

EVALUATION OF TRANSPORT MECHANISMS IN THE RECTAL GLAND OF *Squalus acanthias*

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Previous studies have demonstrated a technique for in vitro perfusion of the rectal gland of the dogfish shark, *Squalus acanthias* (Bull. MDIBL, 13:45, 1973; Am. J. Physiology, 226:1188, 1974). Using this model, preliminary data suggested active transport of both cations and anions, since Na and Cl were secreted against an electrochemical gradient of $+5.7 \pm 1.4$ mV and -13.6 ± 1.2 mV, respectively. To further evaluate the characteristics of this transport process experiments were performed to examine changes in transepithelial PD and the rate of Na secretion when sodium and chloride concentrations of the perfusate are altered independently using non-transported cations or anions.

Rectal glands were obtained and studied by methods previously described (Am. J. Physiology, 226:1188, 1974). The perfusate was gently bubbled with 99 percent O_2 and one percent CO_2 in all experiments. The electrical potential difference, PD, which reflects the potential, lumen negative, between the perfusate reservoir and lumen of the external duct, was determined using methods previously described (Am. J. Physiology, 226:1188, 1974). To minimize junctional asymmetry, specific salt bridges were prepared for each alteration in either Na or Cl content of the perfusate. At the start and termination of each experiment the asymmetry PD between the salt bridges and calomel cells was measured and averaged 0.5 ± 0.2 mV for all experiments. Net PD of each study period was determined by subtracting the mean asymmetry PD from the measured PD.

TABLE I

Experiment	Number of Glands Studied	Sodium, Chloride and Osmolarity of Perfusate Solution During Experimental Period		
		Na mEq/l	Cl mEq/l	OSH mOSM/kg
Control (Shark Ringer's)	5	294.7	242.2	878
TMA-25	6	235.3	272.7	872
TMA-50	6	161.8	232.3	876
TMA-100	7	15.2	274.3	870
High Na (Na ₂ SO ₄)	5	431.3	242.4	875
SO ₄ -50	6	299.1	112.5	890
SO ₄ -100	6	289.2	20.1	885
High Cl (TMA)	6	286.6	351.9	860
SCN (10mM NaSCN)	6	305.2	240.7	890

The following protocol was used to study 53 glands employing nine different perfusate solutions: During Period I (control) the transepithelial PD and rate of sodium secretion were determined while the gland was perfused with standard shark Ringer's solution of 15 minutes; Period II (experimental), similar measurements were made 10 minutes after changing the perfusate to one of the experimental solutions. This period was 20 minutes in duration; Period III (recovery), similar measurements were made 10 minutes after re-starting standard shark's Ringer's solution and lasted 15 minutes. During

TABLE II

TRANSEPITHELIAL ELECTRICAL POTENTIAL DIFFERENCE

Experiment	Period I	Period II		Period III	
	PD mV	PD mV	Δ PD mV ^a	PD mV	Δ PD mV ^b
Control	-10.54±0.65	-10.06±0.57	+0.48±0.23	-9.94±0.55	+0.60±0.14
TMA-25	-11.17±0.85	-14.68±0.69	-3.52±0.63*	-9.42±0.75	+1.85±0.48
TMA-50	-9.62±0.83	-17.25±1.05	-7.63±0.81*	-10.12±0.74	-1.30±0.98
TMA-100	-9.56±0.97	-16.51±0.44	-6.94±0.84*	-3.87±0.51	+5.69±0.59*
High Na	-10.72±0.41	-8.08±0.54	+2.64±0.93**	-9.72±0.98	+1.00±0.98
SO ₄ -50	-10.32±0.21	-14.58±1.21	-4.27±0.85*	-6.93±0.91	+3.83±0.99*
SO ₄ -100	-9.48±0.88	-5.87±0.82	+3.62±0.60*	-3.62±0.41	+5.87±1.17*
High Cl	-11.25±0.57	-12.60±0.67	-1.35±0.65**	-11.72±0.62	-0.43±0.50
SCN	-10.30±0.91	-9.18±0.87	+1.02±0.57	-9.22±0.60	+2.08±0.80

All values are Mean ± SEM

^a Δ PD = PD Period II - PD Period I

^b Δ PD = PD Period III - PD Period I

*P < 0.01, compared to Control

**P < 0.05, compared to Control

each period the PD was recorded at five-minute intervals and glandular fluid was collected for estimation of volume and electrolyte concentration. The measured sodium and chloride concentrations and osmolality of all perfusate solutions are shown in Table I. Tetramethylammonium chloride (TMA) was employed as a cation substitution for Na and as a means to increase Cl concentration independently of Na. In a similar way sodium sulfate (SO_4) was used as a substitute for Cl and to increase Na concentration independently of Cl. In the final series of experiments 10 mM NaSCN was added to shark Ringer's during the experimental period. All experimental perfusate solutions were maintained isotonic to shark Ringer's by adjustment of the urea concentration.

The effect of changes in the perfusate concentration of Na on PD and sodium secretion rate are shown in Table II and Figure 1. The rate of sodium secretion during Period I was similar in each group of experiments and ranged from 2.52 ± 0.59 to 3.09 ± 0.86 $\mu\text{Eq}/\text{min}/\text{gm}$ gland weight. Reduction of the perfusate Na concentration by approximately 25% (TMA-25) resulted in a significant increase in PD of 3.52 ± 0.63 mV ($p < 0.01$) and decrease in sodium secretion to 1.46 ± 0.36 $\mu\text{Eq}/\text{min}/\text{gm}$ ($p < 0.05$). During recovery, Period III, PD and sodium secretion rate returned to control levels. Further reductions in perfusate Na concentration by 50% (TMA-50) and 100% (TMA-100) resulted in greater increases in PD, which reached a plateau of approximately -17 mV, and progressive reductions in the rate of Na secretion occurred. Complete recovery of PD and Na secretion occurred after perfusion with TMA-50 but not after TMA-100 perfusion. Conversely when perfusate Na concentration was increased above control levels (High Na) by the addition of Na_2SO_4 , net PD was reduced by 2.64 ± 0.93 mV ($p < 0.05$) and Na secretion rose markedly to 4.64 ± 1.18 $\mu\text{Eq}/\text{min}/\text{gm}$, ($p < 0.01$). The effect of increased perfusate Na

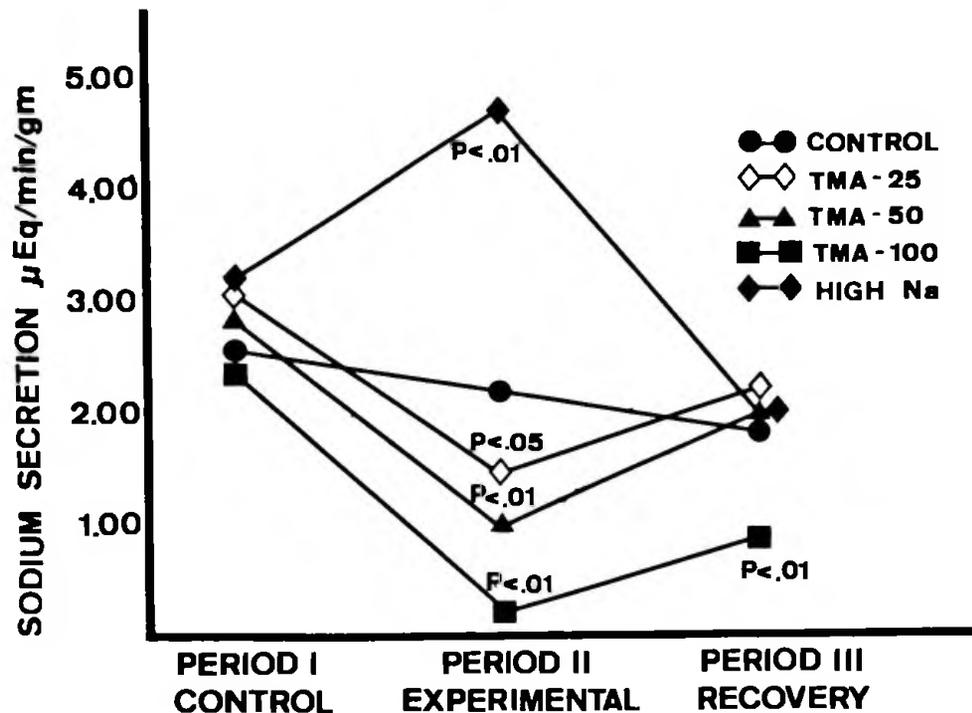


Figure 1: The effect of alterations in perfusate sodium concentration on the rate of sodium secretion during in vitro perfusion of the rectal gland of *Squalus acanthias*

concentration was completely reversible. During the recovery period both net PD and sodium secretion rate returned to control levels. Although not shown the Na concentration in glandular fluid in each of these experiments and in all subsequent studies remained elevated in the range of 450 to 550 mEq/L. Changes in the rate of sodium secretion, therefore, were caused by changes in glandular fluid flow rate.

The effects of changes in the perfusate concentration of chloride are shown in Table II and Figure 2. Perfusion with SO_4 -50 and SO_4 -100 caused a significant decline in Na secretion which did not recover. Enigmatic changes

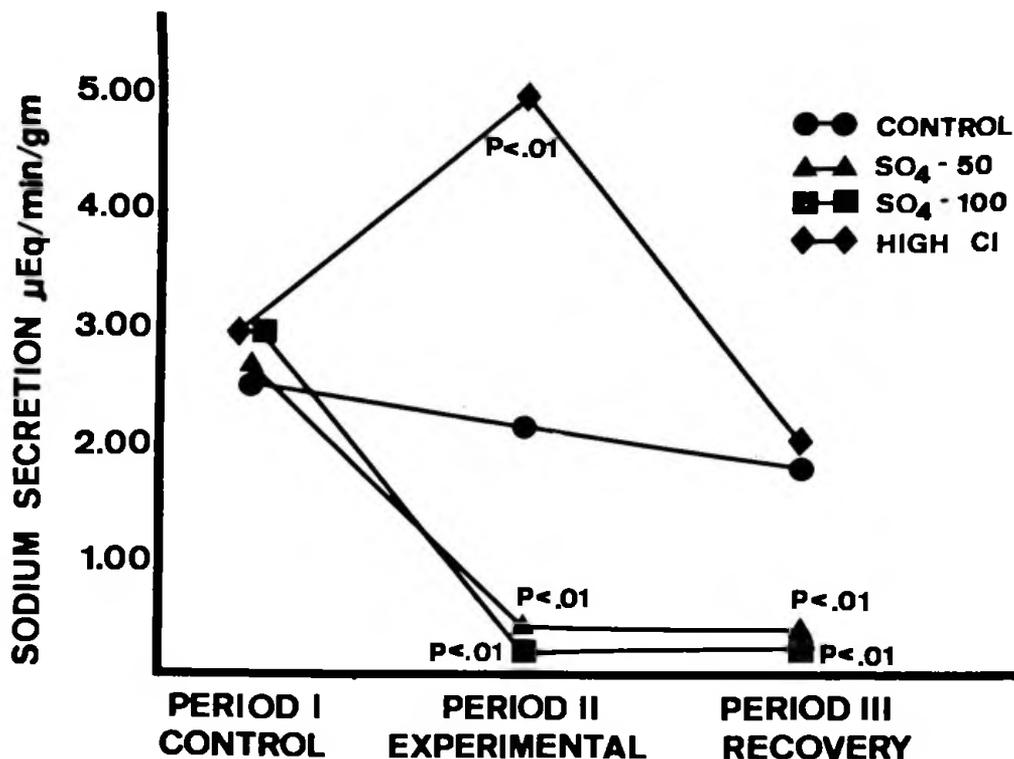


Figure 2: The effect of alterations in perfusate chloride concentration on the rate of sodium secretion during in vitro perfusion of the rectal gland of *Squalus acanthias*

in net PD however occurred. During perfusion with SO_4-50 , PD increased 4.27 ± 0.85 mV. In contrast PD declined by 3.62 ± 0.60 mV during perfusion with SO_4-100 . In further experiments in which the Cl concentration was increased above control levels, both the secretion rate of Na and net PD rose markedly. These changes were completely reversible.

Since previous studies had suggested that 10mM NaSCN reduced sodium secretion by inhibiting chloride transport (Bull. MDIBL, 13:45, 1973), a final series of glands were studied in which this agent was added to the glandular perfusate during Period II. Although there was a marked and

significant reduction in sodium secretion rate during Period II (3.02 ± 0.75 $\mu\text{Eq}/\text{min}/\text{gm}$, Period I, vs. 0.77 ± 0.22 $\mu\text{Eq}/\text{min}/\text{gm}$, Period II; $p < .01$), no significant change in transepithelial PD ($\Delta\text{PD} = 1.02 \pm 0.57$ mV, $p = \text{NS}$) was noted. The effect of 10 mM NaSCN on Na secretion was not reversible and sodium secretion rate remained low during Period III (0.57 ± 0.18 $\mu\text{Eq}/\text{min}/\text{gm}$).

On the basis of these data it is evident that the rate of Na and Cl transport is dependent upon the availability of the respective ions since there was a direct correlation between NaCl movement and the concentration of either Na or Cl in the perfusate. With the exception of the enigmatic findings during SO_4 -50 perfusion there was an inverse correlation between net PD (lumen negative) and perfusate Na concentration and an increased rate of transport during high Cl experiments in association with a further rise in PD. These findings provide strong support for the existence of an active chloride transport mechanism. Since a reversal in net PD (lumen positive) did not occur during Cl substitution there was no direct evidence for an independent electrogenic sodium pump. These data however do not distinguish between 1) separate electrogenic pump mechanisms for Cl and Na; 2) an electrogenic Cl pump and linked passive Na movement, and 3) a non-electrogenic NaCl pump. The inhibition of the rate of secretion, in the absence of a change of net PD by NaSCN, provides support for either the second or third model.

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