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Central Effect of Somatostatin on the Secretion of Growth Hormone in the Anesthetized Rat[1] (40345)

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Somatostatin was isolated and characterized by Brazeau *et al.* (1) as a hypothalamic tetradecapeptide that inhibits the secretion of growth hormone (GH) from the anterior pituitary. Subsequent studies have revealed that somatostatin is widely distributed in the central nervous system (CNS) (2) and localized subcellularly in nerve ending, synaptosome, in the rat (3). Recently it was also demonstrated in the cerebrospinal fluid in man (4).

Somatostatin has been reported to prolong pentobarbital anesthesia time (5), decrease spontaneous motor activity (6), lower the LD50 of barbiturates and increase strychnine LD50 (7). These results are in contrast to those obtained with thyrotropin releasing hormone (TRH) (5–7). It is possible, therefore, that somatostatin might have a role in the CNS opposite to that of TRH.

We have previously reported that TRH has a dual effects on GH secretion in the anesthetized rat; one is stimulating effect acting directly on anterior pituitary, and another is inhibitory action through the CNS (8). The present study was performed to examine the central effect of somatostatin on GH secretion in the rat.

Materials and methods. Male Wistar rats (Japan Animal Co., Osaka) weighing 200–250 g were used throughout the experiment. The animals were maintained in a light (14 hr light and 10 hr dark) and temperature (25 ± 1°) controlled room and fed Oriental Laboratory Chow (Oriental Yeast Co., Tokyo) and water *ad lib.*

After overnight fasting, they were anesthetized with urethane (150 mg/100 g body wt ip) in the morning on the experimental day. Synthetic somatostatin (supplied by Dr. N. Yanaihara) was dissolved in physiological saline containing 0.24% Fast Green FCF (Chroma Co., Stuttgart) as a dye marker and injected into a lateral ventricle or a pituitary portal vessel of the rat.

In the first experiment, somatostatin (0.5 μg and 5 μg/rat) was injected into the right lateral ventricle in a volume of 10 μl per rat as described previously (9). The same volume of saline solution alone was injected in control animals.

In the second experiment, somatostatin (5 μg/rat) of vehicle solution was injected into the lateral ventricle in rats with or without extensive hypothalamic destruction, which was performed two weeks before the experiment with a special knife (stirrup shaped, vertical 2.0 mm, diameter 3.0 mm) as described previously (10) using a modification of the method of Arimura *et al.* (11). The basal medial hypothalamus including the arcuate nuclei and the ventromedial nuclei were necrotically destroyed by interrupting the vascular supply from the ventral surface of the brain.

In the third experiment, somatostatin was injected into a single portal vessel using a modification of the method described by Porter *et al.* (12). Median eminence and pituitary stalk was exposed by the parapharyngeal approach, and fine curved glass cannula was inserted into one of main portal vessels using a micromanipulator. Through the cannula, somatostatin was perfused for 20 min at a flow rate of 25 ng/2 μl/min.

In each experiment, immediately before the injection of test materials and at 10–40 min intervals thereafter blood samples of 0.6 ml were withdrawn from the jugular vein using a heparinized syringe as described previously (12).

Plasma GH levels were determined by a specific radioimmunoassay (14) with the ma-

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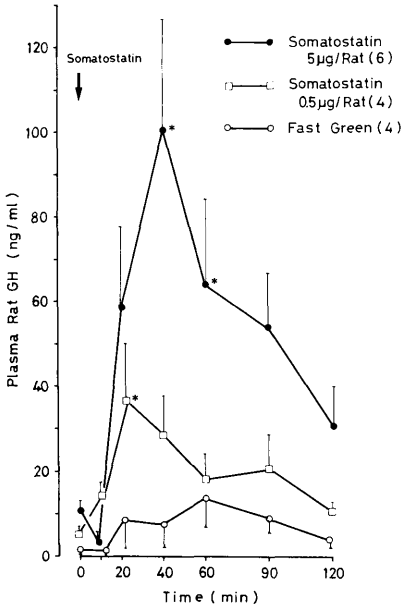

FIG. 1. Effect of intraventricular injection of somatostatin (0.5 and 5 μg/rat) on rat plasma GH. Means ± SE are shown. Control group was injected with saline containing Fast Green solution alone. The number of animals in each test group is indicated in parentheses. Statistical difference (*vs* control) is shown by asterisk: * $P < 0.05$.

terial supplied from the National Institute of Arthritis, Metabolism and Digestive Diseases. NIAMD-rat GH-RP-1 was used as the reference preparation. Student's *t* test was used for the statistical evaluation.

Results. As shown in Fig. 1, injection of somatostatin (0.5 and 5 μg/rat) into the lateral ventricle caused a significant and dose-related increase in plasma GH with a peak response at 20–40 min. Initial decrease of plasma GH at 10 min was observed by the injection of a large dose of somatostatin (5 μg) but not by a smaller dose (0.5 μg).

As shown in Fig. 2, the response of plasma GH induced by intraventricular injection of somatostatin (5 μg/rat) was partially, but significantly blunted by extensive hypothalamic ablation compared with those obtained in sham-operated animals (peak GH value: 50.1 ± 8.1 ng/ml *vs* 100.4 ± 23.6 ng/ml, $P < 0.05$).

Infusion of somatostatin into a stalk–portal vessel for 20 min (25 ng/min) resulted in a significant decrease of plasma GH during the infusion period and no significant change of plasma GH was observed thereafter until 120 min (Fig. 3).

Discussion. In the present study, we observed that intraventricular injection of somatostatin resulted in a significant and dose-related increase of plasma GH in urethane-anesthetized rats. GH release induced by intraventricular injection of somatostatin is not restricted to rats anesthetized with urethane, since stimulating effect of somatostatin in-

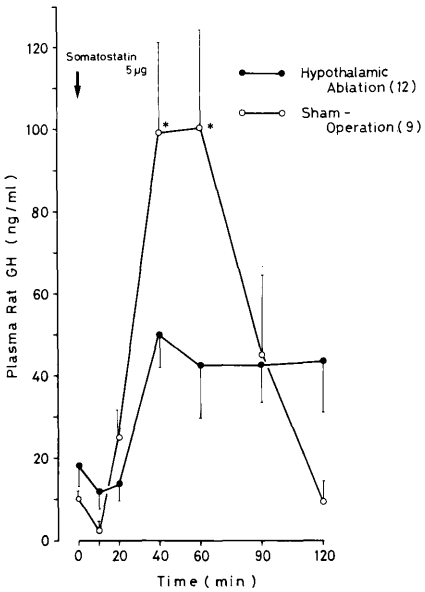

FIG. 2. Effect of hypothalamic ablation on GH release induced by intraventricular injection of somatostatin (5 μg/rat). Means ± SE are shown. The number of animals in each group is indicated in parentheses. Statistical difference (*vs* sham-operated group) is shown by asterisk: * $P < 0.05$.

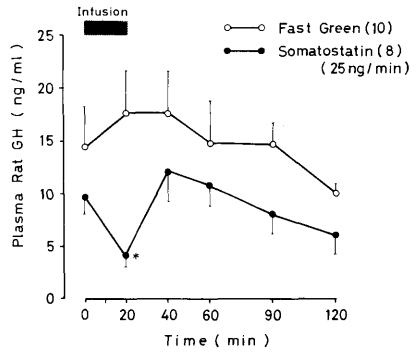

FIG. 3. Plasma GH levels following the infusion of somatostatin (25 ng/min) into a stalk portal vessel for 20 min. Fast Green solution was infused in a control group. Means ± SE are shown. The number of animals in each group is shown in parentheses. Statistical difference (*vs* control) is shown by asterisk: * $P < 0.01$.

jected centrally was also observed in rats anesthetized with pentobarbital or chloral hydrate (unpublished observation). In contrast, injection of somatostatin into a stalk-portal vessel failed to induce GH release. Initial decrease of plasma GH was observed following the administration of somatostatin either intraventricularly or into the portal vessel. The rise in plasma GH following the intraventricular injection of somatostatin cannot be accounted for by a rebound phenomenon following the initial suppression, because the infusion of the peptide into the pituitary portal vessels caused only a slight rebound phenomenon. It appears, therefore, that somatostatin inhibits GH secretion at the pituitary but rather stimulates GH release through the CNS.

These observations are in contrast to the results obtained with TRH (8). TRH stimulated GH release at the pituitary in rats, whereas it has an inhibitory action on GH secretion probably in the CNS. Different CNS effects of these peptides were also previously demonstrated in studies on behavior (7).

The exact mechanism by which intraventricular injection of somatostatin stimulates GH release remains to be investigated. The fact that hypothalamic ablation blunted GH release induced by intraventricular injection of somatostatin suggest that the hypothalamus may play a role, at least in part, in the central effect of somatostatin.

The ventromedial nucleus, which was destroyed by the ablative procedure used in the present experiment, is known to be closely related to GH releasing activity (15). Delayed and long duration of GH response to intraventricular injection of somatostatin is quite compatible to that of various behavioral response which was induced by somatostatin injected into the CNS (16). Cohn *et al.* (17) reported that intraventricular injection of somatostatin induced deep sedation or unusual rotation, which was blocked by atropine. Rezek *et al.* (16, 18) showed that administration of somatostatin into rat amygdaloid or hippocampal formation caused the various behavioral and electrophysiological change. Somatostatin applied by microiontophoresis caused a depressant effect on some central neurons and influenced calcium transport of cortical synaptosomes (19). These results suggest that somatostatin like other hypothalamic peptides, have a variety of effects on the CNS probably as a neurotransmitter.

It is concluded, therefore, that somatostatin may act somewhere in the CNS as a neurotransmitter to elicit GH release possibly influencing GH releasing activity in the hypothalamus, although physiological significance of this central effect of somatostatin must await further clarification.

Summary. Injection of somatostatin into the lateral ventricle caused a significant and dose-dependent increase in plasma GH in urethane-anesthetized rats. Increases in plasma GH induced by intraventricular injection of somatostatin were significantly blunted in rats with hypothalamic destruction. Somatostatin infusion into the pituitary portal vessel significantly lowered plasma GH. These results suggest that somatostatin has dual effects on GH secretion: one is inhibitory effect on the pituitary and another is stimulating action possibly through somewhere in the CNS.

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