NOREPINEPHRINE (NE) AND VASOACTIVE INTESTINAL PEPTIDE (VIP) HAVE OPPOSITE EFFECT ON $[Ca^{2+}]i$ IN RECTAL GLAND ARTERY AND RECTAL GLAND OF *SQUALUS ACANTHIAS*.

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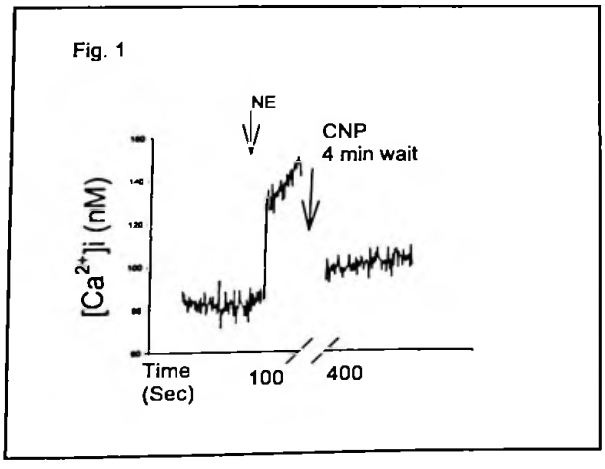
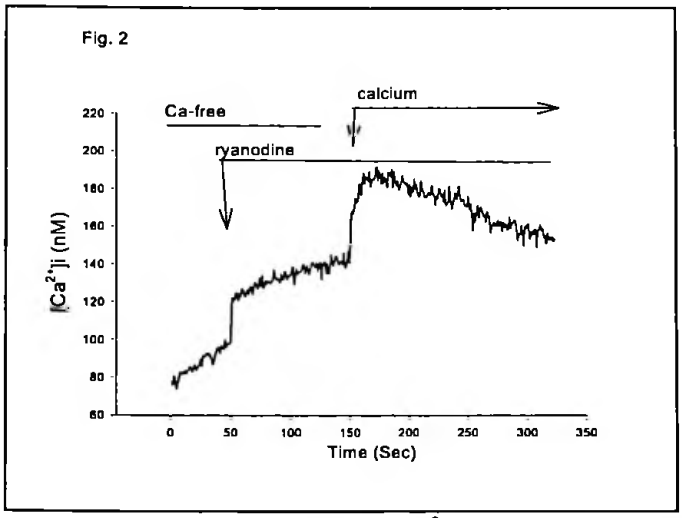
Blood flow to the artery of the rectal gland (RGA) varied from less than 1% in some study animals to 2-7% in others, suggesting a pattern of intermittent blood flow (Kent and Olson, *Am. J. Physiol.* 243:R296-R303, 1982), that may reflect changes in extracellular fluid volume brought about by feeding cycles. NE and endothelin are constrictors and atriopeptin and prostaglandins dilators of shark aorta (Evans et al., Am. J. Physiol. 165:659-664,1999, 274:R1050-1057, 1998, *J. Exp Biol* 157:551-555, 1991). Both VIP and CNP are well known to stimulate secretion of Na, K, 2Cl in the rectal gland (RG). In studies of the effect of NE and VIP on efferent perfusion flow and secretion in the isolated perfused RG, Shuttleworth (*J. Exp. Biol.* 103:193-204, 1983) demonstrated reversal of the vasoconstrictive effect of NE by VIP. During NE vasoconstriction, secretion was absent but increased markedly about 5 minutes after the infusion of VIP, Greger et al. have shown an increase in $[Ca^{2+}]i$ in RG tubules in response to carbachol, adenosine and VIP (*Pflugers Arch.* 436:133-140, 1998). No studies of calcium signaling in shark VSM and in particular, RGA have been previously performed. Hypothesis: Vasoconstrictor agonists should increase $[Ca^{2+}]i$ in the RGA, VIP and CNP should inhibit the $[Ca^{2+}]i$ response in RGA, and these two stimulators of salt secretion by the RG should increase $[Ca^{2+}]i$ in the rectal gland.

RGA was isolated, cleaned of omentum, minced in shark buffer containing trimethylamine oxide (TMAO), pH 7.8. RGA vascular smooth muscle (VSM) was loaded with fura 2AM at 12° C. for 1 hr and at 17 degrees for 30 mins. RG tubules were obtained from longitudinal slices of the gland which were loaded with fura similarly. All studies were then conducted at 18 degrees. $[Ca^{2+}]i$ was measured using a Photon Technology International (PTI) dual excitation wavelength Delta-Scan equipped with dual monochrometers and a chopper. Calibration of $[Ca^{2+}]i$ was based on the signal ration at 340/380.



Baseline $[Ca^{2+}]i$ in RGA was 107 ± 30 nM, rose to 180 ± 26 nM after stimulation with NE (10^{-6} M) and remained elevated as long as NE was present. Addition of CNP and a wait of ~ 4 minutes caused a fall in $[Ca^{2+}]i$ to 138 ± 24 nM (n = 6). $[Ca^{2+}]i$ did not fall abruptly in experiments in which CNP or VIP was not added. VIP required only a delay of ~2 mins. Fig.1 shows a representative recording of RGA stimulated with NE and then given CNP.

Similar experiments were performed with groups of tubules isolated from RG. Baseline values of $[Ca^{2+}]i$ were much more variable than in the RGA. Both CNP and VIP stimulated an increase in $[Ca^{2+}]i$ of 131 ± 28 nM (n = 7). In 2 preliminary experiments, NE appeared to reverse the stimulatory effect of CNP. More experiments are planned for the future to document this finding and investigate whether or not RG tubular cells possess alpha adrenergic receptors.



My previous work in rat preglomerular resistance vessels showed the presence of ryanodine receptors (RyR) in VSM cells (Fellner, *Kidney Internat.*, 58:1686-1694, 2000). Hence ryanodine was tested in RGA at a concentration known to stimulate (3-5 μM) rather than inhibit (>10 μM) the RyR. Studies were performed in nominally calcium-free buffer in order to examine the mobilization of calcium from the SR without the contribution of calcium entry. Baseline $[Ca^{2+}]i$ of 78 ± 14 nM rose to 115 ± 22 nM after the addition of ryanodine. Following the addition of extracellular calcium (2.5 mM), there was a further increase of $[Ca^{2+}]i$ of 93 ± 19 nM, representing calcium entry. Figure 2. shows a typical tracing of fresh RGA VSM in nominally calcium free buffer.

Whereas the presence of RyR in cardiac and skeletal muscle has been known for many years, only recently has it been looked for and found in VSM. In polarized cells, the RyR and IP_3 receptors are spatially distinct (Dranoff, Nathanson, et al. *Biochem J.* 337:305-309, 1999). The subcellular distribution of IP_3R and RyR in VSM have not yet been studied. The mechanism (s) by which calcium enters the cell after stimulation with ryanodine and following restoration of extracellular calcium to normal levels is the subject of further investigation.

In summary, we have shown that: 1.) shark RGA possesses a RyR, which, when stimulated, mobilizes Ca^{2+} from the SR and 2.) that RGA responds to stimulation by NE by increasing $[Ca^{2+}]i$ as one would anticipate. That VIP and CNP inhibit this response, is consonant with their putative vasodilatory action. In contrast, VIP and CNP stimulate salt secretion by RG tubular cells via a mechanism that involves elevation of $[Ca^{2+}]i$. These results are in agreement with the postulated mechanisms of flow and secretion in the isolated perfused RG proposed by Shuttleworth in 1983. Future studies will be directed at examining the mechanisms of calcium entry and calcium mobilization in shark RGA. Supported by a New Investigator Award, MDIBL, Salisbury Cove, ME 04672. Laurel Parker was a Hancock County Scholar.