EVIDENCE FOR A ROLE OF CIRCULATING SHARK C-TYPE NATRIURETIC PEPTIDE IN THE RESPONSE OF THE EXPLANTED RECTAL GLAND OF SQUALUS ACANTHIAS TO VOLUME EXPANSION

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We have previously demonstrated that volume expansion of the intact shark results in an increase in chloride secretion of an explanted rectal gland (Solomon et al., Am. J. Physiol. 246:R63, 1984). This gland is connected to the intact shark via its blood supply only. Therefore, any change in the chloride secretion by this explanted gland reflects changes in the constitution of the blood, presumably a secretory hormone. We have also previously found, using an antibody to human A-type natriuretic peptide, that the plasma level of immunoreactive natriuretic peptide increase in the blood of the intact shark following volume expansion in parallel with the changes in chloride secretion by the explanted gland (Epstein et al., Bull. M.D.I.B.L. 27:72, 1988). However, direct proof that the major hormonal stimulus to rectal gland secretion is a circulating natriuretic peptide has not yet been provided. We have used HS-142, a specific inhibitor of the biologically active natriuretic peptide receptor coupled to guanylate cyclase, to explore the causal relationship between circulating natriuretic peptide and volume expansion induced rectal gland chloride secretion.

Explanted rectal glands were prepared as previously described (Solomon et al., op cit.). After perfusion of the gland was established, the donor fish was infused with 50 ml/kg wt of shark Ringer's over 30 minutes. Simultaneous with the volume load, a constant infusion of either shark Ringer's or shark Ringer's with HS-142 (500 µg/ml) was begun into the arterial catheter connected to the explanted rectal gland. The infusion rate was maintained at 1/10th the arterial blood flow rate for 150 minutes. Rectal gland duct excretion was collected every 30 minutes for a total of 210 minutes and chloride concentration measured by amperometric titration.

To determine the dose of HS-142 sufficient to block the stimulation of chloride secretion by natriuretic peptides, we used isolated fresh rectal gland tubules and measured ouabain-inhibitable oxygen consumption in the presence of varying doses of HS-142 and CNP. Tubules were prepared as previously described (Silva et al., Am. J. Physiol. 265:R439, 1993), a dose response curve to C-type natriuretic peptide (CNP) established, and the concentration of competitive inhibitor, HS-142, necessary to block a submaximal dose of CNP determined. Table 1 depicts the effect of 500 µg/ml of HS-142 only. This dose completely blocked the stimulatory effect of CNP 5x10-8M.

Table 1. Inhibitory effect of HS-142 (500 µg/ml) on ouabain-inhibitable oxygen consumption (QO2, µM/min*mg) in isolated rectal gland tubules. (n)=number of tubule preparations.

	Dose (M)		
	10-8	5 x 10-8	10-7
CNP	84±11	137±17	127±13
(n)	(40)	(16)	(30)
CNP+HS-142		14±17	
(n)		(9)	

In the explanted rectal gland, perfusion with 500 µg/ml of HS-142 completely inhibited the increase in chloride secretion observed following volume expansion of the

donor fish. Following cessation of the HS-142 infusion, there was an increase in rectal gland chloride secretion toward the levels found in the explanted gland which had received vehicle only. There was no difference in blood flow rate to the explanted rectal gland (37±4 vs 39±3 ml/h*g peak flow in HS-142 group vs shark Ringer's respectively) or perfusion pressure (46.7±2.7 vs 43.5±0.9 cmH2O peak pressure in HS-142 group vs shark Ringers respectively) to the explanted gland.

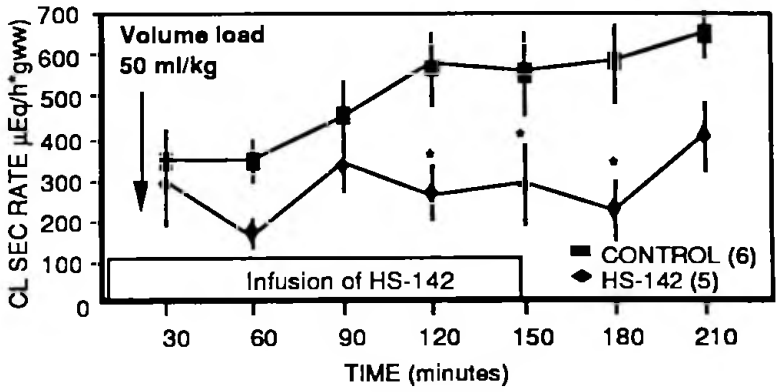

Figure 1.

Perfusion of the explanted rectal gland with HS-142 during volume expansion of the donor fish resulted in significant inhibition of chloride secretion. [n] is the number of explanted rectal glands in each group, * $p<0.05$ by t-test.

HS-142 specifically inhibits the binding of CNP to rectal gland membranes and blocks CNP-induced increase in guanylate cyclase activity in these same membranes (Gunning et al, unpublished results). Thus, these experiments provide direct evidence that the natriuretic peptide receptor coupled to guanylate cyclase, GC-A and GC-B, is involved in mediating the effects of volume expansion on rectal gland function.

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