CONTROL OF RECTAL GLAND SECRETION IN THE DOGFISH (SQUALUS ACANTHIAS): STEPS IN THE SEQUENCE OF ACTIVATION

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SUMMARY

1. We measured the venous and arterial pressure, as well as the rate of secretion and content of cyclic AMP and high energy phosphate compounds, of the rectal gland of the anaesthetized dogfish, Squalus acanthias (L.).

2. Intravenous infusion of isotonic solutions produced a very large increase in the rate of secretion by the rectal gland. The increase in secretion was preceded by an increase in venous blood pressure, but arterial blood pressure was not modified.

3. Injections of small doses of veratridine stimulated gland secretion when given in the vicinity of the heart but not when given in the dorsal aorta.

4. During volume expansion the creatine phosphate and ATP content of the gland were markedly reduced, while ADP and AMP as well as cyclic AMP content were increased.

5. We conclude that: (a) volume expansion leads to the release of a message that activates adenyl cyclase in the gland; (b) the increased venous pressure may be the initial signal in the sequence that leads to the release of the activating messenger; (c) there is a receptor mechanism in the atrial and cardiac region that triggers the sequence that activates glandular secretion; (d) the reduction in the content of high energy phosphate compounds during volume expansion is caused by an increase in energy expenditure, probably due to gland secretion.

INTRODUCTION

The rectal gland of the dogfish secretes a solution composed mainly of NaCl that is nearly isotonic with the plasma of the fish. Although this secretion appears to play an important role in the regulation of body fluids and ion composition (Burger & Hess, 1960; Burger, 1962, 1965) the mechanisms involved in its control are not fully understood.

Most of the progress in understanding rectal gland function has been made by studying the isolated gland (see, for example, Stoff et al. 1977) while advances using the whole animal have been relatively sparse. We have therefore undertaken

Key words: volume expansion, rectal gland, elasmobranchs, cyclic AMP.
experiments on the whole animal to examine the following specific questions. Can we develop a preparation in which it is possible to identify the physiological variable that will consistently trigger secretion by the gland? Does a particular region of the circulation contain a sensory mechanism whose activation initiates the sequence of events that finally leads to increased secretion by the rectal gland? Is there any indication that activation of secretion in the whole fish has a mechanism akin to that found in the isolated perfused glands?

To examine the first question we continued from the point where Burger (1962) left off. He measured the effects of injecting different solutions on the rate of rectal gland secretion in fishes swimming freely in a tank. The major limitation of this approach is that handling of the fish triggers many autonomic responses (Lutz, 1930), which may modify rectal gland function. Moreover, the need to handle the free-swimming fish limits the frequency of sample collection. The low frequency of sampling may distort the measurement of secretory rates because a single sample collected during a long period may pool liquid produced while the gland was secreting at quite different rates. In addition, these problems are greatly compounded when it is necessary to record vascular pressures or obtain blood and tissue samples. We have overcome some of these problems by using anaesthetized fish.

To examine the second question, we adopted a technique that stems from the observations of Von Bezold & Hirt (1867) and consists of injecting locally small amounts of veratrine into different regions of the vascular system. Since veratrine alkaloids markedly increase the firing rate of nerve cells, the finding that injections of small amounts of veratrine in a particular vascular bed trigger marked responses in a remote area of the organism suggests the activation of a reflex pathway. Dawes & Comroe (1954) gave a detailed account of the uses of this technique.

The third question is of interest because it is important to be able to use the data obtained in the perfused gland to understand the control of secretion in the whole animal. One of the major points to be clarified is whether the mechanisms of activation that operate in the perfused tissue are responsible for activation of the in situ gland. For example, in the isolated, perfused gland of Squalus acanthias, adenosine and vasoactive intestinal peptide (VIP) stimulate secretion, probably through a cyclic AMP-mediated process (Stoff et al. 1979; Erlij, Silva & Reinach, 1978), and thus it would be important to know whether the concentrations of cyclic AMP vary in the gland in situ when secretion rates are modified. To examine this problem, we determined whether there was any indication that activation of secretion in the whole animal was associated with changes in the content of cyclic AMP, the hydrolysis of high energy phosphates and adenosine formation in the gland.

Some of the experiments described here have been published in preliminary form (Erlij, Silva & Rubio, 19806; Rubio, Berne, Silva & Erlij, 1980).

MATERIALS AND METHODS

The experiments were carried out on dogfish (Squalus acanthias) taken by hook and line in Frenchman's Bay, Maine, and kept captive in marine livecars in the pier
of the laboratory. The animals were placed in a tank with running sea water, and anaesthetized by an injection of nembutal (20–30 mg kg\(^{-1}\)) into the caudal artery. This dose of nembutal did not stop spontaneous gill movements. The animals were then placed, in a supine position, in the bottom of a shallow tank and the gills were perfused at a rate of 2 l min\(^{-1}\) with sea water equilibrated with air (at 15°C). After opening the abdomen with a small midline incision, the rectum was opened near the insertion of the rectal gland and a polyethylene catheter was tied in the orifice of the rectal gland duct to collect the secreted fluid. For intravenous infusions, a fine needle (23 gauge) was introduced into either the posterior intestinal vein or the lateral abdominal vein.

We used three procedures to compare the effects of injecting veratridine in different sites. (a) A fine polyethylene catheter was inserted into the lateral abdominal vein and advanced until, judging by the length of tubing inserted, it was reasonable to assume that the tip was localized at the atrium; this localization was verified by dissection at the end of the experiment. (b) A fine needle was introduced into the dorsal aorta above the branching of the rectal artery. (c) The conus arteriosus was exposed and injections were performed through a fine needle.

Arterial pressure was measured by cannulating the central end of the posterior intestinal artery using either a mercury manometer or a Statham pressure transducer connected to a Sanborn recorder. Venous pressure was measured with a water manometer connected to a catheter that was introduced into the lateral abdominal vein all the way to the atrium. Care was taken to level the zero of the manometer with the atrium.

Secretion samples were collected from the cannula in the duct at 5- or 10-min intervals in preobrated vials. Volume of secreted fluid was determined by weighing the vials with 0.1 mg precision. Chloride content in the secretion was determined by amperometric titration with a Cotlove Chloridometer.

To collect samples of the rectal gland, we introduced a set of Wollenberger clamps, pre-equilibrated with liquid nitrogen, into the abdominal cavity, taking care not to interrupt the glandular circulation at any time. Immediately afterwards, the glands were clamped and instantaneously frozen \textit{in situ}. Then, the fragment of gland contained within the jaws of the Wollenberger clamp was immersed in liquid nitrogen, weighed, pulverized, homogenized and extracted with 10 volumes (v/w) of 0.5 mol l\(^{-1}\) perchloric acid.

After centrifugation and neutralization with KOH, all the supernatants were analysed for adenosine, inosine and hypoxanthine, using well-established enzymatic spectrophotometric procedures (Dobson, Rubio & Berne, 1971). In addition, the gland extracts were analysed enzymatically for creatine phosphate (CP), adenosine monophosphate (AMP), diphosphate (ADP) and triphosphate (ATP) following the changes in absorbance of NADH or NADPH (for details see Lamprecht, Stein, Heinz & Weiser, 1974; Jaworek, Gruber & Bergmeyer, 1974).

The elasmobranch Ringer solution had the following composition (in mmol l\(^{-1}\)): Na, 280; K, 5; Cl, 270; HCO\(_3\), 8; Ca, 2.5; Mg, 1.2; phosphate, 1; sulphate, 0.5;
urea, 350; glucose, 5. All solutions were gassed with 99% O₂ and 1% CO₂ and their tonicities were altered by raising either urea or NaCl concentrations.

Results concerning groups of experiments are given as the mean ± S.E. mean. The one-tailed Student's t-test for paired variables was used to determine the level of significance. Values of $P<0.05$ were considered significant.

RESULTS

Effects of composition and site of injection

We wished to determine: (a) whether it was possible to reproduce Burger's results in the anaesthetized fish; (b) whether gland secretion was triggered by either the injection of hypertonic solutions or volume expansion; and (c) whether responses could be more readily elicited by injecting solutions into the intestinal venous circulation, the route by which ingested salt enters the fish, rather than into the lateral abdominal vein that collects blood from the systemic circulation.

To determine whether rectal gland secretion can be activated by volume expansion, by salt or by hypertonic solutions we compared the effects of infusing three types of solutions: (a) an isotonic solution similar to shark's Ringer solution, except that all the urea had been substituted isosmotically with NaCl; (b) Ringer solutions in which NaCl was excluded but osmolarity had been maintained either by addition of sucrose or urea; and (c) a hypertonic solution in which the NaCl was about four times as concentrated as in the shark's plasma. Infusion of all three solutions enhanced secretion by the rectal gland (Fig. 1). After NaCl infusion there was a delay of about 20 min before there was any increase in secretion. Similar delays were observed in all other fishes (range 17-49 min). The injection of fluid was interrupted once a clear-cut increase of fluid secretion had been reached. Usually, the secretion continued to increase after suspending the infusion, finally stabilizing at a high plateau that lasted for 1-2 h and then declined towards resting level. In five out of the six animals in this series that were infused with NaCl Ringer, the rate of secretion was increased. The rate changed from $0.79 \pm 0.35 \text{ ml h}^{-1} \text{g}^{-1}$ of gland wet weight (range 0.240-1.682) to $9.47 \pm 1.33 \text{ ml h}^{-1} \text{g}^{-1}$ (range 5.96-13.6). The sixth fish that was spontaneously secreting at a rate of $4.2 \text{ ml h}^{-1} \text{g}^{-1}$ did not respond to saline infusion (total 200 ml) with an increase in secretion. In seven fishes in which all NaCl had been substituted by either urea or sucrose the rate of secretion increased from $0.65 \pm 0.28 \text{ ml h}^{-1} \text{g}^{-1}$ of gland wet weight to $4.72 \pm 1.85 \text{ ml h}^{-1} \text{g}^{-1}$. In four fishes, infusion of hypertonic salt solutions also stimulated gland secretion satisfactorily. The injected volume required to produce a response was in the same range as when isotonic solutions were used, although the responses appeared to be smaller. We suspect that infusions of large amounts of hypertonic saline may be nocive to the fishes since two animals died during the infusion.

There was no clear-cut enhancement in the response by making the infusions into the posterior intestinal vein. In four fishes this route was used for infusion; three received isotonic Ringer while one received hypertonic Ringer. The volumes required to produce a response and the responses themselves were of similar
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magnitude to those observed with lateral abdominal vein injections. This finding suggests that no major receptor signals are triggered by salt and fluid entering through the intestine. However, it does not rule out the possibility that entry through other pathways, like the gills, may trigger significant signals.

**Arterial and venous pressures**

These variables were measured in another series of experiments to determine whether they were modified by infusions of isotonic saline that markedly stimulated secretion. In all five fishes in this series, infusion markedly increased secretion. The temporal relationships between vascular pressures and gland secretion are illustrated in Fig. 2. Infusion of isotonic solution did not modify arterial pressure in any of the five experiments, whereas venous pressure started to increase rapidly with a latency ranging between 10 and 15 min after the beginning of the injection. The increase in venous pressure always preceded the increase in secretion by a short interval.

**Localization of activating receptors**

The results in the previous sections indicate that increases in extracellular space volume trigger an increase in the rate of fluid and salt secretion by the rectal gland of

![Graph](image-url) **Fig. 1.** Effects of intravenous fluid infusion on secretion by the rectal gland of the anaesthetized dogfish. Fluid was infused between the periods indicated by the arrows. The filled circles are results from a fish that received isotonic NaCl Ringer while the empty circles are from a fish that received urea solution.
the dogfish. Can the stimulation of secretion be elicited by activating a receptor in a particular area of the organism?

A group of preliminary experiments showed that the injection of veratridine near the atria frequently produced an increase in the rate of fluid and salt secretion by the rectal gland. However, in some instances the injection had no effect or produced a biphasic response. We also observed that the injection of veratridine was always followed by a marked decrease in the heart rate and therefore suspected that the variability in the secretory response could be related to the haemodynamic effects of veratridine. Indeed, when we recorded the arterial pressure during the injections of veratridine (Fig. 3A) we found that it produced a marked slowing of the heart which considerably reduced the blood pressure. Since, in other organisms, the veratrine-induced cardiac slowing is due to vagal discharge we decided to pretreat the animals with atropine (20 μg kg⁻¹). As expected, veratridine administration after the injection of atropine did not change heart rate and the blood pressure was not reduced (Fig. 3C). The effects of atropine treatment on the secretory response are shown in
Fig. 4. The first dose of veratridine, before the addition of atropine, was followed immediately by a drop in secretion rate and then by an increase that decayed spontaneously. After the injection of atropine, the rate slowly drifted down and when veratridine was given again a large increase in secretion was observed. Since the

![ Diagram of blood pressure and heart rate changes ]

Fig. 3. Effects of veratridine and atropine on the blood pressure and heart rate of an anaesthetized dogfish. (A) 10 μg of veratridine was injected into the lateral abdominal vein. Between parts A and B we waited 15 min. Then 20 μg kg⁻¹ of atropine was injected (B). After another interval of 10 min, the injection of veratridine was repeated (C).

![ Graph of rectal gland secretion rates ]

Fig. 4. Modification of veratridine action on rate of rectal gland secretion by atropine treatment. The first arrow indicates the moment when 10 μg of veratridine were injected into the atrium. Then atropine (20 μg kg⁻¹) was injected. Finally the injection of veratridine was repeated.
Fig. 5. Dependence of the effects of veratridine on rate of rectal gland secretion on the dose and site of injection. The experiment illustrated in the upper panel shows the effects of injecting three different doses of veratridine (1, 2.5 and 10 μg) in the lateral abdominal vein of an anaesthetized fish. The individual injections are indicated by the arrows. In the experiment illustrated in the lower panel, the effects of injecting 40 μg of veratridine into the dorsal aorta are compared with the effects of the same dose injected into the atrium.

Injection of veratridine into the lateral vein of atropinized animals consistently caused a stimulation of rectal gland secretion, all subsequent experiments were performed on atropinized animals.

Fig. 5A shows that the secretory response caused by injection of veratridine into the atria was dependent on the dose used. The threshold for veratridine action varied among different fish ranging between 2.5 and 10 μg. Since veratridine can directly stimulate the secretion of fluid by the isolated perfused rectal gland (Erlij, Lodenquai & Rubio, 1981), it was important to determine whether its effects on the whole
animal were produced by directly activating the gland. To examine this point we compared the effects of injections of veratridine in the dorsal aorta at a point proximal to the rectal artery with injections in the atria. If the same amount of veratridine is injected in both sites the injection into the dorsal aorta ought to produce a much higher concentration of veratridine in the extracellular fluid of the gland than an injection in the atria, since the latter would be more diluted and possibly trapped by tissue receptors before it reaches the rectal gland. Fig. 5B illustrates one out of three experiments carried out to compare injections in both sites. First 40 μg of veratridine were injected into the dorsal aorta without causing any response. However, when the same dose was subsequently injected into the atrium the characteristic large increase in secretion rate was observed.

In three other experiments we determined the effects of injecting veratridine into the conus arteriosus. These injections produced increases in rectal gland secretion that were similar to those observed when veratridine was injected into the atria.

In all the present experiments we measured chloride concentration in the fluid collected from the rectal gland duct. The average chloride concentration during the control periods was 517 ± 43 mequiv l⁻¹. We never detected a difference in chloride concentration between fluids collected during resting conditions with those obtained during stimulation by either fluid infusion or veratridine injection (average 559 ± 69 mequiv l⁻¹).

**Effects on the content of cyclic AMP and high energy phosphate compounds**

For the control group of fish, the spontaneous rate of secretion was determined for at least 1 h before freezing the gland. In the stimulated group, infusion of saline into the lateral abdominal vein at the usual rates was first initiated and once a clear-cut increase in secretion had been reached the glands were promptly frozen (see Fig. 2). The content of cyclic AMP in the control group was 152·64 ± 9·4 pmol g⁻¹, whereas the volume-expanded fishes contained 352·87 ± 46·7 pmol g⁻¹ of cyclic AMP. This difference was significant (P < 0·01).

Table 1 summarizes the effects of volume-loading the fish on the content of creatine phosphate, ATP, ADP and AMP in the rectal gland. Creatine phosphate is

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control (μmol g⁻¹ tissue)</th>
<th>Fluid-loaded (μmol g⁻¹ tissue)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine phosphate</td>
<td>0·477 ± 0·112</td>
<td>0·050 ± 0·015</td>
<td>&lt;0·01</td>
</tr>
<tr>
<td>ATP</td>
<td>1·655 ± 0·104</td>
<td>0·734 ± 0·144</td>
<td>&lt;0·01</td>
</tr>
<tr>
<td>ADP</td>
<td>0·101 ± 0·023</td>
<td>0·219 ± 0·046</td>
<td>&lt;0·05</td>
</tr>
<tr>
<td>AMP</td>
<td>0·064 ± 0·014</td>
<td>0·259 ± 0·037</td>
<td>&lt;0·01</td>
</tr>
</tbody>
</table>

Control and fluid-loaded values are the mean ± s.e.m. Student’s t-test was used to determine the P value, N = 5.
dramatically reduced during the secretory response to volume loading. ATP is also
reduced significantly, whereas ADP and AMP are significantly increased. The
pattern of these changes suggests that during secretion there is an enhanced
utilization of ATP that clearly exceeds the rate of synthesis.

Effects on the content of adenosine and its metabolites

Table 2 shows that during volume expansion there is also a significant increase in
the gland’s adenosine content, while inosine and hypoxanthine are not modified
significantly.

DISCUSSION

Our results show that volume expansion induced by isotonic solutions is a
reproducible method of activating gland secretion in anaesthetized fish, even when
the injected solutions are devoid of NaCl. Moreover, hypertonic solutions appear to
be less effective than isotonic solutions in provoking secretion. Altogether, it seems
that receptor mechanisms activated by pressure or volume rather than by salt or
hypertonicity are involved in the response. This conclusion is in agreement with the
recent findings of Solomon et al. (1985) using the explanted gland and with our
initial suggestion (Erlij, Rubio & Silva, 1980a; Rubio et al. 1980). One difference,
which may be important, concerns the maximum rates of secretion attained in both
groups of experiments. The maximum rates observed in the explanted glands were
much lower than those observed in the anaesthetized fish. Evidently, the physio-
logical state of the anaesthetized fish and the pithed animals used in the two groups of
experiments may be quite different.

It is interesting to compare the behaviour of the elasmobranch’s rectal gland with
that of another gland involved in ion and fluid regulation: the salt-secreting nasal
gland of some birds. Secretion by the nasal gland is activated by the injection of
hypertonic solutions, while volume expansion is without any direct effects on the rate
of secretion. However, in the nasal gland, volume expansion seems to modify the
threshold for stimulation by hypertonic solutions (Hammel, Simon-Opperman &
Simon, 1980). Nevertheless it is difficult to separate hypertonic from volume-

Table 2. Effect of saline infusion on the content of adenosine and its metabolites in
the rectal gland of the dogfish

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control (nmol g⁻¹ tissue)</th>
<th>Fluid-loaded (nmol g⁻¹ tissue)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>0·36 ± 0·07</td>
<td>0·76 ± 0·06</td>
<td>&lt;0·01</td>
</tr>
<tr>
<td>Inosine</td>
<td>9·69 ± 1·62</td>
<td>11·72 ± 4·02</td>
<td>NS</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>15·97 ± 2·94</td>
<td>12·41 ± 4·56</td>
<td>NS</td>
</tr>
</tbody>
</table>

Control and fluid-loaded values are the mean ± s.e.m.; NS, not significant.
Student’s t-test was used to determine the P value; N = 5.
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expansion effects, because in the former case the injected solute will drag water into the vascular space leading to its expansion.

A useful feature of the design of our experiments is that they allow us to follow closely the time course of the changes in fluid secretion and measure other cardiovascular parameters simultaneously. This allowed us to detect that the stimulation of secretion occurred shortly after the venous pressure had increased and that the arterial pressure had not been modified during fluid infusion. It is therefore possible that the increase in venous pressure that follows the infusion of isotonic fluid is the signal that triggers the sequence of events that leads finally to an enhanced rate of secretion.

The experiments in which veratridine was injected into the atria and conus arteriosus suggest that within the vicinity of the heart lies a receptive mechanism, perhaps afferent nerve terminals, whose activation initiates the sequence of events that leads to gland secretion. Evidently, the effect is not due to direct activation by veratridine of structures within the gland, since injections into the dorsal aorta near the gland did not affect the rate of secretion. The exact localization of the receptors involved in the response was not resolved by the present experiments since injections into the conus arteriosus stimulated secretion, thus indicating that structures rostral to the atrium may trigger glandular secretion. We cannot yet tell whether these sensory elements are located in the conus, the gills, the blood vessels that irrigate the brain or the brain itself. We cannot yet affirm that the structures activated by veratridine are the same as those involved in initiating the glandular response caused by volume expansion. The results with veratridine are nevertheless of considerable interest because they strongly suggest that activation of a structure in the heart or in its close vicinity can eventually lead to stimulation of secretion by the rectal gland.

As far as the efferent pathway is concerned, the evidence is still quite scanty. On the one hand, Solomon et al. (1984b) found that volume expansion of donor fish that perfuse an explanted gland can lead to stimulation of secretion by the isolated organ. This finding suggests the presence of a hormonal pathway involved in the activation of the rectal gland. On the other hand, Erlij et al. (1981) found that perfusion with veratridine can activate secretion by the isolated rectal gland. This finding suggests that the tissue contains a pool of an activating agent that can be released by veratridine. The possibility that structures within an organ can contain stores of activators of glandular secretion is supported by the observations of Shuttleworth & Thorndyke (1984) who found that the intestine of Scyliorhinus canicula contains an endogenous peptide that may be responsible for the control of rectal gland secretion. Whether volume expansion releases a mediator from a storage site in the gland or in another organ and whether release is activated by either nerve or hormonal impulses remains to be determined.

Another point that emerges from our experiments is that dogfish have a reflex mechanism with afferents in the cardiovascular system that control heart rate through activation of vagal discharge, since veratridine injected in the vicinity of the heart produced an atropine-sensitive slowing of the heart. The observation that volume expansion does not slow the heart, whereas veratridine produces a marked
bradycardia, suggests that the nerve elements involved in triggering the reflex changes in heart rate are not identical with the structures that activate gland secretion.

Cyclic AMP content was significantly higher in glands from volume-expanded animals than in glands of control animals. It is now well established that secretion is stimulated by increasing the level of cyclic AMP within the secretory cells. Thus, the isolated perfused gland is activated only by the addition of cyclic AMP or by agents that increase cyclic AMP in the cell. The two agents that have received most attention as possible secretagogues are VIP and adenosine. Even though these agents may not be the universal secretagogues, since VIP does not stimulate the rectal gland of *Scyliorhinus canicula* (Shuttleworth, 1983), they both seem to act by increasing cyclic AMP (Stoff et al. 1979; Erlij et al. 1980b). More direct evidence comes from experiments with the isolated perfused tubule of the rectal gland, which responds to cyclic AMP and agents that modify its content in a manner identical to the perfused gland (Greger, Schlatter, Wang & Forrest, 1984). Our determinations of cyclic AMP in the gland have a limitation natural to measurements in whole organs, namely that more than one cell type is being sampled, but given the evidence discussed above, we feel that it is reasonable to suggest that the increases in cyclic AMP and secretion rate found during volume expansion are linked. Apart from secretory cells, the other group of cells that could provide a reasonable site for the changes in cyclic AMP, since they are present in great abundance, are vascular cells. We feel that, for the time being, vascular cells are a weaker candidate for the site of increases in cyclic AMP, particularly if one wishes to suggest that this is the sole site of the increase. Indeed, so far there is no evidence of increases in cyclic AMP content in the vascular cells of the rectal gland during activation of secretion. Moreover, even though it has been shown that stimulation of secretion is associated with increased blood flow through the gland (Shuttleworth, 1983), there is no evidence that increased perfusion can by itself increase secretion.

A marked reduction in the content of high energy phosphate compounds was observed during the activation of secretion by volume expansion. This observation is best explained by suggesting that the reduction in high energy phosphate content is linked to an activation of an energy-requiring process. Another possible cause for the fall in the content of high energy phosphates could be a curtailment of blood flow through the gland during volume expansion. However, others (Solomon et al. 1984a) found that blood flow through the gland actually increases during volume expansion. In addition to a reduction in high energy phosphate compounds, the activation of secretion leads to a marked increase in oxygen uptake (Silva et al. 1980). Since the former reflects energy utilization while the latter enhanced synthesis of high energy compounds, it is evident that we are dealing with a marked increase in the rate of energy turnover. As in the case of the cyclic AMP changes, the interpretation of these findings has the limitations natural to measurements in organs composed of various cell types. However, we suggest that the changes in high energy phosphate compounds are likely to be linked to the secretory process, because this is
the major energy-requiring process that is activated in the gland during volume expansion.

The increase in adenosine concentration during secretion may simply be due to the enhanced ATP hydrolysis. The concentration of adenosine in the gland during marked stimulation of transport is close to the levels that will activate secretion in the isolated perfused gland. The threshold for activation of gland secretion is reached when adenosine concentration in the perfusate is somewhere around $10^{-7}$–$10^{-6} \text{mol l}^{-1}$. Thus, if adenosine plays a role in the regulation of secretion by the in situ gland, it is very likely to be a minor one, since the concentrations found in highly stimulated glands are barely high enough to stimulate secretion.

In summary, our results indicate that volume expansion may be the physiological modification that leads to glandular secretion. There is a receptor mechanism in the vicinity of the heart that upon activation initiates an unidentified sequence of events whose ultimate signal arrives at the gland, elevating cyclic AMP. We suggest that this increase in cellular cyclic AMP activates the secretory ionic mechanisms. This process increases energy utilization and thus enhances adenosine production.

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