

Release of Somatostatin-like Immunoreactivity from Incubated Rat Hypothalamus and Cerebral Cortex: *EFFECTS OF GLUCOSE AND GLUCOREGULATORY HORMONES*

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Somatostatin (SRIF) is localized in the hypothalamus, extrahypothalamic brain, and throughout the gastrointestinal tract. Release of gastrointestinal SRIF-like immunoreactivity (SRIF-LI) is under nutrient regulation but the effect of nutrients on neural SRIF-LI is unknown. The present studies examined the effects of glucose uptake and metabolism and hormones influencing glucose disposition on SRIF-LI release from medial basal hypothalamus (MBH) and cerebral cortex (Cx) incubated in Krebs-Ringer bicarbonate containing bacitracin. After a preincubation to achieve stable secretion, tissues were incubated for 20 min in 14 mM glucose (basal) and then, for 20 min in fresh medium with test materials. MBH SRIF-LI release was inversely related to medium glucose concentration with release in the absence of glucose (235±42 pg/MBH per 20 min) more than five times that in the presence of 25 mM glucose (46±4 pg/20 min). In the presence of 14 mM glucose MBH SRIF-LI release was stimulated above basal by agents interfering with glucose uptake including 3-*O*-methyl-d-glucose (42 mM; 70±5 vs. 42±3 pg/20 min, $P < 0.05$), phlorizin (50 mM; 351±63 vs. 29±2 pg/20 min, $P < 0.001$) or cytochalasin B (20 μM; 110±7 vs. 22±2 pg/20 min, $P < 0.001$). Inhibition of glucose metabolism by 2-deoxy-d-glucose resulted in dose-related stimulation of MBH SRIF-LI release (maximal at 28 mM; 201±28 pg/20 min vs. 32±4 pg/20 min, $P < 0.001$). Viability of [...]

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Release of Somatostatin-like Immunoreactivity from Incubated Rat Hypothalamus and Cerebral Cortex

EFFECTS OF GLUCOSE AND GLUCOREGULATORY HORMONES

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ABSTRACT Somatostatin (SRIF) is localized in the hypothalamus, extrahypothalamic brain, and throughout the gastrointestinal tract. Release of gastrointestinal SRIF-like immunoreactivity (SRIF-LI) is under nutrient regulation but the effect of nutrients on neural SRIF-LI is unknown. The present studies examined the effects of glucose uptake and metabolism and hormones influencing glucose disposition on SRIF-LI release from medial basal hypothalamus (MBH) and cerebral cortex (Cx) incubated in Krebs-Ringer bicarbonate containing bacitracin. After a preincubation to achieve stable secretion, tissues were incubated for 20 min in 14 mM glucose (basal) and then, for 20 min in fresh medium with test materials. MBH SRIF-LI release was inversely related to medium glucose concentration with release in the absence of glucose (235 ± 42 pg/MBH per 20 min) more than five times that in the presence of 25 mM glucose (46 ± 4 pg/20 min). In the presence of 14 mM glucose MBH SRIF-LI release was stimulated above basal by agents interfering with glucose uptake including 3-O-methyl-D-glucose (42 mM; 70 ± 5 vs. 42 ± 3 pg/20 min, $P < 0.05$), phlorizin (50 mM; 351 ± 63 vs. 29 ± 2 pg/20 min, $P < 0.001$) or cytochalasin B (20 μ M; 110 ± 7 vs. 22 ± 2 pg/20 min, $P < 0.001$). Inhibition of glucose metabolism by 2-deoxy-D-glucose resulted in dose-related stimulation of MBH SRIF-LI release (maximal at 28 mM; 201 ± 28 pg/20 min vs. 32 ± 4 pg/20 min, $P < 0.001$). Viability of MBH was unimpaired by incubation in the absence of glucose or following exposure to 2-deoxy-D-glucose as determined by reten-

tion of SRIF-LI responsiveness to stimulation by potassium (60 mM) or neurotensin (5 μ M). In contrast, Cx SRIF-LI release was slightly inhibited by decreases in medium glucose and unaffected by inhibition of glucose uptake or metabolism. These results provide evidence for nutrient regulation of MBH but not Cx SRIF-LI release and may explain inhibition of growth hormone seen in the rat in response to hypoglycemia. Insulin (10 nM–1 μ M) stimulated MBH but not Cx SRIF-LI release while glucagon was without effect. Our previous demonstration that MBH SRIF-LI release was stimulated by somatomedin-C, but not insulin at physiologic concentrations, is consistent with an action of insulin through the somatomedin-C receptor at the doses studied. Our studies indicate a regional specificity for the control of SRIF secretion within the brain and suggests the possibility of a role for hypothalamic SRIF in metabolic regulation.

INTRODUCTION

Somatostatin (SRIF),¹ the inhibitory hypothalamic regulator of pituitary growth hormone (1, 2) and thyrotropin (3, 4) release, is one of a group of peptides present in high concentration in the central nervous system (CNS), the pancreas, and gastrointestinal tract (5). Although the highest concentration of CNS SRIF-like immunoreactivity (SRIF-LI) is present in the median eminence (6), reflecting its hypophysiotropic role, the total content of SRIF-LI is considerably greater in the cerebral cortex (6). SRIF-LI is also present in D cells of the pancreatic islets (7) and throughout the gastrointestinal tract, with highest concentrations in the stomach (5, 6).

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¹ Abbreviations used in this paper: CNS, central nervous system; SRIF, somatostatin; SRIF-LI, somatostatin-like immunoreactivity.

Pancreatic and gastrointestinal SRIF exert an important role in nutrient homeostasis (8). Pancreatic SRIF inhibits the release of insulin (9) and glucagon (10), possibly through a local or paracrine effect, thereby influencing nutrient disposition. Gastric SRIF regulates acid secretion directly (11) and through local inhibition of antral gastrin release (12). In addition, gastrointestinal SRIF appears to have an inhibitory hormonal influence on nutrient, especially lipid, absorption (13). The demonstration that nutrients, in turn, stimulate the release of gastrointestinal SRIF-LI (14, 15) further supports a role for SRIF in nutrient regulation. Glucose stimulates pancreatic SRIF-LI (14) while lipids, especially free fatty acids, stimulate intestinal SRIF-LI release (15).

The role of CNS SRIF-LI in nutrient homeostasis is unknown. While hypothalamic SRIF-LI release has been demonstrated in response to a variety of stimuli including membrane depolarization (60 mM K⁺) (16, 17), biogenic amines (dopamine) (17), peptides (neurotensin) (17), and hormones [growth hormone (18, 19), somatomedins (20, 21), triiodothyronine (22)], and cerebral cortical SRIF-LI release occurs in response to depolarizing stimuli (23), there is a paucity of information available concerning the response of hypothalamic or extrahypothalamic CNS SRIF-LI to nutrients.

The present studies were designed to investigate the effects of glucose uptake and metabolism and of the hormonal regulators of glucose utilization on SRIF-LI release in vitro from incubated rat hypothalamus and cerebral cortex.

METHODS

Experimental animals. Male Sprague-Dawley rats, weighing 150–200 g were housed under constant temperature (24°C) and light (14 h)–dark (10 h) cycles and were provided free access to laboratory rat chow and water. Animals from a single supplier (Locke Erikson, Melrose Park, IL) were used in all experiments.

Animals were decapitated with minimum handling and stress and the brain rapidly (<2 min) removed to an ice-cold surface. The medial basal hypothalamus (defined by the lateral hypothalamic sulci, the mammillary bodies, and the optic chiasm; weight 22±3 mg; *n* = 10) was removed with curved-bladed scissors to a depth of ~2 mm (level of the fornix). A cut made in the coronal plane at the level of the optic chiasm and a parallel cut 3 mm rostral to the first yielded a slice of brain from which a single strip of cerebral cortex could be defined (ventrally by the corpus callosum and its radiations and dorsally by the brain surface) and removed (weight; 124±4 mg; *n* = 24).

Hypothalamic and cerebral cortex incubation. Incubations were performed in 0.5 ml Krebs-Ringer bicarbonate buffer containing bacitracin as previously reported (19, 21, 22) with varying concentrations of glucose and other additions as described below. The sodium chloride concentration

of the medium was varied as appropriate to provide a constant osmolality. After a preincubation to achieve stable secretion (60 min for hypothalamus, 80 min for cerebral cortex), tissues were incubated for 20 min (basal) in fresh medium containing 14 mM glucose. At the end of the basal period medium was removed, fresh medium containing the stimulus under investigation was added and the tissue was incubated for an additional 20 min. In some experiments one or two additional 20-min incubations were performed in medium containing 14 mM glucose with or without added stimuli. Media were removed after each incubation period, boiled for 5 min, centrifuged and the supernatants stored at –20°C until assayed.

Drugs. Stimuli used in the studies described included 60 mM KCl, neurotensin (5 μM, kindly provided by J. Rivier, Salk Institute), 3-*O*-methyl-*D*-glucose (42 mM), phlorizin (50 mM), and 2-deoxy-*D*-glucose (3–42 mM) (Sigma Chemical Co., St. Louis, MO), cytochalasin B (20 μM, Aldrich Chemical Co., Inc., Milwaukee, WI), cytochalasin D (20 μM, Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA), crystalline porcine insulin and bovine-porcine glucagon (10 nM–1 μM, provided by Eli Lilly Research Laboratories, Indianapolis, IN).

Radioimmunoassay. SRIF radioimmunoassay was performed as previously described (22). The assay sensitivity was 1–2 pg SRIF/tube; 12–18 pg SRIF produced 50% displacement from maximal binding. The interassay variation was 12% and the intraassay variation was 9%.

Statistics. Comparisons between groups were performed using Student's nonpaired *t* test and, where appropriate, an analysis of variance.

RESULTS

Effect of medium glucose concentration on SRIF-LI release from hypothalamus and cerebral cortex

Following a 20-min basal incubation period in medium containing 14 mM glucose, media were removed and replaced with fresh medium without glucose or with glucose concentrations ranging from 3 to 25 mM. Hypothalamic SRIF-LI release was inversely related to medium glucose concentration (Fig. 1A) with release in the absence of glucose (235±42 pg/hypothalamus per 20 min) more than five times that in the presence of 25 mM glucose (46±4 pg/20 min). SRIF-LI release from incubated cerebral cortex (Fig. 1B) exhibited a response opposite to that of the hypothalamus. Increasing glucose concentration resulted in a significant enhancement in SRIF-LI release that was maximal in the presence of 14 mM glucose.

In two experiments hypothalamic and cerebral cortical SRIF-LI responses to potassium (60 mM) or neurotensin (5 μM) stimulation were compared during incubation in the absence or presence of 14 mM glucose. Hypothalamic SRIF-LI release in the presence of 14 mM glucose was decreased compared with that in the absence of glucose without additional stimuli (29±2 vs. 149±20 pg/20 min, *P* < 0.001) and in response to

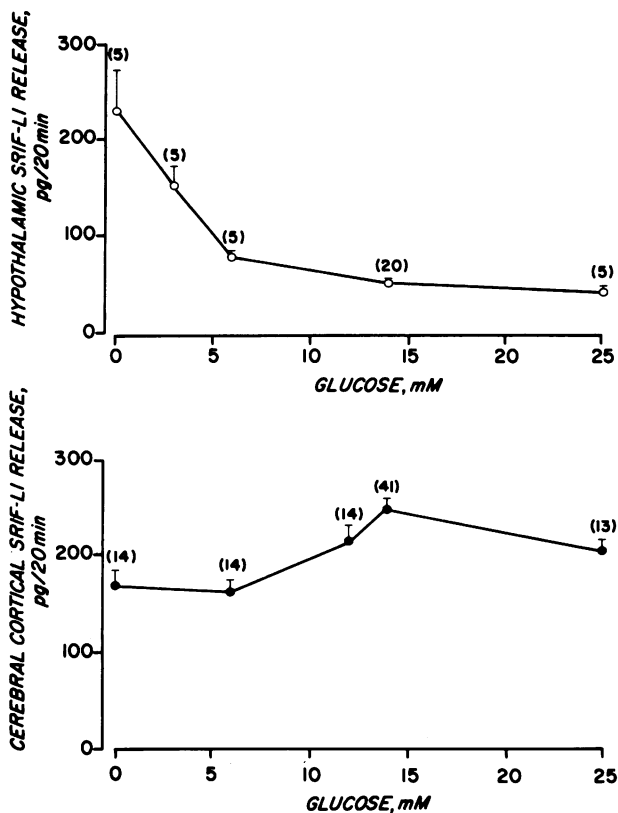


FIGURE 1 Effect of medium glucose concentration (0–25 mM) on SRIF-LI release from incubated hypothalamus (upper panel) and cerebral cortex (lower panel). Release is shown as the mean \pm SE. The number of incubations is in parentheses.

potassium (419 ± 16 vs. 576 ± 37 pg/20 min, $P < 0.001$) or neurotensin ($1,026 \pm 28$ vs. $1,170 \pm 24$ pg/20 min, $P < 0.001$). However, the incremental rise of hypothalamic SRIF-LI release above basal was unaffected by either stimulus. Cerebral cortical SRIF-LI release in response to both stimuli were unaffected by medium glucose concentration (Fig. 2).

Effects of agents influencing cellular glucose uptake or utilization on SRIF-LI release from hypothalamus and cerebral cortex

In all experiments involving agents interfering with glucose metabolism hypothalamic and cerebral cortical incubations were performed in the presence of 14 mM glucose.

(a) *3-O-Methyl-D-glucose*. Hypothalamic SRIF-LI release was stimulated by 3-O-methyl-D-glucose at a concentration of 42 mM as compared with basal release (70 ± 5 vs. 42 ± 3 pg/20 min, $P < 0.05$) (Fig. 3).

(b) *Phlorizin*. Hypothalamic SRIF-LI release was markedly stimulated in the presence of 50 mM phlorizin as compared with basal release (351 ± 63 vs. 29 ± 2 pg/20 min, $P < 0.001$) (Fig. 3).

(c) *Cytochalasin B and D*. Hypothalamic SRIF-LI release was stimulated fivefold in the presence of 20 μ M cytochalasin B (110 ± 7 vs. 22 ± 2 pg/20 min, $P < 0.001$) while release from the cerebral cortex was unaffected (107 ± 10 vs. 152 ± 28 pg/20 min). Hypothalamic SRIF-LI release was unaltered by exposure to cytochalasin D, 20 μ M (Fig. 3).

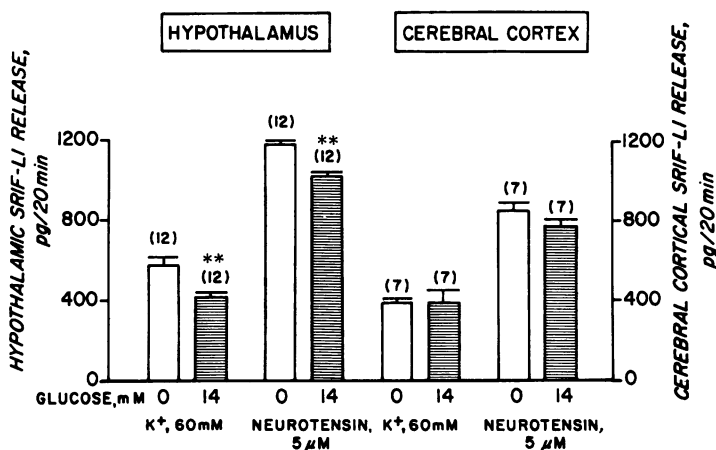


FIGURE 2 Effect of medium glucose concentration (0 or 14 mM) on SRIF-LI release from incubated hypothalamus and cerebral cortex. Release in response to stimulation by 60 mM K⁺ and neurotensin (5 μ M) is shown as the mean \pm SE. **, $P < 0.001$. Basal hypothalamic SRIF-LI release: 0 mM; 149 ± 20 , 14 mM; 29 ± 2 pg/20 min. Basal cerebral cortical SRIF-LI release: 0 mM; 83 ± 8 , 14 mM; 105 ± 6 pg/20 min.

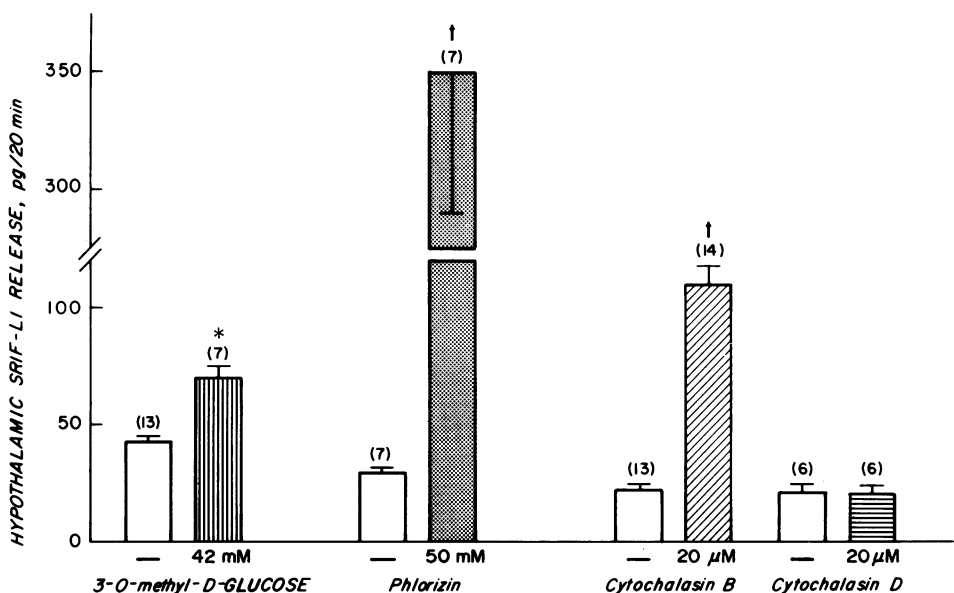


FIGURE 3 Effect of medium 3-O-methyl-D-glucose (42 mM), phlorizin (50 mM), cytochalasin B (20 μ M), and cytochalasin D (20 μ M) on SRIF-LI release from incubated hypothalamus*, $P < 0.05$; †, $P < 0.001$. See Fig. 1 for details.

(d) *2-Deoxy-D-glucose*. Hypothalamic SRIF-LI release showed a dose-related stimulation in response to 2-deoxy-D-glucose that was maximal at 28 mM (201 ± 28 pg/20 min) and represented a greater than sixfold increase over basal release (32 ± 4 pg/20 min). 2-Deoxy-D-glucose produced no significant effect on SRIF-LI release from the cerebral cortex in two experiments (Fig. 4).

To determine the effects of prior exposure to 2-deoxy-D-glucose on hypothalamic and cerebral cortical SRIF-LI release, a further series of incubations were performed. Tissues were first incubated for 20 min in medium containing 14 mM glucose alone or with 2-deoxy-D-glucose at concentrations ranging from 3 to 14 mM (period 1). They were next incubated for 20 min in medium containing 14 mM glucose (period 2). A dose-related stimulation of hypothalamic SRIF-LI release occurred in the presence of 2-deoxy-D-glucose as in the previous experiment (period 1, data not shown). The stimulatory effect persisted following the removal of medium containing 2-deoxy-D-glucose (period 2, Fig. 5). Cerebral cortical SRIF-LI release was unaffected by exposure to 2-deoxy-D-glucose. Tissues were next incubated for 20 min in medium containing 14 mM glucose together with 60 mM K^+ (period 3). Hypothalamic SRIF-LI release was stimulated in the presence of 60 mM K^+ during period 3 to a level that correlated with responses seen in presence of (period 1) and following (period 2) 2-deoxy-D-glucose.

Cerebral cortical SRIF-LI release in response to 60 mM K^+ was unaffected by prior exposure to 2-deoxy-D-glucose up to 6 mM. SRIF-LI release from hypothalamus and cerebral cortex in response to 60 mM K^+ was diminished following prior exposure to 14 mM 2-deoxy-D-glucose.

Effect of hormones influencing glucose disposition on SRIF-LI release from hypothalamus and cerebral cortex

Insulin stimulated hypothalamic SRIF-LI release in a dose-related manner. The lowest insulin concentration resulting in significant stimulation was 0.1 μ M (49 ± 4 vs. 35 ± 1 pg/20 min, $P < 0.05$). Cerebral cortical SRIF-LI release was unaffected by insulin (Fig. 6; upper panel). Glucagon, in concentrations of 10 nM to 1 μ M failed to influence SRIF-LI release from either hypothalamus or cerebral cortex (Fig. 6; lower panel).

DISCUSSION

The present study demonstrates that glucose is an important regulator of hypothalamic SRIF-LI release. Reductions in medium glucose concentration to levels below those normally present in rat plasma (5–6 mM) resulted in increased hypothalamic SRIF-LI release, while increases in medium glucose concentration resulted in an inhibition of hypothalamic SRIF-LI re-

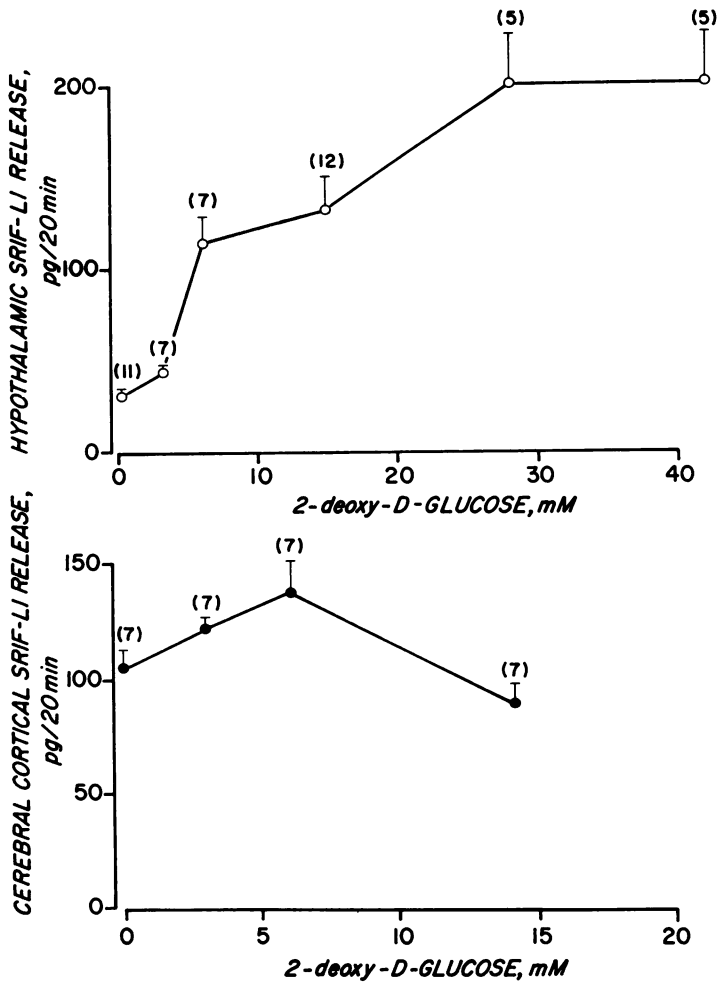


FIGURE 4 Effect of medium 2-deoxy-D-glucose (0–42 mM) on SRIF-LI release from incubated hypothalamus (upper panel) and cerebral cortex (lower panel). See Fig. 1 for details.

lease. Incremental hypothalamic SRIF-LI responses to depolarizing stimulation and to neurotensin were unchanged in the absence of medium glucose suggesting that there was no loss of tissue viability during the short-term (20 min) incubation. To identify the mechanism of glucose-mediated inhibition of hypothalamic SRIF-LI release, the effect of various substances influencing cellular glucose uptake or metabolism was evaluated. Drugs that competed with (3-O-methyl-D-glucose) (24) or blocked (phlorizin) (25) glucose uptake stimulated hypothalamic SRIF-LI release, suggesting that glucose entry into cells is necessary for the inhibitory effect. Cytochalasin B, which inhibits glucose uptake as well as intracellular transport systems (26), stimulated hypothalamic SRIF-LI release. In contrast, cytochalasin D, which has similar effects on intracellular transport but is devoid of effects on glucose up-

take (27), was without effect on SRIF-LI release supporting the hypothesis that glucose uptake into hypothalamic neurons is necessary to inhibit SRIF-LI release. The dose-related stimulatory effect of 2-deoxy-D-glucose on hypothalamic SRIF-LI release indicates that glucose metabolism beyond the level of glucose-6-phosphorylation is required to inhibit SRIF release. Prior exposure to 2-deoxy-D-glucose had no effect on subsequent hypothalamic SRIF-LI responses to depolarizing stimulation though the influence of 2-deoxy-D-glucose persisted for at least 20 min after its removal from the medium as demonstrated by continued elevated basal hypothalamic SRIF-LI release.

The site of glucose action in the hypothalamus has not been defined in the present experiments. A direct suppressive effect on SRIF-containing neurons could result from glucose uptake and metabolism. Alter-

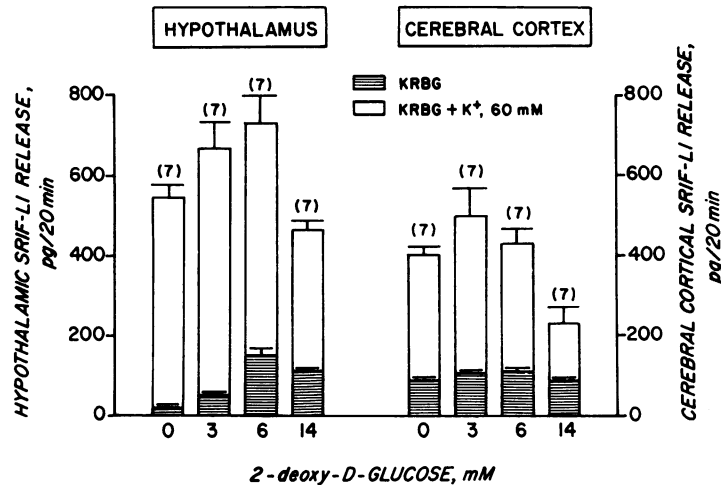


FIGURE 5 Effect of prior incubation in medium containing 2-deoxy-D-glucose (0–14 mM) on SRIF-LI release from incubated hypothalamus and cerebral cortex. Consecutive 20-min incubations in medium alone (Krebs-Ringer bicarbonate buffer with glucose, hatched bars; period 2) then medium + 60 mM K⁺ (Krebs-Ringer bicarbonate buffer with glucose + K⁺, open bars; period 3) are shown as the mean ± SE. See Text for details.

nately, glucose activation of the hypothalamic glucoreceptor could stimulate SRIF-LI release directly, or through interneurons.

Our studies have demonstrated a marked CNS specificity of the effect of glucose on neural SRIF-LI; changes in the opposite direction in cerebral cortical and hypothalamic SRIF-LI release were produced by altering medium glucose concentration. Decreasing medium glucose concentration resulted in decreased cerebral cortical SRIF-LI release, a phenomenon that could represent a metabolic substrate energy-dependent process. A similar phenomenon may have occurred with hypothalamic SRIF-LI release that was masked by the area specific stimulatory effect of low medium glucose.

While the present studies provide the first evidence for nutrient regulation of hypothalamic SRIF-LI release, such regulation of gastrointestinal and pancreatic SRIF-LI has been well described. These observations raise the possibility of an integrated role for hypothalamic, gastrointestinal, and pancreatic SRIF in nutrient homeostasis. Evidence for such a role first appeared with the description of the inhibitory effects of exogenous SRIF on intestinal motility (28), gastric acid secretion (29), pancreatic exocrine secretion (30), and splanchnic blood flow (31). These effects result in delayed or decreased nutrient absorption (13) and complement the inhibitory action of SRIF on hormones important in nutrient absorption (cholecystokinin) (32) or disposition (insulin and glucagon) (33). In subsequent reports nutrients, most notably fatty

acids (15), were shown to stimulate gastrointestinal and pancreatic SRIF-LI release, while glucose and amino acids stimulated predominantly pancreatic SRIF-LI release (14). Further support for a physiologic role of SRIF in fatty acid absorption was provided by experiments where passive immunization with anti-SRIF serum resulted in increased fatty acid absorption, suggesting that endogenous SRIF inhibits lipid absorption (34). Similar experiments have suggested that SRIF also acts physiologically to inhibit hormones important in nutrient homeostasis including gastrin (12), insulin (9), and glucagon (10).

While the present studies have documented an effect of ambient glucose concentration on hypothalamic SRIF-LI release, we have previously demonstrated that lesions of the ventromedial hypothalamus result in increased pancreatic SRIF-LI content and release indicating hypothalamic regulation of pancreatic SRIF (35). It thus appears that peripheral nutrient homeostasis can influence hypothalamic SRIF-LI whereas hypothalamic centers influence pancreatic hormone secretion and thus, in turn, nutrient disposition.

The effect of insulin and glucagon on CNS SRIF-LI release was determined to assess the effect of hormones influencing glucose disposition. Insulin resulted in a dose-related stimulation of hypothalamic SRIF-LI release that was apparent only at concentrations of 0.1 μM and greater. We have previously reported that insulin at physiologic concentrations (4 nM) has no effect on hypothalamic SRIF-LI release (21). The high levels of insulin required to achieve stimulation of SRIF-LI

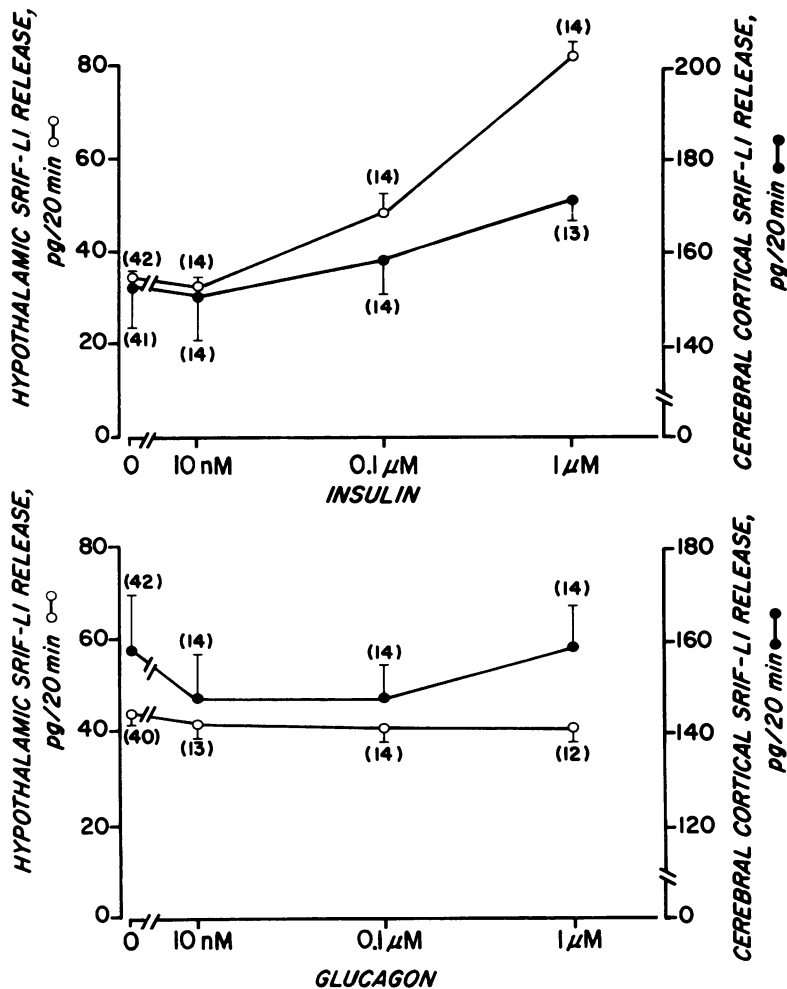


FIGURE 6 Effect of medium insulin (10 nM–1 μ M; upper panel) and glucagon (10 nM–1 μ M; lower panel) on SRIF-LI release from incubated hypothalamus (open circles) and cerebral cortex (filled circles). See Fig. 1 for details.

release could possibly be explained by binding of insulin to a somatomedin-C receptor, an effect previously described in other tissues (36). We have previously demonstrated that somatomedin-C stimulates SRIF-LI release at physiologic concentrations (5–50 ng/ml) (21). The lack of stimulation of cerebral cortical SRIF-LI release by the same insulin concentrations provides further documentation of area specificity of the response.

In summary we have documented an inverse dose-related effect of glucose on hypothalamic but not cerebral cortical SRIF-LI release. The effect required the entry of glucose into cells and its subsequent metabolism. Insulin had no effect on hypothalamic SRIF-LI release at physiologic concentrations but exhibited a stimulatory effect at high concentrations. This effect

is not mediated by glucose entry into cells (which decreases SRIF-LI release) but may result from binding of insulin to a hypothalamic somatomedin-C receptor. These data provide an explanation for the observation of a decrease in serum growth hormone levels in rats during insulin-induced hypoglycemia (37). In man, however, the same stimulus results in growth hormone stimulation suggesting either that SRIF has a more prominent role in growth hormone regulation in the rat or that regulation of hypothalamic SRIF release differs in the two species.

Note added in proof. We have recently determined that synthetic neurotensin prepared by Peninsula Laboratories Inc., San Carlos, CA and by Bachem Laboratories, Inc., Torrance, CA fails to stimulate CNS SRIF-LI release. The ac-

tivity responsible for the elevation of neural SRIF-LI seen in response to synthetic neurotensin provided by Dr. Jean Rivier, Salk Institute, has been found to reside in a contaminant of the material and thus data obtained with this preparation should be disregarded.

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