The methylmethacrylate casting material used is thought to be vasoactively inert. It is replacing an active biological tissue (blood) and the consequences of this on the resulting vascular compartment sizes are not known. This study shows that large discernible differences in the effects of acetylcholine and epinephrine on the microcirculation can be seen using this casting material and technique. This work was supported by departmental funds from the Department of Surgery, Mt. Sinai School of Medicine and by V.A. research funds.

40 ELECTROLYTE TRANSPORT BY THE ALKALINE GLAND OF THE LITTLE SKATE, RAJA ERINACEA. MECHANISM OF LUMINAL ALKALINIZATION

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Prior studies with the alkaline gland of the little skate Raja erinacea demonstrated that this tissue: (1) maintains a lumen negative potential difference of 16 mV with respect to the serosal solution; (2) actively transports Cl from the serosal to mucosal solution under short circuit conditions; and (3) alkalinizes the luminal contents to a pH of 9.2 (Maren et al., Comp. Biochem. Physiol. 10:1, 1963). To further investigate the transport properties of this tissue, we measured: (1) transepithelial unidirectional fluxes of Na and Cl across short-circuited alkaline gland in vitro; (2) luminal alkalinization using a pH stat technique with alkaline gland mounted in Ussing chambers; and (3) transapical membrane potentials using conventional intracellular microelectrode techniques.

METHODS

Male skates, Raja erinacea, were caught in nets in Frenchman Bay and maintained in running seawater tanks until used. Skates were sacrificed by transection of the spinal cord, the pair of alkaline glands removed from their surrounding connective tissue using fine curved forceps, opened along the epididymal border and placed in ice cold Ringer solution containing (mmols/l): Na,290; K,5; Ca,3.8; Mg,3.3; Cl,299; HCO_3,10; urea,350; and glucose,5. The pH was 8.4 when gassed with air.

Transepithelial Na and Cl fluxes were determined under short-circuit conditions as previously described (Field et al., J. Memb. Biol. 41:265, 1978). Luminal alkalinization was measured with tissues mounted in standard Ussing chambers and bathed by 10 ml of normal Ringer on the serosal surface and 10 ml of HCO_3-free Ringer on the mucosal surface (NaCl substituted for $NaHCO_3$ buffered with 0.5 mM HPO_4-H_2PO_4). The serosal solution pH was 7.6 when gassed with 1% CO_2 in O_2. The luminal bathing solution was maintained at pH 5.5 using a pH stat (Radiometer, Copenhagen). The titrant was 3.98 mM H_2SO_4.

Measurement of the electrical potential profile was similar to that described by Welsh et al., (submitted for publication).

RESULTS AND DISCUSSION

Electrical and flux measurements. Alkaline glands bathed on both surfaces by normal Ringer, bubbled with air and maintained at 15°C had a transepithelial potential (ψ_t) of 6.9 ± 0.6 mV (n = 28) serosa positive with respect to the luminal solution. ψ_t increased during the first 45-60 min. after mounting and remained stable for up to 8 hrs.

Unidirectional and net Na and Cl fluxes, short-circuit current (I_{sc}) and transepithelial conductance (G_t) are presented in Table 1.

Under short-circuit conditions, Na fluxes were equal in both directions and I_{sc} could be accounted for entirely by a net movement of Cl from serosa to mucosa. Table 1 also shows that Cl secretion is dependent on the presence of Na in the bathing solutions. These results suggest that Cl secretion by alkaline gland may involve a neutral coupled NaCl cotransport process at the basolateral membrane consistent with the model described by Frizzell et al., (Am. J. Physiol., 236:F1, 1979). Support for this hypothesis is provided by the finding that ouabain (10^{-4}M) added to the serosal bathing medium completely abolished I_{sc}.

Table 1. Na and Cl fluxes across the alkaline gland of the skate.

condition	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	I_{sc}	G_t
control (6)	4.1±0.8	3.7±0.8	0.4±1.0	--	--	--	1.6±0.1	7.0±0.6
control (6)	--	--	--	2.3±0.5	4.2±0.5	-1.8±0.5	1.6±0.2	7.2±0.6
Na-free (5)	--	--	--	2.9±0.7	2.8±0.6*	0.1±0.6*	0.2±0.4*	3.6±0.4*

All values are in $\mu Eq/h \cdot cm^2$ except G_t which is in mS/cm^2, mean ± S.E.M. for n animals.
*$p < 0.05$

Consistent with prior studies (Maren et al., Comp. Biochem. Physiol., 10:1, 1963), we found that the gland fluid pH was significantly higher than the pH of the plasma (gland fluid pH = 9.4 vs plasma pH = 7.4). To determine the rate of luminal alkalinization, we measured the amount of acid required to maintain the luminal pH constant at 5.5 when bathed with normal Ringer on the serosal surface and HCO_3-free Ringer on the mucosal surface. Under control conditions, in vitro preparations of alkaline gland required 3.0 ± 0.2 $\mu Eq/hcm^2$ (n = 6) of H^+ to maintain the pH at 5.5. Replacement of Na with choline in both Ringer solutions produced no change in the rate of luminal alkalinization. However, when Cl was replaced with either gluconate or SO_4 and mannitol, or when the CO_2 content of the gas mixture was reduced by gassing with air, the rate of luminal alkalinization decreased to 1.3 ± 0.3 (n = 6) and 2.0 ± 0.1 $\mu Eq/h \cdot cm^2$ (n = 4), respectively. These results indicate that luminal alkalinization is Cl dependent, non-electrogenic and may involve an anion exchange mechanism.

Measurement of the electrical potential profile and relative membrane resistances. Advancement of the microelectrode from the luminal solution into an epithelial cell resulted in an abrupt negative deflection which remained stable for several minutes.

A histogram of the values of ψ_a obtained from 31 cellular impalements in 6 glands under control conditions revealed that the values of ψ_a are distributed normally about a mean of -39.6 mV.

Control values for the electrical properties of alkaline gland and the effects of unilateral replacement of Cl with either gluconate or SO_4 and mannitol are shown in Table 2. Replacement of Cl in the mucosal solution resulted in an increase in ψ_t, a depolarization of ψ_a and ψ_b and an increase in R_t and fractional apical membrane resistance ($f_r = \frac{\Delta\psi_a}{\Delta\psi_t}$).

Table 2. Effects of serosal or mucosal Cl-replacement on transepithelial and cell membrane potentials, and fractional and transepithelial resistances.

condition	ψ_t (mV)	ψ_a (mV)	ψ_b (mV)	R_t (ohms cm^2)	f_r
control (5,107)	5.4±0.4	-44.7±2.9	50.0±2.8	103±12	0.39±0.09
Cl-free M (5,26)	18.6±2.0	2.3±5.2	16.3±4.0	143±20	0.43±0.09
Cl-free S (5,25)	-3.5±1.2	-42.7±3.2	39.2±4.1	154±19	0.18±0.04

Numbers in parentheses represent the number of glands and number of impalements, respectively. See text for composition of solutions.

Replacement of Cl in the serosal solution resulted in a small decrease in ψ_b, a decrease in ψ_t and f_r and an increase in R_t. Measurements of ψ_a and ψ_t were taken several minutes after the solution change to allow ψ_t to stabilize. The changes in ψ_t and R_t can be explained by the diffusion potential produced in the shunt due to the different permeabilities to Cl and gluconate or SO_4 (Cl > SO_4 or gluconate). Changes in f_r and the cell potentials produced by mucosal or serosal Cl replacement are consistent with the presence of a large Cl conductance at the apical membrane and a small but significant Cl conductance at the basolateral membrane.

The effects of replacing Na with choline are shown in Table 3. Replacement of Na in the mucosal solution

Table 3. Effects of serosal or mucosal Na-replacement on electrical properties of alkaline gland.

condition	ψ_t (mV)	ψ_a (mV)	ψ_b (mV)	R_t (ohms cm^2)	f_r
control (4,114)	5.8±0.7	-45.2±3.7	50.9±3.5	103±16	0.38±0.13
Na-free M (4,17)	-14.4±4.4	-40.8±4.7	26.5±8.4	172±40	0.29±0.14
control (5,129)	5.3±0.5	-43.0±3.7	48.2±3.7	95±15	0.37±0.07
Na-free S (5,21)	17.2±2.2	-33.9±3.8	51.1±5.2	127±22	0.21±0.03

Numbers in parentheses represent the number of glands and number of impalements, respectively. See text for composition of solutions.

resulted in a decrease in ψ_t and ψ_b, an increase in R_t with no significant change in either ψ_a or f_r. Serosal solution Na replacement resulted in an increase in ψ_t and R_t, a decrease in ψ_a and f_r and no significant change in ψ_b. The effects of Na replacement in either solution on ψ_t and R_t can be explained by the bionic potential produced in the shunt due to the different permeabilities to Na and choline (Na > choline). The finding that no change in ψ_a or ψ_b occurred with Na replacement in the mucosal or serosal solution, respectively, suggests that there is no significant conductance to Na in either membrane. The changes in ψ_b and ψ_a produced by Na replacement in the ipsilateral solution may be due to a peculiar effect of choline as noted by Reuss and Finn (J. Memb. Biol. 25:115, 1975) in Necturus gallbladder.

The effects of increasing mucosal or serosal K concentration from 5 to 50 mM by replacing Na with K and the effects of 1 mM Ba in the serosal bathing solution on the electrical properties of the alkaline gland are shown in Table 4. Increasing mucosal solution K concentration from 5 to 50 mM had no significant effect on any of the electrical properties measured. However, increasing serosal K concentration resulted in a decrease in ψ_t, ψ_a and ψ_b and an increase in f_r consistent with the presence of a K conductance in the baso lateral membrane of the alkaline gland. Further support for a K conductqnce at the basolateral border is provided by the decrease in ψ_t, ψ_a and ψ_b produced by the addition of 1 mM Ba to the serosal bathing solution.

A model consistent with these results is shown in Figure 1. The major features of this model include: (1) a coupled NaCl cotransport mechanism at the basolateral border; (2) Na which enters with Cl is recycled back to the serosal solution by the Na/K pump mechanism; (3) Cl which enters with Na presumably accumulates inside the cell above its electrochemical equilibrium and exits across the luminal membrane by a conductive process; (4) K which enters the cell in exchange for Na, exits across the basolateral membrane via a Ba sensitive

Table 4. Effects of serosal or mucosal K concentration or Ba on electrical properties of alkaline gland.

condition	ψ_t (mV)	ψ_a (mV)	ψ_b (mV)	R_t (ohms cm^2)	f_r
control (4,95)	5.3±0.7	-45.2±3.7	50.4±3.6	102±16	0.39±0.09
50 mM K on M (4,13)	4.4±0.5	-42.1±2.9	46.6±2.6	108±21	0.27±0.08
control (5,107)	5.4±0.4	-44.7±2.9	50.0±2.8	103±12	0.39±0.09
50 mM K on S (5,21)	2.7±0.2	-29.0±1.4	31.7±1.2	90±10	0.63±0.13
control (3,70)	4.6±0.1	-44.3±6.6	48.9±6.7	74±13	0.44±0.10
Ba 1mM on S (3,18)	3.5±0.6	-35.3±5.1	38.9±5.6	79±16	0.41±0.07

Numbers in parentheses represent the number of glands and number of impalements, respectively. See text for composition of solutions.

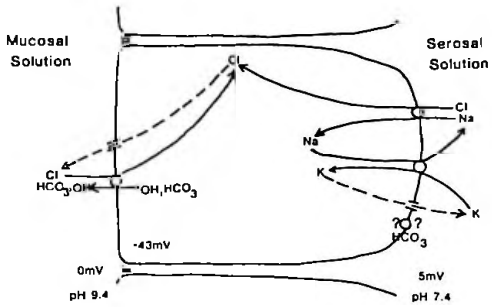

Figure 1.--Model of electrolyte transport by the alkaline gland.

K conductance and may largely determine the value of ψ_b; and (5) luminal alkalinization is accomplished by an increase in luminal solution HCO_3^-/CO_3^{-2} (OH^-) concentration which is Cl dependent. The exact mechanism of this process may involve an anion exchange mechanism at the apical membrane. Cl entering the cell by this process could recycle back to the mucosal solution thru the Cl conductance in this membrane. Whether the HCO_3^-/CO_3^{-2} (OH^-) which accumulates in the lumen arises from a net movement of HCO_3^-/CO_3^{-2} (OH^-), from cellular metabolism or from some combination of these processes is not known.

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SODIUM CHLORIDE COTRANSPORT BY SQUALUS ACANTHIAS RECTAL GLAND: POTENTIAL SITES OF REGULATION BY CYCLIC AMP

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INTRODUCTION

Experiments performed on isolated perfused rectal glands have demonstrated that secretion of sodium chloride is markedly stimulated by cyclic AMP or vasoactive intestinal peptide (Stoff et al., J. Exp. Zoology