Insulin and Growth Hormone Secretion in Rats with Ventromedial Hypothalamic Lesions Maintained on Restricted Food Intake

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SUMMARY

Adult female rats were trained for one week to consume a subnormal amount of food (12 gm.) in two one-hour feeding sessions per day: 4 gm. between 8 and 9 a.m. and 8 gm. between 6 and 7 p.m. At the end of the training period the animals were subjected to either bilateral electrolytic destruction of the ventromedial hypothalamus (VMH) or to a sham operation. The restricted feeding regime was continued for forty-eight hours postoperatively, when comparative studies were made. Plasma insulin response to intravenous Glibenclamide was greater in the rats with VMH lesions than in the pair-fed, sham-operated controls. The former rats also exhibited decreased initial plasma glucose levels, degranulation of the beta cells and reduced pancreatic insulin content. In addition to marked islet enlargement, similar though more severe signs of islet hyperfunction were observed in rats with VMH lesions fed ad libitum, which became hyperphagic immediately after the operation.

VMH lesions did not significantly affect the plasma and pituitary levels of growth hormone (GH). The restricted feeding regime, however, caused an increase in plasma GH, responsiveness to hypoglycemic stimulation and a tendency to decreased pituitary GH content both in injured and sham-operated rats.

These findings indicate that VMH lesions per se enhance the responsiveness of the pancreatic islets to insulin secretory stimulation independent of changes in food intake, meal size and meal frequency. The resulting hyperinsulinism might play an essential role in the occurrence of hypothalamic hyperphagia and obesity following VMH injury. In hyperphagic rats with VMH damage, the islet stimulation is magnified, possibly due to the combination of a direct hypothalamic effect plus the secondary effect of increased food intake. Defective secretion of GH does not appear to be primarily involved in the induction of the hypothalamic syndrome. DIABETES *23*:203-08, March, 1974.

Preliminary reports of this work have appeared in abstract form (see references 1 and 2).

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Bilateral destruction of the ventromedial nuclei of the hypothalamus (VMH) results in hyperphagia, obesity and increased levels of circulating insulin.[3-5] The hyperinsulinism has been interpreted as secondary to overeating.[3] This view has been questioned lately because hyperinsulinism also occurs in weanling rats which do not become hyperphagic after injury to the VMH.[6] Furthermore, when the VMH lesions were produced in hypophysectomized adult rats fed by gavage to avoid hyperphagia, increased levels of plasma insulin were demonstrated between five and twenty-eight days after operation.[7] More recently, hyperinsulinism was observed as early as two days after VMH injury in adult rats on restricted food intake.[8] These findings suggest the existence of a direct hypothalamic influence on insulin secretion.

Here we report the effects of VMH lesions on the insulin secretory response to Glibenclamide stimulation, the pancreatic insulin content, the pancreatic islet mass and the pituitary and plasma growth hormone content forty-eight hours after VMH destruction in adult rats kept under a controlled feeding regime before and after the operation.

MATERIALS AND METHODS

Wistar female rats weighing about 220 gm. were individually caged and housed at a constant temperature of 22° C. and subjected to light for periods of twelve hours. The animals were divided in four groups; groups A and B were trained during one week to eat 12 gm. of food per day in two one-hour periods, 4 gm. between 8 and 9 a.m., and 8 gm. between 6 and 7 p.m. Groups C and D were allowed to eat ad libitum throughout the twenty-four hours. The food (consisting of compressed bars of approximately 50 gm. each made of 1 kg. powdered rat chow, 100 ml. glycerine and 170 ml. water) was dispensed to all animals by a metal tube with a small window near the bottom end. This system reduced food scattering to a

minimum and allowed accurate measurements of food intake.[4] All groups had free access to water.

At the end of the one week training period a bilateral electrolytic lesion of the VMH was made in all animals in groups B and C; the animals in group A had sham operations, while those in group D remained as normal controls fed ad libitum. The lesions were produced with a stereotaxic instrument by passing an anodal current of 1.5 mA for fifteen seconds through a stainless steel electrode, which was coated with varnish except for the tip. Sham-operated rats were identically treated but no current was applied. The operation was performed with ether anesthesia, and the lesions were based on coordinates previously determined for rats of this size.

Forty-eight hours after the operation, and three hours after removal of food, all animals, under light Nembutal anesthesia, were injected intravenously with Glibenclamide, 100 μg./1 kg. body weight. Blood samples were collected from the retro-orbital plexus in heparinized tubes before (zero time) and ten and thirty minutes after the injection. After the last sample was taken, half the rats in each group were kept alive while the other half were killed by decapitation. The surviving animals were allowed free access to food and water for the following three days to assess the efficacy of the lesions. From the killed rats the brain, pituitary and pancreas were removed. The brain and pieces of pancreas from the splenic, gastric and duodenal regions were fixed with Bouin's fluid for histologic examination. The remaining pancreas and the pituitary were immediately frozen in liquid nitrogen and stored at -20° C. for insulin and growth hormone extraction, respectively.

Plasma glucose was measured with a Technicon AutoAnalyzer adapted to the ferricyanide method.[9] Immunoreactive insulin (IRI) in plasma and pancreatic extracts was determined by radioimmunoassay according to Yalow and Berson,[10] using specific antirat insulin guinea pig serum and 131-I-labeled bovine insulin. Crystalline rat insulin* (21 I.U./mg.) was used as standard. The insulin content of the pancreas was determined in acid alcohol extracts.[11]

Growth hormone (GH) in plasma and pituitary extracts was measured by a double antibody immunoassay.[12] Purified rat GH (NIAMD-Rat GH-1-1, biological potency 1.5 I.U./1 mg.)* was labeled with 125-I to a specific activity of 75 mCi./1 mg. The Ellis preparation of rat GH (III-41-E) with a potency of 3.5 I.U./1 mg. served as standard.† GH values in plasma samples were corrected for nonspecific displacement caused by hypophysectomized rat plasma. Pituitary extracts for GH determinations were prepared by extracting the homogenized whole gland of individual rats with 2 ml. 0.25 M ammonium sulfate as described by Garay et al.[12]

The extension of the hypothalamic lesions was assessed on histologic sections of the brain stained with Weigert's hematoxylin. Pancreatic sections stained with aldehyde-fuchsin were used to determine the volume of the islets of Langerhans as percentage of the total pancreatic tissue.[13] The total islets mass was calculated by the following formula:

Islet/acinar x pancreatic weight/100

RESULTS

Body weight and food intake. After the second day of training, animals in the two pair-fed groups (A and B) adjusted to eating the daily ration of 12 gm. within the two scheduled one hour periods. This amount of food was less than the amount consumed per day by the animals fed ad libitum (about 18 gm.). However, such a restriction was necessary to ensure an equal food intake in the pair-fed groups; the achievement of this aim is depicted in figure 1.

The body weight of animals in pair-fed groups A and B showed insignificant differences between groups at any given time until the day of the test (table 1). Rats fed ad libitum (groups C and D) grew faster during the training period, and the rate of body weight increase rose sharply in animals of group C after destruction of the VMH. The VMH lesions produced immediate hyperphagia in rats fed ad libitum, while rats in groups A and B were kept at the preoperative level of restricted food intake. After the Glibenclamide test, when the surviving rats of groups A and B were allowed to eat ad libitum, their food intake increased and those with VMH lesions (group B) became hyperphagic.

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*Rat GH (NIAMD-Rat GH-1-1) was a gift from the National Institute of Arthritis and Metabolic Diseases, Rat Pituitary Hormone Program, through the courtesy of Dr. A. F. Parlow, Los Angeles.

†This rat growth hormone was a gift from Dr. S. Ellis.

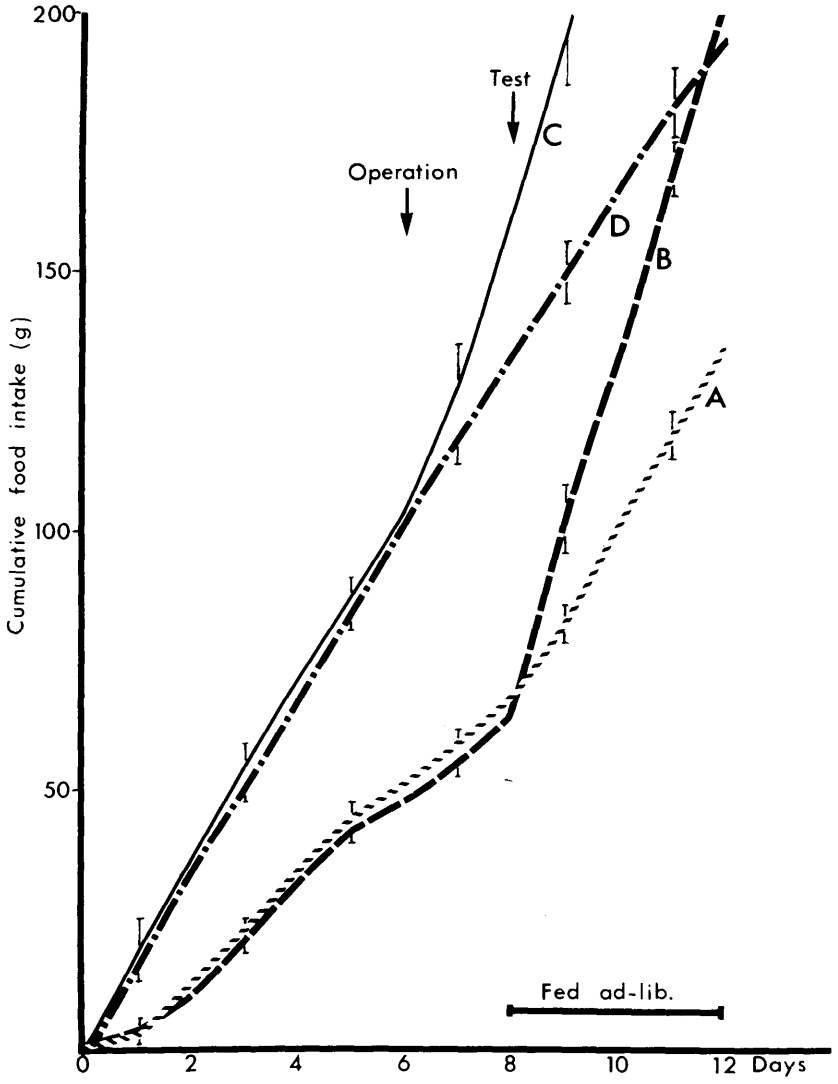

FIGURE 1

Cumulative food intake in pair-fed rats on restricted food intake (groups A and B) and ad libitum fed (groups C and D). After a training period of six days the ventromedial hypothalamus was destroyed in rats of groups B and C. Ad libitum feeding was allowed to the surviving animals of all groups forty-eight hours postoperatively, immediately after the Glibenclamide test was performed. The effectiveness of the lesions in groups B and C is demonstrated by the ensuing hyperphagia.

TABLE 1

Body weight changes in rats with VMH lesions and control animals before and after operation (Mean ± S.D.)

	Days					
	0	2	4	6 (Operation)	8 (Test)	12
Groups:						
A–Sham operated	225 ± 2.0	210 ± 6.2	214 ± 6.0	216 ± 5.0	212 ± 6.4	221 ± 6.1
B–VMH lesions	226 ± 1.8	210 ± 5.8	215 ± 5.5	215 ± 4.8	211 ± 6.0	273 ± 10
C–VMH lesions	227 ± 2.3	227 ± 3.0	229 ± 3.8	230 ± 8.2	250 ± 3.4	280 ± 9.5
D–Normal control	223 ± 2.6	221 ± 2.8	225 ± 1.8	227 ± 5.1	229 ± 4.0	234 ± 12

Groups A and B were pair-fed (see text). Groups C and D were fed ad libitum. All the animals were fed ad libitum from the eighth to the twelfth day.

Ad libitum fed animals (groups C and D) consumed 15 to 30 per cent of their total daily food during the periods of light (from 7 a.m. to 7 p.m.) for seven days preceding the operation. The diurnal distribution was unchanged in group C after the VMH lesions were made, which indicates that the circadian rhythm is independent of the hypothalamic center. Neither did the VMH lesion affect the rate of food intake in trained rats on restricted food intake (group B) during the one hour feeding sessions.

Plasma glucose and insulin concentrations. Fasting levels of glucose (three hours' fasting) were significantly lower in both hypothalamic groups than in sham-operated controls (table 2). After Glibenclamide administration the glucose values fell parallelly in all groups. Fasting insulin levels were higher only in hypothalamic rats fed ad libitum. Following Glibenclamide stimulation plasma insulin levels rose to higher levels in the pair-fed rats with hypothalamic lesions (group B) than in the sham-operated controls (group A). The hypothalamic rats fed ad libitum (group C) had the highest insulin response of all four groups.

Pancreas, pancreatic islets and insulin content. The weight of the pancreas was the same in all groups. The islet mass was larger in the ad libitum fed rats with VMH lesions (group C) than in the sham-operated (group A) and normal (group D) controls (16.2 ± 1.6 mg. vs. 12.6 ± 2.2 and 8.7 ± 1.0, respectively). A similar tendency was observed in the pair-fed injured animals (13.9 ± 1.3 mg.), but the difference did not attain statistical significance.

The insulin content of the pancreas was decreased in the injured animals: 2.0 ± 0.1 vs. 2.8 ± 0.3 mU./1 mg. in the pair-fed groups; 1.3 ± 0.2 vs. 1.8 ± 0.2 mU./1 mg. in the ad libitum fed groups. This finding was in agreement with histologic observation of degranulation of the beta cells, which was most pronounced in the hypothalamic rats fed ad libitum.

The consistent finding of an unusually large number of single cells, as well as clusters of cells with tinctorial characteristics of beta cells (mostly in the proximity of pancreatic ductules), was suggestive of new islet formation.

Plasma GH and pituitary GH content. Data on the plasma and pituitary GH content are given in table 3. The somewhat lower plasma GH level in injured animals, as measured at the beginning of the Glibenclamide test, was not statistically significant as compared with respective control groups. However, the circulating GH was significantly higher in animals, both with and without lesions, on restricted food intake. In these animals the GH levels reached a peak at thirty minutes coincident with the maximal decrease in blood glucose. The lowering of blood sugar had no effect on plasma GH concentration in animals fed ad libitum. Conversely, the pituitary GH content was lower in the pair-fed groups.

DISCUSSION

Previous reports have shown a stimulatory effect of ventromedial hypothalamic lesions on the pancreatic islet.[6-8,14] Nevertheless, the question remained whether such an effect was due to the increased glycemia, as observed in the study of Han and Frohman,[7] or to increased meal size (decreased meal frequency) as suggested by the study of Hustvedt and Løvø.[8] A direct relationship between increased meal size and plasma insulin response to glucose was found in rats adapted to a feeding schedule.[15]

The present study indicates that none of these factors are involved in the increased responsiveness of the pancreatic islets with VMH lesions. Under the strict

TABLE 2

Blood glucose and plasma insulin response to intravenous Glibenclamide injection. Mean ± S.E.

Groups:	Number of animals		Blood glucose (mg./100 ml.)			Plasma insulin (μU./ml.)		
			0	10 min.	30 min.	0	10 min.	30 min.
A–Sham operated	13		162 ± 3.7	135 ± 3.6	73 ± 4.7	20 ± 1.7	57 ± 3.5	36 ± 4.8
		P	<0.005	<0.02	NS	NS	<0.02	<0.05
B–VMH lesions	12		143 ± 5.0	117 ± 5.6	74 ± 4.0	24 ± 4.3	82 ± 9.4	55 ± 7.9
C–VMH lesions	13		133 ± 6.1	111 ± 4.9	63 ± 3.5	60 ± 8.3	224 ± 33.5	140 ± 21.9
		P	NS	NS	NS	<0.005	<0.001	<0.006
D–Normal control	8		138 ± 11.4	112 ± 7.7	61 ± 2.4	25 ± 9.0	98 ± 5.6	49 ± 9.6

Groups A and B were pair-fed (see text). Groups C and D were fed ad libitum.

In all groups the test was performed forty-eight hours after the operation. *P* values were calculated by Student's *t* test, comparing each test group with their corresponding control.

TABLE 3

Plasma and pituitary GH levels in rats with VMH lesions and their corresponding controls either pair-fed on a restricted food intake or fed ad libitum. Mean ± S.E.

Groups:	Number of animals		Plasma growth hormone (ng./ml.) 0	10 min.	30 min.	Pituitary GH (μg./mg.)
A–Sham operated	5		19 ± 4.1	14 ± 7.3	43 ± 34.7	7.4 ± 1.1
		P	NS	NS	NS	NS
B–VMH lesioned	8		13 ± 1.8	14 ± 5.1	57 ± 24.1	9.7 ± 0.7
		P	<0.01			NS
C–VMH lesioned	12		2 ± 1.7	<1	<1	11.6 ± 0.9
		P	NS			NS
D–Normal control	9		7 ± 2.6	5 ± 2.6	1 ± 0.4	10.7 ± 1.1

Groups A and B were pair-fed (see text). Groups C and D were fed ad libitum.

Plasma GH was determined in samples taken during the Glibenclamide test. The pituitaries were removed immediately after the test was completed. Mean values <1 indicate that they were not different from the values obtained with hypophysectomized rat plasma.

control of total food intake, meal size and feeding frequency, the VMH lesions in our trained meal-fed rats caused decreased plasma glucose levels, increased plasma insulin response to Glibenclamide stimulation and decreased insulin content of the pancreas forty-eight hours after the lesions were made. The basal level of plasma insulin was not increased at this time, while Han and Frohman[7] were able to demonstrate in their tube-fed rats with VMH lesions a progressive rise from the fifth postoperative day on. However, at the same time interval, similar but more pronounced changes were observed in hyperphagic VMH-injured rats fed ad libitum. Hence, hyperinsulinism in hyperphagic rats might be the combined result of a direct hypothalamic effect plus the secondary effect of increased food intake on the islets of Langerhans.

In the injured rats on restricted food intake we found a decreased insulin content, islet degranulation and a tendency to enlargement of the islets as early as forty-eight hours after operation. The observation of islet enlargement in tube-fed hypophysectomized rats at twenty-nine and thirty-eight days after production of VMH lesions[7] would indicate that this is an early and progressive change, independent of hyperphagia. Hyperphagia, however, considerably magnifies the primary effects of VMH lesions on islet function and morphologic features. The concomitant occurrence of decreased insulin content, beta cell degranulation and increased responsiveness to stimulation suggests that, at the early stage considered in our experiments, insulin synthesis was falling behind insulin release.

We did not find a decreased plasma GH level in injured adult animals, as described by Frohman and Bernardis in weanling rats.[6] This may be due either to the age of the animals or to the longer period of time (two to three weeks) after operation considered in the study of these investigators. On the other hand, our results indicate that food restriction and meal feeding, two variables that were not explored before, caused a rise in the basal plasma GH level in the rat. Under this feeding regimen, GH secretion also became responsive to hypoglycemia. This effect was not altered by the VMH lesions. Since similar lesions caused immediate hyperphagia in ad libitum fed rats, these observations indicate that defective secretion of GH is not primarily involved in induction of hypothalamic hyperphagia and obesity.

The effect of VMH destruction on the islets of Langerhans draws attention to the role of insulin in the establishment and maintenance of the hypothalamic syndrome. That insulin might be a key factor in this process was suggested by the observations that VMH lesions failed to induce hyperphagia and obesity in rats made diabetic by either pancreatectomy, alloxan or streptozotocin unless insulin was given.[16-18] Furthermore, in rats with established hypothalamic hyperphagia, reduction of insulin secretion by alloxan or streptozotocin administration resulted in decreased hyperphagia and slower gain in body weight.[18,19]

MacKay et al.[20] and May and Beaton[21] were able to induce hyperphagia and obesity in intact rats receiving insulin over long periods of time. Therefore, we may postulate that the enhanced responsiveness of the pancreatic islets is a primary consequence of the VMH lesion. Enhanced islet sensitivity would operate as a positive feedback mechanism, since sustained hyperinsulinism will stimulate lipogenesis and de-

crease lipolysis, thereby reducing the ability of the organism to use and mobilize ingested calories for energy requirements. This would lead to increased food intake, which in turn would further increase insulin secretion. The metabolic consequences of the basic disturbance would be amplified by additional changes in feeding behavior leading to increased meal size. The circle would operate actively during the dynamic phase, when the animal enlarges its fat depot, until it reaches a new state of equilibrium (static phase), when the pancreas has reached its maximal insulin secretory capacity.

The mechanism of the hypothalamic stimulation of the endocrine pancreas is not yet known. It can be envisioned, however, that lesions of the VMH may either eliminate an inhibitory effect of this region on the pancreas or indirectly trigger neurogenic or humoral signals from other parts of the central nervous system, which would stimulate the islets. A humoral mechanism in the latter sense is suggested by the recent observation that insulin secretion from isolated islets in vitro is significantly stimulated by extracts of the ventrolateral hypothalamus.[22,23]

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