phosphodiesterases with methylated xanthines. We therefore sought to determine if changes in the phosphorylation state of proteins could be detected as Ilyanassa eggs underwent polar lobe formation and cytokinesis. Ilyanassa eggs, in spherical stages and in two stages of polar lobe constriction and cytokinesis, were lysed with solutions of Triton X-100, separated into yolky and non-yolky fractions, and incubated with (^{35}S)-adenosine 5'-(γ-thio)triphosphate (^{35}S-thioATP). Incubations were carried out for 30 min on ice or at 15°C in the presence of 5 μM ATP, 25 mM HEPES (pH 7.4), and 1.5 mM Mn Cl_2 or $MgCl_2$ according to Cassel and Glaser (1982. Proc. Nat. Acad. Sci. USA. 79:2231). Reactions were stopped by addition of SDS to 2.3% and heating to 60°C for 10 min. Such samples were subjected to electrophoresis in polyacrylamide gradient slab gels followed by autoradiography with X-ray film at - 80°C. Results indicated the thiophosphorylation of a high molecular weight area of protein near the top of the running gel in both yolky and non-yolky fractions of all samples. No qualitative differences were seen between samples from eggs in different degrees of polar lobe constriction.

Mytilus spawning. It is not convenient to get large numbers of Ilyanassa eggs to develop synchronously. Blue mussels, Mytilus edulis, however, release large numbers of gametes which are fertilized in open sea water and then undergo the same series of cell shape changes (polar lobe formation) that Ilyanassa eggs display. It has been difficult to study Mytilus embryos, however, because no reproducible method has been devised that elicits spawning (injection of 0.5 M KCl causes gamete shedding in echinoderms, but not in Mytilus). We therefore subjected freshly collected mussels to sea water containing a variety of hormones and neurotransmitters in an attempt to trigger spawning. Animals were placed in individual fingerbowls of sea water containing one of the following: 10^{-5}, 10^{-6} M 1-methyladenine: 10^{-5}, 10^{-6} M γ-amino-n-butric acid; 10^{-5}, 10^{-6} M acetylcholine chloride; 10^{-5}, 10^{-6} M progesterone in 1% or 0.1% DMSO; 10^{-6}, 10^{-7} M somatostatin; 10^{-5}, 10^{-6} M epinephrine; 1, 10 I. U./ml insulin; 10^{-5}, 10^{-6} M dexamethasone in 1% or 0.1% DMSO. Results: None of the treatments above elicited spawning in Mytilus. Supported by NIH Grant No. HDO7193.

ISOLATED RECTAL GLAND CELLS: OXYGEN CONSUMPTION STUDIES

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Cells were separated from rectal glands by collagenase and hyaluronidase digestion of minced rectal glands using the technique previously described (MDIBL Bull. 20:38-39, 1980), with only minor modifications. The cells were kept on ice until used in a buffer of the following composition (in mM): Na 280; K 5; Cl 295; Mg 3; Ca 2.5; SO_4 0.5; phosphate 1; Urea 350; Hepes 40, pH 7.6. The same buffer was used for measuring oxygen consumption. When sodium was removed from the bath it was replaced by lithium. When chloride was removed it was replaced by nitrate. When both sodium and chloride were removed they were replaced by lithium nitrate. Cells that were stored in buffer containing sodium and chloride were washed twice in buffer of the same composition as that used for measuring oxygen consumption. Oxygen consumption was measured in a constant temperature (25°C) chamber containing 2 ml of buffer and 4 to 10 mg of cells using a Clark type polarographic oxygen electrode (YSI) connected to a recorder. The electrode was calibrated using the known solubility of oxygen at 25°C and the barometric pressure. The rate of oxygen consumption was calculated from the tangent of the recorded slope of the oxygen consumption, the volume of buffer in the measuring chamber, and the wet weight of a measured aliquot of the cells removed from the measuring chamber at the end of the experiment. Glucose 5×10^{-3} M, pyruvate 10^{-2} M, and acetate 2.5×10^{-3} M were used as metabolic substrates. Theophylline 2×10^{-3} M, dibutyryl cyclic AMP 10^{-3} M, ouabain 10^{-4} M, and bumetanide 10^{-4} M (final concentrations), were all added in a volume not exceeding 1% of the volume of the measuring chamber to avoid dilutional problems. Results are expressed as micromoles of O_2 consumed per hour per gram wet weight of cells. The results are summarized in Table 1.

TABLE 1: Oxygen consumption in isolated rectal gland cells: Requirements for sodium and chloride and effects of Ouabain and Bumetanide

	Basal	Theophylline 2 mM + dibutyryl cyclic AMP 2 mM		
		-	Ouabain 10^{-4} M	Bumetanide 3×10^{-5} M
Control	35.1±3.8(20) 36.0±5.3(13)	67.0±6.4(23)* 74.9±8.8(13)*	30.8±3.1(23)**	39.2±4.9(13)**
Lithium chloride (No sodium)	35.2±6.2(7) 37.9±7.3(6)	38.4±5.9(8)** 32.5±5.9(6)	33.8±5.1(8)	39.0±7.1(6)
Sodium nitrate (No chloride)	34.0±5.0(8) 22.9±7.3(5)	35.1±4.8(9)*** 25.6±7.2(5)	34.9±4.4(9)	26.0±8.4(5)
Lithium nitrate (No sodium or chloride)	26.9±3.9(7)* 25.5±5.0(9)	26.6±3.9(7)** 27.0±5.0(9)	26.3±4.4(7)	26.1±5.0(9)

Values are mean ± SEM micromoles O_2/hr/g wet wt. *Significantly different from basal control. **Significantly different from stimulated control.

The basal oxygen consumption averaged 35.5 micromoles O_2/hr/g ww in 25 isolated rectal gland cell preparations. This rate of consumption was increased by 160% by the addition of 5×10^{-6} M 2,4-dinitrophenol. Oxygen consumption was completely abolished by the addition of KCN 10^{-5}M.

The rate of oxygen consumption remained stable following the initial one or two minutes after the measuring chamber was closed, until the oxygen tension in the chamber reached 7.7±1.7(3)mm of Hg for cells incubated under basal conditions and 19.6±0.3 (3) mm Hg in stimulated cells.

Oxygen uptake was stimulated an average of 97% by cyclic AMP and theophylline. After stimulation, QO_2 was inhibited to or below the basal level by ouabain or bumetanide. Omission of Na^+ or Cl^-, or both, from the bathing medium prevented stimulation of oxygen uptake. Basal QO_2, on the other hand, was little affected by the presence or absence of sodium or chloride. That oxygen consumption depends on the entry of sodium into the cell can be demonstrated under basal conditions by increasing the leak of sodium into the cell using the ionophore monensin. At a final concentration of 3×10^{-5} M monensin increased oxygen consumption by an average of 95% from 22.1±5.6 (7) to 36.2±5.7 (7) micromoles O_2/hr/g ww. Doubling the concentration of monensin did not increase oxygen consumption any further. Stimulation of the cells with theophylline and dibutyryl cyclic AMP did not change oxygen consumption substantially after monensin, the average value remaining at 31.6±5.2 (7). On the other hand, the addition of 3×10^{-5} M monensin after stimulation with theophylline and dibutyryl cyclic AMP increased oxygen consumption further, from 38.8±8.0 (4) to 55.0±10.6 (4) or 43±8%. In the absence of sodium, monensin did not increase oxygen consumption but decreased it in two of the three isolated cell preparations. The average oxygen consumption was 25.5±4.7 in the absence of sodium and after monensin 15.9±4.0, a decrease that was not statistically significant even by 'paired t' test. Ouabain, 10^{-4} M, after monensin, reduced oxygen consumption to basal levels a fact that is compatible with secondary increase in the activity of Na-K-ATPase induced by an increased leak of sodium into the cell. Bumetanide 10^{-4} did not alter oxygen consumption after it had been stimulated with monensin indicating that the increase in oxygen consumption induced by monensin is due to entry of sodium to the cell via a pathway that is not sensitive to bumetanide.

Veratridine (veratrine), an agent that depolarizes nerve cells by opening sodium channels, has been shown to stimulate secretion in perfused rectal glands (Erlij et al., MDIBL Bull. 21:74-76, 1981). The effect of veratridine might

be due either to stimulation of rectal gland nerves or depolarization of the cell membrane of the secretory cells that would activate chloride secretion. Veratrine at concentrations ranging from 0.005 to 0.5 mg/ml did not alter basal oxygen consumption in four different isolated cell preparations (38.8±6.8 in basal conditions vs 39.3±6.8 after veratrine). Theophylline and dibutyryl cyclic AMP stimulated oxygen consumption after veratrine by an average of 85%, a value not different from that observed in the absence of veratrine, this stimulation was returned to basal levels by 10^{-4} M ouabain.

Summary. Isolated rectal gland cells are able to maintain a stable rate of oxygen consumption until the oxygen tension is very low. As expected, the critical pO_2 in the medium for stimulated cells is higher than that seen under basal conditions. Oxygen consumption is linked to ion transport in a way consistent with coupled transport of both sodium and chloride into individual rectal gland cells across their plasma membrane. The failure of veratrine to stimulate oxygen uptake in isolated rectal gland cells devoid of neural connecting suggests that its stimulating effect in the whole perfused gland operates via rectal gland nerves and is due to the release of a neurotransmitter.

SOMATOSTATIN INHIBITION OF RECTAL GLAND SECRETION

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Somatostatin has been shown to inhibit the action of several peptide hormones in different systems. In the rectal gland somatostatin (1.4×10^{-7}M) completely inhibits the stimulatory effect of vasoactive intestinal peptide (VIP) on chloride secretion by isolated perfused rectal glands and on the accumulation of cAMP by rectal gland slices (Stoff et al., Am. J. Physiol., 237:F138-F144, 1979). The present experiments were undertaken to evaluate the effect of somatostatin on rectal gland secretion induced by other agents, including veratrine, adenosine, cAMP and theophylline, and forskolin. Fresh isolated rectal glands of Squalus acanthias were perfused as previously described (Silva et al., Am. J. Physiol., 233:F298, 1977).

In confirmation of Erlij et al (Bull. MDIBL, 21:74-76, 1981), perfusion with either veratridine, 2×10^{-5}M or comparable amounts of veratrine (3 mg/100 ml) stimulated chloride secretion to about 4 x basal levels. The increase in secretion was most marked during the first 10 min period after the addition of the agent, with progressive reduction thereafter. When veratrine was superimposed on somatostatin, 1.7×10^{-7}M in 4 experiments, no stimulation was observed (Figure 1).

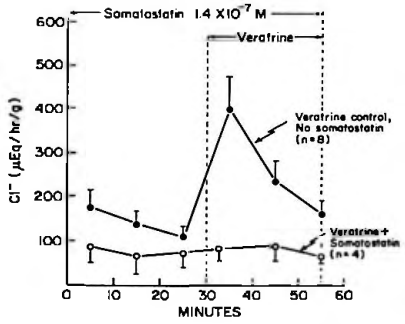

Figure 1.--Effect of somatostatin on rectal gland stimulation by veratrine. In the control experiments without somatostatin, 4 experiments were done with veratridine, 2×10^{-5}M and 4 with veratrine, 3 mg/100 ml. Because there was no significant difference between the two preparations, these results were combined. Values are mean ± s.e.

By contrast, adenosine (10^{-5}M) elicited a prompt increase in rectal gland secretion when infused in the presence of somatostatin at a concentration of either 1.4×10^{-7}M (n=3) or 1.4×10^{-6}M (n=2) Figure 2). When the administration of somatostatin was interrupted and that of adenosine continued, secretion remained the same or fell in 3 experiments, but increased slightly in 2 others.

Forskolin (10^{-5}M), a compound that directly activates adenylate cyclase (Seamon et al, PNAS, 78:3363-3367,