The pressure is derived from the passive and active wall tension, T_p and T_a, respectively, using Laplace's law:

$$P = (T_p + T_a)/r$$

The passive tension is only a function of the local radius. The active tension was assumed to be the product of two factors of which one depends on the radius and the other on the time after the onset of excitation. This assumption is justified by previously reported experiments (Cleemann, Dillon & Morad, MDIBL Bull. 18:54-56, 1978). The expressions for length-tension relations and for the time course of the activation are approximations of previously measured curves (Cleemann, Morad & Dillon, MDIBL Bull. 16:8-13, 1976). The curves in panels D, E, and F of Figure 3 are obtained by step-wise integration of these nonlinear partial differential equations. The initial volume of the heart in the three panels was chosen to match approximately the magnitude of the resting and active pressures in panels A, B, and C. The agreement between measured and calculated pressures is satisfactory. The computer simulation shows that transmission delay is most prominent when the heart is least distended. The first contraction at the stimulated end is transmitted with some delay (d) to the other end and the contraction here is to some degree reflected back (r) to the stimulated end. As transmission velocity increases with increasing filling pressure the reflected wave fuses with the earlier pressure peak. The pressure difference between the two ends of the heart is related to the viscous resistance to backflow through the traveling constriction of the heart. The calculations assume a uniform radius of the relaxed heart. The narrow section present near the center of the heart may account for some of the differences between measured and calculated pressures. However, the calculations are sufficiently realistic to confirm that the pressure difference between the two ends of the heart remains of the order of 0.1 cm of water even when the increased filling pressures produce much larger total pressures.

Experiments with the whole heart were also performed under conditions where the lumen and the outside were perfused with different solution. Epinephrine was found to be effective only when added to the luminal perfusate. Epinephrine increased the strength of contraction and reversed the conduction block which often developed at the narrow central section of the heart at the end of the experiment.

While applying a subthreshold pulse through the cannula at one end of the heart and advancing an electrode through the cannula at the other end of the heart it was possible to measure the electrical space constant of the whole heart. The resistivity of the myocardial wall calculated from these measurements was not larger than that obtained from the bulge clamped preparation. This finding may suggest that damaged edges of the bulge-clamped preparation do not compromise electrical resistance measurements.

EFFECT OF "LOOP DIURETICS" ON SODIUM TRANSPORT BY PLASMA MEMBRANE VESICLES ISOLATED FROM THE RECTAL GLAND OF SQUALUS ACANTHIAS

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Chloride reabsorption in the renal thick ascending limb of Henle's loop (TALH) in mammals and chloride secretion in the rectal gland of the shark have many similarities. Both chloride transport mechanisms are active, sodium dependent and inhibited by ouabain (1,2). Additionally, it was found that furosemide, a potent diuretic, inhibits salt transport in the rectal gland of Squalus acanthias as well as Scyliorhinus canicula (2,3). In the latter animals the diuretics bumetanide and piretanide also decrease the rate of chloride secretion in the perfused in situ rectal gland (3).

Recently it has been demonstrated that plasma membranes isolated from the rectal gland of Squalus acanthias contain a sodium-chloride cotransport system as indicated by the chloride dependence of sodium transport in these vesicles (4). The chloride-dependent sodium transport in the vesicles was also inhibited by furosemide (4). The

aim of the present investigation was to study in more detail the interaction of the various diuretics that have been shown to inhibit chloride secretion in the intact gland and the TALH of the kidney with the sodium-chloride contransport system in the isolated membranes. Such studies should provide further evidence for the involvement of the sodium-chloride cotransport system in the active chloride secretion; they can also contribute to the knowledge of the mechanism of action of the diuretics at the cellular and subcellular level.

Spiny dogfish were caught by hook and line in Frenchman Bay and killed by segmental transsection of the spinal cord. After removal from the animal, the rectal glands were perfused with dogfish Ringer containing 0.05 mM dibutyryl cyclic AMP and 0.25 mM theophylline in order to maximally stimulate chloride secretion. Plasma membranes were prepared from two rectal glands by differential centrifugation (4). Sodium uptake by the vesicles was studied by a rapid filtration method as described previously (4).

The effect of diuretics on sodium uptake by isolated rectal gland plasma membrane vesicles is shown in Fig. 1.

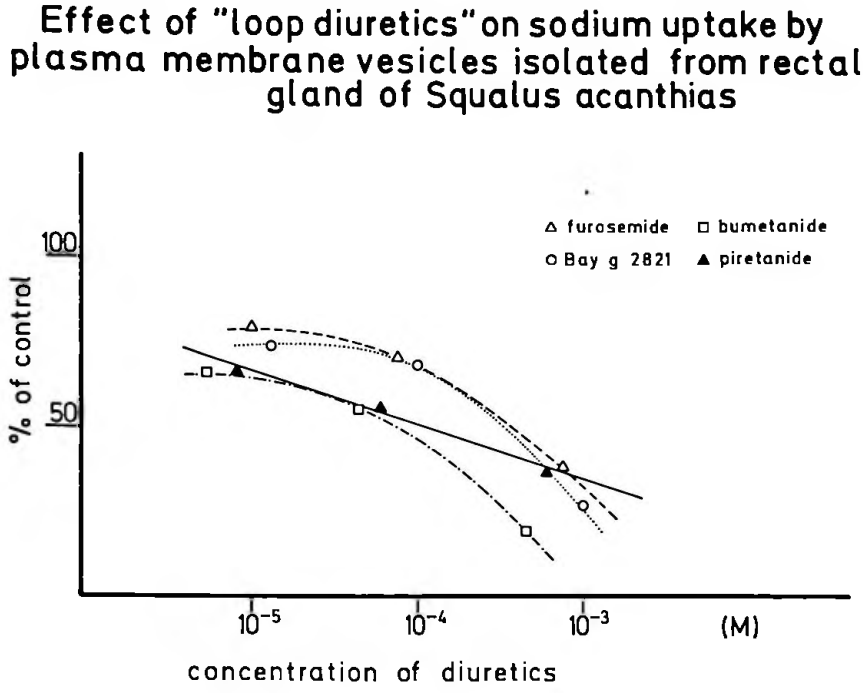

Figure 1. Effect of anion gradients and bumetanide on sodium uptake into rectal gland plasma membrane vesicles. The membranes were prepared in (in mM): mannitol 100, $Mg(NO_3)_2$ 1.2, Tris-HEPES 20 (pH 7.6). The transport medium contained in general (in mM): mannitol 100, $Mg(NO_3)_2$ 1.2, Tris-HEPES 20 (pH 7.6), Na salt 2, K salt 98. ^{22}Na or ^{3}H mannitol uptake into the vesicles was studied in the presence of a NaCl-KCl gradient (□), a $NaNO_3$-KNO_3 gradient (○) or in the presence of 6×10^{-4}M bumetanide plus NaCl-KCl gradient (□) or bumetanide plus a $NaNO_3$-KNO_3 gradient (○) at 15°C. The bumetanide inhibition of ^{22}Na or ^{3}H mannitol uptake was investigated in plasma membrane vesicles preincubated for 5 min at 0°C with 10^{-5}M bumetanide. A single experiment is shown.

Two points are important: one, the sodium uptake by the vesicles is stimulated markedly by chloride in comparison to nitrate and two, the diuretic, in this case bumetanide, inhibits sodium uptake only in the presence of chloride but not in the presence of nitrate. The uptake of mannitol, used as a control to assure that the intravesicular space and general membrane permeability remain unchanged, is neither affected by the anion replacement nor by the addition of the diuretic. Table 1 summarizes the results obtained with the diuretics bumetanide, piretanide, Bay G 2821 and furosemide in the concentrations 10^{-5}, 10^{-4} and 10^{-3}M. All diuretics inhibit the chloride

TABLE 1: EFFECT OF DIURETICS ON CHLORIDE DEPENDENT SODIUM UPTAKE BY PLASMA MEMBRANE VESICLES ISOLATED FROM THE RECTAL GLAND OF SQUALUS ACANTHIAS

Diuretic	Concentration (M)	Sodium Uptake	
		% of Control	% Inhibition
Bumetanide	6.5×10^{-4}	19.5 ± 3.8	80.5
	6.5×10^{-5}	50.8 ± 4.5	49.2
	7.5×10^{-5}	64.2 ± 3.2	35.8
Bay g 2821	1.0×10^{-3}	22.0 ± 4.2	78.0
	1.0×10^{-4}	63.7 ± 10.0	36.3
	1.2×10^{-5}	69.9 ± 5.0	30.1
Piretanide	7.9×10^{-4}	34.0 ± 2.3	66.0
	7.9×10^{-5}	56.0 ± 2.0	44.0
	9.1×10^{-5}	61.4 ± 4.1	38.6
Furosemide	8.7×10^{-4}	39.6 ± 1.6	60.4
	8.7×10^{-5}	72.2 ± 3.1	27.8
	1.0×10^{-5}	76.9 ± 2.2	23.1
Ouabain	8.7×10^{-4}	78.0 ± 4.1	22.0
Diamox	8.7×10^{-4}	76.0 ±6.8	24.0

The values represent the means ± S.D. from 2 to 3 experiments, each value is the average of the uptake measured at 1, 1.75 and 2.5 min. Prior to the transport experiment, the membranes were incubated for 5 min at 0°C with 10^{-5} M of the diuretic under investigation.

dependent sodium uptake in a concentration dependent manner. The highest inhibition is observed with 10^{-3}M bumetanide, which inhibits approximately 80 per cent of the chloride dependent sodium uptake. At the same concentration, furosemide is the least potent inhibitor, inhibiting sodium uptake by 40 per cent. In Fig. 2 the dose response curves for the four diuretics are shown. The shape of the curves for bumetanide, Bay g 2821 and furosemide is similar and the sensitivity of the sodium-chloride transport system is in the order bumetanide Bay g 2821 = gurosemide. A half maximal inhibition is observed at approximately 8×10^{-5}M, 5×10^{-4}M and 5×10^{-4}M, respectively. The dose response curve for piretanide is flatter, the concentration needed for half-maximal inhibition of the chloride dependent sodium flux is about 1×10^{-4}M. Also shown in Table 1, ouabain and Diamox at 10^{-4}M had only a small, insignificant effect on the sodium transport.

Qualitatively, the same order of potency for the diuretics is seen for the plasma membrane vesicles of Squalus acanthias and the sensitivity of the chloride secretion rate in the rectal gland of Scyliorhinus canicula. Thus, the conclusion seems to be valid that the sodium-chloride cotransport system found in the vesicles is involved in chloride secretion and is one target for the diuretics. Qualitatively, however, there is a large difference between the concentration of diuretics needed for half-maximal inhibition in isolated membrane vesicles and in the intact gland. In the isolated plasma membrane vesicles, 5 to 10 times higher concentrations are necessary for inhibition of sodium

Effect of bumetanide on solute uptake by plasma membrane vesicles isolated from rectal gland of Squalus acanthias

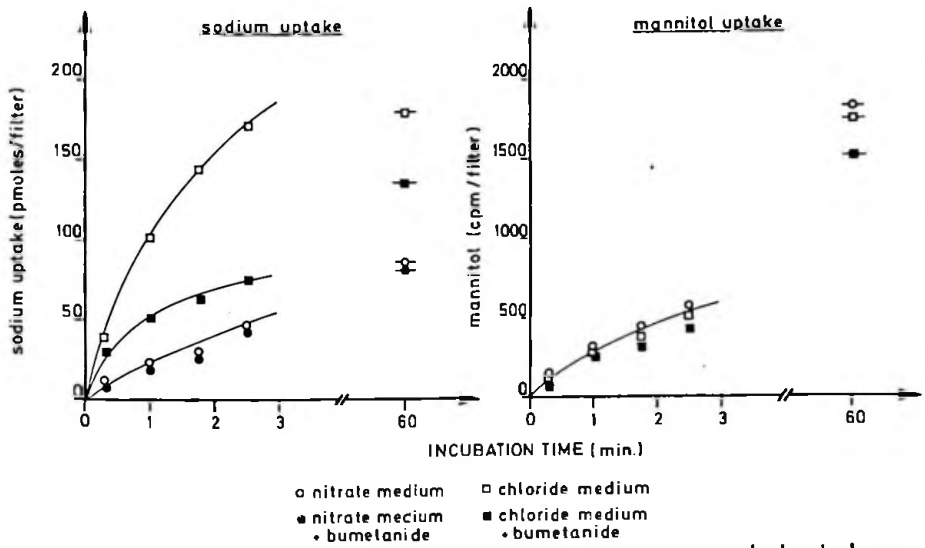

Figure 2. Effect of diuretics on the chloride dependent sodium uptake into rectal gland plasma membrane vesicles. The vesicles were prepared as in Fig. 1. The transport medium contained (in mM): mannitol 100, $Mg(NO_3)_2$, Tris-HEPES 20 (pH 7.6), NaCl 2, KCl 98 plus the diuretics in increasing concentrations from 10^{-5} to 10^{-3}M. The calculated inhibitory constants (K_i) for the diuretics are: bumetanide 8×10^{-5}M, piretanide 1×10^{-4}M, Bay g 2821 5×10^{-4}M and furosemide 5×10^{-4}M. The values represent the means of 2 to 3 experiments and are the average of uptake values measured at 1, 1.75 and 2.5 min. Prior to the transport experiment, the membrane vesicles were preincubated for 5 min at 0°C with 10^{-5}M of the diuretic.

transport. Similarly, in the TALH, the order of potency of the diuretics is identical but a two order of magnitude difference of sensitivity exists.

Several factors may play a role, first, the diuretics may act at additional sites in the cell thus explaining the higher diuretic sensitivity of the intact cell. Secondly, the transport experiments are performed at a low sodium concentration (1 mM compared to 280 mM in the perfusion fluid of the intact gland). This low sodium concentration may affect the affinity of the diuretics to the sodium-chloride cotransport system, similar to the effect of sodium on the affinity of the renal sodium-glucose cotransport system to phlorizen (5). Experiments such as binding studies of diuretics to isolated membranes could clarify this point. Supported in part by USPHS Grant AM 05841 and the Am. Heart Assn.-Maine Affiliate.

PIGMENTED SINUSOIDAL LINING CELLS IN ELASMOBRANCH LIVER

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Elasmobranch livers range in color from gray to a dark brownish-green. Histological examination of liver tissue fixed in 10% formalin and stained with hematoxylin and eosin revealed isolated brown pigmented cells which were situated within the region of the endothelial cells of the hepatic sinusoids, and were particularly prominent in livers from the small skate, Raja erinacea (Fig. 1A). Tissue was prepared for transmission electron microscopy by perfusing livers in the isolated state from 1 kg male skates as previously described (Reed, J.S., N.D. Smith, N. Tavoloni and J.L. Boyer, Bull. Mt. Desert Island Biol. Lab., 16:83-84, 1976). After a 30-minute perfusion with elasmobranch Ringers at 15°C, the livers were fixed by portal vein perfusion with Doyle's modification of Karnovsky's solution at