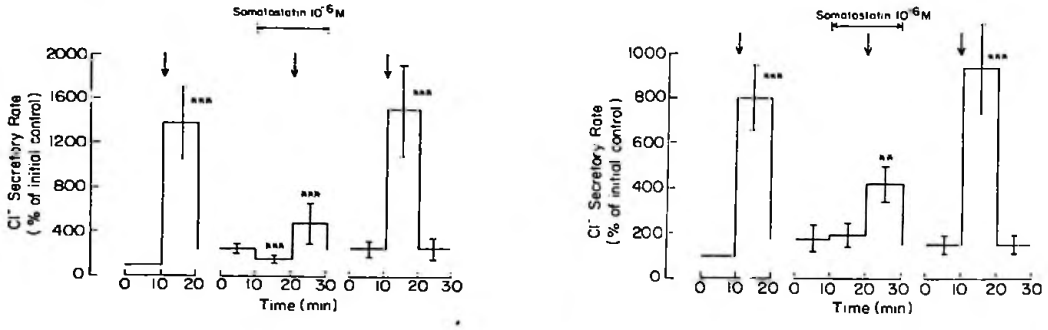

Figure 3.--Effect of somatostatin on rectal gland stimulation by forskolin. This figure summarizes the results of six experiments. Values are mean + SEM.

Figure 4.--Effect of somatostatin on rectal gland stimulation by dibutyryl cyclic AMP-methyl isobutyl xanthine (MIX). Dibutyryl cyclic AMP and methyl isobutyl xanthine were given together, both at a final concentration of 5×10^{-4}M. Values are mean + SEM. (n=4)

Finally, we repeated the experiment giving a bolus of dibutyryl cyclic AMP 5×10^{-4} M together with methyl isobutyl xanthine 5×10^{-4}M. As seen in Figure 4, this mode of stimulation was also inhibited by somatostatin.

We conclude that the action of somatostatin to inhibit the secretory response of the rectal gland to diverse stimulating agents is non-specific and localized at a site distal to the generation of cyclic AMP.

EFFECTS OF MONENSIN ON INTRACELLULAR ELECTROLYTES. OXYGEN CONSUMPTION AND OUABAIN BINDING IN SLICES OF SQUALUS ACANTHIAS RECTAL GLAND

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The present experiments were undertaken to test the hypothesis that the increase in ouabain binding that occurs when rectal gland cells are stimulated with theophylline and cyclic AMP is the result of the entry of sodium into the cell with consequent increase in activity of the sodium pump. Monensin, an ionophore with an ion specificity of Ag > Na > K > Rb > Cs > Li > NH4, was used to promote the entry of sodium into the rectal gland cell. Intracellular electrolytes and oxygen consumption were measured to ascertain that the effect of monensin on the rectal gland was consistent with the admission of sodium into the cells, so as to stimulate active transport.

Rectal glands were removed from Squalus acanthias and placed on ice. Slices were prepared from chilled tissue as previously described (MDIBL Bull. 19:72, 1980) and stored in ice cold shark Ringers. Slices were incubated in 5.0 ml of dogfish shark Ringers of the following composition (in mM): Na(280); K(5); Cl(295); Mg(3); Ca(2.5); SO_4(0.5); PO_4(1); urea(350); glucose(5) along with ^{14}C-inulin, for the determination of the extracellular space, in a Dubnoff water bath under a 99% O_2 and 1% CO_2 gas phase at 25 C° for 15 or 60 minutes. Following the incubation, the slices were blotted dry on filter paper, weighed and either placed in the oven to obtain dry weights or homogenized in 500 microliters of distilled H_2O. The homogenates were placed in 1.5 ml Eppendorff centrifuge tubes and centrifuged for 5 minutes. Samples of the supernatant were used for both the determination of electrolytes and of inulin. Values for the intracellular electrolytes are expressed as mEq/L intracellular tissue water. Table 1 summarizes the effects at 15' and 60' of stimulation with theophylline 10^{-3}M and dibutyryl cAMP 10^{-3}M, monensin 1.5×10^{-5}, ouabain 10^{-4}M and monensin plus ouabain on intracellular electrolytes in the slices of shark rectal gland.

Oxygen consumption was measured in a constant temperature 25°C chamber using a Clark type polarographic oxygen electrode (YSI) as previously described (MDIBL Bull. 22:9, 1982). The incubation solution had the following

Table I

Effect of stimulation with theophylline 10^{-3}M, dibutyryl cAMP 10^{-3}M, monensin 1.5 x 10^{-5}M, ouabain 10^{-4} and ouabain plus monensin on intracellular electrolytes in slices of shark rectal gland at 15′ and 60′.

	15′			60′		
	Na	K	Cl	Na	K	Cl
Control	154±41	126±30	119±26	108±45	139±51	96±21
Stimulated	161±54	97±18[b]	102±19[a]	135±36[a]	73±23[b]	87±14
Monensin	166±37	121±20	122±18	139±61[a]	98±23[a]	112±18[a]
Ouabain	175±45[a]	102±29[b]	119±16	195±58[b]	56±11[b]	112±16
Monensin plus Ouabain	202±46[a]	85±15[b]	129±27	252±18[b]	40±13[b]	131±30[a]

Values are mean + SD. p values were calculated by "paired t" test.
a $p < 0.05$ compared to control
b $p < 0.005$ compared to control

composition (in mM): Na(280); K(5); Cl(295); Mg(3); Ca(2.5); SO_4(0.5); PO_4(1); Urea(350); Hepes(40); Glucose(5); pH 7.6. Single slices kept in ice cold Ringers were placed in 2.0 ml of above solution and oxygen consumption was determined from the tangent of the slope of the oxygen tension recorded against time, the volume of buffer in the chamber, the solubility coefficient of oxygen and the wet weight of the slice. Theophylline 2 x 10^{-3}M, dibutryl cyclic AMP 10^{-3}M, ouabain 10^{-4}M and monensin 10 micrograms/ml were added in a volume not greater than 1% of the incubation volume to avoid dilutional problems. Results were expressed as micromoles of O_2 consumed per hour per gram wet weight of rectal gland slice. The results are summarized in Table 2.

TABLE II

Effect of stimulation with theophylline 10^{-3}M, dibutyryl cAMP 10^{-3}M, monensin 1.5 x 10^{-5}M, ouabain 10^{-4} and ouabain plus monensin on oxygen consumption by slices of shark rectal gland.

	Oxygen consumption micromoles O_2/min/gWW	
Control	40.3 ± 13.3	n=12
Stimulated	70.9 ± 22.2[d]	n=12
Ouabain	36.6 ± 11.3[c]	n= 9
Control	34.6 ± 11.3	n=10
Monensin	55.7 ± 27.4[b]	n=10
Ouabain	32.8 ± 7.3[a]	n= 5

Values are mean ± SD. p values were calculated by "paired t" test.
a $p < 0.05$ compared to monensin.
b $p < 0.01$ compared to control.
c $p < 0.005$ compared to stimulated.
d $p < 0.001$ compared to control.

Since monensin was dissolved in alcohol, an equal volume of alcohol was added to a separate set of slices to determine whether alcohol had any effect on their oxygen consumption. In this series of experiments the oxygen consumption of the slices under basal conditions was 29.2 ± 8.3 with an n=8.

Ouabain binding was measured using the technique previously described (Bull MDIBL 21:103, 1981). Monensin was added at concentration of .15, 1.5 and 15 x 10^{-5} M. The results are summarized in Table 3.

Table III

Effect of monensin .15, 1.5 and 15 x 10^{-5}M and stimulated with theophylline 10^{-3}M plus dibutyryl cyclic AMP 10^{-3}M on ouabain binding by shark rectal gland slices.

	Monensin x 10^{-5}		
	.15	1.5	15
Control	122.4±19.8	127.2±20.3	156.0±20.6
Stimulated	170.1±70.6[a]	189.4±41.9[b]	208.8±15.0[a]
Monensin	137.7±31.4[d]	148.8±29.5[a,d]	100.6± 9.1[a,c]
Alcohol	133.0±27.0	142.9± 5.0[a]	153.2±12.5

Values are mean ± SD. p values were calculated by "paired t" test.
a $p < 0.05$ compared to control
b $p < 0.005$ compared to control
c $p < 0.05$ compared to alcohol alone
d not significantly different from alcohol alone

Monensin, an ionophore that increases the entry of sodium into the cell, does not increase ouabain binding to slices of shark rectal gland. This result contrasts with the effect of theophylline and cyclic AMP which regularly stimulate ouabain binding. The mechanism of the stimulation of ouabain binding by theophylline and cyclic AMP is therefore unlikely to be an increase in activity of the sodium pump, since monensin, which causes a 30% increase in the intracellular sodium concentration and a 66% increase in the rate of oxygen consumption, does not evoke an increase in the binding of ouabain to slices of rectal gland. These results are consistent with our previous findings that cyclic AMP and theophylline stimulated ouabain binding in the absence of sodium and chloride as well as in the presence of bumetanide or furosemide. Thus, an increased transcellular movement of electrolytes is not necessary for the induction of increased ouabain binding by cAMP, and an increase in the sodium pump activity produced by monensin, rather than by cyclic AMP, does not enhance ouabain binding.

EVIDENCE THAT SODIUM IS SECRETED VIA THE PARACELLULAR PATHWAY IN THE SHARK RECTAL GLAND

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The current model for chloride secretion by the rectal gland of the shark can be described as follows. Two chlorides enter the cell across the basolateral cell membrane together with a sodium and a potassium through a neutral chloride sodium, and potassium cotransport system. The energy for this entry step is provided by the downhill gradient for sodium directed into the cell. The gradient for sodium is maintained by Na-K-ATPase. Chloride leaves the cell via a conductive pathway in the luminal membrane. Potassium recycles across the basolateral membrane through a potassium channel that can be blocked by barium. Sodium cannot cross the luminal