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A Role for Somatostatin in the Control of Hamster Growth

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BORER, K. T., B. SHAPIRO AND A. I. VINIK. *A role for somatostatin in the control of hamster growth.* BRAIN RES BULL 11(6) 663–669, 1983.—Concentrations of somatostatin-like immunoreactivity (SRIF-LI) were measured in cerebral cortex, hippocampus, septum-POA, median eminence, gastric antrum, fundus and pancreas in adult female hamsters to determine whether changes in somatostatin could be related to increased growth hormone (GH) secretion and somatic growth that follow bilateral transections of hippocampus (n=18; 17 controls). In addition, choline acetyltransferase (CAT) activity was measured in the four brain regions in hippocampectomized (n=10) and control hamsters (n=10) to gain insight into the relationship between these two neurotransmitters. Hippocampal transections induced: significant acceleration of somatic growth; increased serum GH concentrations; increased concentrations of SRIF-LI in septum-POA and gastric antrum; reduced concentrations of SRIF-LI in hippocampus and pancreas; and reduced CAT activity in the hippocampus. These results suggest that somatostatinergic and cholinergic projections to hippocampus via fornix suppress GH and somatic growth in adult hamsters and that reduced release of SRIF-LI in the gastric antrum may contribute to the acceleration of somatic growth through facilitated nutrient digestion and entry.

Hippocampus Septum-POA Median eminence Gastric antrum Fundus Pancreas
Choline acetyltransferase

SUPPRESSION of growth hormone (GH) secretion, of nutrient absorption [58,59], and inhibition of feeding [40,43] are some of the biological functions of somatostatin (SRIF). Thus, basal serum GH concentration rises when somatostatin action is blocked by active [65] or passive [70] immunization. Immunoneutralization of somatostatin restores the high amplitude of GH secretory pulses in rats in which GH release has been inhibited by stress [4,71], starvation [67], or diabetes [66]. The central site of somatostatinergic suppression of GH secretion is uncertain. It is thought that this action is mediated by the dense cluster of somatostatinergic neurons in the periventricular hypothalamus [27,33] and their terminations in the median eminence [5, 18, 21, 22, 29, 33, 52, 63], but the correlation between the control of GH secretion and the SRIF content of the median eminence following their damage is poor [22]. It is possible that the extra-hypothalamic SRIF neurons with terminations in the hypothalamus [23,29] or that the hypothalamic SRIF neurons with terminations in limbic forebrain [49] play a role in the control of GH release.

As the food passes through the gastrointestinal tract and as the nutrients enter into the systemic circulation, SRIF is released into the stomach from cells in the gastric antrum [58, 61, 62] and fundus [60] and into the splanchnic circulation from D cells in pancreatic islets [27, 35, 51, 60, 72]. As a consequence, nutrient absorption [58,59] and secretion of gut hormones [8,9] is suppressed, splanchnic blood flow is reduced [6,35], and food consumption is inhibited.

This evidence suggests that SRIF could influence somatic growth by controlling the release of GH from the pituitary as well as by controlling the ingestion and the availability of nutrients for anabolism. Evidence for such dual involvement of SRIF in the control of somatic growth comes from two lines of experiments. On one hand, when functional coupling of GH secretion and nutrient entry is disturbed by hypophysectomy, meals no longer elicit SRIF release [78]. On the other hand, chronic administration of pharmacologic doses of SRIF to rapidly growing animals suppresses growth [16, 53, 73], but may not represent the biological function of this peptide. We sought evidence for such dual role of SRIF in the control of somatic growth by looking for changes in somatostatin concentration in regions of hamster brain and gastrointestinal tract following growth-inducing hippocampal transections [11]. In this model of experimental acceleration of growth, there are concommitant increases in the rate of ponderal and skeletal growth, in food consumption, and in concentrations of serum GH and insulin [11].

We have also measured changes in brain acetylcholine in the same animals to gain insight into possible cholinergic-somatostatinergic interactions. Both neurotransmitters are affected in Alzheimer's disease [24,25], and there are conflicting data about their interaction in the brain [20,55].

METHOD

Animals and Maintenance

Female golden hamsters (*Mesocricetus auratus* Waterhouse) over 10 weeks old and weighing 90–100 g were obtained from Engle Laboratory Animals, Farmersburg, IN. Animals were housed individually in light (12L:12D) and temperature-controlled rooms (22°C) on a diet of Formulab 5008 Purina Chow and water ad lib.

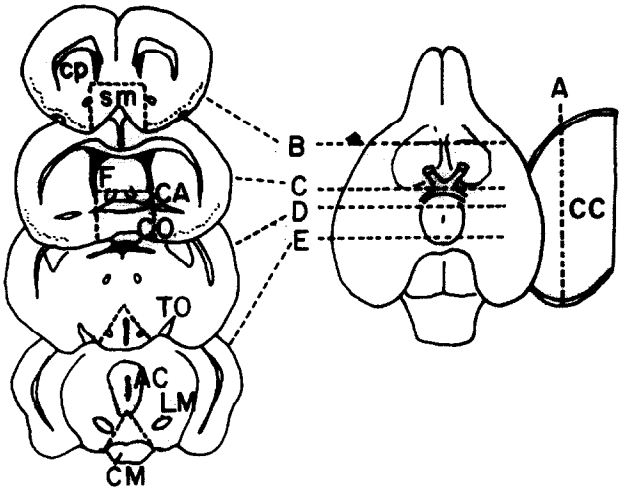

FIG. 1. Dissection of the hamster brain. Ventral view of the brain. Broken lines (right) indicate the location of coronal cuts defining cerebral cortex (A), septum-POA (B and C), and median eminence (D and E). Horizontal, parasagittal and oblique boundaries of these tissue blocks are outlined with broken lines (left) in representative histological sections in the indicated planes. Legend: AC—Cerebral aqueduct of Sylvius; CA—Anterior Commissure; CM—Mammillary Bodies; CO—Optic Chiasm; cp—caudate putamen; LM—Medial Lemniscus; F—Columns of the fornix; sm—medial septum; TO—optic tract.

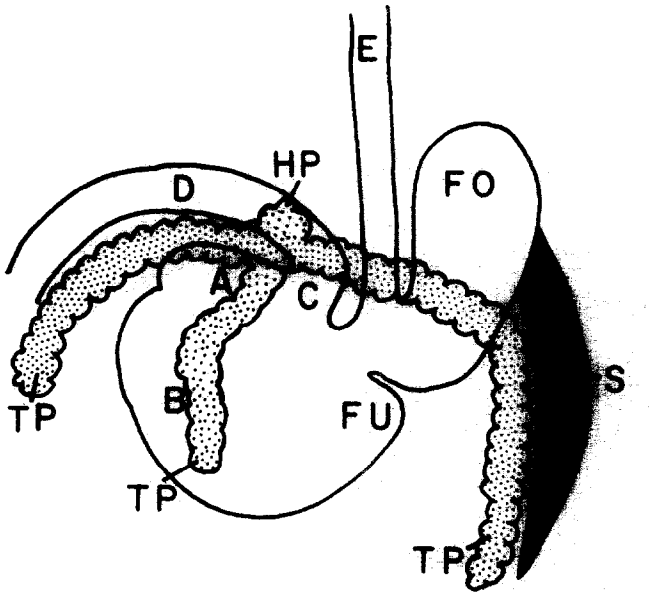

FIG. 2. Dissection of hamster upper gastrointestinal tract. Legend: A—Antrum; B—Body; C—Cardia; D—Duodenum; E—Esophagus; FO—Forestomach; FU—Fundus; S—Spleen; HP—Head of Pancreas; TP—Tail of Pancreas.

Experiments

We determined the tissue concentrations of somatostatin (n=35) and choline acetyltransferase (n=20) in hamsters which received bilateral hippocampal transection (HT, n=18) and in sham-operated controls (C, n=17). Experiment was done twice; hamsters were killed on postoperative days 9 (10 HT, 10C) and 14 (8 HT, 7C). We also determined tissue concentrations of gastrin in five regions of hamster gut to clarify their correspondence with regions of rat gut. This identification seemed warranted because of the subdivision of hamster stomach into a forestomach and a glandular stomach [26] in contrast to the more simple situation in the monogastric rat. We also determined serum GH concentrations in C and SH animals.

Hippocampal Transections

Hamsters were anesthetized with sodium pentobarbital (75 mg/kg IP). Dorsal hippocampus was transected [11] on both sides of the brain with a retractable wire microencephalotome in a plane perpendicular to its long axis. With the skull horizontal between bregma and lamda, the retracted knife was positioned 2.8 mm posterior to bregma and 0.5 mm lateral to midline. The knife was lowered 3.5 mm below dura, extended to a length 3.1–3.5 mm at a 45° angle in the anteriolateral direction and raised to the surface. Sham surgery followed identical procedure except that the knife was lowered 0.5 mm below dura.

Tissue Dissection

Animals were sacrificed by decapitation during the second hour of daylight. Blood was collected into chilled tubes. Serum was stored at −20°C for subsequent determination of growth hormone concentrations. The body and the head were kept on ice. The brain was rapidly removed onto a slab of aluminum maintained at 2°C.

Brain Tissues

The brain was dissected by a modification of the technique of Brownstein *et al.* [17]. Four regions of the brain were collected (Fig. 1). A 3-mm wide strip of cerebral cortex was separated (A) from the corpus callosum by cuts along and parallel to the longitudinal fissure bilaterally. The hippocampus was severed at its transition into dorsal fornix and removed distal to the cuts in HT, bilaterally. The brain was then placed ventral surface uppermost and divided by a series of coronal cuts. The septum-preoptic area (septum-POA) was contained in a block of tissue between cuts B (through the anterior third of the olfactory tubercle) and C (through the optic chiasm and anterior commissure), lateral cuts passing through the lateral ventricles and a dorsal cut placed 1 mm dorsal to the anterior commissure. This was then bisected sagittally. Median eminence was contained in a wedge-shaped block of tissue formed by coronal cuts D and E and two oblique cuts 1.5 mm to either side of the midline meeting at a point halfway between the aqueduct of Sylvius and the ventral surface of the brain. Cortex and hippocampus from one side of the brain, one half of the septum-POA and the median eminence were used for determination of immunoreactive somatostatin concentration. The other cortex, hippocampus, and the other half of the septum-POA were used for determination of choline acetyltransferase concentration.

Gastrointestinal Tissues

The viscera were dissected (Fig. 2) to provide samples of gastric fundus, antrum and pancreas. An additional eight unoperated animals were dissected to provide samples of

TABLE 1

TISSUE CONCENTRATIONS OF IMMUNOREACTIVE GASTRIN AND SOMATOSTATIN IN THE UPPER GASTROINTESTINAL TRACT OF THE GOLDEN HAMSTER (n=8)

	Gastrin-LI (pg/mg Wet Weight)	SRIF-LI (pg/mg Wet Weight)	SRIF-LI (pg/μg Protein)
Esophagus	15.6 ± 3.4*	4.3 ± 2.9	0.031 ± 0.061
Forestomach	7.3 ± 1.9	0.0 ± 0.0	0.00 ± 0.00
Fundus	54.1 ± 28.9	2418.9 ± 405.8	17.361 ± 2.950
Body of stomach	21.1 ± 15.1	1986.9 ± 325.0	14.415 ± 2.089
Antrum	179.4 ± 15.1	156.9 ± 45.9	1.316 ± 0.380

*Mean ± S.E.M.

esophagus, forestomach, gastric fundus, body, and antrum for determinations of gastrin and somatostatin concentration.

Sample Preparation

All tissue samples were wrapped in pre-weighed pieces of aluminum foil, rapidly frozen in solid CO_2 and weighed on a Cahn torsion electrobalance (Cahn Instruments, Cerritos, CA).

For somatostatin and gastrin determinations, tissues were homogenized 24 hr after tissue collection in 2 ml of 2 M acetic acid, immersed in a boiling water bath for 10 minutes to inactivate proteolytic enzymes and rehomogenized. Four ml of 2 M acetic acid was added to all homogenates for a final volume of 6 ml except for the median eminence which was adjusted to a final volume of 3 ml. The tissue homogenates were centrifuged at 700 g for 20 minutes. The supernatants were stored at −20°C for protein determination [44].

For somatostatin determination, aliquots of extracts were lyophilized and reconstituted to the original volume in assay buffer, immediately before assay.

Assays

Somatostatin-like immunoreactivity (SRIF-LI) was measured by radioimmunoassay [75,76] utilizing an antiserum raised in rabbits to synthetic cyclic SRIF 1–14 conjugated to whelk hemocyanin by the carbodiimide reaction. This antiserum (1374 Cape Town) was used in a dilution of 1:4000 and has been shown to be specific for the 6–11 region of SRIF 1–14. It had 75% crossreactivity with SRIF 1–25, and 60% crossreactivity with SRIF 1–28. The label used was ^{125}I-Tyr-1-SRIF prepared by the chloramine-T iodination, and purified by chromatography on a CM 52 cellulose ion exchange column. The specific activity was approximately 750 Ci/mole. The standard reference preparation was synthetic cyclic SRIF 1–14 (Ayerst AY 24910, Ayerst Laboratory, Rouses Point, NY). The assay was performed in 50 mM ammonium acetate buffer at pH 5.6. Separation of antibody-bound from free labeled somatostatin was achieved by the use of dextran-coated charcoal. The assay had an interassay coefficient of variation of 12% and an intraassay coefficient of variation of 5% with a limit of sensitivity (2 standard deviations above the assay buffer control tubes) of 2 pg/tube. Tissue extracts were assayed in multiple dilutions in triplicate to determine the parallelism of extracted SRIF-LI with the standard curve. To determine recovery of somatostatin, each tissue was bisected along the sagittal plane and 400 ng of synthetic SRIF 1–14 was added to one half of the tissue prior to homogenization and determination of SRIF concentrations.

Gastrin-LI was determined by radioimmunoassay (Becton-Dickinson Co., Orangeburg, NY) using an antibody raised in rabbits in a dilution of 1:100,000. This antiserum recognized all forms of gastrin [7]. Synthetic heptadecapeptide gastrin was used for standard and was labeled with ^{125}I by the chloramine T method for tracer. Separation of bound from free tracer was achieved by the use of an ion exchange resin (Amberlite CG4B). The level of sensitivity was 1.6 pg/tube and the intra-assay coefficient of variation was 5%.

For choline acetyltransferase determination by the method of Fonnum [29], tissues were homogenized at 1:50 dilution in 0.5% Triton in 10 mM EDTA, pH 7 [29]. Growth hormone concentration was measured with a homologous radioimmunoassay for hamster GH [15].

Data Analysis

Ponderal growth rate was determined with least-squares linear regression of weight as a function of time. Student's *t*-test (two tailed) was used to evaluate differences between experimental groups. To normalize GH distribution, statistical analysis was performed on logarithmic transformation of the data.

RESULTS

The recovery of synthetic SRIF-LI 1–14 after the extraction procedure was 90.4±8.4%, and the data were not corrected to account for the percent of recovery. In all instances, SRIF-LI in tissue extracts diluted in parallel with the SRIF-LI 1–14 standard. A comparison of SRIF-LI and gastrin-LI in the upper gastrointestinal tract is presented in Table 1. Concentration of gastrin-LI was highest in the antrum. The fundus and body of stomach had 13 to 50 times more somatostatin than the antrum. There was about 50 to 200 times less somatostatin in the median eminence of sham-operated hamsters as compared to the values usually found in the ME of rats [2, 17, 23, 50, 68, 69].

Hippocampal cuts induced significant acceleration of somatic growth as reflected in four-fold increase in the rate of ponderal growth in the lesioned hamsters relative to the values obtained from the sham-operated animals (2.1±0.2 vs. 0.5±0.2 g/day, $p<0.001$). Serum GH concentration was six times higher in lesioned than in sham-operated hamsters (36.2±12.3 vs. 6.5±1.0 ng/ml, $p<0.01$). Hippocampal transections induced a significant 39% depletion of hippocampal

TABLE 2

CONCENTRATION OF SRIF-LI IN REGIONS OF THE HAMSTER BRAIN AND GUT FOLLOWING HIPPOCAMPAL TRANSECTIONS (n=18) OR SHAM SURGERY (n=17)

	(pg/mg wet weight)		(pg/μg protein)	
	Hippocampal transection	Sham surgery	Hippocampal transection	Sham surgery
Cerebral cortex	185.4 ± 28.0§	161.0 ± 22.3	1.7 ± 0.3	1.4 ± 0.2
Hippocampus	247.5 ± 30.1	406.0 ± 58.0*	2.6 ± 0.3	4.3 ± 0.5‡
Septum-POA	470.3 ± 36.1	319.8 ± 42.7†	3.9 ± 0.3	3.3 ± 0.3
Median eminence	506.9 ± 82.6	414.3 ± 39.5	4.4 ± 0.9	3.8 ± 0.4
Antrum	54.6 ± 6.1	34.7 ± 3.7†	0.5 ± 0.1	0.3 ± 0.02*
Fundus	714.0 ± 102.3	899.4 ± 96.2	6.6 ± 1.2	5.5 ± 0.7
Pancreas	151.6 ± 25.4	256.8 ± 35.7*	1.0 ± 0.1	1.7 ± 0.3†

§Mean ± S.E.M.
*$p<0.05$.
†$p<0.02$.
‡$p<0.01$.

TABLE 3

CONCENTRATION OF CHOLINE ACETYLTRANSFERASE IN REGIONS OF THE HAMSTER BRAIN FOLLOWING HIPPOCAMPAL TRANSECTIONS (n=10) OR SHAM SURGERY (n=10)

	(pM/mg wet weight)		(nM/mg protein)	
	Hippocampal transection	Sham surgery	Hippocampal transection	Sham surgery
Cerebral cortex	2302.6 ± 111.2‡	2456.1 ± 111.9	20.1 ± 1.6	22.0 ± 1.1
Hippocampus	556.2 ± 92.6	3105.8 ± 100.5*	5.3 ± 0.9	28.0 ± 1.0†
Septum-POA	5350.0 ± 113.0	4651.5 ± 192.3	51.8 ± 7.4	49.9 ± 5.0

‡Mean ± S.E.M.
*$p<0.01$.
†$p<0.001$.

somatostatin (Table 2) and 82% depletion of hippocampal acetylcholine (Table 3). Hippocampal transection induced an accumulation of SRIF-LI in septum-POA (Table 2). Furthermore, there was a significant accumulation of SRIF-LI in the antrum and depletion of SRIF-LI from the pancreas (Table 2).

DISCUSSION

In this study we have investigated a possible role of somatostatin in the control of hamster growth through concurrent suppression of GH secretion and nutrient entry from the gastrointestinal tract. We found that the acceleration of somatic growth by bilateral hippocampal transection which involves increased skeletal growth, increased serum GH and insulin concentrations, and transient increase in food consumption [11], is associated with regional changes in SRIF-LI concentration in the hamster brain and gastrointestinal tract.

Growth-inducing hippocampal transections were associated with a significant depletion of hippocampal SRIF-LI, an accumulation of SRIF-LI in septum-POA, and no change in the SRIF-LI concentration in the median eminence (Table 2).

This pattern of change in cerebral SRIF-LI concentration following growth-inducing hippocampal transections, has the following implications with respect to distribution of SRIF fibers in the hamster brain, and their role in the control of growth and GH release in the mature hamster. First, there appears to be a species difference in the extent of somatostatinergic innervation of the median eminence, but not of septum (and hippocampus) in the hamster and the rat. We found evidence (Table 2) that at least 40% of hippocampal SRIF-LI in the hamster is of extra-hippocampal origin and arrives to this target via the septum-POA. Likewise, about 70% of septal SRIF-LI depends on connections originating in the periventricular hypothalamus in the rat [48,77]. Some of the hippocampal SRIF-LI content in the rat is of local origin [52]. In contrast to this congruence of SRIF distribution in the limbic forebrain in the two species, rat appears to have a relatively greater distribution of somatostatinergic fibers from the periventricular to the median eminence areas [1, 5, 18, 21, 23, 29, 33, 52, 63] than the hamster judging from the 100 times lower concentration of SRIF-LI in the median eminence of the hamster (Table 2) than the values reported for the rat [2, 17, 23, 50, 68, 69].

This distribution difference is paralleled by a similar species difference in the role of SRIF in the control of GH secretion and of somatic growth between the hamster and the rat. In the rat, somatostatinergic fibers arriving to the median eminence from the periventricular nucleus appear to play a role in the suppression of growth, in the maintenance of low basal serum GH concentrations [21, 22, 46, 47], and in the suppression of GH release in response to

stress [21,22]. In the hamster, suppression of growth is mediated by a septohippocampal neuronal circuit [13, 14, 15, 16], which includes somatostatinergic fibers with terminations in hippocampus but not in the median eminence area (Table 2). Furthermore, hamster is not likely to have the component of SRIF-LI projection to the median eminence which suppresses GH secretion in response to stress in the rat [4, 21, 22, 69, 71], because it responds to ether stress with an increase in GH release [15].

Thus it would appear that the control over GH secretion in the hamster is mediated by somatostatinergic innervation of the hippocampus and by neurons communicating with the median eminence via transmitters other than SRIF-LI.

Our data imply that somatostatin and acetylcholine may act in concert in the control of GH secretion and somatic growth in the hamster. Our cuts most probably damage the well-known cholinergic projection to hippocampus and cerebral cortex from cells located in medial septum and in the brainstem [36, 41, 42, 64]. Acetylcholine has been implicated in facilitation of sleep-related GH secretion in the man [45] and GH secretion in the rat [74], but conflicting results on the interactions between acetylcholine and somatostatin [20,55] make it uncertain whether this effect is mediated by cholinergic inhibition of somatostatinergic neurons. Our findings of parallel depletions of SRIF-LI and acetylcholine in hippocampus are in concert with the findings of parallel depletions of these two neurotransmitters in the forebrain of patients with Alzheimer's disease [24,25] and prompt further experimental scrutiny of the functional relationship between these two neurotransmitters.

Changes in the regional concentration of SRIF-LI in hamster gastrointestinal tract during rapid growth suggest that this peptide may play a role in nutrient entry in this species. We tentatively interpret the increases in antral SRIF-LI concentrations as evidence of reduced somatostatin release into the antrum and of possible reduced paracrine action of this peptide on adjacent [32] gastrin cells. An antagonistic interaction between somatostatin and gastrin has previously been shown in the stomach of the rat [56,57].

We have also uncovered another apparent species difference in the regional distribution of SRIF in this rodent. Hamster fundus contains 15 times more SRIF-LI than the antrum (Table 1) in contrast to the rat in which SRIF concentration in the antrum has been reported to be similar to that in the fundus [3] or ten times higher [22,38]. We hypothesize that high fundic SRIF-LI concentration may have functional significance for the control of nutrient transit between the forestomach and glandular stomach [26] and for the remarkable constancy of hamster meal size and frequency under the conditions of variable nutrient need [14].

Depletion of pancreatic SRIF-LI in rapidly-growing hamsters suggests that lesions induced oversecretion of this peptide from pancreatic D cells. This putative oversecretion of SRIF may reflect altered pattern of central nervous control over pancreatic islets as was reported to occur in rats with lesions of the ventromedial hypothalamus [30]. Alternately, putative oversecretion of pancreatic SRIF may be a reactive endocrine reflex to increases in concentration of circulating GH [31] or to increased consumption of food [34,51].

Thus, our data suggest, but do not prove, that in the hamster, somatostatin exerts an inhibitory influence over GH secretion through nerve terminals innervating the hippocampus, and over nutrient entry through cells located in gastric antrum.

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