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Effect of Treatment with Growth Hormone on Somatostatin in the Median Eminence of Hypophysectomized Rats[1, 2] (39919)

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A releasing factor and a release-inhibiting factor produced by the hypothalamus are postulated (1) to modulate the secretion of growth hormone by the pituitary gland. Although the existence of a releasing factor is supported by substantial evidence (2), its isolation has not been achieved. On the other hand, a growth hormone release-inhibiting factor (somatostatin) has been isolated by Brazeau *et al.* (3) from ovine hypothalami, and by Schally *et al.* (4) from porcine hypothalami. Subsequently, somatostatin was synthesized (5, 6). Utilizing antisera to somatostatin with immunocytochemistry, several investigators have described the distribution of somatostatin in the median eminence of the rat (7-9) and guinea pig (10). They are in general agreement regarding the distribution of somatostatin in axons, but the location in the brain of neuronal cell bodies that give rise to the somatostatin-containing axons remains unclear.

Baker and Yu (11) recently reported that the store of somatostatin in axons traversing, and/or terminating in, the median eminence is diminished greatly by hypophysectomy. Subsequently, this observation was corroborated by Wakabayashi *et al.* (12), who assessed the amount of somatostatin in the median eminence by radioimmunoassay. If somatostatin disappeared from the hypothalamus because insufficient growth hormone was present to elicit its formation, or prevent its release, one might postulate that the concentration of circulating growth hormone exerts a "short-loop" feedback control over the accumulation of somatostatin in the median eminence. The specific objective of this study was to evaluate this hypothesis by observing the capacity of exogenous growth hormone to prevent loss of somatostatin from the median eminence after pituitary ablation and to effect its restoration some time after the operation.

Materials and methods. Adult, female Sprague-Dawley rats were caged individually in a room with controlled temperature and a lighting schedule of 14 hr of light/10 hr of darkness. Food and water were available *ad libitum*.

Hypophysectomy was performed by the parapharyngeal approach. Two experiments were carried out. In Experiment I, treatment with growth hormone began the day after hypophysectomy and continued for 10, 20, or 31 days. In Experiment II, at least 169 days elapsed after hypophysectomy before initiation of growth hormone treatment, which continued for 10 or 32 days. For each period of treatment in each experiment, there were three groups of rats with three rats per group: (a) hypophysectomized, growth hormone-treated rats (Somar A, bovine, Abbott; 1.25 μg in 0.1 ml of water, twice daily, subcutaneously); (b) hypophysectomized rats, injected with distilled water (0.1 ml, twice daily); and (c) intact controls, injected with distilled water as for (b). Body weights were recorded every third day until the end of treatment.

At termination of the experiments, the rats were decapitated. A portion of the brain encompassing the hypothalamus and superior portion of the pituitary stalk was excised, fixed in Bouin's fluid for 48 hr, dehydrated in ethanol, embedded in Paraplast, and sectioned serially at 5 μm. Sections from the cephalic, middle, and caudal

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median eminence, including the proximal infundibular stem, were stained immunocytochemically for somatostatin with the peroxidase-antiperoxidase (PAP) procedure of Petrali *et al.* (13). The primary antiserum was raised in rabbits against synthetic somatostatin, and evidence for its specificity in the PAP technique has been described previously (9, 11). Specificity was indicated, also, by loss of effectiveness of the antiserum in immunocytochemical staining following preabsorption with synthetic somatostatin and by continued effectiveness after preabsorption with thyrotropin-releasing hormone, gonadotropin-releasing hormone, vasopressin, or oxytocin. The amount of somatostatin observed in the median eminence of each rat was estimated on a scale of 0 to 4, with 4 representing the greatest amount observed in the intact controls injected with water. Completeness of hypophysectomy was verified by microscopic examination of serial sections of the pituitary capsules, stained with the Masson procedure. Biological activity of the growth hormone treatment was indicated by an increase in body weights during treatment and by widening of the proximal epiphyseal cartilages in the tibias, as determined by measurement in microscopic sections. The value obtained for the epiphyseal cartilage of each rat represented the average of 10 measurements made in areas selected at random. To acquire evidence regarding possible contamination of the growth hormone preparation with other pituitary hormones, ovaries, adrenals, and uteri were weighed at termination of the experiment.

Results. Experiment I. As compared with the intact controls, hypophysectomized rats injected with water showed depletion of somatostatin from the cephalic, middle, and caudal portions of the median eminence at 10, 20, and 31 days after pituitary ablation (Figs. 1A and B; Table I). This effect was most marked at 31 days. Reduction in the magnitude of this depletion by treatment with growth hormone was not evident after 10 and 20 days of treatment, but at 31 days the amount of somatostatin retained in the middle and caudal segments of the median eminence of hypophysectomized-growth hormone-treated rats (Fig. 1C) was greater than the amount in the hypophysectomized group injected with water (Fig. 1B; Table I, mean estimate of 2.2 vs 0.8 and 2.6 vs 0.8, respectively.). However, the amount of somatostatin retained in the median eminence as a result of treatment with growth hormone did not approximate that observed in the intact controls.

The effectiveness of growth hormone therapy was shown by the greater body weights and thicker epiphyseal cartilages of the hypophysectomized-growth hormone-treated rats, as compared with those of the hypophysectomized rats injected with water, when therapy was continued for 10, 20, or 31 days (Table II).

Experiment II. When many weeks had elapsed after hypophysectomy, a profound depletion in the somatostatin content of the median eminence was observed (Figs. 2A and B). Ten days of growth hormone therapy, begun 169 days posthypophysectomy, failed to effect a restoration of somatostatin (Table III). However, 32 days of treatment, initiated 224 days after pituitary ablation, brought about a significant restoration (Figs. 2B and C), although the amount of somatostatin remained far below the level observed in the intact controls (Fig. 2A). For the middle zone of the median eminence, the mean estimate of somatostatin content was 3.5, 0.3, and 1.3 for the intact controls, hypophysectomized rats given water, and hypophysectomized-growth hormone-treated rats, respectively (Table III). General effectiveness of growth hormone treatment was demonstrated by significant increases in body weight and epiphyseal cartilage width in the hypophysectomized-growth hormone-treated rats as compared with those in hypophysectomized rats injected with water (Table IV).

In Experiments I and II, no neuronal perikarya that contained somatostatin were observed in any animal in the hypothalamus or preoptic area. In both experiments, the weights of ovaries, adrenals, and uteri were reduced greatly by hypophysectomy. In the hypophysectomized groups treated with growth hormone, the mean absolute weights for these organs were increased in relation to their weights in the hypophysectomized groups injected with water. However, when

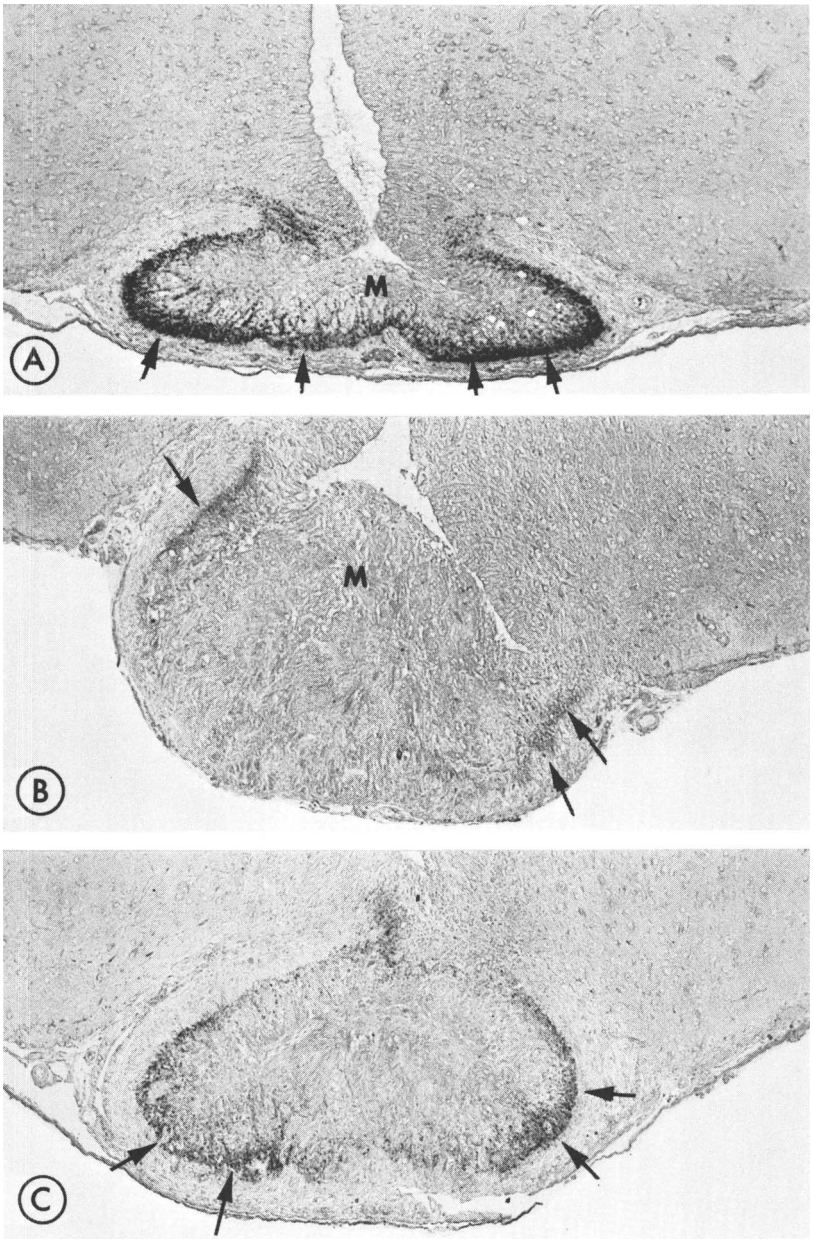

FIG. 1. Somatostatin (arrows) in the caudal median eminence (M) 31 days after treatment which was begun immediately following hypophysectomy. (A) Intact-water-treated rats; (B) hypophysectomized-water-treated rats; (C) hypophysectomized-growth hormone-treated rats. Distortion and thickening of the median eminence resulted from regeneration of magnocellular hypothalamo-infundibular nerve fibers after hypophysectomy. ×110.

these organ weights were related to body weights, no significant increases were evident.

Discussion. The conclusion that growth hormone effected significant retention or restoration of somatostatin in the median eminence and infundibulum of hypophysectomized rats is supported by several observations. Hormones secreted by retained pituitary fragments did not contribute to the

TABLE I. EFFECT OF GROWTH HORMONE TREATMENT BEGUN AT HYPOPHYSECTOMY ON SOMATOSTATIN IN THE MEDIAN EMINENCE.

Treatment	Mean estimate of somatostatin content (0 to 4)		
	Cephalic	Middle	Caudal
10 Days posthypophysectomy			
Hyp-GH[a]	1.6 ± 0.9[b]	3.1 ± 0.3	3.9 ± 0.3
Hyp-H_2O	1.1 ± 0.3	2.9 ± 0.5	2.7 ± 0.6
Intact-H_2O	4.0 ± 0	4.0 ± 0	4.0 ± 0
20 Days posthypophysectomy			
Hyp-GH	1.0 ± 0.9	1.5 ± 0.5	2.8 ± 0.3
Hyp-H_2O	0.7 ± 0.6	1.5 ± 0.5	1.7 ± 0.3
Intact-H_2O	3.2 ± 0.7	4.0 ± 0	4.0 ± 0
31 Days posthypophysectomy			
Hyp-GH	1.0 ± 0.8	2.2 ± 0.3	2.6 ± 0.8
Hyp-H_2O	0.8 ± 0.7	0.8 ± 0.3	0.8 ± 0.3
Intact-H_2O	2.8 ± 0.3	3.5 ± 0.5	4.0 ± 0

[a] Hyp, hypophysectomized; GH, growth hormone.
[b] Mean ± standard deviation, $n = 3$.

TABLE II. EFFECT OF GROWTH HORMONE ON BODY WEIGHT AND WIDTH OF THE EPIPHYSEAL CARTILAGE, WITH TREATMENT BEGUN AT HYPOPHYSECTOMY.

Treatment	Mean body wt (g)		Mean width of epiphyseal cartilage (μm)
	Initial	Final	
10 Days posthypophysectomy			
Hyp-GH[a]	175 ± 6[b]	197 ± 12	150 ± 25
Hyp-H_2O	174 ± 3	160 ± 2	85 ± 13
Intact-H_2O	163 ± 8	187 ± 5	139 ± 14
20 Days posthypophysectomy			
Hyp-GH	171 ± 9	232 ± 2	171 ± 8
Hyp-H_2O	182 ± 8	170 ± 5	84 ± 7
Intact-H_2O	168 ± 12	218 ± 25	130 ± 19
31 Days posthypophysectomy			
Hyp-GH	174 ± 9	247 ± 9	193 ± 14
Hyp-H_2O	182 ± 11	163 ± 13	76 ± 12
Intact-H_2O	167 ± 4	225 ± 17	113 ± 6

[a] Hyp, hypophysectomized; GH, growth hormone.
[b] Mean ± standard deviation, $n = 3$.

effect, because completeness of hypophysectomy was established by microscopic examination of the pituitary capsule. The growth-stimulating capacity of the growth hormone preparation was verified by the marked increase in body weight and growth of the epiphyseal cartilage that occurred in treated, hypophysectomized rats. The lack of involvement of pituitary hormones other than growth hormone is indicated by failure of the adrenals, ovaries, and uteri of treated, hypophysectomized rats to increase out of proportion to the increase in body weight.

It seems likely that growth hormone increases the amount of somatostatin in the median eminence–infundibular stem by a "short-loop" feedback (1). Evidence favoring this hypothesis was obtained by Elde and Parsons (14), who, by transplanting a growth hormone/prolactin-secreting tumor into rats, demonstrated somatostatin-containing neuronal cell bodies in the periventricular area of the hypothalamus, while somatostatin was not found in perikarya of such neurons in control rats. This observation might indicate that growth hormone caused an accumulation of somatostatin in the cell body of origin by stimulating its synthesis or by inhibiting its release. Conversely, removal of growth hormone from the circulation might cause depletion of somatostatin from the hypothalamus, as observed by Baker and Yu (11) and Wakabay-

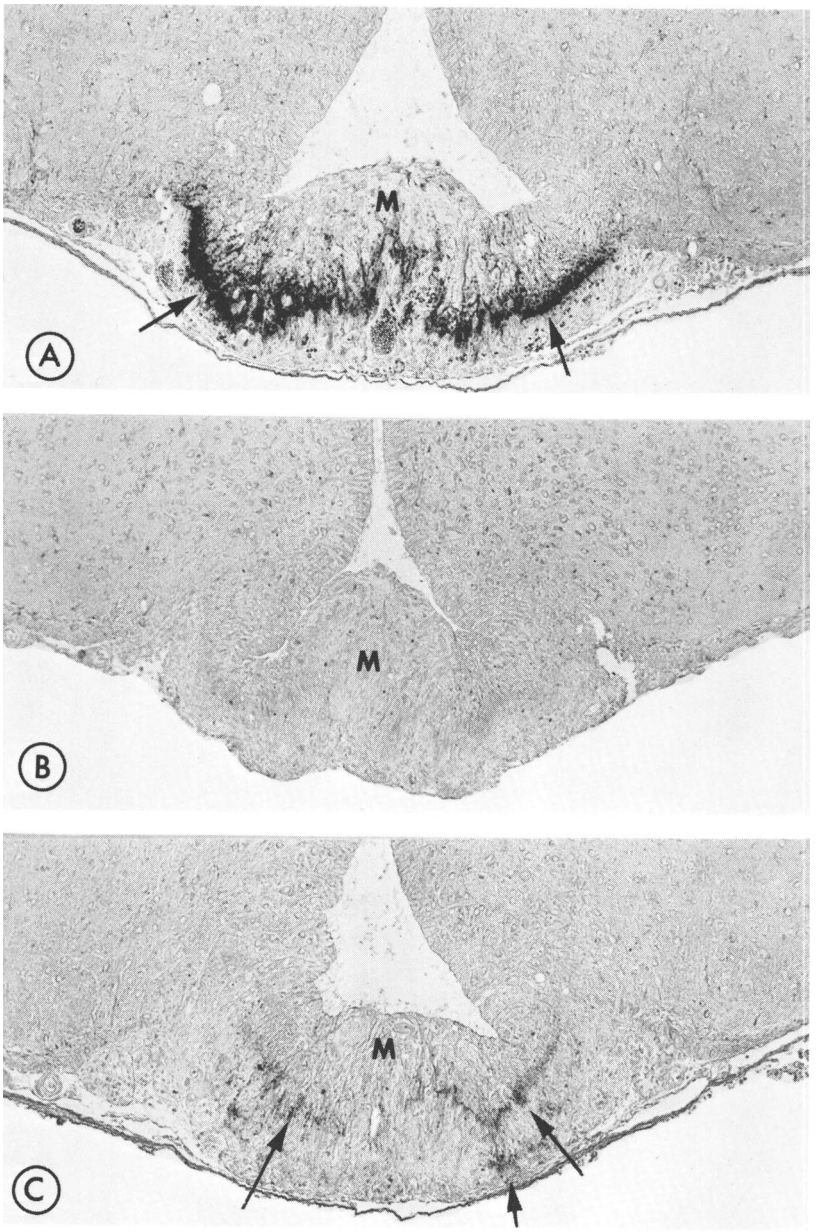

FIG. 2. Distribution of somatostatin (arrows) in the middle portion of the median eminence (M) 32 days after treatment which was begun 169 days after hypophysectomy. (A) Intact-water-treated rats; (B) hypophysectomized-water-treated rats; (C) hypophysectomized-growth hormone-treated rats. ×110.

ashi *et al.* (12) in hypophysectomized animals. Failure of growth hormone to effect full retention or restoration of somatostatin may have been due to less-than-optimal conditions for treatment or to the lack of other hormones, e.g., thyroxine and corticosteroids, whose synergistic support is necessary for manifestation of the full biological activity of growth hormone. Finally, it is unclear whether growth hormone acts directly on the somatostatin-secreting system or indirectly, through an intermediate compound such as somatomedin.

Summary. Treatment of rats with growth

TABLE III. EFFECT OF GROWTH HORMONE ON THE AMOUNT OF SOMATOSTATIN IN THE MEDIAN EMINENCE, WITH TREATMENT BEGUN AFTER A LONG POSTHYPOPHYSECTOMY PERIOD.

Treatment	Mean estimate of somatostatin content (0 to 4)		
	Cephalic	Middle	Caudal
10 Days of therapy, begun 169 days after hypophysectomy			
Hyp-GH[a]	0.7 ± 0.3[b]	0.8 ± 0.3	0.7 ± 0.3
Hyp-H_2O	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0.2
Intact-H_2O	3.0 ± 0	3.7 ± 0	3.7 ± 0
32 Days of therapy, begun 224 days after hypophysectomy			
Hyp-GH	0.3 ± 0.2	1.3 ± 0.3	1.8 ± 0.3
Hyp-H_2O	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.2
Intact-H_2O	3.3 ± 0.6	3.5 ± 0.5	4.0 ± 0

[a] Hyp, hypophysectomized; GH, growth hormone.
[b] Mean ± standard deviation, $n = 3$.

TABLE IV. EFFECT OF GROWTH HORMONE ON BODY WEIGHT AND WIDTH OF THE EPIPHYSEAL CARTILAGE, WITH TREATMENT BEGUN AFTER A LONG POSTHYPOPHYSECTOMY PERIOD.

Treatment	Mean body wt (g)		Mean width of epiphyseal cartilage (μm)
	Initial	Final	
10 Days of therapy, begun 169 days after hypophysectomy			
Hyp-GH[a]	190 ± 6[b]	209 ± 8	
Hyp-H_2O	188 ± 11	167 ± 2	
Intact-H_2O	186 ± 3	281 ± 10	
32 Days of therapy, begun 224 days after hypophysectomy			
Hyp-GH	191 ± 10	260 ± 12	209 ± 27
Hyp-H_2O	196 ± 3	174 ± 8	96 ± 11
Intact-H_2O	178 ± 3	280 ± 24	103 ± 4

[a] Hyp, hypophysectomized; GH, growth hormone.
[b] Mean ± standard deviation.

hormone partially prevented depletion of somatostatin from the median eminence when therapy was begun immediately following hypophysectomy, and partially restored somatostatin when treatment was initiated after a prolonged posthypophysectomy interval. These observations suggest that growth hormone tends to increase the store of somatostatin in the median eminence.

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