The effects of growth hormone administration to adult rats with lesions of the ventromedial hypothalamus *

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Adult rats with lesions of the VMH were given daily injections of saline, 0.2 mg of growth hormone (GH) per rat, or 1.0 mg GH per rat. One lesioned group and one unoperated control group were not injected. GH was no more effective than saline in reducing weight gain, reactivity to a pencil thrust, or quinine suppression. Rats receiving the higher GH dose had increased linear growth and decreased body fat compared to the other lesioned animals, but they still differed from the normal animals on these measures. It was concluded that changes in GH levels are not a primary factor in producing the physiological and behavioral effects associated with VMH lesions.

Lesions of the ventromedial hypothalamus (VMH) of rats produce a decrease in growth hormone (GH) levels and a decrease in linear growth (Bernardis, 1966; Frohman & Bernardis, 1968; Hales & Kennedy, 1964). Using weanling rats with VMH lesions, studies have found no weight changes as a function of GH administration but an increase in linear growth approaching that of normal animals (Goldman, Schnatz, Bernardis, & Frohman, 1970; Han, 1967). Han (1967) reported a reliable decrease in body fat in GH-injected VMH-lesioned rats, while Goldman et al (1970) reported statistically nonsignificant decreases in body fat. The differences between the results of the two studies may be due to the fact that Han injected his rats for 40 days, while Goldman et al (1970) injected for 15 days. Since weanling rats with VMH lesions do not demonstrate a greater than normal increase in body weight (Han, Lin, Chu, Mu, & Liu, 1970), while adult rats with VMH lesions show large weight increases (Marks & Brown, 1971; Marks & Remley, 1972; Stevenson, 1969; Thomas & Mayer, 1968), the effect of GH on VMH lesion-induced weight gain is unknown.

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Rats show a number of behavioral changes following VMH lesions. A marked increase in aggressiveness and irritability to touch is almost always reported following VMH lesions (Goldman et al, 1970; Hales & Kennedy, 1964; Marks & Brown, 1971; Marks & Remley, 1972). In addition, a replicable reduction in quinine intake in VMH-lesioned rats has been demonstrated (Corbit, 1965; Corbit & Stellar, 1964; Marks & Brown, 1971). No studies have attempted to investigate the effects of GH administration on the behavioral changes associated with VMH lesions. This study attempted to determine the effects of GH on weight gain, linear growth, and body fat, as well as reactivity to a pencil and quinine consumption in adult rats with VMH lesions.

METHOD

Sprague-Dawley-derived female rats (Cherokee Laboratories, Atlanta, Ga.) were used. The rats were approximately 100 days old at the time of surgery, with preoperative body weights ranging from 217 to 235 g. The rats were housed in a temperature-controlled room ($24^{\circ} \pm 2^{\circ}\text{C}$) with free access to food and water at all times, with the exception of 18-24 h postoperatively.

All rats were anesthetized with sodium pentobarbital (Nembutal, 60 mg/kg). In 16 rats, bilateral lesions of the VMH were produced by passing a direct current of 1.5 mA for 30 sec through the uninsulated tip of a 0.6-mm-diam stainless steel electrode. The stereotaxic coordinates were AP = +5.9 mm, L = 0.6 mm, and DV = 0.7 mm from the base of the skull.

The lesioned rats were assigned randomly to one of four groups. Three groups received daily 0.40-cc subcutaneous injections of physiological saline, 0.2 mg of GH/rat, or 1.0 mg of GH/rat. The other

lesioned group and an unoperated control group were not injected. After 28 days, one animal from each group (the rat gaining the least amount of weight) was no longer injected. The other rats continued to be injected for an additional 17 days.

Just prior to the daily injections, the animals were weighed and rated, on a 5-point scale, for reactivity to a pencil thrust toward them (Table 1).

A measure of linear growth, nasal occipital (N-O) length, was made immediately prior to surgery and again at the time of sacrifice. Three days prior to sacrifice, water intake was measured for 24 h, followed by a single 24-h measure of quinine (0.0005% quinine HCl) consumption. Only one measure of quinine consumption was made, since a reduction in fluid intake when only quinine was available would naturally alter body weight and potentially alter body fat.

On the day following the last injection, all animals were sacrificed with an overdose of sodium pentobarbital, perfused with physiological saline, and their brains removed and stored in 10% Formalin. The brains were embedded in cellodion, sectioned at 30 microns, and stained with thionin for histological verification of lesion placement.

The procedure for determining body fat was adapted from Han (1967). Each decapitated carcass was eviscerated. The viscera were cleaned of all food and fecal material and returned to the carcass. The carcass and viscera were then towel-dried, autoclaved for 15 min at 15 psi, and homogenized in a Waring blender. A 10%-20% aliquot by weight was oven-dried to a constant weight at $70^{\circ}\text{--}75^{\circ}\text{C}$. The dried fat and residue were placed in a glass extraction thimble and placed in a soxhlet apparatus containing petroleum ether. The fat extraction process continued until a constant thimble weight was obtained.

RESULTS

With the exception of one animal in the 1.0-mg-GH group, all lesioned

Table 1
Rating Scale for Reactivity to a Pencil

Scale	Behavior
1	Ignores or sniffs pencil
2	Chews on pencil but does not bite
3	Bites pencil but does not attempt to maintain hold on it
4	Attacks and bites pencil but quickly terminates attack
5	Attacks and bites pencil, does not terminate attack, pencil must be forcefully removed

Fig. 1. Weight increases during the 45 postoperative injection days.

Table 2
N-O Length Increase and Body Composition as a Function
of Surgical Condition and Injection Group

Group	N-O Increase (mm)	Percent Fat*	Weight of Fat (Gm)
Unoperated Control	4.2 ± 0.14†	9.84 ± 0.49	21.7 ± 0.96
VMH Control	2.5 ± 0.10	56.88 ± 3.01	281.6 ± 6.79
VMH Saline	2.5 ± 0.17	50.00 ± 3.22	215.4 ± 8.35
VMH 0.2 mg GH	2.9 ± 0.56	49.71 ± 2.89	208.7 ± 8.19
VMH 1.0 mg GH	3.5 ± 0.21	39.60 ± 3.76	164.4 ± 8.92

*Percent fat is the percentage of ether-extracted lipids of the wet weight of the carcass.
†Mean ± SD

Fig. 2. Changes in reactivity toward a pencil thrust at the animal during the 45 postoperative injection days.

animals had extensive damage in the VMH and showed the weight gain characteristic of VMH-lesioned rats. The one animal whose lesion invaded the lateral hypothalamic and posterior hypothalamic areas unilaterally and the VMH and anterior hypothalamic areas contralaterally did not show the weight gain or reactivity of the other lesioned animals and was excluded from the statistical analyses. The saline and 0.2-mg-GH rats that were not injected after 28 days were included in the statistical analysis, since no behavioral or physiological differences were found between them and the other animals in their respective groups. The 1.0-mg-GH rat that was not injected after 28 days was the animal found to have the inappropriate lesion placement.

While all lesioned groups gained more weight than the unoperated control group ($p < .01$), GH injections were no more effective in suppressing weight gain than saline injections (Fig. 1). All injected groups gained reliably less weight than the noninjected, lesioned group ($p < .05$) and reliably more than the noninjected, unoperated control group ($p < .01$).

Linear growth of the lesioned rats was smallest for the noninjected, saline-treated, and 0.2-mg-GH injected groups. The 1.0-mg-GH group showed a reliably greater linear growth rate than the former groups ($p < .025$), but a reliably smaller linear growth rate than the unoperated control group ($p < .05$; Table 2).

Saline and 0.2-mg-GH injections had little effect on the percentage of carcass fat, as these groups were not statistically different from the VMH-lesioned noninjected group. All of the injected groups had reliably smaller absolute amounts of carcass fat than the noninjected VMH group ($p < .01$). The differences in the amount of body fat appear to reflect the differences in body weight. The 1.0-mg GH group had a reliably smaller percentage of carcass fat than the VMH control ($p < .01$) of the other injected groups ($p < .05$) but a greater percentage of carcass fat than the unoperated control group ($p < .001$; Table 2).

Measures of reactivity to the pencil indicated a rapid reduction in reactivity for all injected animals. After the first day of injections, there were no statistically reliable differences between the injected groups and the unoperated control group (Fig. 2). The VMH control group showed a small drop in reactivity after the first day, but from that point to the termination of the study, they appeared stable in their response to the pencil. After the first 24 days, the VMH control group was

reliably more reactive than both the injected groups and the unoperated control group ($p < .05$).

Nonlesioned control animals showed essentially no quinine suppression, while all other groups produced reliable percentage suppression scores ($p < .05$). No statistically reliable differences were found in quinine suppression scores among any of the lesioned groups, nor were there any reliable differences among any of the groups in terms of the absolute amounts of either water or quinine consumed (Table 3).

DISCUSSION

All of the physiological measures reported in this paper are in agreement with Han (1967). Linear growth deficits accompanying VMH lesions are partially compensated by injections of GH. Fat deposition is partially inhibited by GH. However, GH has no more effect on body weight than saline, suggesting that food intake is not altered by GH in VMH rats. The fact that Goldman et al report no evidence of alterations of lipogenic activity and that Han and we, ourselves, have found changes in percentage body fat with GH injections suggests that an increase in lipolysis accompanies GH administration in the VMH rat. In the intact rat, GH increases lipolysis without significantly altering lipogenesis (Vaughn, 1964; Vaughn & Steinberg, 1963). Growth hormone, then, would appear to have the same effect in both intact and VMH-lesioned rats: an increase in lipolysis with no change in lipogenesis.

This proposed change in lipolytic activity may be a function of the dose rate of GH (as shown by the differences between the 0.2-mg-GH and the 1.0-mg-GH groups in this study) and/or the length of time the animals are injected. From the results of Goldman et al (1970), Han (1967), and this study, the age and sex of the animal do not appear to be critical variables. We suggest that a direct measure of lipolytic activity, such as free fatty acid release rate from adipose tissue, would more satisfactorily answer the question of lipolytic alterations in the GH-injected VMH rat.

It is questionable as to whether the lipolytic effect of GH is sufficient, in and of itself, to account for the changes found in VMH-lesioned rats, since the lesioned group injected with 1.0 mg of GH had almost eight times the amount of fat as the normals. In addition, the fact that weight increases were essentially identical for the saline, 0.2-mg-GH, and 1.0-mg-GH groups strongly

Table 3
Quinine Suppression as a Function of Surgical Condition and Injection Group

Group	Water Intake*	Quinine Intake*	Percent Suppression†
Unoperated Control	28.5	28.4	0.4
VMH Control	39.7	26.1	34.3
VMH Saline	34.3	24.2	29.4
VMH 0.2 mg GH	37.0	27.6	26.4
VMH 1.0 mg GH	39.9	30.9	24.5

*Intake in milliliters during a 24-h test

†Suppression was determined by the formula: (water-quinine)/water

suggests that whatever lipogenic effects GH has, these do not directly affect the weight gain of the VMH-lesioned rat. While it is possible that higher dose rates of GH would have produced a more marked change toward normal physiological levels, greater amounts of GH would probably exceed normal physiological levels.

Our behavioral data indicate that GH was not demonstrably effective in producing behavioral changes in VMH-lesioned rats. Quinine suppression was not statistically different for any of the lesioned groups. While there appears to be a slight reduction in quinine suppression among the injected groups, compared to the VMH control group, the reduction can most probably be attributed to the effects of the injection itself.

Reactivity to the pencil thrust was apparently unaffected by GH. The only effect was due to the amount of handling accompanying the injection, since the VMH control group was more reactive than any of the injected groups. While the finding of a rapid dissipation of reactivity appears to contradict previous reports, the amount of handling in this study was considerably greater than that reported in previous works (Malick, 1970; Singh, 1969).

The replacement of GH in the VMH rat alters its rate of linear growth and its degree of fat deposition. However, since the 1.0-mg-GH group still showed a smaller than normal linear growth rate and a significantly greater than normal degree of adiposity, as well as no change in either of the behavioral measures, GH lack is most probably not primarily responsible for the effects of VMH lesions, either behavioral or physiological, although it undoubtedly plays some role in the complex syndrome of effects found with VMH-lesioned rats.

REFERENCES

BERNARDIS, L. L. Development of hyperphagia in female rats with ventromedial lesions placed at four

different ages. *Experientia*, 1966, 22, 593-599.
CORBIT, J. D. Hyperphagic hyperreactivity to adulteration of drinking water with quinine HCl. *Journal of Comparative & Physiological Psychology*, 1965, 60, 123-124.
CORBIT, J. D., & STELLAR, E. Palatability, food intake, and obesity in normal and hyperphagic rats. *Journal of Comparative & Physiological Psychology*, 1964, 58, 63-67.
FROHMAN, L. A., & BERNARDIS, L. L. Growth hormone and insulin levels in weanling rats with ventromedial hypothalamic lesions. *Endocrinology*, 1968, 82, 1125-1131.
GOLDMAN, J. D., SCHNATZ, J. J., BERNARDIS, L. L., & FROHMAN, L. A. Adipose tissue metabolism of weanling rats after destruction of ventromedial hypothalamic nuclei: Effect of hypophysectomy and growth hormone. *Metabolism*, 1970, 19, 995-1005.
HALES, C. N., & KENNEDY, G. C. Plasma glucose, nonesterified fatty acid and insulin concentrations in hypothalamic hyperphagic rat. *Biochemical Journal*, 1964, 90, 620-623.
HAN, P. W. Hypothalamic obesity in rats without hyperphagia. *Transactions of the New York Academy of Science*, 1967, 30, 229-243.
HAN, P. W., LIN, C. H., CHU, K. C., MU, J. Y., & LIU, A. C. Hypothalamic obesity in weanling rats. *American Journal of Physiology*, 1970, 209, 627-631.
MALICK, J. B. A behavioral comparison of three lesion-induced models of aggression in the rat. *Physiology & Behavior*, 1970, 5, 679-681.
MARKS, H. E., & BROWN, G. E. The effects of VMH lesions in Charles River rats. *Psychonomic Science*, 1971, 23, 117-119.
MARKS, H. E., & REMLEY, N. R. The effects of type of lesion and percentage body weight loss on measures of motivated behavior in rats with hypothalamic lesions. *Behavioral Biology*, 1972, 7, in press.
SINGH, D. Comparison of hyperemotionality caused by lesions of the septal and ventromedial hypothalamic area in the rat. *Psychonomic Science*, 1969, 16, 3-4.
STEVENSON, J. A. F. Mechanisms in the control of food and water intake. *Annals of the New York Academy of Science*, 1969, 157, 1069-1083.
THOMAS, D. W., & MAYER, J. Meal taking and regulation of food intake by normal and hypothalamic hyperphagic rats. *Journal of Comparative & Physiological Psychology*, 1968, 66, 624-653.
VAUGHN, M. The metabolism of adipose tissue *in vitro*. *Journal of Lipid Research*, 1964, 5, 293-316.
VAUGHN, M., & STEINBERG, D. Effect of hormones of lipolysis and esterification of free fatty acids during incubations of adipose tissue *in vitro*. *Journal of Lipid Research*, 1963, 4, 193-199.