30 minutes following IV tracer injection. Three regions of brain were analyzed, cerebellum, medulla oblongata and telencephalon. $k_i$ was measured in isosmotic control skates; in skates rendered hyperosmotic with NaCl (56 mosmoles/kg 1M) or fructose (56 mosmoles/kg 1M); and, to test if NaCl influx across the BBB is carrier mediated, in control and hyperosmotic skates pretreated with methazolamide (100 mg/kg IV), furosemide (10 mg/kg IV) or bumetinide (1 mg/kg IV). There were five skates in each experimental group and measurements of $k_i$ in the hyperosmotic skates were made during the first 5 to 35 min of hypertonicity.

Value of $k_i$ (in units of $10^{-4}$ $min^{-1}$; mean $\pm$ SE) for $^{22}Na$ in isosmotic control skates were 14.0 $\pm$ 3.8, 7.0 $\pm$ 1.0 and 6.6 $\pm$ 0.8 in cerebellum, medulla and telencephalon, respectively. In skates rendered hyperosmotic with NaCl or fructose, $k_i$ increased linearly with plasma osmolality in all three brain regions (four-fold per 100 mOsm), to a degree sufficient to account for the Na loaded during hypernatremia. Furosemide and bumetinide inhibited the increase in $k_i$ by an average of 41%. Neither drug affected the isosmotic $k_i$. Methazolamide did not affect the increase in $k_i$.

The NaCl influx into brain in hypernatremic skates is associated with an inwardly directed NaCl concentration gradient (i.e., from plasma towards interstitium). The results summarized in Table 1 suggest this gradient is needed for

TABLE 1

LOSS OF EXTRACELLULAR ELECTROLYTES FROM BRAIN DURING FRUCTOSE HYPERTONICITY

| Condition | Telencephalon | | Medulla | | Cerebellum | |
|---|---|---|---|---|---|---|
| | Na | Cl | Na | Cl | Na | Cl |
| Control | 470 $\pm$ 15 | 432 $\pm$ 7 | 350 $\pm$ 10 | 298 $\pm$ 21 | 453 $\pm$ 21 | 428 $\pm$ 19 |
| Hypertonic 35 min | 470 $\pm$ 16 | 437 $\pm$ 12 | 332 $\pm$ 14 | 267 $\pm$ 11 | 405 $\pm$ 6* | 382 $\pm$ 5* |
| Hypertonic 2 hr | 455 $\pm$ 23 | 386 $\pm$ 14* | 316 $\pm$ 12 | 243 $\pm$ 14 | 402 $\pm$ 13* | 345 $\pm$ 12* |

Brain electrolyte concentrations in mEq/kg dry weight. Plasma osmolality increased by 55$\pm$7 mOsm and 67$\pm$5 mOsm after 35 min and 2 hr, respectively, of fructose-induced hypertonicity. Values are means $\pm$SE and N=5 in each experimental group.

NaCl loading. With fructose-induced hypertonicity, plasma [Na] and [Cl] fall and brain ISF [Na] and [Cl] rise together setting up outwardly directed [Na] and [Cl] gradients. Associated with this outwardly directed gradient, there is a loss of brain NaCl, increasing with time and most marked in the cerebellum.

The necessity for inwardly directed [Na] and [Cl] gradients, localization to the BBB, and partial inhibition by "loop" diuretics suggest that ISF volume regulation in the skate brain during hypertonicity is due to a passive increase in BBB permeability, in part carrier mediated. Supported by PHS NS 11050.

## SOMATOSTATIN INHIBITS THE STIMULATION OF CHLORIDE SECRETION IN THE SHARK RECTAL GLAND AT A STEP BEYOND ADENYLATE CYCLASE

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Previous reports have shown that somatostatin blocks the stimulatory effect of VIP or veratrine on chloride

secretion by the isolated perfused shark rectal gland, though stimulation by adenosine, forskolin, or CAMP and theophylline was not prevented. (Stoff. et al., Am. J. Physiol. 237:F138-F144, 1979, Epstein et al., MDIBL Bull. 22:11-12, 1982.) These results were difficult to interpret because the duration of the stimulus delivered by VIP and veratrine was brief, in the form of a bolus, whereas the other agents were given as a continuous infusion. Accordingly, the present experiments were designed in which stimulation by a variety of agents, at various points in the hormone-adenylate cyclase-effector chain, was delivered as a bolus superimposed upon a rectal gland already exposed to somatostatin, or in its native basal state.

Rectal glands were perfused as previously described (Silva et al. Am. J. Physiol. 233:F298-F306, 1977). After a control period during which a stable basal secretory rate of 130 + 28 μEq/min/g was established, a bolus of a stimulating agent. VIP ($3 \times 10^{-7}$ M), adenosine ($10^{-5}$M), forskolin ($10^{-5}$M) or dibutryl cyclic AMP ($5 \times 10^{-4}$M), plus methyl isobutyl xanthine MIX ($5 \times 10^{-4}$M), was given over a period of one minute. Following return of the secretion rate to its basal level (217 + 45 μEq/gm/min) somatostatin ($10^{-6}$M) in shark Ringers was infused for the ensuing twenty minutes. Ten minutes after the beginning of the somatostatin infusion another bolus of the stimulating agent was injected. Ten minutes later the infusion of somatostatin was stopped. After the secretion rate had again stabilized at 192 + 41 μEq/gm/min, a third bolus of the stimulating agent was given.

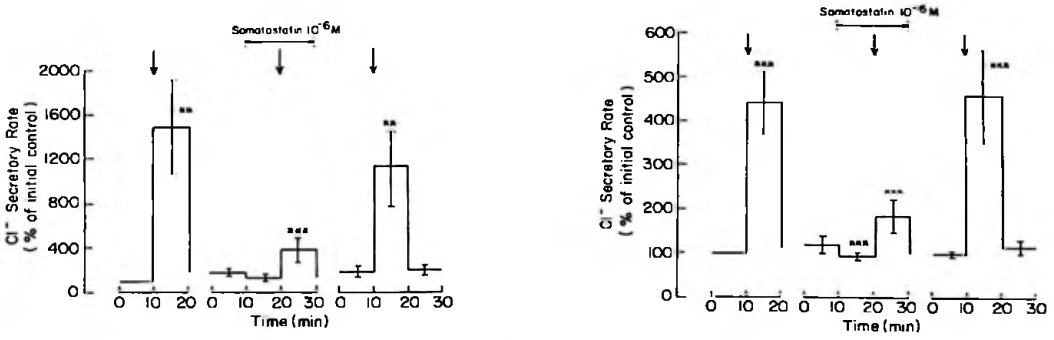


Figure 1.--Effect of somatostatin on rectal gland stimulation by VIP. Each column represents a ten minute period. The time intervals between each of the events during which secretion rate was allowed to return to basal levels varied between 30 and 60 minutes. VIP at a final concentration of $3 \times 10^{-7}$ M was given as a bolus for a period of one minute beginning at the times indicated by the arrows. Somatostatin was infused for 20 minutes at a final concentration of $10^{-6}$ M at the time indicated by the box. Values are mean + SEM. n=7

Figure 2.--Effect of somatostatin on rectal gland stimulation by adenosine. The results summarize 7 complete sequences in five glands. Values are mean + SEM.

Somatostatin substantially inhibited the effect of VIP (Figure 1). Before and after somatostatin, VIP stimulated chloride secretory rate to 1500% and 1100% of control respectively, but in the presence of somatostatin VIP stimulated secretion to only 226% of control.

The inhibitory effect of somatostatin on adenosine stimulation was entirely similar to that on VIP (Figure 2). This result stands in contrast to our previous experiments, in which rectal gland stimulation by a continuous infusion of adenosine $10^{-5}$ M was not prevented by somatostatin at either $1.4 \times 10^{-7}$ M or $1.4 \times 10^{-6}$ M. It appears that a continuous infusion of adenosine can break through the somatostatin block while a bolus infusion is appreciably suppressed.

While both VIP and adenosine activate adenylate-cyclase via a membrane receptor, forskolin is thought to activate the enzyme directly. We tested the effect of somatostatin on forskolin $10^{-5}$M (Figure 3). Somatostatin also inhibited the effect of forskolin, indicating an action at or distal to the catalytic subunit of adenylate cyclase.

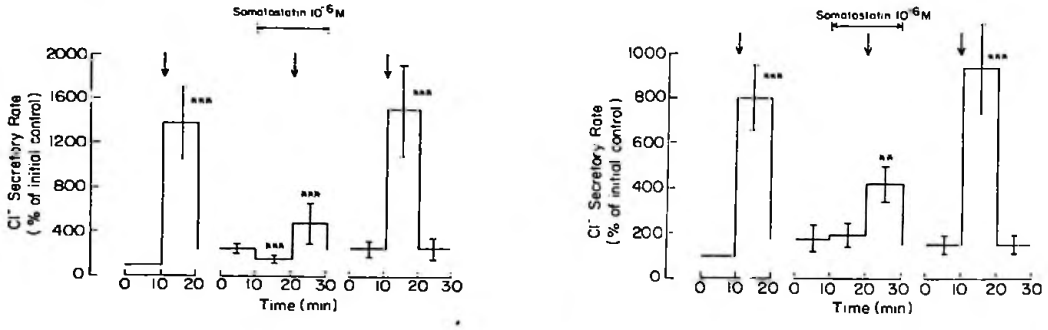


Figure 3.--Effect of somatostatin on rectal gland stimulation by forskolin. This figure summarizes the results of six experiments. Values are mean + SEM.

Figure 4.--Effect of somatostatin on rectal gland stimulation by dibutyryl cyclic AMP-methyl isobutyl xanthine (MIX). Dibutyryl cyclic AMP and methyl isobutyl xanthine were given together, both at a final concentration of $5 \times 10^{-4}$M. Values are mean + SEM. (n=4)

Finally, we repeated the experiment giving a bolus of dibutyryl cyclic AMP $5 \times 10^{-4}$ M together with methyl isobutyl xanthine $5 \times 10^{-4}$M. As seen in Figure 4, this mode of stimulation was also inhibited by somatostatin.

We conclude that the action of somatostatin to inhibit the secretory response of the rectal gland to diverse stimulating agents is non-specific and localized at a site distal to the generation of cyclic AMP.

## EFFECTS OF MONENSIN ON INTRACELLULAR ELECTROLYTES. OXYGEN CONSUMPTION AND OUABAIN BINDING IN SLICES OF SQUALUS ACANTHIAS RECTAL GLAND

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The present experiments were undertaken to test the hypothesis that the increase in ouabain binding that occurs when rectal gland cells are stimulated with theophylline and cyclic AMP is the result of the entry of sodium into the cell with consequent increase in activity of the sodium pump. Monensin, an ionophore with an ion specificity of Ag > Na > K > Rb > Cs > Li > NH4, was used to promote the entry of sodium into the rectal gland cell. Intracellular electrolytes and oxygen consumption were measured to ascertain that the effect of monensin on the rectal gland was consistent with the admission of sodium into the cells, so as to stimulate active transport.

Rectal glands were removed from Squalus acanthias and placed on ice. Slices were prepared from chilled tissue as previously described (MDIBL Bull. 19:72, 1980) and stored in ice cold shark Ringers. Slices were incubated in 5.0 ml of dogfish shark Ringers of the following composition (in mM): Na(280); K(5); Cl(295); Mg(3); Ca(2.5); $SO_4$(0.5); $PO_4$(1); urea(350); glucose(5) along with $^{14}$C-inulin, for the determination of the extracellular space, in a Dubnoff water bath under a 99% $O_2$ and 1% $CO_2$ gas phase at 25 C° for 15 or 60 minutes. Following the incubation, the slices were blotted dry on filter paper, weighed and either placed in the oven to obtain dry weights or homogenized in 500 microliters of distilled $H_2O$. The homogenates were placed in 1.5 ml Eppendorff centrifuge tubes and centrifuged for 5 minutes. Samples of the supernatant were used for both the determination of electrolytes and of inulin. Values for the intracellular electrolytes are expressed as mEq/L intracellular tissue water. Table 1 summarizes the effects at 15' and 60' of stimulation with theophylline $10^{-3}$M and dibutyryl cAMP $10^{-3}$M, monensin $1.5 \times 10^{-5}$, ouabain $10^{-4}$M and monensin plus ouabain on intracellular electrolytes in the slices of shark rectal gland.

Oxygen consumption was measured in a constant temperature 25°C chamber using a Clark type polarographic oxygen electrode (YSI) as previously described (MDIBL Bull. 22:9, 1982). The incubation solution had the following