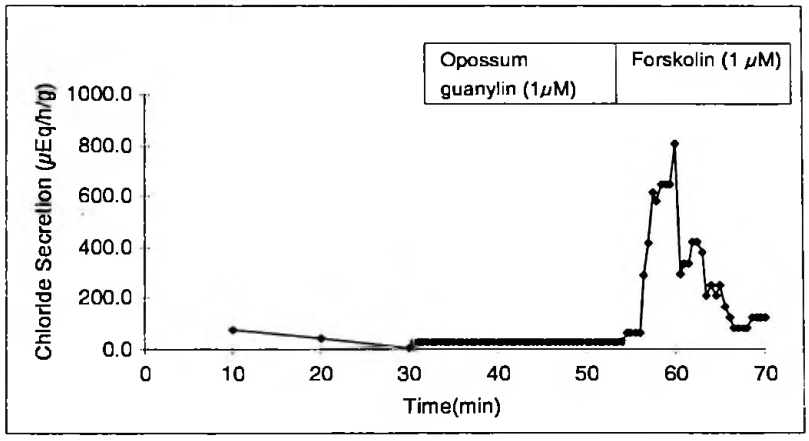


Figure 2. Opossum guanylin (1μM) added at t=30 for a duration of twenty minutes did not increase $Cl^-$ secretion of the rectal gland above basal values. Forskolin (1μM) added at t=50 stimulated $Cl^-$ secretion, indicating that the gland was able to respond to a secretagogue. The figure is representative of two experiments.

We next examined the effect of *E. coli* heat stable enterotoxin (STa) in the perfused gland. In these experiments, a control group (no guanylin peptide) was included. After 30 minutes of basal secretion, glands were either perfused for an additional 20 minutes with or without STa. At 70 minutes of perfusion the glands were perfused with forskolin alone (control group) or STa +forskolin. (Figure 3). STa (400 nM) alone did not stimulate chloride secretion above basal values. Furthermore, in contrast to known secretagogues (including C-type natriuretic peptide, vasoactive intestinal peptide, pituitary adenylate cyclase activating peptide, and adenosine), STa was not additive or synergistic with forskolin.

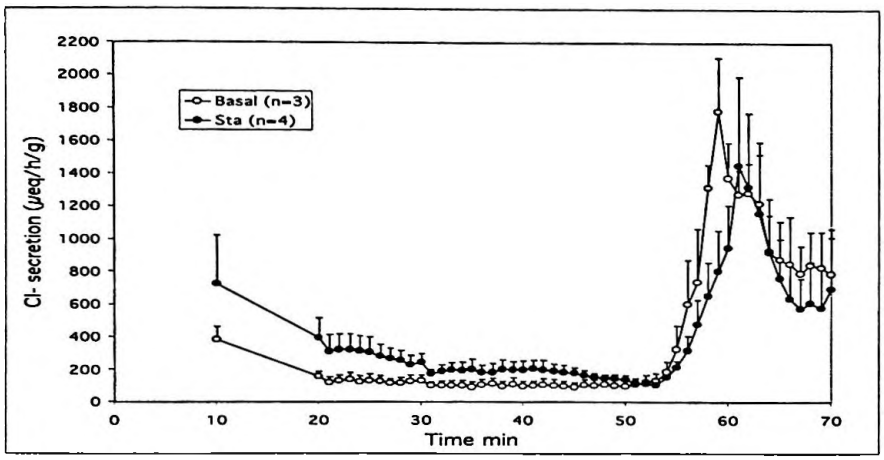


Figure 3. All glands were first perfused for 30 minutes with Shark Ringer's only (basal secretion). In one group (n=4) STa was then added to the perfusate at a concentration of 400 nM while the other group was perfused in the basal state without STa (n=3). Heat stable enterotoxin (STa) did not increase chloride secretion above basal levels. At 50 minutes of perfusion, forskolin was added to the perfusate of both groups. Chloride secretion in the STa + forskolin group was not different from the forskolin only group.

The failure of forskolin to enhance the responsiveness to STa is further evidence that the gland does not respond to this peptide (Figure 3). Finally, we also examined the effects of these peptides on chloride secretion in cultured monolayers of rectal gland tubular cells. None of the agonists applied to the apical or basolateral solutions stimulated chloride secretion (measured by short circuit current) in monolayers that were responsive to forskolin.

We conclude that the guanylin family of cGMP-regulating peptides does not regulate chloride secretion in the elasmobranch shark rectal gland.

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