of the hemodynamic effects of parasympathetic inhibition by Kent et al., documented a 30% decrease in cardiac output with a 12 mg/kg dose of atropine and a smaller decrease following surgical vagotomy (Bull. MDIBL 16:66, 1976). In contrast, Opdyke et al., found only minor decreases in blood pressure with either atropine (0.1 mg/kg) or hexamethonium (10 mg/kg) (Bull. MDIBL 18:32, 1978). In the present studies, a combination of both atropine and hexamethonium at doses intermediate to those employed by Kent and Opdyke were administered. Such dosages appear to inhibit rectal gland secretion in response to saline infusion, but the experiments do not allow distinction between a direct inhibitory effect on gland secretion and an indirect effect secondary to decreased cardiac output and rectal gland flow.

Similarly, the enhanced rectal gland secretion in response to saline infusion may be mediated directly by increased rectal gland blood flow or indirectly by activation of a hormonal or neurogenic stimulus to rectal gland secretion. Though blood pressure does not increase with either 150 ml of isotonic shark Ringers (Kent, et al., Bull. MDIBL. 14:55, 1974) or 30 ml of 1 M NaCl (Opdyke, et al., Bull. MDIBL. 12:73, 1972) in Squalus acanthias, it is not known whether cardiac output is altered by such maneuvers. It seems unlikely, however, that the three-fold increase in rectal gland blood flow can be entirely accounted for by an increase in cardiac output and furthermore that a three-fold increase in rectal gland blood flow can induce a 10-20 fold increase in the rate of secretion of the rectal gland.

ISOTONIC SOLUTIONS STIMULATE SECRETION BY THE "IN SITU" RECTAL GLAND OF THE DOGFISH

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The rate of secretion of the rectal gland of the dogfish varies over a wide range of values. The identification of the hormones or transmitters that control secretion rate requires prior knowledge of the variables in the internal environment of the animal that are capable of provoking secretion. The pioneer studies of Burger (Physiol. Zool. 35: 205-217, 1962) in which he measured the effect of injecting different solutions on the rate of secretion of fishes swimming in a tank provide a method to search for clues of the nature of the variables. His studies on free swimming fish, however, have some limitations. For example, the frequency with which samples can be collected is restricted, thus distorting the determinations of the time course of any effect. In turn, the low frequency of sampling may also distort the assessment of secretion rates because a single sample collected during a long period may pool liquid produced while the gland was secreting at quite different rates. Finally, the same problems apply to the collection of samples of venous and arterial blood.

In this communication we describe experiments in anesthetized dogfish that allow us to follow closely the time course of the changes produced by different experimental modifications, Dogfish (Squalus acanthias) weighing between 2 and 3.8 kg. were injected in the caudal artery with nembutal (20 mg/kg). The gills were perfused at a rate of 2L/min. with sea water equilibrated with air (T = 15°C). After opening the abdomen with a midline incision, the rectum was opened near the insertion of the rectal gland. A polyethylene catheter was tied in the orifice of the rectal gland duct to collect continuously the secreted fluid. For intravenous injections, a fine needle (23 ga.) was introduced into the posterior intestinal vein. The NaCl solution infused into the animals was a shark Ringer solution in which all urea had been replaced with NaCl. The composition (in millimoles per liter) was NaCl,

445; KCl, 5; $NaHCO_3$, 8; $CaCl_2$, 2.5; $MgSO_4$, 1.0; Na_2HPO_4, 1.0; pH was 7.6 after equilibrating with 99% O_2 and 1% CO_2. The urea and sucrose solutions infused were prepared by substituting all NaCl for 890 millimoles of either urea or sucrose.

Figure 1 illustrates two different experiments in which the effects of infusing an isotonic solution into the

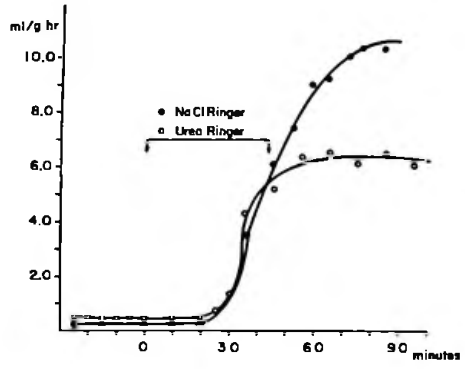

Figure 1.--Comparison of the effects of injecting isotonic solutions in which the principal solute was either NaCl or urea on the rate of fluid secretion by the "in situ" rectal gland of the dogfish. Fluid was injected during the period indicated by the horizontal bar. Ordinate: rate of fluid secretion in ml/hr · g of rectal gland wet weight.

posterior intestinal vein were studied. In the experiment represented by the filled circles, NaCl solution was injected, the shark weighed 1.65 kg and 150 ml were injected over a 45 min. period, (i.e., around 2 ml/kg min.). A delay of about 20 minutes elapsed before any increase in secretion was observed. Long delays were also observed in all other fishes (range 17-49 min.). The injection of fluid was interrupted once a clear cut increase of secretion had been achieved. Since all fishes were injected at nearly equal rates, the total injected volume (expressed per kg of weight) varied somewhat among different animals. Usually, the secretion continued to increase after suspending the injection of fluid, finally stabilizing at a high plateau that lasted between 1 and 2 hours and then declined rapidly towards resting level. In 5 animals the rates increased from 0.79 $\pm$ 0.35 ml/hr.g. (range .240 to 1.682) to 9.47 $\pm$ 1.33 ml/hg.g of gland wet weight (range 5.96 to 13.6 ml/hr.g). The chloride content in the secreted fluid was not significantly different in resting (505 $\pm$ 43 mM) and nonstimulated glands (497 $\pm$ 38 mM). A sixth fish that spontaneously was secreting at a rate of 4.2 ml/hr.g did not respond to saline infusion (total 200 ml) with an increase in secretion.

Three other animals were infused with urea-Ringer. In the three fishes injected with urea solution, the resting rates -0.514, 1.12 and .230 were increased to 6.47, 4.87 and 3.63 ml/hr.g respectively. A fourth animal was infused with sucrose solution. The sucrose infusion increased secretion from 0.67 to 4.35 ml/hr.g. As shown in the experiments of Figure 1 the general features of the response to the urea solution (empty circles) were similar to those observed when NaCl solution was used. The chloride concentration of the secretions of the fishes during stimulation with nonelectrolyte solutions (557 $\pm$ 69 mM) was not significantly different from that found during the control period (504 $\pm$ 47 mM).

The first point that emerges from these results is that the rectal gland can be stimulated to secrete by simple volume expansion since, as shown here the infusion of nonelectrolyte solutions were capable of inducing secretion. The larger responses obtained with sodium chloride solutions suggest the possibility of some specific effect of NaCl, however, the number of experiments is too small to be conclusive.

The effects of fluid injection cannot be ascribed to a simple modification of hemodynamic parameters in the fish since, as described in a companion note (Rubio, Berne, Silva and Erlij; This Bulletin), the ATP levels were significantly reduced in the glands stimulated to secrete, indicating the involvement of an energy requiring process.

Our findings confirm and extend the original observations of Burger (1962). In addition they provide a simple and reliable system to analyze the regulation of rectal gland secretion since the time course and magnitude of the effects of modifying the internal envrionments on the rate of secretion of the rectal gland can be followed with greater ease. Moreover, repeated sampling of arterial and venous blood, to analyze for hormone levels and electrolyte composition, as well as injection in different vascular beds can be carried out without difficulty. The information obtained with such measurements should permit more detailed speculation on the mechanisms regulating rectal gland secretion. This work was supported by Grants from the New York Heart Association and the NIH (AM 24064 and HLB 10384).

EFFECT OF STIMULATING SECRETION ON PURINE DERIVATIVES IN THE RECTAL GLAND AND BLOOD OF THE DOGFISH

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We have previously shown that extremely low concentrations of adenosine and some of its derivatives, such as ATP and ADP, stimulate markedly the rate of fluid and chloride secretion by the perfused rectal gland of the dogfish (Erlij, Silva and Reinach, Bull. MDIBL, 18: 92-93, 1978). As these purine derivatives are widely distributed in animal cells and fluids it is of interest to determine their concentration in the rectal gland and blood of the dogfish. It is also of interest to determine whether the increase in the rate of secretion caused by injecting saline solution can be ascribed to changes in concentrations in blood or tissue of these purine derivatives. Finally, the data on the content of high energy compounds can inform on the metabolic burden due to the high rates of secretion.

The experiments were carried out in anesthetized dogfish (Squalus acanthias). The samples of blood and glands were obtained from either animals secreting at a spontaneous resting rate or after the high rate of secretion induced by the injection of saline solution had reached a stable level. The data on the increase in secretion induced by saline solution injection in the glands used in this communication are described in an accompanying paper. (Erlij, Rubio and Silva, This Bulletin). The arterial blood was drawn from the posterior intestinal artery and venous blood from the vein of the rectal gland. The rectal glands were rapidly excised and immediately frozen and flattened with a set of Wollenberger clamps pre-equilibrated with liquid nitrogen. The glands were immersed in liquid nitrogen, pulverized, homogenized and extracted with perchloric acid. The samples of blood were also extracted following a similar procedure. After centrifugation and neutralization all the samples were analyzed for adenosine, inosine, and hypoxantine by an enzymatic method that follows uric acid production (Dobson, Rubio and Berne, Cric. Res. 29: 375-384, 1971). In addition the gland extracts were analyzed for creatine phosphate (CP), adenosine monophosphate (AMP), diphosphate (ADP) and triphosphate (ATP) following the changes in absorbance of NADH or NADPH (for details see Lamprech, Stein, Heinz and Weisser, Methods of Enzymatic Analysis U. Bergmeyer Ed. Vol. 4. pp 1777-1781, New York, Academic Press Inc., 1974, and Jaworek, D. Gruber, W. and Bergmeyer, U. in Methods in Enzymology U. Bergmeyer Ed. Vol. 4. pp 2127-2131, New York, Academic Press Inc., 1974).

So far we have carried out successful determinations in 3 non-stimulated animals and in 4 stimulated animals. The results are summarized in Tables 1 and 2. One of the interesting findings is that glands of both resting and stimulated animals contain similar levels of adenosine. These levels are an order of magnitude larger than those that activate the perfused gland. In contrast, the venous and arterial blood had no detectable concentration of adenosine in animals secreting at either low or high rates. As far as the products of adenosine metabolism - inosine and hypoxantine - are concerned they were abundantly present in both the gland and