AN INCREASE IN INTRACELLULAR CALCIUM IS ASSOCIATED WITH INHIBITION AND NOT STIMULATION OF THE RECTAL GLAND OF SQUALUS ACANTHIAS

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A number of second messengers are thought to be of importance in the stimulation of rectal gland chloride secretion. We used the calcium sensitive fluorescent probe, Indo-1, to assess the effects of stimulatory and inhibitory maneuvers on intracellular calcium, $[Ca]_i$ in freshly prepared rectal gland tubules. To determine whether the observed changes in $[Ca]_i$ were associated with functional effects, oxygen consumption of paired rectal gland tubule preparations or chloride secretion in the isolated perfused rectal gland were also measured.

Rectal gland tubules were freshly prepared as previously described (Silva et al., Am. J. Physiol. 265:R439, 1993). Tubules were incubated with the acetoxymethyl-ester of Indo-1 (Molecular Probes I1223) for 60 minutes at 15 °C in shark Ringer's-Hepes containing 10% fetal calf serum. The tubules were then diluted 1:10 in shark Ringer's-Hepes and placed in 2ml cuvettes for measurement of emission fluorescence using the ratio method (405 nm/475 nm). The ratio of emissions was determined every 10 seconds using a Perkins-Elmer LS-5B luminescence spectrometer. At the end of each experimental maneuver, the maximum fluorescence was obtained using saponin (75 µg/ml) and the minimum fluorescence was obtained by the addition of EGTA (10 mM). Intracellular calcium was calculated using the method of Grynkiewicz, J. Biol. Chem. 260: 3440, 1985) after correction for autofluorescence.

Intracellular calcium in unstimulated rectal gland tubules varied from 100-500 nM. The reason for this wide variation was unclear. However, there was a tendency for the $[Ca]_i$ to rise with time after the preparation of the rectal gland tubules, possibly as a consequence of ischemic or other damage to the tubules. This was supported by the observation that there was a parallel and progressive loss of the ability of secretagogues to increase oxygen consumption by the same tubules. In all experiments, therefore, $[Ca]_i$ was measured in parallel with QO_2 in order to ensure that the tubules were viable.

Vasoactive intestinal peptide ($5x10^{-9}$ M to 10^{-7}M) and C-type natriuretic peptide (10^{-8}M to 10^{-7}M) when added to freshly prepared tubules produced a dose dependent increase in oxygen consumption (QO_2) (Table 1). VIP produced a maximum 28 fold increase in QO_2 at 10^{-7}M while CNP produced a maximum 11 fold increase in QO_2 at $5x10^{-8}$M. Of note, these effects paralleled the increases in chloride secretion observed in the isolated perfused rectal gland preparation during perfusion with similar concentrations of the agonist. The effect on $[Ca]_i$ was examined at maximal doses of agonists. No increase in $[Ca]_i$ over baseline was observed during 10 minutes of continuous measurements .

Table 1. Dose response curve for VIP and CNP in rectal gland tubules (* $p<0.05$).

	DOSE OF AGONIST (mean±sem)			
	$5x10^{-9}$	10^{-8}	$5x10^{-8}$	10^{-7}
Vasoactive Intestinal Peptide (n)				
% Change QO2	1033*(11)	1475*(27)	2030*(16)	2820*(10)
% Change $[Ca]_i$				6±4 (8)
C-type Natriuretic Peptide (n)				
% Change QO_2		490*(40)	1142*(16)	943*(22)
% Change $[Ca]_i$				9±6 (10)

Thapsigargin, an inhibitor of Ca-ATPase in the endoplasmic reticulum, resulted in a rise in intracellular calcium within 1 minute of exposure. In 5 experiments, thapsigargin 2.5-5.0 μM resulted in a fourfold increase in $[Ca]_i$ from an average of 377 to 1243 nM.

In tubules from the same preparation, thapsigargin, 5 μM, inhibited the increase in ouabain-inhibitable oxygen consumption produced by VIP or CNP (Figure 1).

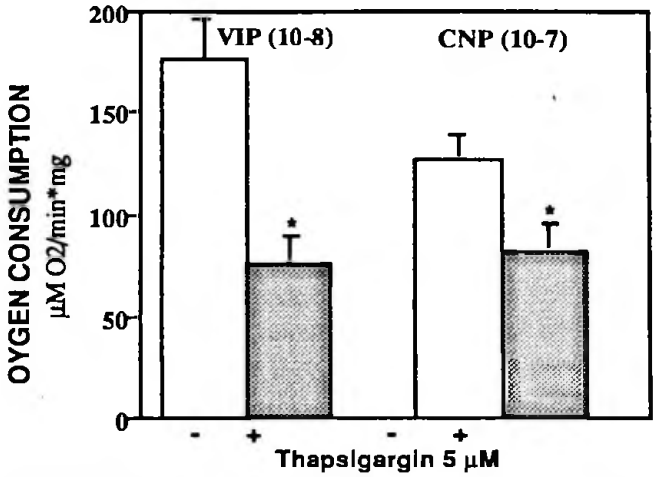

Figure 1. Thapsigargin inhibits oxygen consumption in rectal gland tubules.

Ionomycin also produced a dose dependent and rapid increase in $[Ca]_i$ (Figure 2). During perfusion of isolated rectal glands with dibutyryl cAMP and theophylline, ionomycin inhibited chloride secretion (Figure 3).

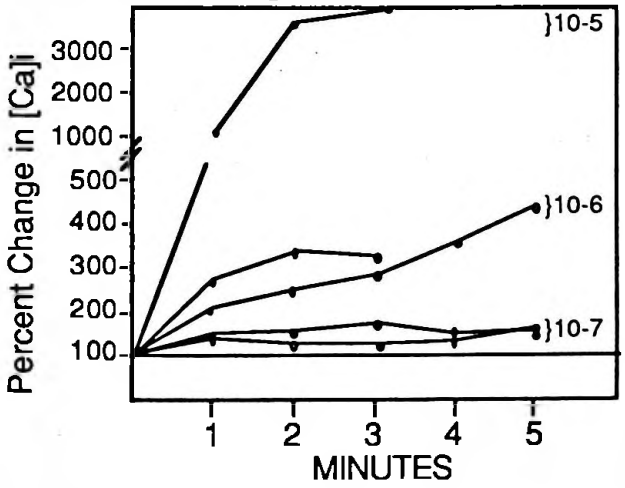

Figure 2. Each line represents a separate tubule preparation.

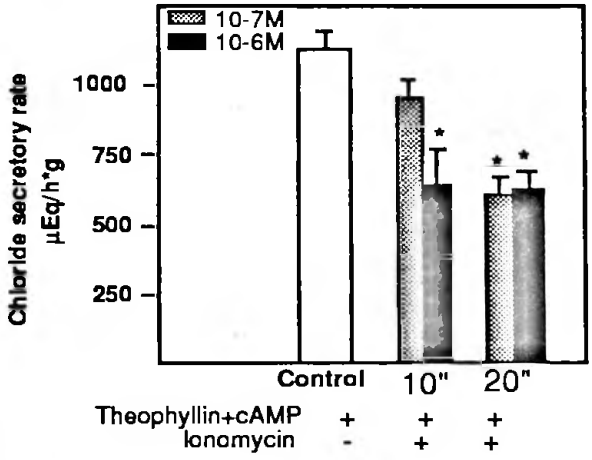

Figure 3. Ionomycin inhibits chloride secretion in the isolated perfused gland.

To determine whether a rise in $[Ca]_i$ was a general feature of chloride secretory inhibitors, we examined the response to somatostatin, neuropeptide Y, and ouabain. Both somatostatin and neuropeptide Y directly inhibited chloride secretion in the isolated perfused rectal gland preparation and oxygen consumption of isolated rectal gland tubules (Silva et al., Am. J. Physiol. 249:R329, 1985 and Silva et al., Am. J. Physiol. 265:R439, 1993 respectively). Neither neuropeptide Y (10^{-7}M), somatostatin (10^{-6}M), or ouabain (10^{-3}M) increased $[Ca]_i$ (data not shown).

We interpret these preliminary observations as follows. An increase in $[Ca]_i$ was not necessary for the stimulation of rectal gland secretion. On the other hand, an increase in $[Ca]_i$ as produced by thapsigargin or ionomycin was associated with inhibition of the secretory effects of VIP and CNP. This increase in $[Ca]_i$ was not necessary for inhibition (eg. by ouabain, neuropeptide Y, or somatostatin).

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