SHORT-CIRCUIT CURRENT IN FLAT SHEETS OF THE RECTAL GLAND OF SQUALUS ACANTHIAS: EFFECTS OF 8-CHLOROPHENYLTHIO CYCLIC AMP, ION SUBSTITUTION AND FUROSEMIDE

John N. Forrest, Jr., David Andrews, and David C. Dawson, Department of Medicine, Yale University School of Medicine, New Haven, Connecticut, and Department of Physiology, University of Michigan, Ann Arbor, Michigan

In the isolated perfused rectal gland of Squalus acanthias, NaCl secretion is stimulated by cyclic AMP and its analogs and is dependent upon the presence of both sodium and chloride in the perfusate. Measurements of potential difference (P.D.) across the perfused gland (from perfusion chamber to rectal gland duct) have shown lumen negativity of 6-7 mV increasing to 15-19 mV on stimulation with theophylline and dibutyryl cyclic AMP (Silva et al., Am. J. Physiol., 233, 1977). Zadunaisky and Silva (Bull. MDIBL 16: 109, 1976) and Zadunaisky and Garretson (Bull. MDIBL 17: 102, 1977) mounted sections of rectal gland as a thick membrane in an Ussing chamber and reported a maximum short-circuit current (I_{sc}) of 16 $\mu A/cm^2$ following stimulation with theophylline and dibutyryl cyclic AMP. Measurement of bidirectional fluxes of ^{36}Cl suggested that this I_{sc} was due to net Cl movement from serosal to mucosa solutions.

The present studies were undertaken to explore further the feasibility of electrical measurements in mounted flat sheets of rectal gland and to evaluate the ionic dependence of I_{sc} in this tissue.

Methods

The rectal gland was removed and kept moist with a gauze soaked in shark Ringers. The proximal and distal ends of the gland were pinned on a soft plastic sheet and a longitudinal incision was made through the capsule on the dorsal surface and the capsule was stripped off with blunt dissection. With this method the entire capsule could be removed in one or two pieces. The initial incision was then extended through the gland to the central duct exposing the mucosal surface. When comparison of capsulated and decapsulated sections were made, adjacent portions were sectioned and the capsule removed from one. Sections for study were taken from the mid-portion of the gland. Free edges of both serosal and mucosal tissue were secured with Eastman 910 adhesive onto thin plastic holders with a central aperture of 0.64 cm^2 and the tissue mounted in a Ussing chamber bathed on both sides with regular shark Ringer (Forrest et al., Bull. MDIBL 18: 10, 1978) containing 10 mM glucose. Both mucosal and serosal solutions were vigorously stirred with a gas mixture of 99%-1% CO_2, at pH 7.4-7.5. All experiments were performed at room temperature (20-22°C). The chamber was equipped with four electrodes; two for monitoring P.D. and two for passing current across the tissue. An electronic voltage clamp maintained the P.D. at 0 mV and tissue conductance was determined from the change in transepithelial current producted by a brief (1 sec.) 5 mV change in change in clamping potential.

Results

Results are summarized in Table 1. When paired sections of capsulated and decapsulated portions of the gland were compared following the addition of dibutyryl cyclic AMP (5×10^{-5}M) and theophylline (2.5×10^{-4}M) to the serosal media, the maximum steady-state I_{sc} was higher in decapsulated sections in 3 of 4 glands (29 vs 21 $\mu A/cm^2$) and maximum stimulation occurred earlier in decapsulated sections. More striking results were seen with the cyclic AMP analog 8-chlorophenylthio cyclic AMP (8 cpt-cyclic AMP). Maximum stimulation with $1-3 \times 10^{-5}$M 8-cpt cyclic AMP + theophylline (2.5×10^{-4}M) was 72± 16 $\mu A/cm^2$ in decapsulated glands vs 15±4 in five paired capsulated portions. Overall, in 19 experiments with 8-cpt-cyclic AMP and theophylline, mean I_{sc} was 83±5 $\mu A/cm^2$. This maximum stimulation occurred within 20-50 minutes and in most experiments persisted

TABLE 1. Short-circuit current (I_{sc}) in flat sheets of rectal gland of Squalus acanthias

Conditions	n experiments	I_{sc} ($\mu A/cm^2$) $\bar{x}$ baseline (15-30 min)	I_{sc} ($\mu A/cm^2$) $\bar{x}$ stimulation (60-120 min)
I. Paired capsulated and decapsulated sections - regular Ringers			
dibutyryl cyclic AMP* + theophylline†	(4)		
capsulated sections		0.92±1.0	21.3±6
decapsulated sections		0.2±0.2	29±5
8-cpt-cyclic AMP†† + theophylline†	(5)		
capsulated sections		1.3±0.8	15±4
decapsulated sections		1.5±0.9	72±16
II. Decapsulated sections - Ringers as specified			
8-cpt-cyclic AMP + theophylline	(19)	3.9±1	83±5.5
regular Ringers			
8-cpt-cyclic AMP + theophylline	(4)		
Na^+ free Ringers		5.3±1.3	8.7±1.6
regular Ringers			67±9
8-cpt-cyclic AMP + theophylline	(5)		
Cl^- free Ringers		2.2±0.9	4.3±2.9
regular Ringers			63±13
+ furosemide (10^{-4}M)			16±3
+ furosemide (10^{-3}M)			5.4±4

* dibutyryl cyclic AMP, 5 X 10^{-5}M, serosal media
† theophylline, 2.5 X 10^{-4}M, serosal media
†† 8-cpt-cyclic AMP, 1-3 X 10^{-5}M, serosal media

for 2-3 hours with a gradual decline with time. When decapsulated sections were bathed on serosal and mucosal surfaces in a Na^+ free (choline) Ringers, the addition of 8-cpt-cyclic AMP and theophylline had no significant effect on I_{sc} (5.3 to 8.7 $\mu A/cm^2$); however, full stimulation (67±9 $\mu A/cm^2$) occurred when the solutions were changed to regular Ringers. Similarly, bathing in a chloride-free Ringers (sulfate substitution) prevented stimulation of I_{sc} whereas return to regular Ringers stimulated I_{sc} to 63±13 $\mu A/cm^2$. In the latter experiments during stimulation in regular Ringers the addition of furosemide to the serosal media resulted in a dose-dependent inhibition of I_{sc} within 5-10 minutes (Table 1). Ouabain (10^{-4}M) in the serosal media similarly inhibited I_{sc}. In every experiment with furosemide, tissue conductance fell from a maximum of 4.3±0.6 mS/cm^2 during stimulation with 8-cpt-cyclic AMP to 3.2±0.6 after furosemide ($p<0.005$).

These experiments demonstrate that 8-cpt-cyclic AMP stimulated I_{sc} in flat sheets of the rectal gland is dependent on the presence of both sodium and chloride in the bathing media and is abolished by furosemide and ouabain. The behavior of the I_{sc} is consistent with a current working hypothesis for active chloride secretion which invokes coupled entry of sodium and chloride into the transporting cells (Silva et al., Am. J. Physiol., 233, 1977). These results suggest that despite the structural complexity of the isolated rectal gland, I_{sc} may provide a direct and instantaneous measurement of active ion transport in this tissue.