that net Cl exit across the basolateral membrane is impaired. The reduction in cell K activity, in spite of a decrease in apical membrane K permeability, suggests inhibition of Na-K-pump activity at the basolateral membrane by reduced pH. This decrease in cell K activity would diminish the rate of KCl co-transport across the basolateral membranes causing cell Cl activity to rise. Finally, we estimate that KCl co-transport across the basolateral membrane could account for as much as 60% of net Cl absorption (Stewart et al., Bull. MDIBL. 20: 1980). It is of interest that this is the approximate degree of inhibition produced by acidification of the bathing media. Supported by grants from NIH (AM 27524 and AM 21345) and from Merck and Co. PLS was supported by a National Research Service Award (AM 05973), MJW by a National Pulmonary Faculty Training Award (HL07159), and RAF by a Research Career Development Award (AM 00713); from the NIH.

INTRACELLULAR CHLORIDE ACTIVITIES IN THE ISOLATED PERFUSED SHARK RECTAL GLAND

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The rectal gland of the spiny dogfish secretes a fluid rich in NaCl in the interest of maintaining salt homeostasis (Burger and Hess, Science, 131:670, 1960). Based on studies of Cl secretion by the isolated perfused rectal gland, Silva et al., Am. J. Physiol., 233:F298, 1977, proposed a model that appears to apply to a wide variety of secretory epithelia (Frizzell et al., Am. J. Physiol., 236:F1, 1979); this model is illustrated in Fig. 1. Two critical

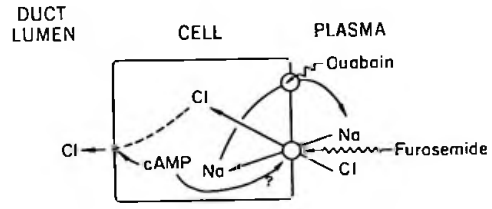

Fig. 1.--Model for chloride secretion. See text for details.

features of this model are an intracellular Cl activity greater than the value expected for electrochemical equilibrium and a Na-coupled entry of Cl into the cell across the basolateral membrane. To test these components of the model for the rectal gland, we used ion-selective microelectrodes to measure intracellular Cl activity under baseline conditions, when secretion was stimulated and when the gland was perfused with Na-free media.

METHODS

Rectal glands were obtained and perfused as previously described (Silva et al., Am. J. Physiol., 233:F298-F306, 1977). The perfusion medium contained (mM): Na, 288; K, 5; Mg, 2.5: Ca, 2.5; Cl, 293; HCO_3, 8; SO_4, 0.5; H_2PO_4, 1; and urea, 350. The solution was bubbled with 99% O_2 - 1% CO_2 and maintained at 15°C. Sodium-free solutions were prepared by substituting choline for Na. Secretion was stimulated by addition of 1 mM theophylline plus 0.05 mM 8-Bromo-cAMP to the perfusate.

A small area of capsule (10-30 mm^2) was removed to permit impalement of the epithelial cells with microelectrodes. The electrical potential difference between periglandular fluid and duct fluid (ψ_t) and the rate of secretion (J_v) were measured as previously described (Silva et al., Am. J. Physiol., 233:F298, 1977). Microelectrodes were advanced manually to the gland surface with a micromanipulator and cellular impalements were made by rapidly advancing the microelectrode with a piezo-electric driver (Physik Instruments, Berlin). The electrical potential

difference across the basolateral membrane (ψ_b) was referenced to the periglandular fluid. Three to seven cellular impalements were made with both conventional and ion-selective microelectrodes during each experimental condition in each tissue. Conventional and Cl-selective microelectrodes were constructed and calibrated, and the intracellular Cl activities were calculated using published formulae (Duffey et al., J. Memb. Briol., 50: 331, 1979).

RESULTS AND DISCUSSION

Results obtained under control and stimulated conditions are shown in Table 1. In agreement with previous

Table 1.--Stimulation of rectal gland secretion: cell Cl activities

Conditions	J_v ($\frac{ml}{hr.\ gm\ wet\ wt}$)	ψ_t (mV)	ψ_b (mV)	a_c^{Cl} (mM)	a_e^{Cl}/a_{eq}^{Cl}
Control	0.1	-3.2	-78	57	6.9
	±0.1	±0.6	± 2	± 6	±0.7
Stimulated	1.9*	-6.1*	-73	52	4.6*
	±0.7	±0.6	± 3	± 7	±0.5

Values are mean ± SEM for 7 glands. Glands were stimulated with 1.0 mM theophylline and 0.05 mM 8-bromo-cAMP.

a_e^{Cl}/a_{eq}^{Cl} refers to the ratio of measured Cl activity to the activity expected for an equilibrium distribution across the basolateral membrane. *$p < 0.05$.

results, the addition of theophylline and cAMP increased J_v almost 20-fold and increased ψ_t. Intracellular Cl activity (a_c^{Cl}) was six times greater than the value predicted for electrochemical equilibrium across the basolateral membrane. Neither ψ_b nor a_c^{Cl} changed significantly following the onset of secretion.

To determine whether the intracellular accumulation of Cl is dependent on the presence of Na in the perfusate, stimulated glands were perfused with Na-free solutions and a_c^{Cl} determined. The results are shown in Table 2. In

Table 2.--Effect of Na-free perfusate on rectal gland Cl activities

Condition	J_v ($\frac{ml}{hr.\ gm\ wet\ wt}$)	ψ_t (mV)	ψ_b (mV)	a_c^{Cl} (mM)	a_e^{Cl}/a_{eq}^{Cl}
Control	2.1	-3.4	-71	67	5.9
	±0.9	±1.1	± 2	± 5	± .3
Na-free	0*	1.5	-68	28*	2.1*
		±1.2	± 1	± 4	± .2
Recovery	1.6	-4.0	-69	-61	4.9
	±0.5	±1.3	± 2	± 8	± 4

Values are mean ± SEM for 4 glands. Theophylline and cAMP were present during all conditions. *$p < 0.05$.

agreement with the findings of Silva et al., perfusion with Na-free solutions decreased the secretory rate and ψ_t. Although ψ_b was not affected by perfusion with Na-free media, a substantial decrease in a_c^{Cl} was observed.

Our findings will be discussed with reference to the model of Cl secretion depicted in Fig. 1. According to this model, Cl enters the cell across the basolateral membrane coupled to Na. This results in accumulation of intracellular Cl to an activity greater than that predicted for electrochemical equilibrium, thus providing the electrochemical driving force for Cl exit across the apical membrane. Intracellular Na activity is maintained at a low value by the Na-K-pump located at the basolateral membrane (Eveloff et al., J. Cell Biol., 83:16, 1979).

We found that intracellular Cl activity was approximately six times higher than the value predicted for electrochemical equilibrium across the basolateral membrane. The observed cell Cl activity (c.a. 60 mM) is in excellent agreement with the value obtained by (Duffey et al., Bull. MDIBL. 18:73, 1978) from preliminary studies and is also in good agreement with chemical determinations of intracellular Cl concentration (Silva et al., Am. J. Physiol., 233:F298, 1977). The transepithelial electrical potential difference and Cl activity of the luminal fluid at the site of secretion are not known with certainty due to the complex architecture of the gland. However, we can estimate the electrochemical driving force for Cl, $\Delta\tilde{\mu}_{Cl}$, across the apical cell membrane based on the Cl activity in the emergent duct fluid (approximately 340 mM), a_c^{Cl}, and the calculated electrical potential difference across the apical membrane ($\psi_a = \psi_t - \psi_b = 75$ mV). Using these values, $\Delta\tilde{\mu}_{Cl}$ across the apical membrane under non-stimulated conditions is 31 mV favoring Cl movement from cell to duct lumen. Thus, a secretagogue-induced increase in the Cl permeability of the apical membrane would permit Cl exit from the cell by diffusion.

During steady-state stimulation of secretion with theophylline and cAMP, neither ψ_b nor a_c^{Cl} changed significantly. During stimulation the calculated $\Delta\tilde{\mu}_{Cl}$ across the apical membrane is approximately 20 mV, directed from cell to lumen. The failure of cAMP and theophylline to markedly affect a_c^{Cl} and $\Delta\tilde{\mu}_{Cl}$ suggests that secretagogues not only stimulate Cl exit across the apical membrane but also enhance Cl entry across the basolateral membrane. In this manner, a reduction in a_c^{Cl}, which would compromise the driving force for diffusional Cl exit across the apical membrane, is obviated. The factors which govern the activity of NaCl co-transport across the basolateral membrane are not known, but it seems reasonable that cAMP may be the controlling factor. This proposal is in accord with the findings of Silva et al. Bull. MDIBL., 49:70, 1979). In rectal glands treated with ouabain to inhibit transport, addition of theophylline and cAMP to the perfusion solution increased Cl entry into the epithelial cells; this effect is consistent with accelerated Cl entry via NaCl co-transport since it was blocked by furosemide.

We also evaluated the effect of Na substitution on a_c^{Cl}. As shown in Table 2, replacement of Na with choline in the perfusion solution decreased a_c^{Cl}. Although a_c^{Cl} was still twice that predicted for electrochemical equilibrium across the basolateral membrane, $\Delta\tilde{\mu}_{Cl}$ across the apical membrane decreased to approximately 4 mV. This indicates that a_c^{Cl} did not differ significantly from the value predicted for electrochemical equilibrium across the apical cell membrane (i.e., $\Delta\tilde{\mu}_{Cl} = 0$) during perfusion with Na-free media. Therefore, the presence of Na in the perfusion media is required for the maintenance of a high intracellular Cl activity, a finding consistent with NaCl co-transport across the basolateral membrane. This conclusion is in agreement with that of Eveloff et al., Pflugers Arch., 378:87, 1978, who demonstrated Cl-dependent Na transport in a vesicle preparation from the rectal gland enriched in basolateral membranes. Supported by NIH (AM-27524) and Merck and Co. MJW was supported by a National Pulmonary Faculty Training Award (HL-07159); PLS by a National Research Service Award (AM-05973) and RAF by a Research Career Development Award; from the NIH.