A CALCIUM SENSING RECEPTOR (CaSR) MODULATES THE FUNCTION OF RECTAL GLAND ARTERY (RGA) AND TUBULES (RGT) IN *SQUALUS ACANTHIAS*

Susan K. Fellner[1] and Laurel Parker[2]
[1] Department of Cell and Molecular Physiology, University of North Carolina at Chapel Hill, 27599. [2] University of Maine, Orono, ME.

Blood flow to the RGA in conscious sharks varies from less than 1% in some animals to 2-7% of the cardiac output in others, suggesting a pattern of intermittent blood flow (Kent and Olson, *Am. J. Physiol.* 243:R296-R303, 1982). Shuttleworth (*J. Exp. Biol.* 103:193-204, 1983) has shown that α-adrenergic catecholamines cause vasoconstriction of the RGA and that agents known to stimulate RGT secretion of salt (Cyclic AMP, vasoactive intestinal peptide (VIP)) abolished the vasoconstriction. Subsequent studies in the shark, *Scyliorhinus canicula L.*, perfused at in vivo pressures showed that reducing perfusion to one third of control values caused a marked decline in sodium excretion by the gland (*J. Exp. Biol.* 125:373-384, 1986). Previous work from our laboratory (Fellner, *MDI Bull.* 40:88-90, 2001) showed that cytosolic calcium ($[Ca^{2+}]_i$) of RGA responded in a dose-dependent fashion to changes in extracellular calcium ($[Ca^{2+}]_e$) and that Ca^{2+} entry was not affected by the L-channel blocker, nifedipine. Thus, in the current study we investigated the mechanism(s) by which changes in $[Ca^{2+}]_e$ might influence calcium signaling in not only in RGA and but also in RGT. In particular, we tested the hypothesis that a calcium sensing receptor (CaSR) might be present in both and contribute to intermittency of function of the rectal gland.

We prepared RGA and RGT and measured $[Ca^{2+}]_i$ with fura-2 ratiometric analysis as previously described (*MDI Bull.* 40:88-90,2001). Rectal gland tubules were teased from thin slices of rectal gland from which the capsule had been removed. RGA segments with intact endothelium were < 0.1 mm in size. To investigate the possibility that a CaSR is responsible for the sensitivity of RGA and RGT to $[Ca^{2+}]_e$, we employed a variety of agonists known to activate the CaSR. Agonist activation of a CaSR stimulates mobilization of Ca^{2+} from the endo- or sarcoplasmic reticulum (ER, SR) followed by Ca^{2+} entry through store-operated calcium entry (SOC) channels (Brown, EM, et al. *Nature.* 366:575-580,1993). Gadolinium, a specific inhibitor of SOC, is an agonist of the CaSR. RGA segments in nominally calcium-free shark Ringer's had a baseline $[Ca^{2+}]_i$ of 165 ± 26 nmol/L which rose to 263 ± 27 nmol/L following the addition of Ca^{2+} (N = 10, P <0.01). Subsequent addition of Gd^{3+} further increased $[Ca^{2+}]_i$ to 314 ± 23 nmol/L (P <0.05), suggesting that Gd^{3+} stimulates additional mobilization of Ca^{2+} from the SR. The response to Gd^{3+} is blunted compared to that achieved by other CaSR agonists likely because of the inhibitory effect on Ca^{2+} entry via SOC.

Spermine and other polyamines are agonists for the CaSR (Quinn, S.J. *Am. J. Physiol.* 273:C1315-23, 1997). Figure 1 compares the response of RGT in calcium-free Ringer's to Ca^{2+} (5 mmol/L) followed by the addition of spermine (0.3 mmol/L) or Gd^{3+} (0.3mmol/L).

The increment in $[Ca^{2+}]_i$ over baseline following the addition of Ca^{2+} was similar in both studies (*, $P < 0.01$), but spermine further increased $[Ca^{2+}]_i$ by 235 ± 94 nmol/L (**, $P < 0.01$) compared to only 60 ± 21 nmol/L for Gd^{3+} (#, $P < 0.05$). These data show that RGT, like RGA, respond to external Ca^{2+} with a step-wise increase in $[Ca^{2+}]_i$ and that both spermine and Gd^{3+} are agonists for a CaSR in the tubules. As well, the diminished response to Gd^{3+} compared to spermine may derive from the inhibition of Ca^{2+} entry via SOC by Gd^{3+}.

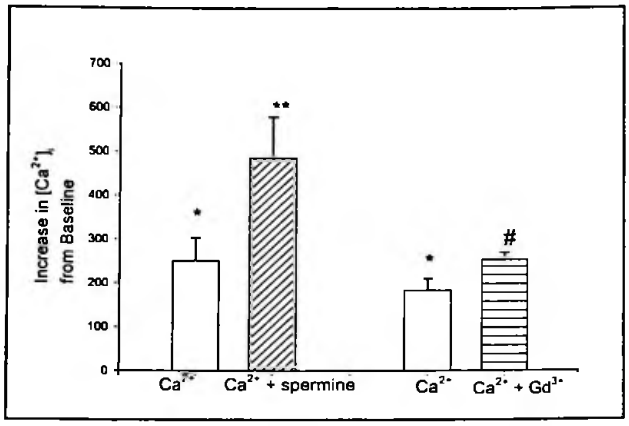

Figure 1. Increment in $[Ca^{2+}]_i$ in RGT in calcium-free Ringer's to external Ca^{2+} (5 mmol/L) followed by the addition of spermine (0.3 mmol/L) or Gd^{3+} (0.3mmol/L).

Because the extracellular domain of the CaSR interacts with polyvalent cations, it was hypothesized that activation of the receptor might occur through the screening of charged side chains of acidic or basic aminoacids. Furthermore, if ionic strength were increased by the addition of salts to the extracellular environment, the ability of polycations to trigger the CaSR should be decreased (Quinn, JS. et al. *J. Biol. Chem.* 273:19579-19586, 1998). In the isolated perfused RGT of *Squalus acanthias*, cAMP cases a biphasic response, the second of which arises from stimulation of the Na-K/2Cl cotransporter (Greger,R. et al. Pfluger's Arch. 438:165-174). Addition of NaCl (150 mmol/L) to the perfusate caused cell shrinkage and a relative increase in cell chloride concentration. In mammalian ascending limb of Henle cells, activation of the CaSR results in diminished cAMP production and increased degradation (Ferreiera, M. *J. Biol. Chem.*273:15192-15102). Thus, inhibition of the CaSR by increasing ionic strength should result in an increase in cAMP and Cl transport.

We propose that the function of a CaSR in shark RGA and RGT is to inhibit tonically blood flow to the gland and to inhibit salt secretion by the tubules during non-feeding periods. When the shark ingests food and seawater, the resultant increase in salt concentration (ionic strength) of the blood and interstitial spaces would then inhibit the CaSR resulting in disinhibition of RGA vascular contraction and reversal of inhibition of salt secretion. Such a control mechanism would permit the animal to efficiently recruit the function of the rectal gland only during periods of feeding.

Future studies will be directed at studying the effect of alterations of ionic strength on salt secretion in the isolated perfused rectal gland and on calcium signaling in cells of RGA and RGT. Supported by a MDIBL New Investigator Award. Laurel Parker was supported by a National Science Foundation fellowship.