VASOACTIVE INTESTINAL PEPTIDE AND FORSKOLIN INCREASE INTRACELLULAR FREE CALCIUM IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

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The role of cyclic AMP as an intracellular messenger of agonist-mediated stimulation and inhibition of chloride transport in the rectal gland has been well established; however, there is little understanding of the role of calcium as a second messenger in this tissue. In other chloride secreting epithelia, evidence is accumulating that Ca^{2+} may play an important role in regulating chloride secretion. In the rabbit intestinal epithelia, Frizzell (J. Membrane Biol. 35:175, 1977) and Bolton et al (J. Membrane Biol. 35:159, 1977) first showed that the Ca^{2+} ionophore A23187 stimulates chloride secretion. Darmsathaphorn and co-workers (J. Biol. Chem. 260:14163, 1985) have shown that A23187 stimulates chloride secretion in the T84 colon epithelia cell line by activating a basolateral K^+ channel that is distinct from a basolateral cAMP activated K^+ channel. They have shown that A23187 (J. Clin. Invest. 76:1837, 1985) and the Ca^{2+} mobilizing agent carbachol (J. Clin. Invest. 77:348, 1986) interact synergistically with vasoactive intestinal peptide (VIP) to stimulate secretion. Using Quin 2, they also attempted to determine if VIP increased intracellular free Ca^{2+}, and found no change with VIP. An increase in intracellular $(Ca^{2+})_i$ was observed with carbachol. They concluded that the effects of VIP on secretion are mediated solely by cAMP.

In the rectal gland, Palfrey et al (MDIBL Bull. 20:146, 1980) demonstrated a calmodulin dependent protein kinase(s) that phosphorylates a large number of cytosolic proteins. Forrest and coworkers have shown that A23187 modestly stimulates secretion (MDIBL Bull. 20:158-160, 1979) and that the calcium channel blockers (Verapamil and D600) inhibit adenosine stimulated secretion (MDIBL Bull. 20:158-160, ,1980). Furthermore, Cantley et al (MDIBL Bull. 24:42-44, 1984) have shown that calcium antagonists (verapamil, diltiazem, and nifedipine) and calmodulin antagonists (W-13 and calmidazolium) inhibit db-cAMP stimulated oxygen consumption in isolated rectal gland cells. They also attempted to determine if dibutyryl cAMP increases intracellular free Ca^{2+} with Quin II and did not see a change.

In the present studies the role of Ca^{2+} in forskolin and VIP stimulated secretion has been examined by using the non-specific Ca^{2+} channel antagonist cobalt (Co^{2+}) and the Ca^{2+} sensitive photoprotein aequorin.

Rectal glands were perfused as previously described. Intracellular Ca^{2+} was measured by the Ca^{2+} sensitive probe aequorin. Thin slices of rectal glands were mounted on a plastic frame support and loaded with aequorin by a modification of the method of Morgan et al described by Takuwa et al (J. Clin. Invest. 80:248, 1987) except that 300 mM potassium gluconate was used to maintain the high osmolality of the shark's cells. After the loading procedure and equilabration, the tissue slices were placed in a photometer and luminescence was measured in response to VIP and forskolin.

To determine if Ca^{2+} is required for VIP and forskolin stimulated secretion in the perfused rectal gland, the effect of the Ca^{2+} channel antagonist cobalt (Co^{2+}) was determined on VIP and forskolin stimulated secretion. From a basal of 162 ± 22, 0.1 nM VIP stimulated secretion to 1686 ± 418. The addition

of 0.1 mM Co^{2+} inhibited the VIP stimulated secretion to 224±125 over 30 minutes ($p < 0.05$, n=4) and upon removal of the Ca^{2+} channel antagonist secretion promptly increased to 1151±114.

Cobalt also inhibited forskolin stimulated secretion; however, unlike the effect of cobalt on VIP stimulated secretion, 0.1 mM cobalt did not inhibit the response to forskolin (1uM). When the concentration was increased to 1 mM, however, the forskolin response was inhibited by 60%. From a basal value of 38±3.4, forskolin stimulated secretion to 248±67 in the presence of Co^{2+}. When the Co^{2+} was removed, secretion increased to 552±99 ($p<0.05$, n=4). The reason for the different sensitivities to Co^{2+} is not clear. The experiments, however, suggest that an influx of Ca^{2+} is important for VIP and forskolin stimulated secretion.

The effect of VIP and forskolin on the intracellular free Ca^{2+} concentration $[Ca^{2+}]_i$ was determined with the sensitive Ca^{2+} indicator aequorin. Figure 1 shows that VIP (100 nM) immediately increased the aequorin luminescence 1.3 fold above basal. The elevation remained until VIP was removed from the perfusate. Figure 2 shows that forskolin also increased the aequorin luminescence. Five minutes after the addition of forskolin (50 uM), aequorin luminescence increased to a maximum of 2.5 fold above basal at 20 minutes, then slowly decreased over the next 30 minutes to 1.3 fold above basal. When the forskolin was removed from the perfusate, the luminescence decreased to baseline values over 20 minutes. The immediate response for VIP and the 5 minute delay for the forskolin stimulated increase in luminescence, and the immediate reversibility of the VIP response but slow reversibility of the forskolin response, are consistent with the effects of these agents on secretion in the perfused gland.

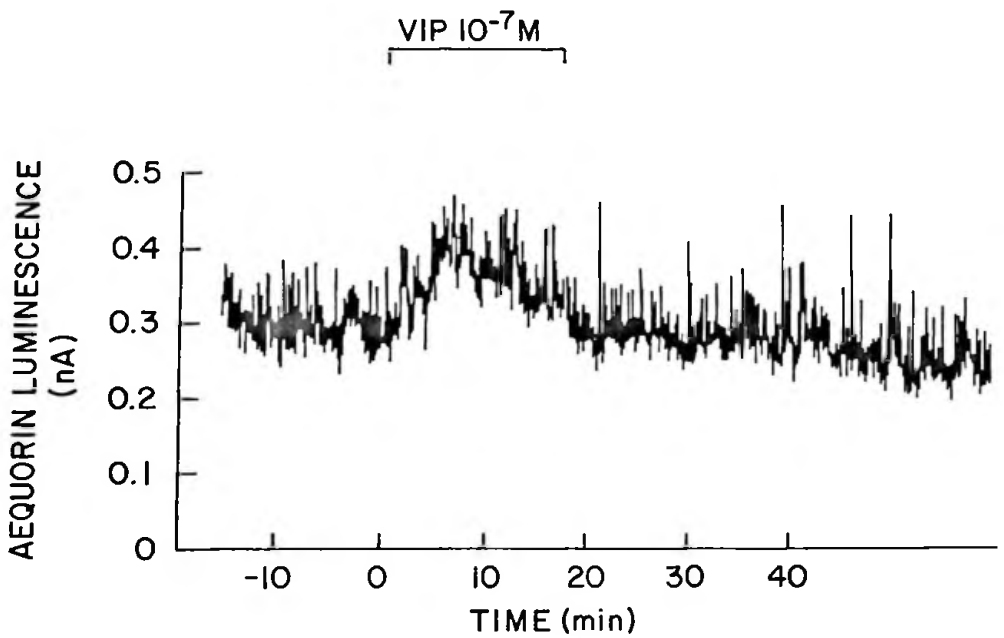

Figure 1. Aequorin luminescence in slices of rectal gland in response to VIP (10^{-7}M), (representative tracing of 4 glands).

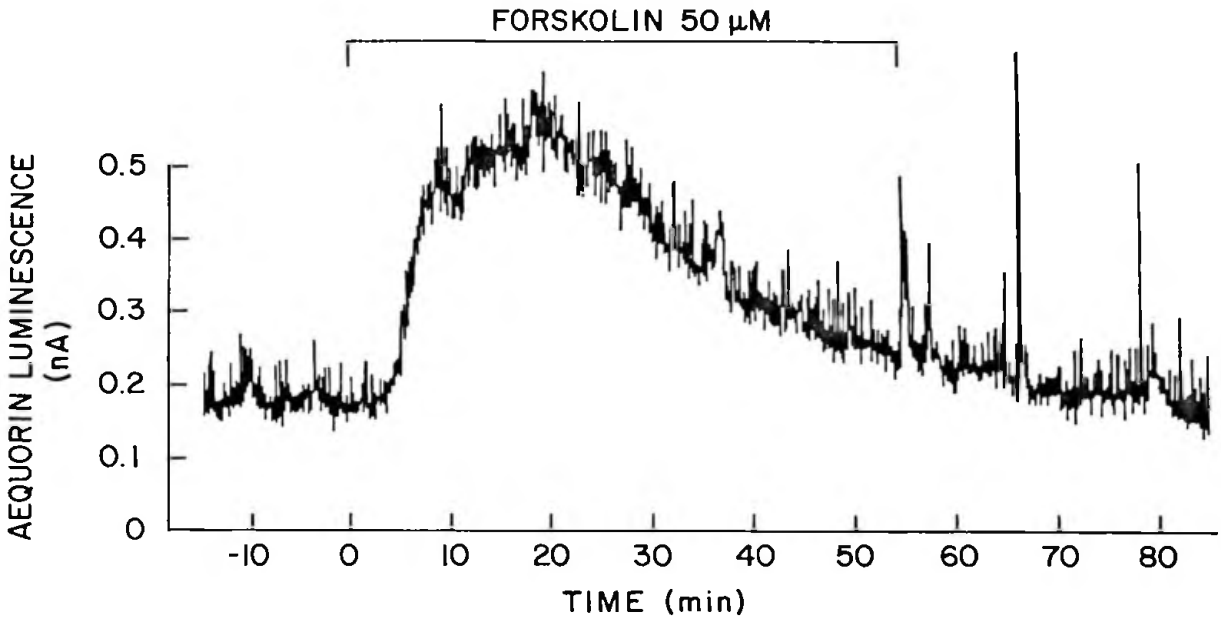

Figure 2. Aequorin luminescence in slices of rectal gland in response to forskolin (50uM) (representative tracing of 3 glands)

These studies indicate that both VIP and forskolin increase $[Ca^{2+}]_i$. The source of this increase is uncertain. However, since Co^{2+} and other calcium channel blockers inhibit secretion, the source of calcium is likely from an extracellular influx and not from intracellular stores.

The mechanisms(s) by which VIP and forskolin increase Ca^{2+} is also uncertain. The observation that forskolin alone increases $[Ca^{2+}]_i$ suggests that cAMP mediates the increase. This hypothesis is consistent with the findings of investigators in other tissues. Using Fura-2, Prentki et al originally showed that the adenylate cyclase stimulating agents forskolin, isoperoternol, and glucagon, increase cystolic free calcium in an insulin secreting clonal pancreatic cell line (HIT-15). These effects were blocked by the calcium channel antagonists verapamil and nifedipine or by removal of extracellular calcium. Using the patch clamp technique it has been demonstrated that cyclic AMP enhances transmembrane Ca^{2+} influx in heart cells (Tsien RW et al. J. Mol. Cardiol. 18:691, 1986) and corticotopin-secreting cells (Luini et al Proc. Natl. Acad. Sci. USA 82: 1985) by increasing the number and opening probability of voltage-sensitive Ca^{2+} channels.

In summary, studies employing Co^{2+} suggest that VIP and forskolin stimulation of chloride secretion is dependent on an influx of extracellular Ca^{2+}. Furthermore, well known stimulators of cAMP accumulation and chloride secretion increase $[Ca^{2+}]_i$. These studies provide evidence that changes in $[Ca^{2+}]_i$ may be important for hormonal stimulation of chloride transport.

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