muscle (4.0 μmoles/min/g of muscle - our unpublished data). In those same experiments the A-V pCO_2 gradient rose from 6 mmHg to 27 mmHg. It is likely that such increases in tissue pCO_2 occur in the stimulated rectal gland and CA may permit more CO_2 to be exchanged across a smaller pCO_2 gradient by facilitating its diffusion and thus preventing critical large decreases in intracellular pH. The complete abolition of secretion in the avian salt gland following methazolamide may be explained by such a critical intracellular drop in pH so that fluid formation could not occur. Metabolic, hemodynamic and acid-base measurements are planned to try to show how carbonic anhydrase and CO_2 balance may be involved in the secretory process of the rectal gland.

In summary, we have shown that carbonic anhydrase inhibition reduces rectal gland secretion in vivo, when appropriate control secretory rates are established. The precise role of this enzyme is enhancing saline secretion in several vertebrate systems remains to be discovered. Supported by NIH - HL 22258.

NERVOUS CONTROL OF SECRETION OF RECTAL GLAND OF THE DOGFISH: PHARMACOLOGICAL EVIDENCE.

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We are interested in determining whether the nerve terminals observed with the electron microscope within the rectal gland of the dogfish play any role in the regulation of secretion (Doyle; Bull. MDIBL, 15:28-30, 1975). Since the pathway through which these fibers enter the gland has not been identified, electrical stimulation cannot be performed readily. To obtain clues whether the nerve fibers may play a regulatory role, we have used a pharmacological approach that is widely employed to depolarize preparations that cannot be stimulated electrically in easily reproducible form. This approach involves the use of agents that selectively activate the Na channel responsible for nerve impulse propagation. The agent most widely used for this purpose is veratridine (Minchin, J. Neurosci. Meth. 2:111-121, 1980). This alkaloid acts by blocking the inactivation of the nerve channels leaving them in a permanently open state. Once veratridine has been used to open the channels, a further test can be made of the specifity of the effect by determining the action of tetrodotoxin, a second agent with extremely high selectivity for blocking the Na channels involved in nerve propagation.

The experiments were carried out in the isolated and perfused rectal gland of the dogfish (Squalus acanthias) following procedures described in previous publications (Silva et al., Am. J. Physiol. 233:F298-F306, 1977; Erlij et al., Bull. MDIBL, 18:92-93, 1978).

The effects of veratridine on the rate of fluid secretion by the rectal gland of the dogfish are shown in Figure 1. Figure A illustrates one out of three experiments in which the concentration of veratridine used was 2×10^{-4}M. After allowing the gland to reach a stable rate of spontaneous secretion the drug was continuously infused. In every case veratridine produced a rapid and large increase in secretion. The rate was markedly increased already 5 minutes after beginning the infusion of the drug. A maximum was reached 15 to 20 minutes afterwards. In the three experiments with 2×10^{-4}M veratridine increased secretion from basal rates of 0.180, 0.060 and 0.045 ml/g.h. to peak values of 2.0, 1.2 and 2.4 ml/g/h/ respectively. In all the experiments with 2×10^{-4}M veratridine the effect peaked rapidly returning to half maximum level within 20 minutes and to a level near the control within 40 to 60 minutes. When lower concentrations were used the effect was more prolonged. One example is shown in Figure 1B. In this case the concentration used was 2×10^{-5}M. The initial effects were also observed rapidly, after the first 5 minutes of infusion the secretory rate was markedly stimulated. The rate increasing then to 1.32 ml/g.h. The initial maximum also was relatively brief but the decay to control levels was much slower; the secretory rate remaining above the resting level for more than two hours. An additional example of the use of this veratridine concentration is shown in Figure 2. On the average (n = 9)

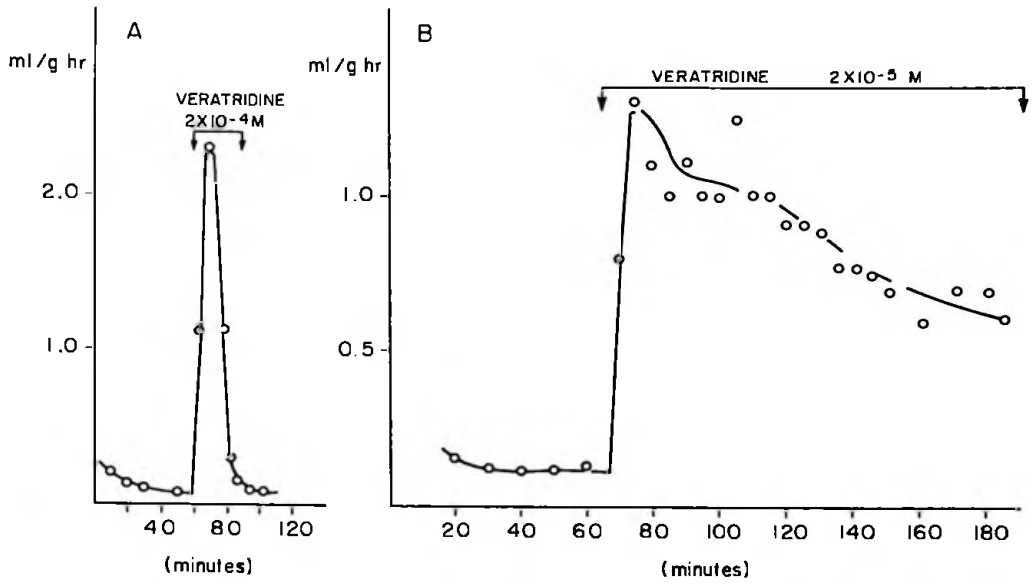

Figure 1.--Effects of two different doses of veratridine on fluid secretion by the perfused rectal gland of the dogfish.

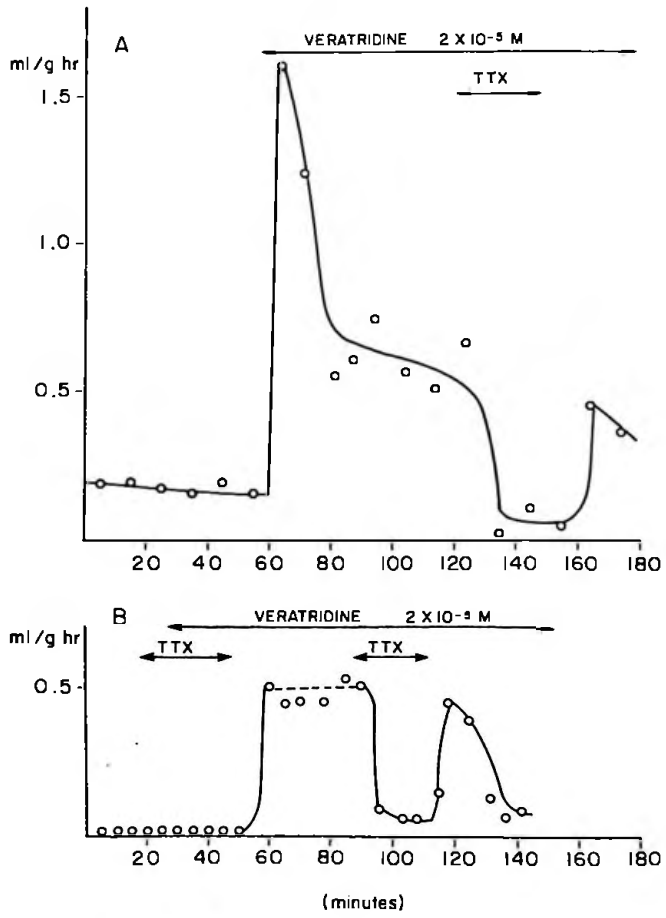

Figure 2.--Effects of tetrodotoxin (10^{-6}M) on the veratridine induced secretion. A) In this experiment tetrodotoxin was added once the veratridine effect had attained a steady level. B) In this experiment the gland was not secreting spontaneously during the initial part of the experiment.

2×10^{-5}M veratridine produced a peak increase to 1.23 ± 0.13 ml/g.h.; the secretory rate two hours after initiating the infusion was 0.565 ± 0.061 ml/g.h., still well above the control level of 0.088 ± 0.011 ml/g.h.

If the effects of veratridine on secretion are due to prolonged opening of Na channels like those involved in nerve propagation, the effect should be blocked by tetrodotoxin. Figure 2 shows two types of experiments carried out to test this point. In the experiment of Figure 2A, an infusion with veratridine (2×15^{-5}M) was initiated after the initial control period. Once the effect of veratridine had reached a stable level, tetrodotoxin (10^{-6}M) was also included in the perfusion fluid. The addition of tetrodotoxin caused a rapid reduction in the rate of secretion. When tetrodotoxin was washed out the rate of secretion increased to levels close to those observed before its addition. In the experiment in Figure 2B after the control period tetrodotoxin was infused alone it was followed by perfusion solution containing both tetrodotoxin and veratridine. In contrast with control experiments the addition of veratridine in the presence of tetrodotoxin did not stimulate secretion for up to 20 minutes. However when the perfusion was switched to a solution containing only veratridine, the rate rapidly increased to values above the control level.

Measurements of Cl^- content in the secreted fluid showed that Cl^- was not different in control and stimulated secretions (450 ± 21 mM and 447 ± 15 mM respectively).

These observations, showing that veratridine stimulates the secretion of the rectal gland through a tetrodotoxin sensitive mechanism, strongly suggest that depolarization of nerve fibers within the gland leads to activation of the Cl^- secretory mechanism. The depolarization very likely would act through the release of a transmitter that in turn activates the Cl^- secretion mechanism. Two alternative hypothesis to explain the effects of veratridine can be suggested: a) the effects are due to depolarization of a paracrine cell and not of nerve fibers; b) The effect is due to an increase of Na permeability of the Cl^- secreting cell itself. These hypothesis are, for the time being, less likely than the nerve depolarization proposal. When we consider the paracrine cell suggestion, it turns out that there is thus far no anatomical evidence showing the presence of such cells within the gland. While the possibility that an increased Na permeability of the secretory cells would, by itself, cause increased secretion runs contrary to our present understanding of the mechanism of Cl^- secretion by the gland (Silva et al., Am. J. Physiol. 233:F298-F206, 1977). Supported by the New York Heart Association.

EFFECTS OF ACETYLCHOLINE AND EPINEPHRINE ON DOGFISH GILL FILAMENT VASCULAR COMPARTMENT SIZE

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Blood entering the afferent filamental arteries of the dogfish gill either perfuses the respiratory lamellae to provide oxygenated blood to the systemic circulation or flows into the interlamellar and collateral vessels which drain back into the venous side of the circulation. The function of the latter pathway is not clear, but the vessels do course beneath the interlamellar filamental and water channel epithelium reported to contain chloride-like cells. A role in osmoregulation is implied. When the fish is exposed to a low oxygen environment, resistance to blood flow across the gill is known to increase (Kent & Pierce, Comp. Biochem. Physiol., 60C:37-44, 1978). This effect is mimicked by acetylcholine and blocked by atropine (Kent et al., MDIBL Bull. 20:109-111, 1980). Epinephrine, on the other hand, does not raise gill resistance, but increases systemic vascular resistance. The present study was undertaken to determine possible anatomical correlates to the change in gill resistance brought about by acetylcholine and epinephrine. The effect on the size of the two major blood compartments in the gill, the lamellar and intrafilamental-collateral, were studied by scanning electron microscopic analysis of methylmethacrylate corrosion replicas of the gill vasculature.