Immunoreactive Somatostatin in the Hypothalamus and Other Regions of the Rat Brain: Effects of Insulin, Glucose, α- or β-Blocker and L-Dopa

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Synopsis

Effects of various hormonal and pharmacological manipulations on somatostatin distribution were investigated to elucidate the physiological significance of somatostatin in the hypothalamus and the other regions of the rat brain. Immunoreactive somatostatin (IRS) was measured by radioimmunoassay newly developed. Insulin induced an increase of hypothalamic IRS and a decrease of plasma RGH, while glucose administration resulted in the opposite responses, which were not significant. Insulin also increased IRS in the thalamus and the brain stem. The insulin-induced increase of hypothalamic IRS was reduced by hyperglycemia. Glucagon reduced IRS initially and then increased it with an elevation of plasma RGH. L-dopa did not affect hypothalamic IRS, although it decreased plasma RPRL. Phentolamine slightly increased plasma RGH and decreased IRS in most regions of the rat brain, while propranolol increased IRS in these regions. Pretreatment with propranolol significantly increased plasma RGH 120 min after insulin administration, and hypothalamic IRS decreased initially by pretreatment with propranolol, and then it increased significantly. When pretreated with propranolol, glucagon markedly increased plasma RGH and decreased IRS significantly.

From these findings it is concluded that hypothalamic IRS may participate in the hormonal regulatory system in correlation to plasma RGH, as observed in studies on plasma GH and hypothalamic IRS following insulin, glucose, propranolol or phentolamine administration, but IRS in other regions of the brain may have some other actions as a neurotransmitter or a modulator, because of no significant correlation between plasma GH or PRL and IRS in these regions following various stimuli. In addition, glucose homeostasis and adrenergic mechanism may be important factors in regulating IRS in the rat brain.

Growth hormone release-inhibiting hormone (somatostatin) was isolated from hypothalamic tissue and its structure determined (Brazeau et al., 1973). It inhibits the secretion of growth hormone (GH) (Brazeau et al., 1973; Hall et al., 1973; Hansen et al., 1973), thyroid-stimulating hormone (TSH) Hall et al., 1973; Lucke et al., 1975), insulin (Alberti et al., 1973), glucagon (Koerker et al., 1974), and a variety of gastrointestinal hormones (Bloom et al., 1974; Boden et al., 1975; Sakurai et al., 1975). In addition, it has been demonstrated to have a number of effects on the central nervous system (Brown and Vale, 1975a; Prange et al., 1974; Segal and Mandell, 1974) including some behavioral effects (Plotnikoff et al., 1974), probably independent of its pituitary inhibitory effects. It has subsequently been shown to be distributed widely throughout not only the brain (Alpert et al., 1976; Brown-
stein et al., 1975) but also other organs; pancreas (Arimura et al., 1975a; Dubois et al., 1975; Luft et al., 1974), the gastrointestinal tract (Arimura et al., 1975; Polak et al., 1975; Rufener et al., 1975) etc. However, the physiological and pathological roles of this widely distributed somatostatin still remain uncertain.

There have been few studies on the correlation between widespread somatostatin throughout the brain and the secretion of pituitary hormones such as GH and prolactin (PRL). In the present study, the correlation between immunoreactive somatostatin (IRS) content in the hypothalamus and other regions of the rat brain and pituitary hormones in plasma was investigated using various hormonal and pharmacological manipulations altering plasma rat GH (RGH) and PRL (RPRL).

Materials and Methods

Animals

Male Wistar rats, weighing 140-180 g, were housed in a temperature (24±2°C) and humidity-controlled laboratory and allowed free access to tap water and lab chow. After a period of 7-14 days for adaptation to the laboratory with a light-dark cycle of 12:12h (lights on at 0700h), rats were injected i.v. with various agents under urethane anesthesia (150 mg/100 g BW, ip, 20 min before the treatments) at 1-2 PM following 20 hour fasting. At varying intervals after injection, the animals were decapitated with a guillotine.

Extraction method

The brains were rapidly removed, frozen and dissected into five fragments, consisting of the hypothalamus, the thalamus, the cortex, the cerebellum and the brain stem, according to the modification of the technique of Glowinski and Iverson (1966). After dissection, the fragments were weighed, homogenized and extracted with 5 ml (per s.a. 0.2 g tissue) of a mixture of 2N acetic acid and methanol (1:2). After centrifugation for 15 min at 2000 rpm, the supernatant was dried in an air stream at 60°C. The dried extracts were dissolved in 1 ml 0.5% bovine serum albumin (BSA)-0.1M Phosphate buffer saline, 0.025 M EDTA-2Na, diluted in an appropriate dilution and their IRS contents were determined by radioimmunoassay (RIA) as described below. The average recovery rate of synthetic somatostatin added to the brain homogenate was more than 90% (Itoh et al., 1978).

In the experiments trunk blood was collected in heparinized tubes and centrifuged immediately. Plasma was frozen for a later analysis of RGH and RPRL after removal of 0.2 ml for plasma glucose, whose concentration was determined by the method using glucose oxidase.

RIA of IRS

RIA of IRS was performed under the modification (Itoh et al., 1977) of the method of Arimura et al. (Arimura et al., 1975b). The anti-somatostatin was prepared by the immunization of rabbits with somatostatin-HSG (a) conjugate. The antibody had no cross-reactivity with TRH, LHRH, pituitary hormones, insulin, C-peptide or glucagon. 125I-Tyr1-somatostatin was prepared by the lactoperoxidase method. The standard curve was constructed using synthetic cyclic somatostatin with a reproducible limit of sensitivity of 10 pg/tube. Intraassay and interassay coefficients of variation were ±8% and ±10% respectively. In one experiment, all samples were determined within the same assay. Hypothalamic and brain IRS were expressed in terms of concentration, i.e., an amount of hormone per mg wet weight.

RIA of RGH and RPRL

Plasma RGH and RPRL were measured using GH and PRL assay kits kindly supplied by the Rat Pituitary Hormone Distribution Program of the Niamdd (reference standard Niamdd-rat GH-RP-1; anti-rat GH-serum 2: Niamdd-rat PRL-RP-1; anti-rat PRL-serum 5).

Drugs

Regular insulin (2, 5 U/rat), glucose (100, 250 mg/rat), glucagon (500 µg/rat), L-dopa (1.25 mg/rat), phentolamine (100 µg/rat), propranolol (100 µg/rat) were dissolved in a volume of 0.5 ml of saline and given intravenously to the tail vein. 0.5 ml Saline was used as a control. In some experiments, glucose (250 mg) or propranolol (100 µg) was injected intraperitonealy prior to insulin or glucagon i.v. injection.

Statistical treatments

Animals were randomly assigned to experimental and control groups. The data were evaluated using the t test for non-paired samples.
Results

Effect of insulin on IRS in the rat brain

Levels of plasma glucose began to decrease 15 min after insulin administration, and then reached the nadir at 30 min. On administration of 5 U insulin, the levels of plasma glucose decreased at the similar rate but remained low even after 60 min. The plasma RGH level decreased at 30 min (33 ± 6 vs 12 ± 2 ng/ml, M ± SE). The decrease of plasma RGH was intensified after 5 U insulin administration and the significant low level was obtained after 15 min (39 ± 12 vs 3 ± 1 ng/ml). Plasma RPRL did not change significantly after 2 or 5 U insulin administration (Fig. 1).

Insulin administration resulted in a significant increase in IRS content in the hypothalamus and the brain stem. In the hypothalamus as indicated in Fig. 1, a significant higher value, 2018 ± 285 pg/mg wet weight was observed 15 min after 2 U insulin administration, as compared to the pre-injection value of 1220 ± 84 pg/mg wet weight. Moreover, an exaggerated increase of hypothalamic IRS content was observed after 5 U insulin administration. IRS content returned to the pre-injection level 60 min after administration. IRS content did not change significantly in the cerebellum, while it increased significantly in the thalamus and slightly in the cortex at 15 min after the injection (Fig. 1).

Effect of glucose on IRS in the rat brain

After 250 mg glucose administration, plasma RGH levels slightly increased after 15 min and then declined. Plasma RPRL levels increased transiently. After 100 mg glucose administration, no significant fluctuations in plasma RGH and RPRL levels were observed, compared to the control (Fig. 3).

Hypothalamic IRS content began to decrease at 5 min. after 250 mg glucose administration, but it was not significantly different from that in the control (Fig. 3). IRS content in the thalamus increased after 250 mg glucose administration and in the brain stem it decreased after 100 mg glucose administration. In the cortex and the cerebellum, however, it did not change significantly after 250 or 100 mg glucose administration (Fig. 4).

Effect of insulin on IRS following glucosc pretreatment

The effect of insulin administration on IRS content in the rat brain following pretreatment with glucose was investigated (Fig. 5, 6). Plasma glucose decreased from 375 ± 45 mg/dl to 94 ± 21 mg/dl after insulin administration and the nadir was delayed by pretreatment with intraperitoneal administration of 250 mg glucose. Plasma RGH slightly decreased after insulin administration, but the values at all periods were not significantly different from 0 time. Plasma RPRL increased 60 min after insulin administration. IRS content in the hypothalamus decreased after insulin administration. IRS in regions other than the hypothalamus also decreased in the thalamus, the cortex and the cerebellum but increased in the brain stem.

Effect of glucagon on IRS in the brain

Glucagon induced a slight increase in plasma glucose. Plasma RGH increased but plasma RPRL did not change after glucagon administration (Fig. 7). As indicated in Fig. 8., IRS increased in the hypothalamus, the thalamus and the brain stem, but the changes were not statistically significant.

Effect of L-dopa on IRS in the rat brain

L-dopa induced an increase in plasma glucose and a decrease in plasma RPRL (Fig. 7). After L-dopa administration, IRS not change in the hypothalamus, the
Fig. 1. Plasma glucose, RGH, RPRL and IR-somatostatin content in the hypothalamus after insulin (2, 5 U/rat) administration. Mean±SE for each group of five (2 U) or four (5 U, saline) rats is shown. Statistical differences (vs. 0 time) are shown by asterisks.
Fig. 2. Effect of insulin administration on IR-somatostatin content in the thalamus, the cortex, the cerebellum and the brain stem. Mean±SE for each group of five (2 U) of four (5 U, saline) rats is shown. Statistical differences (vs. 0 time) are shown by asterisks.
Fig. 3. Plasma glucose, RGH, RPRL and IR-somatostatin content in the hypothalamus after glucose (100, 250 mg/rat) administration. Mean±SE for each group of four rats is shown. Statistical differences (vs. 0 time) are shown by asterisks.
Fig. 4. Effect of glucose administration on IR-somatostatin content in the thalamus, the cortex, the cerebellum and the brain stem. Mean±SE for each group of four rats is shown. Statistical differences (vs. 0 time) are shown by asterisks.
Fig. 5. Effect of the pretreatment with glucose (250 mg/rat, i.p.) on plasma glucose, RGH, RPRL and IR-somatostatin content in the hypothalamus after insulin (2 U/rat, i.v.) administration. Mean±SE for each group of five rats is shown.
Fig. 6. Effect of the pretreatment with glucose on IR-somatostatin content in the thalamus, the cortex, the cerebellum and the brain stem after insulin administration. Mean±SE for each group of five rats is shown.
cortex and the cerebellum, but it increased significantly in the thalamus and the brain stem (Fig. 8).

**Effect of phentolamine or propranolol on IRS in the rat brain**

Administration of phentolamine or propranolol did not change plasma glucose level significantly. Plasma RGH slightly increased after phentolamine and decreased after propranolol. Plasma RPRL increased after propranolol administration (Fig. 7). After phentolamine administration IRS decreased significantly in the hypothalamus (1141 ± 91 vs 606 ± 129 pg/mg wet weight) and also slightly decreased in the thalamus the cerebellum and the brain stem. On the contrary, hypothalamic IRS increased initially after propranolol administration and then declined. The value at 60 min was significantly lower than the pre-injection value (1313 ± 142 vs 889 ± 69 pg/mg wet weight). IRS in regions other than the hypothalamus increased after propranolol administration, compared to the value obtained by phentolamine administration (Fig. 9).

**Effect of glucagon or insulin on IRS following propranolol pretreatment**

The pretreatment with propranolol prevented glucagon-induced hyperglycemia and enhanced insulin-induced hypoglycemia. Plama RGH level was elevated 120 min after glucagon or insulin administration. Plasma RPRL level was not influenced by glucagon administration, while it was elevated significantly after insulin administration (Fig. 10). After the pretreatment with propranolol, insulin administration induced a decrease of hypothalamic IRS after 30 min followed by a significant increase, and it also induced a slight decrease in the thalamus 15 min after admi-

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**Fig. 7.** Plasma glucose, RGH and RPRL after phentolamine, propranolol, L-dopa or glucagon administration. Arrows indicate time of injection of drug. Mean ± SE for each group of four rats is shown. Statistical differences (vs. 0 time) are shown by asterisks.
Fig. 8. Effect of glucagon (500 µg/rat) or L-dopa (1.25 mg/rat) administration on IR-somatostatin content in each region of the rat brain. Mean±SE for each group of four rats are shown. Statistical differences (vs. 0 time) are shown by asterisk.
Fig. 9. Effect of phentolamine (100 μg/rat) or propranolol (100 μg/rat) administration on IR-somatostatin content in each region of the rat brain. Mean ± SE for each group of four rats is shown. Statistical differences (vs. 0 time) are shown by asterisks.
Fig. 10. Effect of the pretreatment with propranolol (100 ug/rat, i.p.) on plasma glucose, RGH and RPRL after glucagon (500 ug/rat, i.v.) or insulin (2 U/rat, i.v.) administration. Mean±SE for each group of five rats is shown.
Fig. 11. Effect of the pretreatment with propranolol on IR-somatostatin content in each region of the rat brain after glucagon or insulin administration. Mean±SE for each group of five rats is shown.
nistration. On the other hand, it induced an increase in the cerebellum. The pretreatment with propranolol enhanced the glucagon-induced changes of IRS in each region of the rat brain and induced a significant decrease in the hypothalamus and the cortex (Fig. 11).

Discussion

There have been many reports in which somatostatin inhibits tropic hormones in vivo (Hall et al., 1973; Hansen et al., 1973; Kato et al., 1974; Martin et al., 1974b; Siler et al., 1973) and in vitro (Brown and Vale, 1975b; Carlson et al., 1974; Stachura, 1976), but there have been only a few reports on the study of the correlation of somatostatin in the brain with pituitary hormone secretion, because it is difficult to evaluate the change of IRS content. Although the change in IRS content would reflect the change in the balance of its synthesis, activation, degradation and release, the exact correlation between IRS content and its release still remains unclear.

Saline injections did not cause significant changes in the plasma glucose level and hypothalamic IRS content under urethane anesthesia. This suggests that experiments under urethane anesthesia were fairly good for the present studies. A significant reduction in plasma RGH and an increase of hypothalamic IRS were observed by insulin-induced hypoglycemia and this change in IRS might be related to a relative increase of synthesis and/or reduction of degradation. On the contrary, a slight increase in plasma RGH and a slight decrease of hypothalamic IRS caused by a glucose administration might be related to a relative decrease of synthesis and/or increase of degradation. The increment of hypothalamic IRS content after insulin administration was cancelled by the pretreatment with glucose. From these data it is probable that the plasma RGH level is related to hypothalamic IRS and also the blood glucose level is related to the changes in plasma RGH and hypothalamic IRS content. On the contrary, Hoffman and Baker (1977) reported that RGH administration increased the hypothalamic IRS content in hypophysectomized rats. Hypophysectomy may cause a destruction of fine structure of median eminence and may decrease transport of somatostatin (Wakabayashi et al., 1976), so the above disagreement would be attributed at least partly to the difference of experimental conditions.

Glucagon administration caused an increase of plasma GH in the human (Mitchell et al., 1969). On the other hand, high dose of glucagon induced the release of IRS from perfused canine pancreas (Patton et al., 1977), which suggests the correlation of glucagon with IRS in the pancreas. But it is still unclear whether hypothalamic IRS is influenced by systemic administration of glucagon or not. Even high dose of glucagon failed to affect hypothalamic IRS significantly in the present investigation. A slight increase of plasma RGH corresponding to a slight decrease of IRS in the early phase and an increase in the later phase indicate that glucagon might influence plasma RGH mediated by IRS in the hypothalamus. But these effects are not prominent compared to hypoglycemia-induced effects which are supposed to be mediated by IRS. Therefore, some other factors may account for the effects of glucagon on plasma RGH.

L-dopa also failed to affect hypothalamic IRS in spite of the reduction of plasma RGH and RPRL. There are some possibilities to explain this result; 1) the dose was not enough to affect IRS content. 2) Urethane for anesthesia modified IRS content. 3) L-dopa directly acted on the adenohypophysis.
Phentolamine caused an increase of plasma RGH level, while propranolol caused a decrease. These observations were compatible with the data observed in the other report (Kato et al., 1973). These changes of plasma RGH also corresponded to changes of hypothalamic IRS content. But in this instance the change of plasma glucose could not induce the change of IRS, so the brain catecholamine might affect hypothalamic IRS content. Catecholamines were shown to be present in axons and nerve terminals in the medial basal hypothalamus (Hökfelt et al., 1973; Palkovits et al., 1974) in which somatostatin was demonstrated to be rich. In addition, it has been indicated that monoamines are considerably important for the central nervous regulation of GH secretion (Martin, 1976). All these reports suggest that the regulation of hypothalamic somatostatin is closely related to the adrenergic mechanism.

It has been also well recognized that β-blockade enhanced glucagon or insulin-induced GH release in the human (Blackard and Heidingsfelder, 1968; Mitchell et al., 1971). The pretreatment with propranolol enhanced the reduction of hypothalamic IRS and exaggerated the increase of plasma RGH level following glucagon administration. These results also indicated that glucagon influenced plasma RGH independent of hypothalamic IRS or glucagon directly acted on the adenohypophysis. On the contrary, the pretratment with propranolol reduced hypothalamic IRS content and then increased it later in case of insulin administration. These result suggested that the insulin-induced increase of hypothalamic IRS was partly mediated through the adrenergic mechanism. Propranolol caused just a slight increase of IRS in the early phase but a marked decrease in the later phase. Thus, propranolol pretreated may inhibit the insulin-induced increase of IRS.

Plasma RPRL was not influenced by glucose, insulin or glucagon that altered hypothalamic IRS content. So no correlation between plasma RPRL and hypothalamic IRS content was observed. Somatostatin inhibited the basal secretion of PRL in vitro (Grant et al., 1974), but the in vitro influence of somatostatin on the secretion rate of PRL was considerably less than on the secretion of GH (Vale et al., 1974). Moreover, the thyrotropin-releasing hormone (TRH)-triggered secretion of PRL was not suppressed by somatostatin in vivo (Drouin et al., 1976; Vale et al., 1974).

Changes of IRS content in other regions of the rat brain than the hypothalamus were obtained as follows. IRS content in the thalamus and the brain stem increased after insulin or glucagon administration, but these changes were not correlated to those in plasma RGH demonstrated in the hypothalamus. In the cortex and the cerebellum no prominent change of IRS content was observed. Interestingly, phentolamine or propranolol administration induced changes of IRS content in all regions of the brain and the same change was observed in the hypothalamus. These correlations suggested the possibility that IRS content in some regions of the brain was affected by brain catecholamines as proposed in the hypothalamus. The extrahypothalamic regulation of GH secretion was pointed out (Eleftheriou et al., 1969; Martin et al., 1974b; Newman et al., 1967), and it was reported that catecholamines were involved in relays of GH release induced by stimulation of extrahypothalamic structures (Martin et al., 1973). Although the regulation of hypothalamic somatostatin may be related to adrenergic mechanisms as mentioned above, the role of extrahypothalamic somatostatin remains to be elucidated. Hökfelt et al. (1977) reported that somatostatin like peptide was contained in the peripheral sympathetic nerve. It is still unknown that catecho-
laminergic nerve in the rat brain contains somatostatin, but the present results may indicate that catecholamine probably related to IRS in extrahypothalamic regions of the rat brain. Therefore, IRS content in regions other than the hypothalamus was proved to be affected by insulin, glucose, glucagon, L-dopa and α- or β-blocker in some cases. However, the exact mechanism by which glucose, catecholamine or glucagon affects IRS content in the rat brain remains to be elucidated. These IRS contained in regions other than the hypothalamus may play a role apart from the regulation of pituitary hormones. This possibility was partly supported by the reports that denervation of the fiber projected into the hypothalamus caused no change in IRS in regions other than the preoptic area that would project their fiber to the median eminence as related to a tropic action. The amygdala is proved to have neural connection with the medial basal hypothalamus, but its IRS content was not affected by the deafferentation (Brownstein et al., 1977; Epelbaum et al., 1977b). As most IRS has been reported to be concentrated in nerve terminals in the brain and presumably released from synapses (Epelbaum et al., 1977a), it may have a role of neurotransmitter or modulator (Martin et al., 1975; Renaud et al., 1975).

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