Somatostatin: An endogenous peptide in the toad urinary bladder inhibits vasopressin-stimulated water flow

(somatotropin release-inhibiting factor/hormone-mediated epithelial transport/8-p-chlorophenylthioadenosine 3',5'-cyclic monophosphate/paracrine)

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ABSTRACT   Somatostatin (somatotropin release-inhibiting factor; SRIF) is a tetradecapeptide present in brain, pancreas, gastrointestinal tract, and thyroid that inhibits the secretion or action of several hormones in these tissues. We observed that the toad urinary bladder contains concentrations of endogenous somatostatin (8.0 pg/μg of protein) comparable to those found in the mammalian pancreas and gastrointestinal tract. To determine if somatostatin directly alters the action of vasopressin we studied the effects of this polypeptide on vasopressin-stimulated transport processes in the toad urinary bladder in vitro. Somatostatin produced a dose-dependent, reversible inhibition of vasopressin-stimulated osmotic water flow; it inhibited theophylline-stimulated water flow but not the water flow stimulated by 8-p-chlorophenylthioadenosine 3',5'-cyclic monophosphate. These data are consistent with an inhibition of both basal and hormone-stimulated adenylate cyclase. Vasopressin-stimulated short circuit current was not inhibited by somatostatin. These studies provide direct evidence for an effect of somatostatin on hormone-modulated epithelial transport in tissues other than the gastrointestinal tract. We propose that endogenous somatostatin may function as a local regulator of the cellular action of vasopressin on osmotic water flow.

Somatostatin (somatotropin release-inhibiting factor; SRIF) is a cyclic tetradecapeptide with various proposed physiological roles, including (i) an inhibitor of secretion of all known circulating gastrointestinal and several pituitary hormones; (ii) a neurotransmittor or neuromodulator in the central and peripheral nervous systems; and (iii) a local regulatory factor (paracrine, cybernin) (1–3). Somatostatin inhibits several hormone-stimulated ion transport processes in the gastrointestinal tract, including secretin-stimulated pancreatic bicarbonate secretion (2), acetylcholine-, histamine-, and gastrin-stimulated gastric acid secretion (2, 4), and vasoactive intestinal peptide (VIP)-stimulated colonic fluid secretion (5). Previous studies of the effects of somatostatin have been limited to tissues known to have high concentrations of this peptide (brain, pancreas, gastrointestinal tract, and thyroid (6–8).

Although immunoreactive somatostatin has not been detected in the mammalian kidney (6), in vivo infusions of somatostatin increase urine flow and decrease urine osmolality during antidiuresis induced by either endogenous or exogenous antidiuretic hormone (ADH) in the dog (9, 10). Because the toad urinary bladder is an established in vitro model for study of the action of ADH, we determined both the content of endogenous immunoreactive somatostatin and the effects of exogenous somatostatin on ADH-stimulated water and solute transport in this tissue.

METHODS

Paired hemibladders were removed from doubly pithed toads (Bufo marinus) (National Reagents, Bridgeport, CT), mounted as sacs on glass cannulae, and bathed in mucosal and serosal solutions as described (11). Net osmotic water flow (J_v) in paired hemibladders was determined gravimetrically. After a 30-min equilibration period, somatostatin was added (in amphibian Ringers as vehicle) to either the serosal or mucosal solution in the experimental group and basal J_v was determined at 10-min intervals for 60 min. Vasopressin (0.5–3 munits/ml), theophylline (20 mM), or 8-p-chlorophenylthioadenosine 3',5'-cyclic monophosphate (8-CPT-cyclic AMP) (10^{-5}–10^{-4} M) was then added to the serosal solution, and J_v was determined at 10-min intervals for an additional 60 min. Values for J_v are reported as mean flow (mg/min) for 60 min.

The effects of somatostatin on ADH-stimulated short circuit current (I_{SC}) and urea permeability were monitored in a double compartment lucite chamber as described (11). After a 30-min equilibration period, [^{14}C]urea (10^6 cpm/ml) was added to the mucosal compartment and was measured in 50-μl aliquots of the serosal fluid sampled every 10 min. After 60 min, somatostatin was added to the serosal solution of the experimental hemibladders, and the incubation was continued for an additional 60 min before addition of ADH. The rate of [^{14}C]urea movement before ADH addition was compared with the rate of its movement observed for 60 min after ADH addition.

To determine the content of immunoreactive somatostatin in the whole toad bladder, hemibladders were mounted as sacs and bathed with undiluted Ringer's solution on both serosal and mucosal surfaces. Following a 30-min incubation, the tissue was quickly removed from its glass cannula, blotted, and prepared for extraction and radioimmunoassay for somatostatin as described (6). Endogenous prostaglandin E₂ (PGE₂) production in the presence and absence of somatostatin was determined by direct assay of the serosal media for PGE₂ using a radioimmunoassay described by Dunn et al. (12).

Results obtained in one hemibladder were compared to those of paired controls by the paired Student's t test. All values are means ± SEM. Synthetic somatostatin was obtained from Wyeth and Sigma; Pitressin (ADH) was obtained from Parke, Davis and 8-CPT-cyclic AMP was obtained from ICN.

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RESULTS

The concentration of endogenous immunoreactive somatostatin in the toad urinary bladder was determined after 30-min incubation in Ringer's solution. Radioimmunoassay demonstrated a remarkably high concentration of endogenous somatostatin in this tissue (8.0 ± 1.1 pg/μg of protein, $n = 8$).

Addition of increasing concentrations of exogenous somatostatin to the serosal medium resulted in a dose-dependent inhibition of ADH-stimulated J_v. At 1 μM, somatostatin had no effect on basal J_v (0.87 ± 0.24 mg/min in somatostatin-treated hemibladders vs. 0.84 ± 0.18 in controls, $n = 8$) or on J_v response to 0.5 munit of ADH per ml (7.6 ± 1.7 mg/min vs. 8.1 ± 2.1 in controls, $n = 8$). At 2.5 μM somatostatin, J_v was inhibited by 44% in response to 0.5 munit of ADH per ml (4.9 ± 1.3 vs. 8.8 ± 1.7 in controls, $n = 8$, $P < 0.01$). At 5 μM somatostatin, J_v was inhibited by 73% in response to 0.5 munit of ADH per ml (2.5 ± 1.6 vs. 8.9 ± 2.5 in controls, $n = 6$, $P < 0.005$) but was not inhibited by 1.0 munit of ADH per ml (19.4 ± 2.3 vs. 17.4 ± 2.1 in controls, $n = 8$). At 50 μM somatostatin, basal J_v was slightly stimulated (1.00 ± 0.35 vs. 0.84 ± 0.36 in controls, $n = 14$, $P < 0.02$) but the ADH-stimulated J_v was inhibited by 70–74% in response to both 0.5 and 1.0 munit of ADH per ml (Fig. 1,
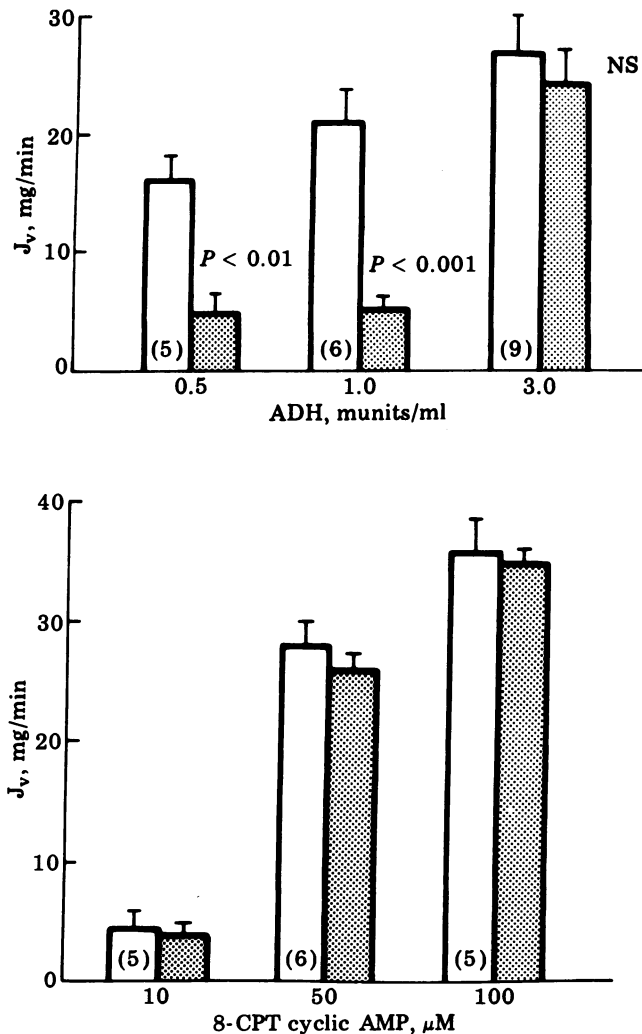
FIG. 1. (*Upper*) Effects of somatostatin (50 μM) in serosal media on ADH-mediated hydroosmotic water flow (J_v) in paired hemibladders. (*Lower*) Failure of somatostatin (50 μM) in serosal media to inhibit J_v stimulated by 8-CPT-cyclic AMP. All values are means $\pm$ SEM with the number of paired somatostatin and control hemibladders given in parentheses. ▨, With 50 μM somatostatin; □, control.
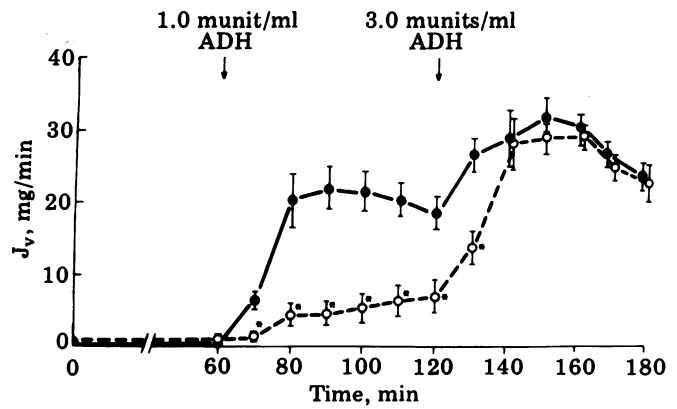
FIG. 2. Time course of somatostatin (50 μM, serosal) inhibition of water flow (J_v) stimulated by 1.0 munit of ADH per ml, with reversal of inhibition by 3.0 munits of ADH per ml. Values are means $\pm$ SEM in six paired somatostatin-treated (O---O) and control (●——●) hemibladders.

Upper). Addition of somatostatin (50 μM) to the mucosal solution did not inhibit ADH-stimulated J_v.

To further characterize the site at which somatostatin inhibited the action of ADH, the effects of somatostatin (serosal medium) on J_v stimulated by theophylline and by 8-CPT-cyclic AMP were compared. J_v stimulated by theophylline (20 mM) was inhibited by 85% in the presence of 10 μM somatostatin (2.2 ± 0.4 vs. 14.8 ± 0.9 mg/min in controls, $n = 8$, $P < 0.001$). In contrast, 50 μM somatostatin had no effect when water flow was stimulated by either submaximal or maximal concentrations of 8-CPT-cyclic AMP (Fig. 1, *Lower*). The inhibitory effect of somatostatin at submaximal doses of ADH (1.0 munit/ml) could be reversed by the addition of near maximal concentrations of ADH (3.0 munits/ml) to the serosal medium (Fig. 2).

The inhibition of ADH-stimulated J_v by somatostatin could not be attributed to increased production of endogenous PGE_2 in the presence of somatostatin. The production of PGE_2 during the 60-min basal period (determined as the release of PGE_2 into the serosal media) was identical in control and in 50 μM somatostatin-treated bladders (4.14 ± 1.27 pmol/min/g of hemibladder vs. 4.25 ± 1.02 in control, $n = 8$).

In contrast to the inhibition of ADH-stimulated J_v, all concentrations of somatostatin studied (up to 50 μM) were without significant effect on ADH-stimulated I_{SC}. Somatostatin (50 μM) had no effect on basal I_{SC} and did not alter the time course of the increase in I_{SC} in response to 0.5 munit of ADH per ml. The ratio of I_{SC} 15 min after ADH treatment to I_{SC} baseline was 1.45 $\pm$ 0.15 in somatostatin-treated bladders and 1.31 $\pm$ 0.01 in controls ($n = 5$, $P < 0.2$) (Fig. 3). Additionally, 50 μM somatostatin had no effect on basal [^{14}C]urea permeability, although a slight decline that was not statistically significant was observed in the rate of [^{14}C]urea movement induced by 0.5 munit of ADH per ml in somatostatin-treated bladders (2.3 ± 0.5-fold vs. 2.8 ± 0.6-fold in controls, $n = 5$, $P < 0.1$) (Fig. 3).

DISCUSSION

The present studies demonstrate that the toad urinary bladder contains remarkably high concentrations of endogenous immunoreactive somatostatin. The concentration of somatostatin found in the toad bladder (8.0 ± 1.1 pg/μg of protein) exceeds that detected by the same assay in all portions of the rat gastrointestinal tract [pancreas, 1.8 ± 0.13 pg/μg of protein; stomach 2.4 ± 0.22; pylorus 6.4 ± 0.92; duodenum and jejunum 1.94 ± 0.2 (6)]. Exposure of the bladder to exogenous somatostatin inhibits osmotic water flow stimulated by submaximal
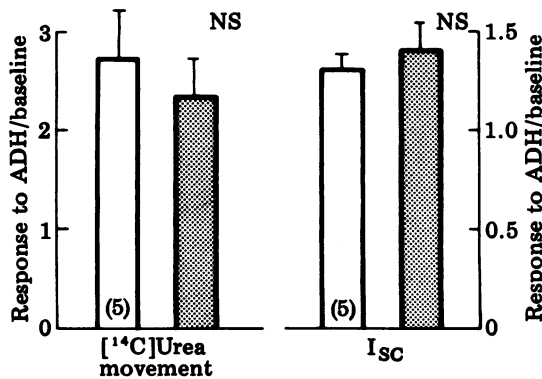
FIG. 3. Absence of an effect of somatostatin (50 μM) on ADH-stimulated urea movement and I_{SC} in response to 0.5 munit of ADH per ml. Values are the mean ratios ($\pm$ SEM) of post-ADH to baseline measurements with the number of experiments given in parentheses. In control tissue 0.5 munit/ml produced a 2.78 $\pm$ 0.57-fold increase in urea permeability; I_{SC} increased from 53.9 $\pm$ 10.6 to 71.8 $\pm$ 15.3 μA/cm^2. ⊞, With 50 μM somatostatin; □, control.

concentrations of ADH and by theophylline but does not inhibit the response to 8-CPT-cyclic AMP, a potent analog of cyclic AMP that mimics the maximum effect of ADH in the toad urinary bladder and mammalian cortical collecting duct (11, 13). These studies provide direct evidence for a role for somatostatin in modulating hormone-mediated epithelial transport outside of the gastrointestinal tract.

While the concentration of exogenous somatostatin required to inhibit ADH-stimulated J_v is higher than that required to inhibit insulin release in the rat pancreas or ion transport in the rabbit ileum, (1–10 nM) (3, 14), the concentration is similar to that required to inhibit VIP-stimulated colonic fluid secretion (10 μM) (5). The variable concentrations of exogenous somatostatin required to inhibit hormone release or action may reflect different local concentrations of this endogenous regulator in different tissues.

In the amphibian urinary bladder and mammalian collecting tubule, ADH-stimulated water flow down an osmotic gradient results from the binding of ADH to specific receptors on the baso-lateral membrane, the stimulation of adenylate cyclase, the cellular accumulation of cyclic AMP, and a series of post-cyclic AMP intracellular events that have not been firmly established. PGE$_2$, calcium ion, and catecholamines have been suggested as regulators of one or more steps in the cellular action of vasopressin (15). The observations that somatostatin (*i*) inhibits J_v stimulated by submaximal but not maximal concentrations of ADH, (*ii*) inhibits theophylline-stimulated J_v, and (*iii*) does not inhibit J_v response to submaximal concentrations of cyclic AMP analogs are identical to the responses observed with exogenous PGE$_1$ or PGE$_2$ (16–18). These data suggest that the inhibitory effect of somatostatin occurs beyond the receptor for ADH since theophylline-stimulated flow is inhibited. As suggested previously for prostaglandins (18), the observations are most consistent with an inhibitory effect of somatostatin on both basal adenylate cyclase (because theophylline-stimulated flow is inhibited) and hormone-stimulated adenylate cyclase. Inhibition of ADH-stimulated water flow by somatostatin is not due to increased production of endogenous PGE$_2$, because basal PGE$_2$ production in somatostatin-treated bladders was identical to controls. Tissue concentrations of cyclic nucleotides *per se* were not measured in the present study because changes in cyclic AMP content and adenylate cyclase activity were not detected in recent studies (19, 20) when toad bladder was exposed to less than 3 munits of ADH per ml.

The concept of somatostatin as a local regulatory factor or

cybernin (1), modulating the release or action of polypeptide hormones, is supported by previous studies in the pancreas and gastrointestinal tract. The localization of somatostatin in the delta cells of pancreatic islets in close proximity to alpha and beta cells (21) and the capacity of somatostatin to lower insulin and glucagon levels in man (22) and to reduce the secretion of both hormones in the isolated perfused pancreas (23) suggest a local or paracrine effect. It is likely that such effects are physiologic because increased insulin and glucagon are released from pancreatic islets in the presence of somatostatin antiserum (24, 25). Somatostatin also inhibits both the release of VIP (26) and thyroid-stimulating hormone (TSH) (27) and the response of target tissues to these hormones. VIP-stimulated fluid secretion in the rat colon (5), VIP-stimulated chloride secretion in the elasmobranch rectal gland (28), and TSH-stimulated triiodothyronine and thyroxin release from the thyroid in man (29) are all inhibited by somatostatin.

Consistent with the present findings in the toad bladder, somatostatin inhibits adenylate cyclase or the increase in cyclic AMP after hormone stimulation in many (5, 30–32) but not all tissues (33) responsive to somatostatin. Additionally, it has been proposed that somatostatin acts by altering the entry and intracellular release of calcium (34, 35). However, somatostatin also blocks calcium ionophore-stimulated hormone release (insulin, glucagon, growth hormone) (36–38). Taken together these data indicate that all biological actions of somatostatin cannot be explained simply by effects of the peptide on either intracellular cyclic AMP accumulation or calcium metabolism.

In summary our studies demonstrate that: (*i*) somatostatin produces a reversible, dose-dependent inhibition of ADH-stimulated J_v but is without effect on ADH-stimulated sodium movement, and (*ii*) the toad bladder contains concentrations of endogenous somatostatin comparable to the highest concentrations measured in the mammalian gastrointestinal tract. These findings suggest that endogenous somatostatin may be an inhibitor of ADH-stimulated water flow, providing local regulatory control of the cellular action of ADH, and that somatostatin may function in the urinary tract in addition to the gastrointestinal tract as a regulator of epithelial transport.

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