GUANYLIN STIMULATES CHLORIDE SECRETION BY THE RECTAL GLAND OF *SQUALUS ACANTHIAS*

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We have previously shown that C-type natriuretic peptide (CNP) and related natriuretic peptides stimulate the secretion of chloride by the isolated perfused rectal gland of the shark (Silva, P., et al. *Am.J. Physiol.* 252: F99-103, 1987; Solomon, R., et al. *Am.J. Physiol.* 249: R348-54, 1985, Solomon, R., et al. *Am.J. Physiol.* 262: R707-R711, 1992). The effect of CNP is mediated by a guanylyl cyclase-linked specific receptor which we have also previously characterized (Gunning, M., et al. *Am.J. Physiol.* 264: F300-305, 1993). Three types of receptors linked to guanylyl cyclase have been described GC-A, GC-B, and GC-C. The first two, GC-A and GC-B, are natriuretic peptide receptors. Atrial natriuretic peptide is the natural ligand for GC-A and CNP is the ligand for GC-B. A natural ligand for GC-C is guanylin, a naturally occurring peptide first isolated from rat jejunum. GC-C also mediates the stimulatory effect of heat stable *E. coli* enterotoxin STa, a case of molecular mimicry that results in the secretory diarrhea induced by enterotoxigenic *E. coli*. We considered the possibility that the rectal gland of the shark expressed GC-C in the cell membrane as well as GC-B. The following experiments were done to ascertain this possibility.

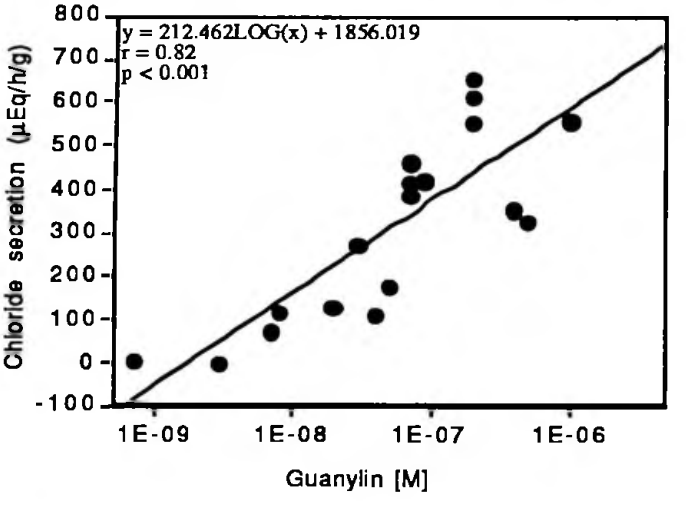

Figure 1. Guanylin dose response. Boluses of guanylin were injected into the arterial line of isolated perfused rectal glands in the amount calculated to provide final concentration ranging from 10^{-9} to 10^{-6}M.

Shark rectal glands were perfused as described in Silva P, et al. Methods Enzymol. 192:754-66, 1990. The secretion of chloride was allowed to achieve a steady state and a bolus of guanylin (human sequence), calculated to achieve final concentrations ranging from 10^{-9} to 10^{-6}M, was injected into the arterial line over a period of one minute. Figure 1 summarizes the results. Guanylin stimulated the secretion of chloride in a dose dependent way. Maximal stimulation was obtained at 2 x 10^{-7} M. Half maximal stimulation was seen at 5 x 10^{-8}M. The effect of guanylin lasted for approximately 20 minutes before declining to baseline.

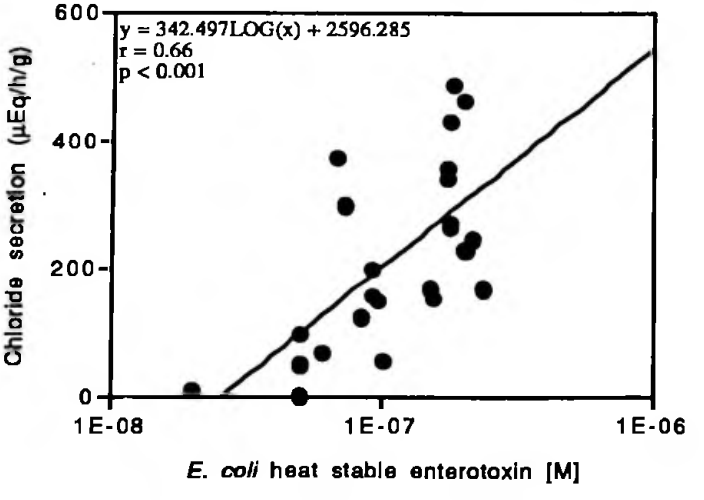

Figure 2. *E. coli* heat stable enterotoxin (STa) dose response. Boluses of STa were injected into the arterial line of isolated perfused rectal glands in the amount calculated to provide final concentration ranging from 5 x 10^{-8} to 2 x 10^{-7}M.

Similar experiments were done using *E. coli* heat stable enterotoxin. The results are shown in Figure 2. Bolus injections of STa induced a dose dependent increase in chloride secretion.

Guanylin and STa stimulate guanylyl cyclase in mammalian tissues with the generation of cyclic GMP. Cyclic GMP, however, has no effect on chloride secretion in isolated perfused rectal glands. Chao et al., EMBO J. 13:1065-1072, 1994, have shown that guanylin and STa activate intestinal chloride secretion via a cyclic AMP-dependent protein kinase. We therefore examined the effect of guanylin on adenylyl cyclase to see whether it could stimulate the production of cyclic AMP in an adenylyl cyclase assay in vitro. Guanylin did not stimulate the activity of adenylyl cyclase in vitro.

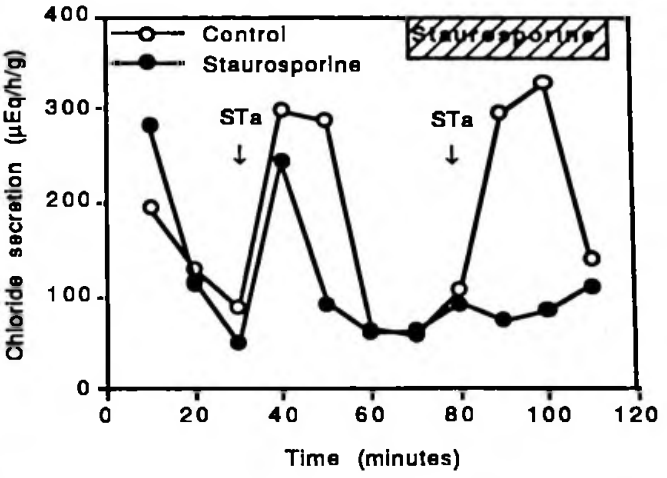

Figure 3. Staurosporine prevents the effect of STa. Repeat boluses of STa, 2 x 10^{-7}M were injected into the arterial line of isolated perfused rectal glands in the presence and absence of 10^{-8}M staurosporine. Control experiments are shown in the open circles. Experiments with staurosporine are shown in the closed circles. The duration of the infusion of staurosporine is indicated by the hatched bar. Staurosporine completely prevented stimulation with STa.

Previous studies in our laboratory have shown that the stimulatory effect of CNP is partially mediated by activation of protein kinase C. The evidence for that conclusion is based on the inhibition of the stimulatory effect of CNP by staurosporine that does not inhibit the stimulatory effect of VIP. To determine whether the effect of guanylin was also mediated by protein kinase C we examined its effect in the presence and absence of staurosporine 10^{-8}M. Staurosporine completely prevented the effect of STa, Figure 3.

The response of the rectal gland to guanylin and STa indicate that the rectal gland has GC-C. Guanylin is a fifteen amino acid peptide that bears no sequence similarity to natriuretic peptides or VIP. It has two double sulfhydryl bonds that confer a helical structure to the molecule. STa is a sixteen amino acid peptide with 56% sequence homology with guanylin and similar conformational structure. The guanylin peptide sequence used in these experiments was that of the human molecule. The structure of the shark molecule is unknown, but must be quite similar to the human peptide.

The intracellular mediators of the action of guanylin appear to be very similar to that of CNP. Like CNP it does not stimulate adenyl cyclase. Like CNP its effect is prevented by staurosporine. Still to be determined is whether it stimulates the release of VIP.

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