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THYROID FUNCTION IN HYPOTHALAMIC OBESE RATS

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ABSTRACT

Bilateral thermal lesions producing hyperphagia and subsequently obesity are made on the hypothalami of rats. Thyroid function of these rats is assessed by measuring, namely, thyroid I^{131} uptake, PBI^{131} concentration, conversion ratio and percentage of I^{131} labeled fractions of thyroid hydrolysate. Femur length and fat content are measured and compared among experimental groups. Except in two special cases of the hypothalamic obese group showing obvious depression of thyroid function, fat accumulation and retardation of growth of the femur are evident in the others of hypothalamic obese rats and the femur change occurs even after thyroidectomy. Histological examination reveals that in one case of the hypothalamic obese rat thermal lesions involve not only ventromedial nuclei but also paraventricular nuclei and anterior hypothalami. Thus, results suggest that fat accumulation does not depend upon the presence of thyroid gland and that depression of thyroid activity in those two special cases may result from lesions being occasionally placed at the nuclei other than the ventromedial ones.

INTRODUCTION

Hypothalamic obesity is probably the most common type of experimental obesities. In general, of course, the most important factor in the production of hypothalamic obesity is an excessive food intake during the active phase of fat accumulation, However, hyperphagia cannot be the sole cause, because Han and Young (6) found that the hypothalamus-lesioned rat still becomes obese even with food intake not in excess of the control. In continuing their studies on the mechanism of production of adiposity in the hypothalamic rat, Han *et al* (7) further observed that the hypothalamic obesity in the rat was associated in some degree with the growth retardation of the femoral bone. The essential problem relating to hypothalamic obesity is therefore whether the lesion directly disturbs one of the mechanisms for regulating food intake (2) or that the lesion affects a more complex one involving not only the central mechanism but also a general endocrine disturbance of etiologic significance (11).

In order to assess the possible endocrine dearrangements in the hypothalamic obese rats, workers in the Laboratory have conducted a series of experiments to study the functional relationship between the production of hypothalamic obesity and the activities of adrenals, gonads, pancreas and the pituitary gland (9, 10, 15, 12). Nevertheless, studies of thyroid function in relating to the production of hypothalamic obesity has not been documented.

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As it is generally known, thyroid gland plays an important role in body growth and lipid synthesis (4). An attempt has hence been made to study thyroid function in hypothalamic obese rats in order to clarify the relationship between the production of adiposity and thyroid activites. Thus, in the present experiments, thyroid function in rats is evaluated by measuring thyroid I^{131} uptake, PBI^{131} concentration, conversion ratio and percentage of I^{131} labeled fractions of hydrolysate. Femoral length and fat content are measured and compared among experimental groups in order to understand better whether the production of obesity in the hypothalamic ones comes from less utilization of energy when body growth is impaired.

MATERIALS AND METHODS

Totally forty male Sprague-Dawley rats from five litters, weighing initially from 81 to 121 grams, were used in this experiment. Animals were fed ad libitum with water and chicken feed of Taiwan Sugar Corporation throughout the experiment, and were maintained in a well-ventilated thermostated room (26°C ±1.0).

On an appropriate date, bilaterally symmetrical lesions were produced in the hypothalami of rats of group 2 (8 rats) by conventional stereotaxic methods. The tip of the coagulating electrode was placed 5.5 mm. anterior to the external auditory meatus and 0.5 mm. on either side of the sagittal suture and 0.5 mm. elevated from skull base. A direct current of 2 milliamperes for 20 seconds duration was used. Total thyroidectomy was done on rats of group 3 (10 rats) and combined operation (thyroidectomy+hypothalamic lesion) was carried on rats of group 4 (10 rats). Rats of group 1 (12 rats), chosen from each litter, served as controls.

Six weeks after operation seven μc of I^{131} was injected intraperitoneally into all rats 24 hours before sacrifice. At autopsy, the thyroid gland was cleanly dissected and weighed on a torsion balance. Plasma was collected through a rapid puncture over the right ventricle of the rat. The thyroid radioiodine uptake and radioactivity in plasma and PBI^{131} were measured in a well-type scintillation counter. Carcass of the rat was treated with petroleum ether to measure total fat content and the length of femoral bone.

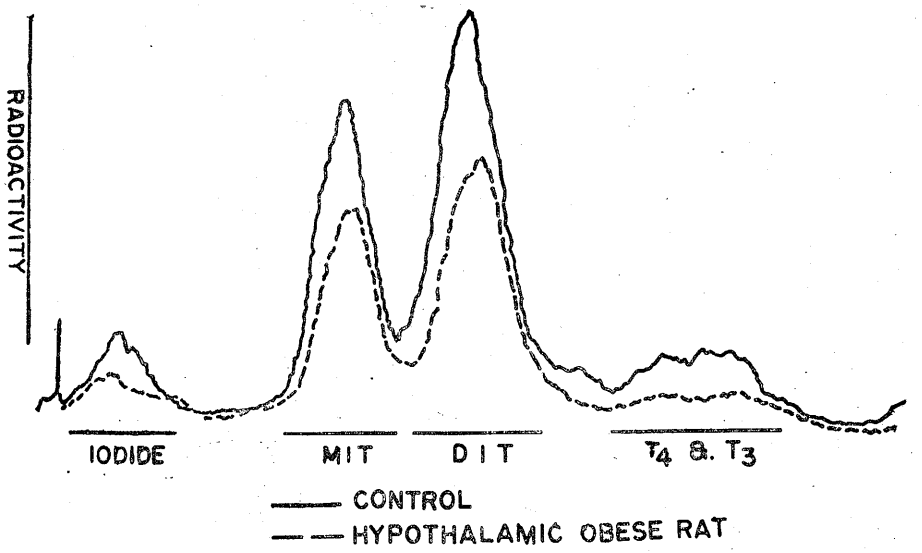
Thyroid glands of groups 1 and 2 were homogenized in 1 *ml* of phosphate buffer *p*H 8.4. Approximately 1×10^4 units of powdered trypsin and one drop of toluene were added to the homogenate. Hydrolysis was allowed to proceed at 38°C for 36 hours. Following digestion, aliquots of each homogenate, as well as plasma with carriers, were applied to paper chromatographic strips and subjected to descending chromatography in a butanol-acetic acid-water system. Iodide, monoiodotyrosine (MIT), diiodotyrosine (DIT), triiodothyronine (T3) and thyroxine

LEGEND OF FIGURES

Fig. 1. Radioactivity of the thyroid hydrolysate. Note that the radioactivity of the thyroid hydrolysate of the hypothalamic obese rat is generally lower than that of the control.

Fig. 2. Diagram showing the possible placement of he destructive lesions in the hypothalami of rats of groups 2 and 4. Legend: Shed area, thermal lesions

PV, Paraventricular nucleus
VM, Ventromedial nucleus
ME, Medium eminence
Ant, Anterior hypothalamus
III V, Third ventricle


RADIOACTIVITY
IODIDE
MIT
DIT
T_4 & T_3
CONTROL
HYPOTHALAMIC OBESE RAT

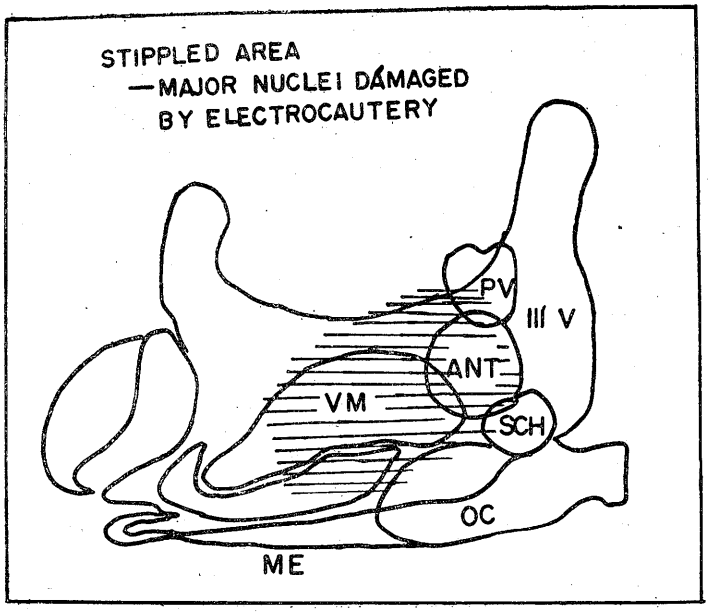

STIPPLED AREA
—MAJOR NUCLEI DAMAGED
BY ELECTROCAUTERY
PV
III V
ANT
VM
SCH
OC
ME

TABLE 1

General data of experimental rats

Group	No. of animal	Av.Bd.Wt. Gm.	Bd. Wt. gain Gm.	Femoral length mm.	Fat content of percent of wet body weight %
1. C	12	270±10.4*	152±10.8	30.4±0.44	7.94±0.63
2. H	8	359± 8.8	239± 9.4	28.0±0.75	40.11±5.70
3. T	10	151± 9.7	69± 8.1	25.9±1.08	7.34±1.34
4. H+T	10	282±10.2	163± 9.5	26.1±0.59	28.20±3.61
P value			0.0001 (1,2)	0.0062 (1,2)	0.0001 (1,2)
			0.0001 (2,3)	— (2,3)	0.0001 (2,3)
			0.0001 (1,3)	0.0001 (1,3)	— (1,3)
			— (1,4)	0.0001 (1,4)	0.0001 (1,4)
			0.0001 (2,4)	0.0480 (2,4)	— (2,4)
			0.0001 (3,4)	— (3,4)	0.0001 (3,4)

Legend: C, Control; H, Hypothalamic obese rats; T, Thyroidectomized rats.
—, P value above 0.05.
* Mean±S.E.

(T4)were employed as carriers. The paper chromatogram was then cut into 4.0 cm. vertical strips for radioactivity measurement in a Model 1002 Actigraph III (Nuclear Chicago Corp., Ill., U. S. A.). Fractionated amino acids on the paper strip were further identified by a spray of Gmelin stain (5).

Histological examinations were carried out on every brain of rats of groups 2 and 4 to identify the exact position of the thermal lesions (Figure 2). Serial tissue section of 40 μ in thickness were made and subsequently stained with hematoxylin-eosin. Thus, the unsuccessfully lesioned rats of groups 2 and 4 (9 rats in total) were not included in this account.

RESULTS

1. Thyroid radioiodine uptake and thyroid hormone synthesis in hypothalamic obese rats:

As shown in Table 2, the thyroid weight in rats of group 2 does not decrease significantly as compared to that of the control.

TABLE 2

Thyroid uptake and hydrolysis of iodoproteins in thyroid tissue of experimental rats

Group	Thyroid weight	Thyroid uptake	% of total radioactivity in thyroid				
			Origin	Iodide	MIT	DIT	T_4+T_3
1. C	21.1±1.62	17.6±3.60	6.7±1.85	4.1±1.22	32.0±1.33	51.3±1.60	5.7±1.07
2. H	20.4±1.55	10.8±1.44	5.7±1.76	4.3±1.08	31.2±2.21	53.2±3.35	5.1±1.75
P value	—	—	—	—	—	—	—

Legend: Same as in Table 1.

The radioiodine uptake by the thyroid in group 2 is approximately normal, but is apparently low (7.9% and 9.4% of the total dose) in two special cases. However, when groups 1 and 2 are compared, there is no statistically significant difference.

There is a marked reduction of the proportion of monoiodotyrosine (MIT) to diiodotyrosin (DIT) in these two special cases, which is illustrated in Figure 1. Under this circumstance, the ratio of MIT/DIT decreases to 0.49 but the ratio was as high as 0.65 in the control. Nevertheless, the similar finding does not frequently appear in other hypothalamus-lesioned obese rats. In other words, among six rats of group 2, generally the ratio of MIT/DIT maintains at a constant figure of 0.63±0.03. When other fractions are compared (i. e., iodothyronines and iodide), no obvious change can be found in groups 1 and 2.

2. Thyroid and plasma radioiodine distribution:

Data in Table 3 indicate that the concentration of protein bound radioiodine in blood, when compared to the control, is significantly lower in group 2 and the lowest in groups 3 and 4. An alternative way of recording the PBI^{131} is the calculation of the "conversion ratio", that is the ratio of the protein bound radioactivity to the total plasma after 24 hours administration. It is interesting to note that the conversion ratio is relatively lower (av. −45%) in hypothalamic obese rats, and the lowest are in thyroidectomized (av. −91.5%) and hypothalamic-thyroidectomized animals (av. −92.2%) as compared to that of the control. By the same token, it may imply that in the hypothalamic obese rats iodoprotein release from thyroid tissues is restricted.

TABLE 3

Data showing I^{131} distribution in thyroid and serum 24 hours after parenteral administration

Group	Thyroid uptake %	PBI^{131} %	Conversion ratio
1. C	17.6±3.60	0.83±0.067	78.0±10.3
2. H	10.8±1.44	0.39±0.045	43.2±8.42
3. T	—	0.19±0.014	6.5±2.08
4. H+T	—	0.16±0.032	6.1±2.84
P value	— (1,2)	0.0001 (1,2)	0.0082 (1,2)
		0.0001 (2,3)	0.0001 (2,3)
		0.0001 (1,3)	0.0001 (1,3)
		0.0001 (1,4)	0.0001 (1,4)
		0.0001 (2,4)	0.0001 (2,4)
		— (3,4)	— (3,4)

Legend: Same as in Table 1.

3. Femoral length and fat content:

Fat accumulation in hypothalamus-lesioned rats (group 2) is evident that the percentage of fat content of these animals increases to 40.1% which is about five times higher than that of the

control. However, in rats of group 3 (thyroidectomized rats) percentage of total body fat remains within a normal range. Obesity can be produced also by placing bilateral thermal lesions in the hypothalami of rats of group 4 even when their thyroid glands are radically ablated.

In fact, fat content of the rat's carcass of group 4 runs up to 28.20% of total body weight. The extent of obesity developed in rats of group 4, therefore, is significantly higher than that of the control, but not statistically different from that of the hypothalamic obese rats.

It is of interest that among littermates receiving different treatments, the hypothalamus-lesioned rats show, to some extent, retardation of growth, and the thyroidectomized rats and the hypothalamus-lesioned thyroidectomized rats almost remain at their initial body length. Measurements of the length of femoral bone in each experimental rat indicate a similar change; the length of femoral bone in the hypothalamus-lesioned rats is shorter than that of the control, but the shortest are those of the thyroidectomized rats and the hypothalamus-lesioned thyroidectomized rats (Table 1, column 4).

4. Histological examinations:

Altogether eighteen brains of groups 2 and 4 have been stained and examined. Thermal lesions on hypothalami, usually 1.2 mm. in diameter, are identified by gross and microscopic examinations. As shown in Figure 3, lesions involved bilateral ventromedial (VM) nuclei of the hypothalamus, but sometimes slightly higher or more anteroposterior to the VM nucleus. It may suggest that damages of different degrees might have incurred upon paraventricular nuclei and anterior hypothalamus.

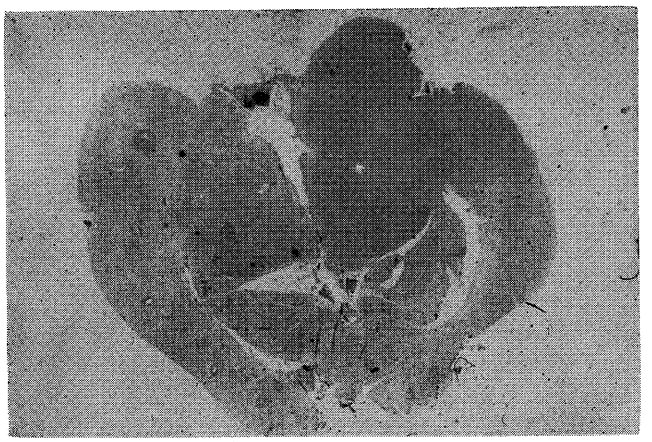


Fig. 3. A sample of the brain cross section of rats of group 2 showing that after thermal cauterization an extensive area in the hypothalamus is destroyed and the placement of thermal lesions is shown by two darken spots in the lower-middle portion of the section. Electrode tracts through the hypothalamus are indistinguishable owing to part of the tissues cracked in the staining manipulation.

DISCUSSION

Results in this experiment suggest that, owing to hyperphagia (av. 37 gm. per day per rat), bilateral thermal lesions placed on the hypothalami of albino rats can produce adiposity, which is independent to radical thyroidectomy. Though to a less extent than rats of group 2, the hypothalamus-lesioned thyroidectomized rats bear 28.20% of their body weight as storing neutral fat. This discrepancy may be resulted from a less food intake among rats of group 4 (av. 21 gm per day per rat). Surely, still it is difficult to neglect Kennedy's argument that it is the degree of adiposity rather than the absolute intake of food that regulated by the VM nucleus (8).

Pertaining to the observation on the thyroid activities of the hypothalamic obese rats, there are depressed thyroid uptake, decreased PBI^{131} concentration and a low conversion ratio of plasma iodoproteins (Table 3, columns 2, 3 and 4), thus favouring a condition of inadequate thyrotropin secretion. When thyroid gland has been ablated such as in groups 3 and 4, hypothalamic lesions do not affect any longer the PBI^{131} concentration and conversion ratio (Table 3, columns 3 and 4).

In their experiments, de Jong and Moll have mapped the rat's hypothalamus to find a level at the paraventricular nucleus (PVN) which controls TSH secretion (3). When applying their findings to our obesrvations, it seems more than a coincidence that the two special obese rats of group 2 bear their lesions more superior to the VM nucleus, namely, probably involving at the level of paraventricular nuclei. In other words, an alteration in TSH release as reflected by thyroid depression may be owing to an extensive destruction on PVN instead of VM nuclei alone.

However, only under the condition when we are possible to make lesions precisely limiting to the VM nuclei by thermal coagulation technique, then one can tell more correctly either PVN and VM or PVN alone is responsible for thyrotropin release mechanism. Besides, this deduction is favorably supported by Schindler's experiments on mice. By a single injection of goldthioglucose into CBA/Ki mice to produce VM nuclei destruction and subsequent obesity, he found that the obesity in CBA/Ki mice is not associated with alterations in TSH release as reflected in thyroid activity (14).

Obesity occurs only if the VM nuclei have been damaged, but the lesions responsible for dwarfing can not be so precisely located. Besides, slowing of growth without obesity has been reported after hypothalamic lesions by many authors (1, 13). In our experiments, results only indicate that thyroidectomy plays the major role in the retardation of growth of the young rat, which does not cause and/or prevent fat accumulation when the hypothalamus is damaged.

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