# SLOW VOLUME EXPANSION: EFFECTS ON RENAL AND RECTAL GLAND FUNCTION IN *SQUALUS ACANTHIAS*

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Salt water elasmobranchs are constantly challenged by salt and water excess. The hypertonicity of extracellular fluid with respect to seawater creates an osmotic gradient for water entry across the relatively permeable gill epithelium. The plasma sodium and chloride concentrations of only 50% that of seawater create a chemical gradient for the entry of sodium and chloride across the gills. In order to maintain salt and water balance, an equal rate of water, sodium, and chloride must be lost through branchial, renal, and rectal gland transport mechanisms. The contribution of each of these organs to the maintenance of resting salt and water balance is not completely understood. Indeed, the role of each organ may varying under different environmental stresses (eating/drinking vs. acclimation to dilute seawater) and experimental maneuvers (hypertonic vs. hypotonic volume expansion, acute vs. chronic volume expansion).

We have previously argued that plasma volume rather than plasma sodium or chloride concentration is the primary stimulus to rectal gland secretion of salt and water. The hormonal messenger coupling extracellular volume expansion to an increase in rectal gland secretion is thought to be a member of the natriuretic peptide family. This hypothesis was based on experiments in which whole animals received acute intravascular infusions of hypertonic fluid but in which plasma volume was maintained constant by the simultaneous removal of blood. Subsequent administration of isotonic fluid with plasma volume expansion led to stimulation of rectal gland secretion [Solomon et al., Am. J. Physiol. 1985;248:R638]. Support for the hypothesis that the rectal gland is the primary effector in this response to volume are the observations that exogenous natriuretic peptides increase rectal gland secretion in vivo. This stimulatory effect on the rectal gland occurs without a stimulatory effect on urinary output of water and salt [Solomon et al., Am. J. Physiol. 1985;249:R348 and Bull. MDIBL 1993; 32:69]. The latter observations also suggest that during states of increased salt and water intake, the rectal gland is the primary organ responsible for maintaining volume homeostasis. In this report, we infused shark Ringer's solution at a slow constant rate and monitored rectal gland and urinary chloride output in an effort to more clearly describe the relative roles of the kidney and the rectal gland to overall salt and water homeostasis.

Live female dogfish (2.5 -5.5 kg) were pithed via insertion of a wire through the spinal column. All fish were spontaneously gilling and oxygenation was maintained throughout the experiments by passing seawater over the gills. Catheters (PE50) were placed in the urinary papilla and the rectal gland duct (after prolapse of the rectum through the anus) for collection of urine and rectal gland duct secretion respectively. Blood pressure was monitored manometrically via a catheter (PE50) inserted into the dorsal aorta percutaneously. Rectal gland secretion and urine were collected at thirty minute intervals. Shark Ringer's solution [Solomon, et al., Am. J. Physiol. 1992, 262:R707] was infused into the dorsal aorta distal to the blood pressure monitoring site at a rate of 5 $ml \cdot kg^{-1} \cdot h^{-1}$ Collections were continued until rectal gland secretion was stimulated to a maximum level and two consecutive rectal gland duct collections did not differ in volume by more than 10%. Chloride was measured in the rectal gland fluid and urine by amperometric techniques. Mean dorsal aortic pressure was recorded every 30 minutes. Blood samples were obtained before the start of the infusion and after peak rectal gland flow was observed.

Nine animals were studied. Rectal gland chloride secretion increased to stable peak levels after 195 ± 27 minutes (mean ± SEM) of continuous infusion (range 90 to 270 minutes). A doubling of rectal gland chloride secretion was observed within 90 minutes in 5/9 animals. Rectal gland chloride secretion increased from 327±149 $\mu Eq \cdot kg^{-1} \cdot h^{-1}$ to 1508±274 $\mu Eq \cdot kg^{-1} \cdot h^{-1}$ ($p<.01$). During the entire period of observation (approximately 300 minutes), there was no increase in urinary flow or urinary chloride excretion (Figure 1). Concomitant with the increase in rectal gland chloride secretion, there were significant increases in mean dorsal aortic pressure (26±1 before and 30±2 mmHg after infusion, $P<.05$ by paired t test) and decreases in hematocrit (24±1% before and 19±1% after infusion).

The total volume of shark's Ringers infused prior to the establishment of peak rectal gland secretion was 16.3±2.3 ml/kg (range 7.5 - 22.5 ml/kg). At the peak of rectal gland secretion, 1.5 $mEq \cdot kg^{-1} \cdot h^{-1}$ of chloride was secreted by the rectal gland and 0.1 $mEq \cdot kg^{-1} \cdot h^{-1}$ was excreted in the urine. Thus the rectal gland accounted for 93% of the combined choride loss through the rectal gland and kidney. The infusion rate of 5 $ml \cdot kg^{-1} \cdot h^{-1}$ represents a chloride input of 1.4 $mEq \cdot kg^{-1} \cdot h^{-1}$ (280 mEq/L x .005 L/h). Thus rectal gland secretion increased to match quantitatively the intake of chloride resulting from the administration of shark's Ringers solution.

These studies confirm our previous observations and support the hypothesis that the physiologic response to volume expansion in the shark is mediated mostly if not exclusively by the rectal gland. The primary observation is that rectal gland chloride secretion increases within hours of mild volume expansion to balance the input of chloride.

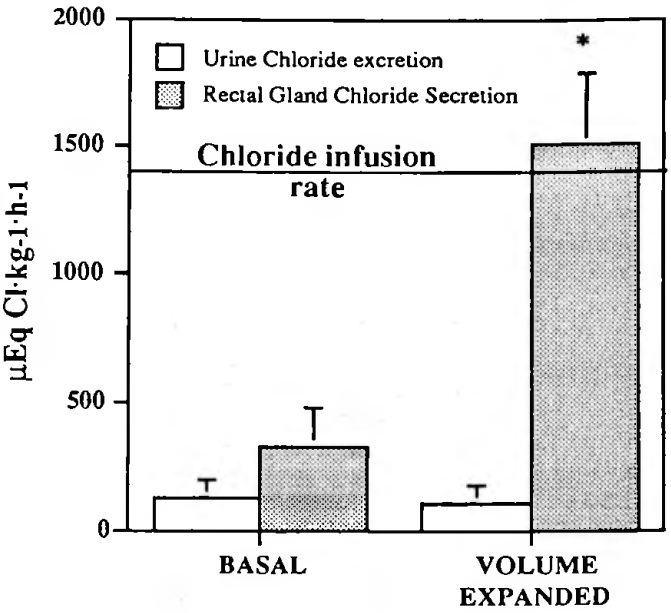


FIGURE 1

Loss of chloride in the urine and rectal gland fluid following volume expansion with intravascular shark's Ringers at 5 $ml \cdot kg^{-1} \cdot h^{-1}$. Rectal gland chloride secretion increased significantly while no change in urinary chloride excretion was observed.

The present experiments presented a total volume load of 16 ml/kg over a 3 hour period. This contrasts with our previous studies which challenged the animals with a load of 30-50 ml/kg given over 15 to 30 minutes. The present load is less likely to produce exaggerated hemodynamic effects although mean dorsal aortic pressure increased even with this load.

Based upon this analysis of net chloride balance, we conclude that the rectal gland plays the primary, if not sole, role in defending the animal against increases in salt and water. While we cannot exclude a contribution of other mechanisms, such as branchial transport of chloride, to the return of chloride balance, the rectal gland alone accounted for complete restitution of chloride (and sodium) balance. Urinary chloride excretion remained unchanged arguing against a physiologic role for the kidneys in maintaining salt balance, at least with this degree of volume expansion.

Unidirectional flux studies, on the other hand, have suggested that the branchial epithelium is capable of significant outward movement of Na (perhaps as high as 1 mEq/kg/h) [reviewed by Evans, in *Comparative Physiology of Osmoregulation in Animals*, Academic Press, 1979;305]. In addition, isotopic flux measurements in a variety of elasmobranchs indicate that the sum of renal and rectal gland sodium loss accounts for at most 50% of the isotopic sodium which leaves the animal [Evans , Ibid]. There are significant methodologic problems confounding the interpretation of these flux measurements. The outward unidirectional movement of sodium is determined by loading the animal with $^{22}Na$ and following the appearance of isotope in the external bath. Because sodium crosses the branchial epithelium in both directions and the specific activity of $^{22}Na$ is usually 1000 fold higher in plasma than in the external bath, net appearance of isotope in the bath is not an accurate estimate of net sodium loss from the animal. Unlabelled sodium from the bath may be exchanged for $^{22}Na$ in plasma without a net gain or loss from the animal. In fact, unidirectional influx of $^{22}Na$ is 4-5 fold greater than unidirectional outward flux of $^{22}Na$ [Horowicz and Burger, Am. J. Physiol. 1968;214:635]. Thus unidirectional flux measurements may significantly overestimate net ion movements and can not be used to quantitate the contribution of the rectal gland and kidney to overall sodium and chloride balance. Finally, these studies are conducted in the basal state and do not address the role of the kidney, rectal gland, and gill during a salt and water challenge. There are no data suggesting that the transport of sodium across the branchial epithelium is regulated in a manner consistent a role in volume homeostasis. Instead, branchial sodium transport appears to be linked to acid-base homeostasis [Evans, in *Osmotic and Ionic Regulation in Animals,* CRC Press, 1993; 279].

An additional argument that is often cited to support a role for non rectal gland mechanisms of sodium and chloride balance is that removal of the rectal gland is not always accompanied by a change in plasma sodium or chloride concentrations [Evans, Ibid]. This argument ignores the observations that the gills of elasmobranchs have a very high water permeability allowing as much as 100% of body water to be exchanged each hour [Evans, Ibid]. This high water permeability will allow maintenance of plasma osmolality (and therefore sodium and chloride concentrations) despite net retention of sodium and chloride resulting from the loss of the rectal gland. Only measurements of plasma volume are likely to reveal the importance of the rectal gland to net sodium and chloride balance under these experimental conditions.

At the peak of rectal gland chloride loss, rectal gland water loss averaged 2.7 $ml \cdot kg^{-1} \cdot h^{-1}$ At the same time, the urinary water loss never exceeded 1 $ml \cdot kg^{-1} \cdot h^{-1}$. Thus although the rectal gland contributed entirely to the restitution of chloride balance, water balance was not achieved by the combined renal and rectal gland outputs during the period of observation. What accounts for the difference between water input (5 $ml \cdot kg^{-1} \cdot h^{-1}$) and water loss (3.7 $ml \cdot kg^{-1} \cdot h^{-1}$ at maximum rectal gland and urine excretion) observed in these experiments? It is possible that there was absolute water retention during the experiment. Mechanisms which might possibly increase water loss, for example, an increase in hypotonic urine output, may have a different time course for activation. Future experiments will address this issue.

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