EFFECT OF CHANGES IN IONIC STRENGTH ON THE CALCIUM SENSING RECEPTOR (CaSR) IN RECTAL GLAND ARTERY (RGA) AND TUBULES (RGT) IN *SQUALUS ACANTHIAS*

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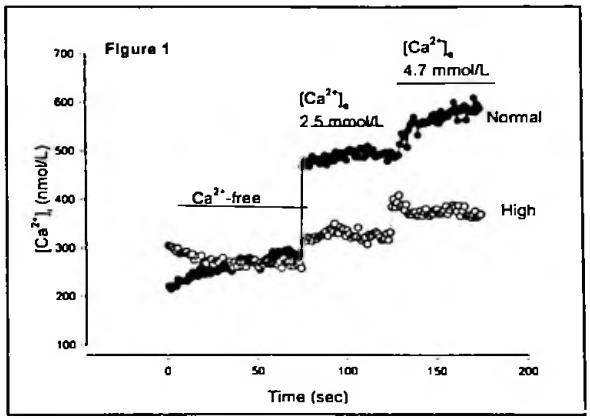
The elasmobranch, *Squalus acanthias* controls plasma osmolality and extracellular fluid volume by secreting a hypertonic fluid from its rectal gland. Previous work from our laboratory (Fellner and Parker, *MDI Bull.* 40:88-90, 2001) showed that cytosolic calcium ($[Ca^{2+}]_i$) of RGA responded in a 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. Subsequently we investigated the mechanism(s) by which changes in $[Ca^{2+}]_e$ might influence calcium signaling not only in RGA and but also in RGT. In particular, we tested the hypothesis that a CaSR might be present in both and contribute to intermittency of function of the rectal gland (Fellner and Parker, *MDI Bull.* 41:5-6,2002). Functional and immunocytochemical studies documented the presence of a CaSR in RGA and RGT (Fellner and Parker, *J. Exp. Biol.* 205:1889-1897, 2002). We postulated that the CaSR stimulates Ca^{2+}-mediated constriction of the RGA and diminishes cyclic AMP-mediated salt secretion in RGT during non-feeding conditions. Because the CaSR is inhibited by high ionic strength (Quinn et al., *J. Biol. Chem.*, 273:19579-19586, 1998), we proposed that an influx of sea water, particularly during feeding, increases blood and interstitial ionic strength, thereby relaxing the RGA and permitting salt secretion by the RGT.

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. Shark Ringers solutions containing trimethylamine oxide (TMAO) (pH 7.7) were prepared with normal, high and low ionic strength, the latter two made 15% different from normal shark Ringers by adding or subtracting sodium chloride and adjusting the amount of urea added to keep osmolality the same (940 ± 20 mOsm/L). We prepared the tissue in normal ionic strength Ringers, changing the solution to high or low ionic strength Ringers before measurement of agonist-stimulated changes in $[Ca^{2+}]_i$.

Intracellular Ca^{2+} of RGT in nominally calcium-free Ringers (normal ionic strength) rose by 110 ± 16 nmol/L when extracellular calcium ($[Ca^{2+}]_e$) was increased to 2.5 mmol/L and by 201 nmol/L when was further increased to 4.7 mmol/L (n = 13 and 10 respectively, $p < 0.01$ for both comparisons). RGT in high ionic strength medium showed an increase of only 45 ± 15 nmol/L to 2.5 mmol/L $[Ca^{2+}]_e$ and 114 ± 15 nmol/L to 4.7 mmol/L (n = 12 and 7). Thus the response of the CaSR to calcium in RGT was diminished by about 50% in high ionic strength Ringers ($p < 0.01$ for both calcium concentrations). Representative tracings are shown in Fig.1. The response of RGT to calcium (4.7 mmol/L) in low ionic strength buffer showed an increase in $[Ca^{2+}]_i$ of 534 ±

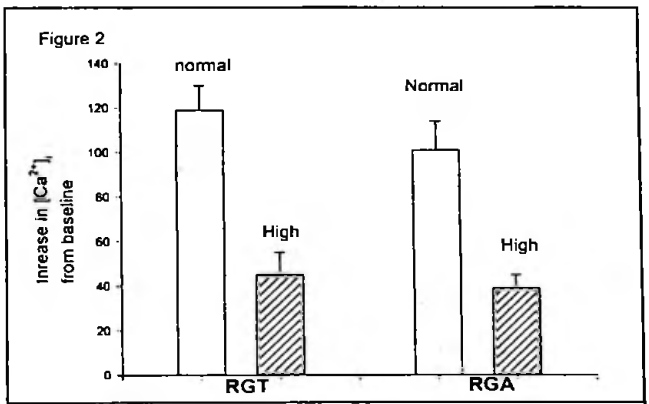
186 nmol/L (n = 8) but was not statistically significant compared to normal ionic strength (n = 8, p = 0.065).

We performed similar studies in RGA. For $[Ca^{2+}]_c$ of 2.5 and 4.7 mmol/L calcium, the increases in $[Ca^{2+}]_i$ in normal ionic strength Ringers were 159 ± 18 and 231 ± 14 nmol/L whereas the increases in high ionic strength buffer were 26 ± 5 and 56 ± 11 nmol/L respectively ($p < 0.01$). The enhanced response of RGA to 4.7 mmol/L $[Ca^{2+}]_e$ in low ionic strength Ringers of 323 ± 44 nmol/l compared to normal ionic strength (231 ±14 nmol/L) was significant ($p < 0.01$).



Comparison of $[Ca^{2+}]_i$ response of RGT to $[Ca^{2+}]e$ in normal vs. high ionic strength Ringers.

Spermine and other polyamines are agonists for the CaSR (Quinn, S.J. *Am. J. Physiol.* 273:C1315-23, 1997). Figure 2 compares the response of RGT and RGA in calcium-free Ringers to spermine (0.5 mmol/L) in normal vs. high ionic strength shark Ringers. The increment in $[Ca^{2+}]_i$ over baseline following the addition of spermine was similar in both tissues. There was an approximately 50% reduction in the $[Ca^{2+}]_i$ response in high ionic strength buffer ($p < 0.01$). Thus, independent of agonist stimulation of the CaSR, a high ionic strength medium diminishes the response.



$[Ca^{2+}]_i$ response of RGT and RGA in normal and high ionic strength Ringers to spermine.

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). Based upon our previous findings (Fellner and Parker, *J. Exp. Biol.* 205:1889-1897, 2002), we proposed 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. The current study documents that activity of the shark CaSR in RGA and RGT is inhibited under conditions of increased in ionic strength.

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