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Hypothalamic Knife Cut Obesity in Hyper or Hypothyroid Rats[1,2]

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LOWELL, B. B., R. M. GOLD AND C. A. ADAMCHUK. *Hypothalamic knife cut obesity in hyper or hypothyroid rats.* PHARMAC. BIOCHEM. BEHAV. 12(6) 837–841, 1980.—Recent evidence indicates that the paraventricular nucleus of the hypothalamus (PVN) contains both neurons that produce thyrotropic releasing hormone (TRH) and neurons that are destroyed or disconnected by the knife cuts that produce hypothalamic hyperphagia and obesity. This, and other evidence, suggested linkage between thyroid regulation and appetite control. As predicted, hyperthyroidism potentiated and hypothyroidism tempered the weight gains of knife cut rats. However, these effects were due entirely to increased and decreased, respectively, linear growth, not to differences in the degree of obesity. Enhanced linear growth and elevated growth hormone levels are a minor component of the enhanced weight gain of hypothalamically knife cut rats. Most of the weight gain is due to fat deposition. Only the enhanced linear growth and growth hormone aspect appear to possibly be mediated via the thyroid. In addition, obesifying knife cuts did not reduce goiterogenesis in PTU treated rats, as would be expected if the elaboration of TRH were blocked by obesifying knife cuts. Thus, neither TRH nor thyroxine is involved in the etiology of hypothalamic obesity.

Thyroid Hypothalamus Paraventricular nucleus Obesity Goiter

A ROLE for the hypothalamus in regulating the thyroid is well established. Thyrotropin releasing hormone (TRH) is found in the hypothalamus [21,35]. Electrical stimulation of the hypothalamus activates the anterior pituitary basophils, decreases thyrotropin (TSH) stores, increases plasma TSH, and thereby stimulates the thyroid [3]. Conversely, lesions of the paraventricular nucleus (PVN) inhibit TSH secretion [1,40]. In addition, systemically injected radioactive thyroxine concentrates in the PVN, indicating the possible presence of a negative feedback loop [6,39]. Interference with this feedback via medial or basal hypothalamic lesions [9,33], hypothalamic island knife cuts [7], or PVN lesions [4]; all prevent the rapid goiter formation that would otherwise follow propylthiouracil (PTU) or methylthiouracil (MTU) administration. Similarly, basal hypothalamic lesions prevent the rapid enlargement of the remaining thyroid lobe after hemithyroidectomy [32].

The time course of goiterogenesis is critical. For eleven days after the administration of PTU, knife cut animals fail to show the expected increase in TSH, but after eleven days an increase does occur, presumably via direct feedback to the pituitary, independent of the hypothalamus [7].

Abnormal thyroid states have long been associated with changes in food intake and body weight [37]. Hyperthyroid animals eat more but tend to lose weight [18, 19, 33, 37], while hypothyroid animals eat less but tend to put on fat [20,37].

The fact that weight loss occurs despite elevated food intake is attributed to an elevated metabolic rate [16,37]. Thyroxine also facilitates lipolysis, which probably contributes to the weight loss. TSH lowers body fat content [28]. Thyroxine increases fatty acid release [38], enhances catecholamine-induced lipolysis [31], increases serum cholesterol [39], and probably increases insulin requirements.

The PVN is the locus of the most discrete brain lesions that prevent goiter, and is therefore presumably the site of TRH production. The PVN has also recently been revealed as being the probable site of the somae of the neurons whose destruction leads to hyperphagia and obesity. This suggests linkage between thyroid regulation and appetite regulation. Damage to the ventromedial region of the hypothalamus (VMH) has long been known to produce hyperphagia and obesity [11,12], but detailed anatomical studies have revealed that the PVN, or its connections, is probably the critical locus for hypothalamic obesity [2, 10, 11, 26]. Recent evidence also suggests that this hypothalamic syndrome may be mediated via disinhibition of neurogenically released pancreatic secretions, most likely insulin. (Insulin injections elicit food intake [29].) Perhaps the most compelling evidence implicating neurally elicited insulin secretion in hypothalamic obesity is the finding that diabetic rats whose pancreatic endocrine function is restored via ectopic non-innervated pancreatic transplants do not respond to what would otherwise be obesifying hypothalamic lesions [24]. Thus, denervation of the pancreas appears to be sufficient to block hypothalamic obesity.

As might be predicted from the preceding account, hypothalamic obesity is blocked by full subdiaphragmatic

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vagotomy [5, 23, 30, 34], suggesting that it is the parasympathetic innervation of the pancreas that is critical to the mediation of hypothalamic obesity. However, more selective parasympathetic denervation of the pancreas via section of just the coeliac branch of the vagus only partially blocks the syndrome, suggesting that part of the prevention of obesity by full vagotomy is due to an inability to process the excess food eaten. Therefore, the sympathetic innervation of the pancreas may also play a major role in the mediation of hypothalamic obesity [27].

It is known that hyperthyroidism favors β adrenergic stimulation of the pancreas, which causes increased insulin release, while hypothyroidism blocks insulin secretion by favoring α adrenergic stimulation [8]. It was therefore posited that hypothalamic hyperphagia is in part mediated via thyroid hormone activation of β adrenergic insulin release.

We therefore predicted that hyperthyroidism might potentiate hypothalamic obesity, while hypothyroidism should reduce it. In addition, we predicted that our obesifying hypothalamic knife cuts would block the ability of PTU to produce goiters.

METHOD

Animals

Charles River CD female albino rats weighing 210–250 g were individually housed at 21±2°C, under cycling light (12 hr on, 12 hr off) with unrestricted access to tap water and to Purina Laboratory Chow pellets. A minimum of 10 days of adaptation to these conditions preceded surgery.

Surgery

Asymmetrical hypothalamic knife cuts were used, as opposed to symmetrical cuts, in order to minimize bilateral damage to neural circuits unrelated to hypothalamic obesity. The presumption is that systems damaged only unilaterally, because they pass through the plane of only one of the two cuts, will continue to function normally. For example, obesity producing asymmetrical cuts do not also elevate water/food ratios. Symmetrical obesity producing cuts often do elevate water/food ratios [13]. The cuts were performed under sodium nembutal anesthesia. A unilateral parasagittal cut was made with a caudally directed 3.0 mm long retracting wire knife [14]. With the incisor bar 3.0 mm below the interaural line, the guide cannula was lowered at 8.4 mm anterior to the ear bars and 0.9 mm lateral to the midline. At this position the wire knife was extended, lowered until it contacted the base of the skull, and raised 3.0 mm before being retracted into its guide cannula. In addition, each rat in the operated group received a contralateral coronal cut [13] with a 2.0 mm long medially directed wire which was lowered 6.2 mm anterior from the ear bars at 2.3 mm lateral to the midline. The knife transected the bottom 3.0 mm of the brain.

Sham operated control animals were treated identically except that the guide cannula was not lowered.

Drug treatment

All drug treatments began postoperatively, 10 days after the rats regained pre-operative weight. To induce hypothyroidism, the rats were injected SC daily with 10 mg of 6,N-propylthiouracil (PTU-Sigma) made soluble in 0.2 cc of bacteriostatic water by the addition of gum acacia. To induce hyperthyroidism the rats were injected SC daily with 7 μg of L-thyroxine (Sigma) per 100 g of body weight. This dosage is sevenfold above normal daily thyroxine turnover, but is not so high as to restrict body weight gain in neurologically intact female rats [17,20]. Euthyroid animals were given daily SC injections of 0.2 cc saline. After the animals had been given the drug treatments for 39 days, the drugs were discontinued for 35 days to return them to the euthyroid state. At that time the drugs were given as above for eleven more days. Eleven days is long enough to cause thyroid enlargement on PTU-treated shams, but not long enough for the pituitary to compensate for an absent hypothalamic feedback loop [7].

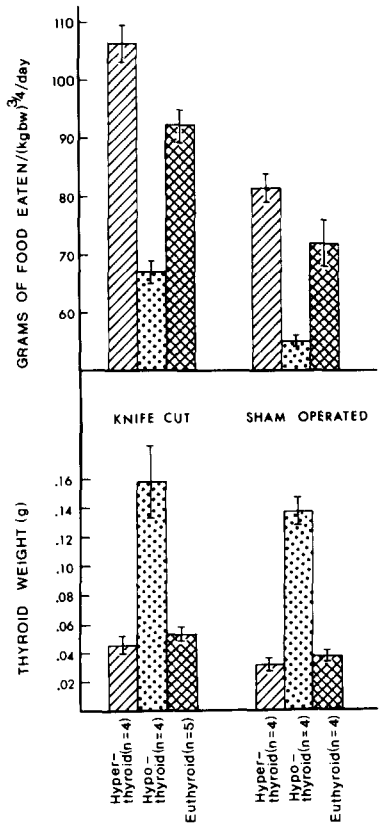

FIG. 1. Mean±SE daily food intakes (top) and mean±SE wet thyroid weights (bottom) for hyperthyroid, hypothyroid, and euthyroid rats with and without obesifying hypothalamic knife cuts. Food intakes are adjusted for body weight as grams eaten per kilogram raised to the $^{3}/_{4}$ power. Lacking an appropriate correction formula for body weight's effect upon thyroid weight, thyroid weights±SE are presented per rat.

Histology

At the end of the experiment the thyroids were dissected out prior to perfusion with saline and 10% Formalin. Horizontal brain sections were examined to verify the locations of the brain cuts.

Statistics

ANOVA was done on the average values of food intake/kg bw $^{3}/_{4}$/day during the 19 day period of drug treatment, on thyroid weights, on body-weight each 3 days, on naso-anal length differences, and on Lee obesity indices.

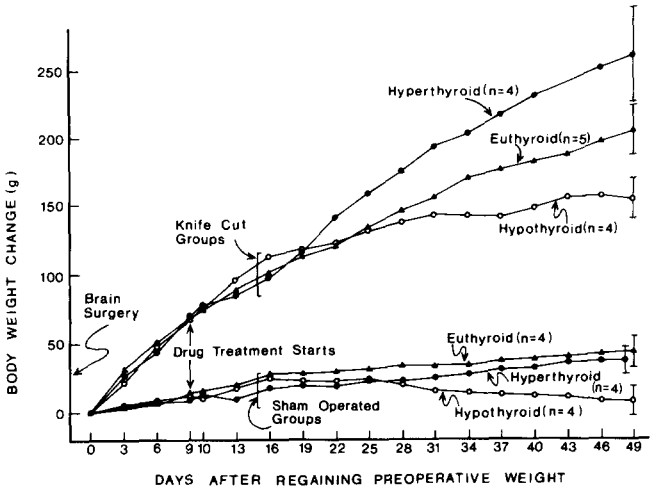

FIG. 2. Mean body weight changes±SE after regaining preoperative weight.

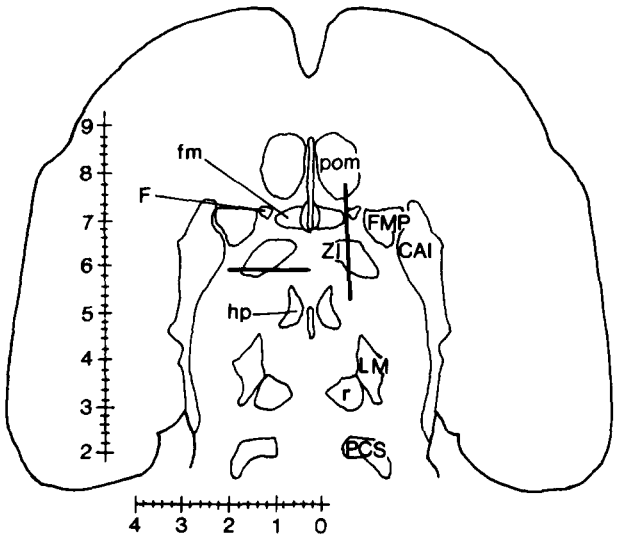

FIG. 4. Parasagitral and coronal knife cuts from a representative rat are shown in this drawing of a horizontal section of the brain. CAI, internal capsule; F, fornix; fm, paraventricular n.; FMP, medial forebrain bundle; hp, posterior hypothalamic n.; LM medial lemniscus; PCS, superior cerebellar peduncle; pom, medial preoptic n.; r, red n; ZI, zona incerta.

RESULTS

As expected, both the knife cut and sham operated hyperthyroid animals ate more than the respective euthyroid controls, and the hypothyroid animals ate less (Fig. 1, $p<0.01$ ANOVA). Food intake was calculated as g eaten/kg of body weight $^{3}/_{4}$/ day to correct for the increased energy demands for heavier animals [13]. The knife cuts increased food intake (Fig. 1, $p<0.01$) and body weight (Fig. 2, $p<0.01$). Also as predicted, hyperthyroidism potentiated the knife cut induced body weight gains while hypothyroidism reduced the weight gains (Fig. 2, $p<0.01$). In the sham knife cut rats hyperthyroidism had no effect upon body weight ($p>0.1$), whereas hypothyroidism produced small weight losses (Fig. 2, $p<0.05$).

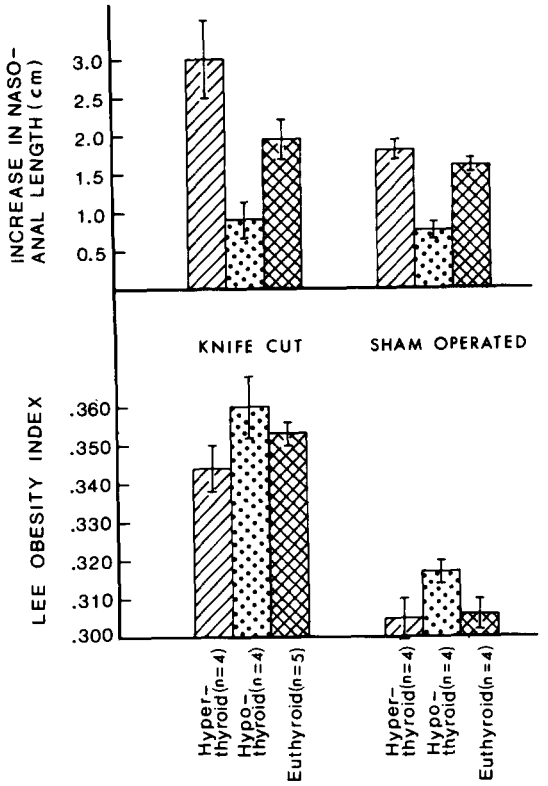

FIG. 3. Mean±SE increases in naso-anal length (top) and mean±SE final Lee obesity indices (bottom) as a function of thyroid state and brain surgery. Naso-anal lengths are presented as mean increases (sacrifice NAL–surgery NAL, in 50 days) to reduce variance, as body sizes varied somewhat at the time of surgery. Lee obesity indices are, however, presented as such, as found at the time of sacrifice, to permit comparisons with values found in the literature (normal=0.300–0.310). In contrast to naso-anal lengths, obesity indices varied little at the time of surgery.

The changes in body weight are, however, not due to differences in obesification, as operationally defined in terms of elevated Lee obesity indices [25]. The body weight effect was due to differences in the lengths of the rats (Fig. 3). Nasal-anal lengths were enhanced by the obesifying knife cuts, this effect being potentiated by hyperthyroidism ($p<0.05$), and prevented by hypothyroidism ($p<0.01$).

The Lee obesity index [25] was used to reveal relative obesities across a range of body lengths. The formula for calculation is:

$$OI = \sqrt[3]{\text{Body Weight (g)}}/\text{Naso-Anal length (cm)}.$$

When the body weight differences were so adjusted via the Lee obesity index (Fig. 3), the hyperthyroid group was not more obese than the euthyroid or hypothyroid groups. Instead, the hyperthyroid group was now slightly less obese than the hypothyroid group ($p<0.05$).

Contrary to our predictions, obesifying knife cuts did not block goiterogenesis. PTU treated knife cut and PTU treated sham-operated rats both had enlarged thyroids (Fig. 1, $p<0.01$).

Examination of the brain sections revealed no consistent differences between groups. The parasagittal cuts were, with one exception, alongside the PVN, 0.5 to 1.0 mm from the midline, 2.8 to 3.0 mm long (AP), and extended to the base of

the brain. The one exception was a hyperthyroid rat whose parasagittal cut was too low, sparing the area alongside the PVN. That rat's data are not reported. The coronal cuts were, without exception, all behind the PVN, and within the range of loci previously found to elicit obesity [13]. Representative asymmetrical cuts are shown in Fig. 4.

DISCUSSION

We conclude that the thyroid does not mediate the development of hypothalamic obesity. Although hyperthyroid knife cut rats do gain more weight than euthyroid knife cut rats, this additional increase is secondary to linear growth, an occurrance which has been found previously [39]. Conversely, hypothyroid knife cut rats gain less weight than euthyroid knife cut rats. This attenuated increase is secondary to an inhibition of linear growth which is consistent with data suggesting that thyroxine promotes growth of bone [39].

The thyroid is also not necessary when eliciting feeding by noradrenergic stimulation of the PVN [26]. Brain lesions produced with gold thioglucose produce obesity with no impairment of thyroid activity [36]. Furthermore, obesifying knife cuts produce no difference in mean daily energy expenditure relative to sham-operated groups [15].

Contrary to our prediction, obesifying knife cuts did not block goiterogenesis in PTU treated rats. Thus, the two syndromes, hypothalamic obesity and goiterogenesis blocking, do not share a common neurocircuitry. Both may still relate to the PVN, but to different populations of cells with different neural connections. We conclude, therefore, that the thyroid does not play a primary role in the development of hypothalamic obesity, but may mediate the enhanced linear growth seen as a small but significant contribution to the weight gain due to hypothalamic knife cut hyperphagia. The hyperphagic rats appear to regulate their degree of obesity, since increased linear growth in hypothalamic hyperphagic rats leads to a proportional increase in fat deposition while the reverse is true for rats with decreased linear growth. In both cases the obesity indices are held relatively constant. Severe stunting, as in hypophysectomy, further reduces absolute fat deposition in knife cut rats, but the obesity indices for hypophysectomized knife cut rats are actually just as high as those of knife cut rats with intact pituitaries [22].

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