to the more dispersed and less organized microfilaments observed in the same region from eggs treated with 5 mM $MgCl_2$. These results suggest that *Ilyanassa* microfilaments are stabilized in the presence of 50 mM $MgCl_2$, another feature characteristic of actin-containing microfilaments.

Recent work (Pollard, T.C. et al., Cell Motility, Vol. B, p. 689, Cold Spring Harbor Laboratory) has demonstrated that tropomyosin can bind to muscle and *Acanthamoeba* F-actin and protect the filaments from fragmentation by osmium tetroxide. Similar studies were performed on *Ilyanassa* microfilaments incubated *in situ* after glycerination. Tropomyosin was extracted from chicken skeletal muscle with 1 mM dithiothreitol and isolated by ammonium sulfate and isoelectric precipitations. Glycerinated *Ilyanassa* eggs were incubated in buffer solutions containing tropomyosin and prepared for electron microscopy. Microfilaments from the lob constriction of tropomyosin-treated eggs often appeared long and straight, whereas microfilaments in control eggs most often assumed a network-like pattern. These results suggest that the actin-like protein in *Ilyanassa* microfilaments is capable of binding muscle tropomyosin, thereby acquiring resistance to OsO_4-induced fragmentation.

Ilyanassa eggs contain a prominent protein which, on SDS-PAGE slab gels, co-migrates with actin prepared from chick skeletal muscle. To determine whether the amount of this protein changes dramatically during polar lobe formation, we collected known numbers of eggs at each of several specific stages before and during the formation of the third polar lobe and concomitant first cleavage. Soluble proteins were extracted from yolk-containing, as well as from yolk-free fractions of detergent-lysed eggs and separated by SDS-PAGE on slab gels. The gels were stained with Coomassie blue and then scanned directly with a Joyce-Loebl double-beam recording microdensitometer (Model MK III C). The results indicated that the amount of soluble actin-like protein per egg rises by about 23% (above that in round eggs) as lobe formation begins, but then falls to about 30% below the level of round eggs when cleavage furrows and polar lobe constrictions display maximal numbers of microfilaments ultrastructurally.

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EFFECTS OF CALCIUM IONOPHORE A23187, CARBAMYLCHOLINE, AND RMI 12330A ON CHLORIDE SECRETION IN THE ISOLATED PERFUSED RECTAL GLAND OF *Squalus acanthias*

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Recent studies have described a cyclic AMP mediated active secretion of chloride in the rectal gland of the dogfish (Stoff et al., J. Expertl. Zool. 199:443-448, 1977; Silva et al., Amer. J. Physiol. 233(4):F298-F306, 1977). In many tissues in which activation of physiologic processes is mediated by cyclic AMP, calcium has been demonstrated to be a second intracellular messenger regulating the physiologic response. The divalent cation ionophore A23187 enhances calcium uptake or exchange in many epithelia and has been used extensively to study the effects of calcium on hormone mediated events. In mammalian intestinal tissue, A23187 has been demonstrated to stimulate chloride secretion in both rabbit ileal mucosa and the rabbit colon, without change in the intracellular level of cyclic AMP in these tissues (Bolton and Field, J. Membrane Biol. 35:159-173, 1977; Frizell, J. Membrane Biol. 35:175-187, 1977). In the present studies we investigated the effects of the calcium ionophore A23187 and carbamylcholine on chloride secretion in the isolated perfused rectal gland. The intestinal secretagogue carbamylcholine (carbachol) was chosen because stimulation of intestinal secretion by carbachol is independent of intracellular cyclic AMP and dependent on extracellular calcium (Bolton and Field, J. Membrane Biol. 35:159-173, 1977) and is thought to be mediated by increased cell permeability to calcium (Jaffer and Mitchell, Biochem. J. 160:163-169, 1976). In addition, we determined the effects of RMI 12330A, an organic cycloalkyl compound which inhibits both adenylate cyclase and cyclic AMP and ionophore A23187

induced changes in mucosal to serosal chloride flux in the rabbit ileum (Guellaen et al., Biochem. Pharmacol. 27:641-645, 1978 and Ilundain and Naftalin, in press).

Rectal glands were obtained from spiny dogfish (*Squalus acanthias*) of either sex weighing 2 to 6 kg. The rectal gland was removed, and cannulae were placed in the rectal gland artery, vein and duct and the gland was perfused by gravity flow from an oxygenated reservoir at a pressure of approximately 3.5-4.0 mm of mercury and a flow rate of 4-9 ml/min with a shark-Ringer perfusion solution containing: Na, 280; K, 5.5; Cl, 270; bicarbonate, 8; Ca, 2.5; Mg, 1.2; phosphate, 1; sulfate, 0.5; urea, 350; glucose, 5 (in millimoles/liter) as previously described (Silva et al., Amer. J. Physiol. 233(4): F298-F306, 1977). Ionophore A23187 was prepared as a 5×10^{-3} M stock solution in ethanol. The ionophore was added to perfusion medium while stirring with a magnetic stirrer. Solution in the reservoir was stirred to prevent precipitation of ionophore at 10^{-5} M and a faint opalescence was occasionally seen at this concentration.

Measurements of rectal gland fluid flow rate, chloride secretion, and rectal gland fluid chloride concentrations were made at ten-minute intervals throughout the experiments. All glands were perfused for 40 minutes until low basal rates of flow and chloride secretion were observed. Concentrations of ionophore of 10^{-6} M (three experiments) or 10^{-5} M (six experiments) were added after the fourth period (40 minutes) and the peak response obtained in the next two periods is given. Perfusion with ionophore was continued to 90 minutes. The perfusion fluid was then changed to contain theophylline (0.25 mM) and dibutyryl cyclic AMP (0.05 mM) without ionophore. Values are provided for the basal period, the peak ionophore response (40-60 min), the response at the end of the ionophore infusion and the peak response following theophylline and dibutyryl cyclic AMP (Table 1).

Table 1. Effects of calcium ionophore A23187 and carbachol on chloride secretion in the in vitro perfused rectal gland.

		secretion rate		
A23187 (10^{-6}-10^{-5}M, n=9)	Time (min)	V µl/h/g wet wt.	Cl^-secretion µEq/h/g wet wt.	RG Fluid [Cl] mEq/L
basal	30-40	184±47	76±17	416±14
peak ionophore response	40-60	448±127^{xx}	191±56^{xx}	416±17
end ionophore response	80-90	994±338	275±96	316±21^{++}
peak theophylline (0.25 mM) and db cyclic AMP (0.05 mM)	110-120	2931±574***	1010±179***	388±14
Carbachol (2 x 10^{-3}M, n=5)				
basal	30-40	166±69	78±29	441±6
peak carbachol response	40-60	476±96^{xxx}	211±35^{xxx}	444±12
end carbachol response	70-80	256±52^{++}	118±17^{++}	430±24
peak theopylline (0.25 mM) and db cyclic AMP (0.05 mM)	100-110	1818±521**	870±223**	462±5

For A23187, xx p<0.02 compared to basal, ++ p<0.02 compared to peak ionophore, *** p<0.001 compared to end ionophore (Student's paried t test)

For carbachol xxx p<0.001 compared to basal, ++ p<0.02 compared to peak carbachol, ** p<0.02 compared to end carbachol (Student's paired t test).

All values are mean ±SEM.

Following 30-40 minutes of unstimulated perfusion, rectal gland fluid flow rate and chloride secretion declined to 184 µl/h/g wet weight and 76 ± 17 µEq/h/g wet weight respectively. During the peak ionophore response, flow rate and chloride secretion increased approximately 2.5-fold to

448 µl/h/g wet weight and 191 ± 56 µl/h/g wet weight (both $p < 0.02$) without change in the chloride concentration of rectal gland fluid. Although mean flow rate and chloride secretion increased further during continuation of the ionophore, rectal gland fluid chloride concentration fell significantly to 316 ± 21 mEq/l. Following stimulation with theophylline and dibutyryl cyclic AMP, both flow rate and chloride secretion increased markedly, approximately 15-fold above baseline (2931 ± 574 µl/h/g wet weight and 1010 ± 179 µEq/h/g wet weight). In four experiments in which external calcium concentration was increased to 10 mM after addition of ionophore, no further increase in rectal gland chloride excretion was observed. Removal of all calcium and magnesium in the perfusion medium resulted in a prompt decline in the rectal gland fluid flow and chloride concentration during stimulation with dibutyryl cyclic AMP and theophylline. Therefore, experiments were carried out with 0.05 mM calcium in the medium. Addition of ionophore at 10^{-6} in the low calcium medium (three experiments) did not result in increased flow or chloride secretion.

The response to carbachol at 2×10^{-3} M indicated a biphasic response (Table 1). A peak response was seen 20 minutes after addition of carbachol as chloride secretion increased from 78 to 211 µEq/h/g wet weight without change in the chloride concentration of rectal gland fluid. Flow rate and chloride secretion declined during continued carbachol administration. In contrast to experiments with A23187, rectal gland fluid chloride concentration did not decline throughout the experiment. Following stimulation with theophylline and dibutyryl cyclic AMP, a marked increase in volume and chloride secretion was again observed. Experiments with carbachol were not performed in the presence of low calcium in the perfusion fluid.

Table 2. Effect of RMI 12330A on theophylline (0.25 mM) - dibutyryl cyclic AMP (0.05 mM) stimulated chloride secretion in the *in vitro* perfused rectal gland. (n=4)

	time (min)	secretion rate V µl/h/g wet wt.	Cl secretion µEq/h/g wet wt.	RG Fluid [Cl] mEq/L
Theophylline + db cyclic AMP	20-40	2437±270	1374±95	490±21
Theophylline + db cyclic AMP + RMI 12330A (5×10^{-4} M)	60-90	410±114^{xxx}	179±63^{xxx}	409±54
Theophylline + db cyclic AMP	110-130	1269±64^{+++}	499±97^{+++}	393±70

xxx $p<0.001$ compared to theophylline - db cyclic AMP; +++ $p<0.001$ compared to theophylline - db cyclic AMP + RMI 12330A.

All values are mean ±SEM.

The effect of RMI 12330A (5×10^{-4} M) on theophylline and dibutyryl cyclic AMP stimulated chloride secretion is shown in Table 2. Flow rate and chloride secretion were markedly inhibited by RMI and this effect was partially reversible when RMI was removed from the perfusion medium. In preliminary experiments using mounted segments of capsule stripped glands, a marked decline in theophylline-dibutyryl cyclic AMP stimulated short circuit current and p.d. was observed following addition of RMI, 10^{-4} M (Forrest and Zadunaisky, unpublished observations).

The present experiments demonstrate a modest but significant stimulation of basal chloride secretion in the dogfish rectal gland following addition of the divalent ionophore A23187 and carbamylcholine, an intestinal secretagogue. Although direct measurements of intracellular cyclic AMP and calcium influx were not made in these experiments, Bolton and Field (J. Membrane Biol. 35:159-173, 1977) have demonstrated that stimulation of intestinal secretion with A23187 and carbamylcholine in

the rabbit ileum is independent of changes in cyclic AMP but dependent on extracellular calcium. The present studies are consistent with the hypothesis that changes in intracellular calcium and cholinergic stimuli may modulate chloride secretion in the dogfish rectal gland.

We emphasize that in contrast to the modest effect of A23187 seen in the present studies, chloride secretion in the rabbit colon following ionophore is quantitatively similar to that observed following cyclic AMP (Frizzel, J. Membrane Biol. 35:175-187, 1977). Indeed the response to ionophore in the dogfish rectal gland is quantitatively much less than that observed in numerous other secretory epithelia (Candia et al., Amer. J. Physiol. 233(2):F94-F101, 1977). The reason for these differences is not apparent from the present studies.

FURTHER STUDIES ON THE MECHANISM OF CHLORIDE TRANSPORT IN THE RECTAL GLAND OF *Squalus acanthias*

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The rectal gland of the spiny dogfish, *Squalus acanthias*, secretes chloride against a steep electrochemical gradient. We have postulated that the secretion of chloride depends on the linked movement of sodium and chloride across the basolateral cell membrane, thus chloride entry into the cell follows the passive movement of sodium down its electrochemical gradient. The gradient for sodium is maintained by the continued activity of NaKATPase. The efflux of chloride from the cell into the duct lumen, completing the transcellular passage, would be effected down an electrical gradient, the lumen being less negative than the cell, and the potential difference overcoming the very steep chemical gradient. Sodium on the other hand would move through intercellular junctions since its extrusion across the luminal membrane against a large electrochemical gradient precludes simple diffusion. This putative model for the active secretion of chloride involves several different steps: (1) The linked entry of chloride and sodium into the cell. (2) The continued activity of NaKATPase. (3) The passive diffusion of chloride across the luminal membrane. (4) The paracellular movement of sodium. Previous reports from our laboratory have given evidence for the involvement of NaKATPase in this process, described the requirement for either sodium or chloride and shown that inhibition of chloride entry into the cells by pharmacological means impairs its transcellular movement. The present report provides additional evidence for the specificity of the carrier system for chloride and presents further support for the notion that the entry of chloride into the cell is necessary for transepithelial transport. In addition, the effect of an attempt at pharmacological blockade of the intercellular pathway for sodium is described.

Dogfish of either sex were taken by hook and line from Frenchman Bay and kept in marine livecars until they were killed, usually within three days of capture. The dogfish were killed by segmental transection of the cord and the rectal gland removed through an abdominal incision. The rectal gland artery, vein and duct were cannulated with PE90 tubing. The glands were then placed in either a plexiglass and aluminum or all glass perfusion chamber and kept at 15°-17°C by running seawater. The rectal glands were perfused by gravity at a pressure of 4 mm Hg and a flow of 1.2 to 7 ml/min. The perfusion medium composition, unless otherwise specified, was (in mM): Na^+ 280; K^+ 5; Cl 290; HCO_3 8; phosphate 1; Ca 2.5; Mg 3; sulfate 0.5; urea 350; glucose 5; pH 7.6 when gassed with 99% O_2 and 1% CO_2. In all experiments the glands were stimulated continuously with 0.25 mM theophylline and 0.05 mM dibutyryl cyclic-AMP. Rectal gland fluid was collected over periods of ten minutes. Changes in the composition of the perfusate were made at the end of two or more collection periods. Experimental periods were usually of 30 minutes duration divided into 10-minute intervals. Determination of chloride was done by amperometric titration in a Buchler-Cotlove chloridometer. Sodium and potassium were measured in an IL 143 flame photometer. Results are expressed as mean ± SEM (n).