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Temporal Adjustments in Sympathoadrenal Activity in Rats with Obesity-Producing Hypothalamic Knife Cuts[1] (42527)

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Abstract. Sympathoadrenal activity was assessed in adult rats with obesity-producing hypothalamic knife cuts prior to and after the onset of gross obesity by measuring urinary excretion of norepinephrine and epinephrine and by determining rates of norepinephrine turnover in selected organs. Urinary excretion of norepinephrine, as an index of overall sympathetic nervous system activity, was approximately doubled throughout the 4-week study in knife-cut rats, as was intake of the high-fat diet. Three days after knife-cut surgery (before the onset of gross obesity) rates of norepinephrine turnover ($ng \cdot organ^{-1} \cdot hr^{-1}$) were 23–33% lower in three of the four organs examined than in the corresponding organs of control rats; rates of norepinephrine turnover were depressed in pancreas, interscapular brown adipose tissue, and abdominal white adipose tissue and unchanged in hearts. Four weeks after surgery when gross obesity was evident, rates of norepinephrine turnover were accelerated in heart (+82%) and pancreas (+63%), but remained low in interscapular brown adipose tissue (−27%) and abdominal white adipose tissue (−28%). Adrenal medullary activity, assessed by urinary excretion of epinephrine, was suppressed within the 1st day after knife-cut surgery and remained suppressed for several weeks. Brown adipose tissue and white adipose tissue appear to be selectively excluded from the generalized activation of the sympathetic nervous system in adult hyperphagic rats with obesity-producing hypothalamic knife cuts. Activation of the sympathetic nervous system was associated with reciprocal suppression of adrenal medullary responses in knife-cut rats.

Bray and co-workers have proposed an autonomic hypothesis which suggests that disturbances in the balance between the sympathetic and parasympathetic limbs of the autonomic nervous system explain many of the metabolic adjustments occurring in the syndrome of hypothalamic obesity (1, 2). The hypothesis predicts that sympathetic nervous system activity would be depressed in animals with hypothalamic obesity; however, available evidence based on assessment of norepinephrine turnover in selected organs to support this hypothesis is inconclusive. Rates of norepinephrine turnover in heart, pancreas, brown adipose tissue, and white adipose tissue are depressed in weanling rats with ventromedial hypothalamic lesions (3). But, rates of norepinephrine turnover have been reported to be accelerated in hearts (4, 5), unchanged in pancreas (4), and increased (4) or decreased (5) in brown adipose tissue of adult rats with hypothalamic obesity. Weanling rats remain normophagic (3) whereas adult rats develop marked hyperphagia after obesity-producing hypothalamic lesions (5). Since norepinephrine turnover in several organs of rats is positively correlated with energy intake (6), the resulting hyperphagia in adult rats with hypothalamic lesions may have contributed to the unpredicted elevation of norepinephrine turnover observed in some organs. Indeed when energy intake is controlled, rates of norepinephrine turnover are not elevated in adult rats with hypothalamic lesions (5). Another possible confounding factor is that several weeks elapsed between the time of placement of the hypothalamic lesions and examination of norepinephrine turnover (3–6). Consequently, rats had already developed gross obesity prior to examination of norepinephrine turnover.

The purpose of the present study is to determine if temporal adjustments occur in sympathoadrenal activity in rats with obesity-producing hypothalamic knife cuts as the obe-

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sity develops. Urinary excretion of norepinephrine is monitored for 4 weeks postsurgery as an indicator of overall sympathetic nervous system activity. Rates of norepinephrine turnover are measured in heart, pancreas, interscapular brown adipose tissue (IBAT), and abdominal white adipose tissue (WAT) prior to (3 days after surgery) and after the onset of gross obesity (4 weeks after surgery) to obtain data on sympathetic nervous system activity in specific organs. The sympathetic nervous system and adrenal medullary responses are often reciprocally regulated (7, 8). We previously reported that urinary epinephrine excretion, an index of adrenal medullary activity, is depressed in obese rats with hypothalamic knife cuts (5). Thus, excretion of epinephrine is also monitored with progressive development of obesity in rats with hypothalamic knife cuts.

Methods. *Animals and diet.* Female, Sprague–Dawley rats (Harlan Industries, Inc., Indianapolis, IN) weighing 173 ± 1 (mean ± SEM) and 201 ± 2 g at the time of surgery (experiments 1 and 2, respectively) were housed individually in metal cages with raised wire-mesh floors. Room temperature was maintained at 23 ± 1°C and lights were on from 0700 to 1900 hr. Water and a semipurified high-fat diet were available *ad libitum* except during the 15 to 20 hr before surgery when food was withheld.

The high-fat diet, described previously (5), contained casein, glucose, corn oil, and tallow to provide protein, carbohydrate, and fat at 20, 20, and 60% of metabolizable energy. Food intake and body weights were measured during each experiment.

Surgery. After an overnight fast (15 to 20 hr), rats were anesthetized with sodium pentobarbital (35 mg/kg body wt, ip) and were given atropine methyl nitrate (1 mg, ip) to reduce respiratory problems during anesthesia. Bilateral, parasagittal wire knife cuts were placed stereotaxically (David Kopf Instruments, Tugunga, CA) between the medial and lateral regions of the hypothalamus as described before using a retractable wire knife (5). With the incisor bar fixed at −3.0 mm, the knife cuts were made 1.0 mm lateral to midline and extended posteriorly 3.0 mm, from 8.5 to 5.5 mm anterior to the ear bars and from the base of the brain dorsally 3.0 mm. Sham-operated rats were treated similarly except the needle containing the retracted wire knife was lowered only 3 to 5 mm beneath the dorsal surface of the brain. Each rat received 20,000 units of penicillin G (im) following surgery to reduce infection.

After surgery rats were fed the high-fat diet *ad libitum.* Knife-cut rats that did not exhibit food intakes at least 1 standard deviation greater than the mean intake of sham-operated rats during the first 2 days after surgery were excluded from the data analysis.

Locations of the knife cuts were verified histologically in brains fixed in 10% buffered formalin, embedded in paraffin, serially sectioned (6 μm), and stained with cresyl violet. The knife cuts were consistently located between the medial and lateral hypothalamic areas. The cuts extended from the ventral surface of the brain dorsally past the dorsomedial hypothalamus and from the preoptic area posteriorly past the paraventricular nuclei and the ventromedial hypothalamic areas (5).

Experiment 1. To measure urinary excretion of norepinephrine and epinephrine during the development of hypothalamic obesity, knife-cut and sham-operated rats were housed in metabolism cages and daily urine samples were collected in light-protected flasks containing 1 ml 6 *N* HCl. Samples were collected on Days 1, 2, 3, 5, 10–12, and 23–24. Samples obtained from individual rats on Days 10, 11, and 12 were pooled before analysis as were samples obtained on Days 23 and 24. Free and total (after acid hydrolysis for 30 min at 95°C) norepinephrine and epinephrine were measured using HPLC and electrochemical detection (5).

Experiment 2. To determine how the obesity-producing, hypothalamic knife cuts affected sympathetic nervous system activity in specific organs, norepinephrine turnover rates were measured at 3 days (prior to onset of gross obesity) and at 28 days (after onset of gross obesity) after surgery in heart, pancreas, interscapular brown adipose tissue, and abdominal white adipose tissue of *ad libitum*-fed knife-cut and sham-operated rats (9). WAT represented the total dissectable white adipose tissue in the abdominal cavity. Briefly, rats were injected ip with tritiated *levo*-[ring-2,5,6-^{3}H]norepinephrine (New England Nuclear, Boston, MA) diluted with saline to provide doses of 34–37 μCi/rat in 0.5 ml total volume. Knife-cut and sham-operated rats were injected between 0900 and 1000 hr either 3 or 28 days after surgery and killed by cervical dislocation at 2, 6, 12, or 24 hr after injection (four to six rats from each treatment group at each time point) (6, 9). The heart, pancreas, IBAT, and 0.5 to 1.0 g WAT were rapidly removed and frozen until later analysis for norepinephrine content and [^{3}H]norepinephrine specific activity.

Norepinephrine content of the tissues was measured as before (9) using HPLC and electrochemical detection. Norepinephrine was collected and counted in a liquid scintillation counter to determine the specific activity of [^{3}H]norepinephrine. Slopes (*b*) of linear regressions describing the disappearance of [^{3}H]norepinephrine from heart, IBAT, WAT, and pancreas were used to calculate the fractional turnover rates (k) where $k = b/0.434$. Total norepinephrine turnover rates per organ were calculated as the product of k and the endogenous norepinephrine content of each organ and are reported as ng • organ^{-1} • hr^{-1}.

Statistical analysis. Slopes of linear regressions describing the disappearance of [^{3}H]-norepinephrine in tissues and fractional turnover rates of norepinephrine were compared using the variance estimated for the difference between slopes (10). All other means obtained from knife-cut and sham-operated rats were compared using Student's t test. Differences were considered significant at $P < 0.05$.

Results. Metabolizable energy intake of knife-cut and sham-operated rats is summarized in Table I. Knife-cut rats consumed nearly 2 times more energy than sham-operated rats. Knife-cut rats also gained more body weight than sham-operated rats (Tables II and III), but most of the excess weight gained by knife-cut rats during the first 3 days after surgery was likely due to increased gut fill resulting from the hyperphagia. However, by 28 days after surgery, knife-cut rats were markedly obese. Their energy intake and weight gain were similar to data reported earlier (5) when knife-cut rats fed the high-fat diet *ad libitum* for 4 weeks gained 10 times more body energy than sham-operated rats.

An indication of total sympathoadrenal activity during the development of obesity resulting from hypothalamic knife cuts was obtained by measuring urinary excretion of nor-

TABLE I. METABOLIZABLE ENERGY INTAKE OF SHAM-OPERATED AND KNIFE-CUT RATS

Days postsurgery	Sham-operated (kcal/day)	Knife-cut (kcal/day)
1–2	49 ± 3	98 ± 8*
3	55 ± 3	101 ± 7*
8–14	54 ± 2	98 ± 5*
15–21	60 ± 3	119 ± 5*
22–28	67 ± 3	144 ± 4*

Note. Means ± SEM of 24 sham-operated and 24 knife-cut rats fed a high-fat diet *ad libitum*. Energy intake was determined by measuring food consumption and assuming metabolizable energy values of 4, 3.64, 9, and 9 kcal/g for casein (90% protein), glucose, corn oil, and tallow, respectively. An asterisk (*) indicates a significant difference ($P < 0.05$) between sham-operated and knife-cut rats.

TABLE II. NOREPINEPHRINE (NE) CONTENT AND TURNOVER 3 DAYS AFTER SURGERY

	Sham-operated	Knife-cut
Final body weight (g)	214 ± 3	242 ± 4*
Heart		
Weight (mg)	757 ± 16	812 ± 13*
Total NE (ng • heart^{-1})	619 ± 50	621 ± 54
NE turnover (ng • heart^{-1} • hr^{-1})	50 ± 4	54 ± 5
Pancreas		
Weight (mg)	898 ± 29	979 ± 32
Total NE (ng • pancreas^{-1})	589 ± 21	517 ± 19*
NE turnover (ng • pancreas^{-1} • hr^{-1})	57 ± 2	38 ± 1*
IBAT		
Weight (mg)	350 ± 18	765 ± 30*
Total NE (ng • IBAT^{-1})	385 ± 18	328 ± 18*
NE turnover (ng • IBAT^{-1} • hr^{-1})	53 ± 3	41 ± 2*
WAT		
Weight (mg)	4517 ± 268	6495 ± 387*
Total NE (ng • WAT^{-1})	312 ± 12	269 ± 18
NE turnover (ng • WAT^{-1} • hr^{-1})	33 ± 1	23 ± 2*

Note. Means ± SEM for 24 sham-operated and 19 knife-cut rats weighing 201 ± 4 and 198 ± 3 g at the time of surgery and gaining 13 ± 1 and 43 ± 3 g during the 3 days after surgery, respectively. IBAT indicates interscapular brown adipose tissue and WAT indicates abdominal white adipose tissue. An asterisk (*) indicates a significant difference ($P < 0.05$) between sham-operated and knife-cut rats.

TABLE III. NOREPINEPHRINE (NE) CONTENT AND TURNOVER 28 DAYS AFTER SURGERY

	Sham-operated	Knife-cut
Final body weight (g)	255 ± 3	385 ± 6*
Heart		
Weight (mg)	864 ± 10	1084 ± 21*
Total NE (ng · heart^{-1})	1008 ± 39	894 ± 47
NE turnover (ng · heart^{-1} · hr^{-1})	60 ± 2	109 ± 6*
Pancreas		
Weight (mg)	981 ± 32	1037 ± 28
Total NE (ng · pancreas^{-1})	714 ± 22	660 ± 17
NE turnover (ng · pancreas^{-1} · hr^{-1})	54 ± 2	88 ± 2*
IBAT		
Weight (mg)	456 ± 15	1467 ± 60*
Total NE (ng · IBAT^{-1})	786 ± 29	664 ± 27*
NE turnover (ng · IBAT^{-1} · hr^{-1})	124 ± 5	91 ± 4*
WAT		
Weight (g)	5.31 ± 0.34	31.26 ± 1.06*
Total NE (ng · WAT^{-1})	366 ± 15	294 ± 26*
NE turnover (ng · WAT^{-1} · hr^{-1})	29 ± 2	21 ± 2*

Note. Means ± SEM for 24 sham-operated rats and 24 knife-cut rats weighing 201 ± 2 and 204 ± 2 g at the time of surgery and gaining 54 ± 2 and 181 ± 6 g, respectively, during the 28 days after surgery. IBAT indicates interscapular brown adipose tissue and WAT indicates abdominal white adipose tissue. An asterisk (*) indicates a significant difference ($P < 0.05$) between sham-operated and knife-cut rats.

epinephrine and epinephrine on Days 1, 2, 3, 5, 10–12, and 23–24 after surgery (Fig. 1). During the first 24 hr after surgery (Day 1) there were no differences in free norepinephrine excretion between knife-cut and sham-operated rats. But on the 2nd and 3rd days after surgery, knife-cut rats excreted considerably more free norepinephrine than sham-operated rats (77 and 111%, respectively). Differences between knife-cut and sham-operated rats were slightly attenuated by Day 5 and then gradually became more pronounced by Days 10–12 and 23–24. In contrast to norepinephrine, excretion of free epinephrine was lower in knife-cut rats than in sham-operated rats during the first 12 days after surgery (Fig. 1).

Significant quantities of norepinephrine and epinephrine are conjugated prior to excretion. The percentage of norepinephrine excreted in the free form averaged 50–60% and was unaffected by knife cuts (Fig. 2). Excretion of epinephrine in the free form averaged 52–74% of total epinephrine excreted and likewise was unaffected by the knife cuts (Fig. 2). Thus, under these conditions excretion of the free catecholamine was representative of total excretion.

To assess activity of the sympathetic nervous system in specific organs before and after the development of gross obesity in knife-cut rats, rates of norepinephrine turnover were measured in heart, pancreas, IBAT, and WAT 3 and 28 days after surgery. Three days after surgery, knife-cut rats had slightly heavier hearts (7%), as well as heavier IBAT (119%) and WAT (44%) depots than sham-operated rats (Table II). Fractional norepinephrine turnover rates (k) were unaffected by knife cuts 3 days after surgery (Fig. 3), but total norepi-

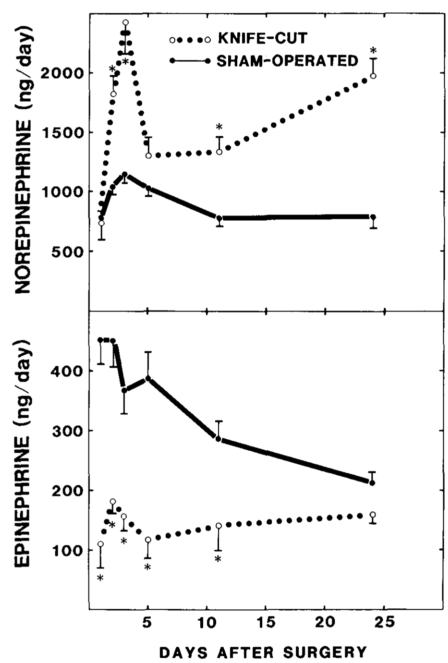

FIG. 1. Free (unconjugated) norepinephrine and epinephrine excretion rates in urine of knife-cut and sham-operated rats measured on Days 1, 2, 3, 5, 10–12, and 23–24 after surgery (experiment 2). Each point represents the mean ± SE for six to nine rats. Individual daily samples collected on Days 10, 11, and 12 and on Days 23 and 24 were pooled before analysis. Asterisks (*) indicate significant differences ($P < 0.05$) between knife-cut and sham-operated rats.

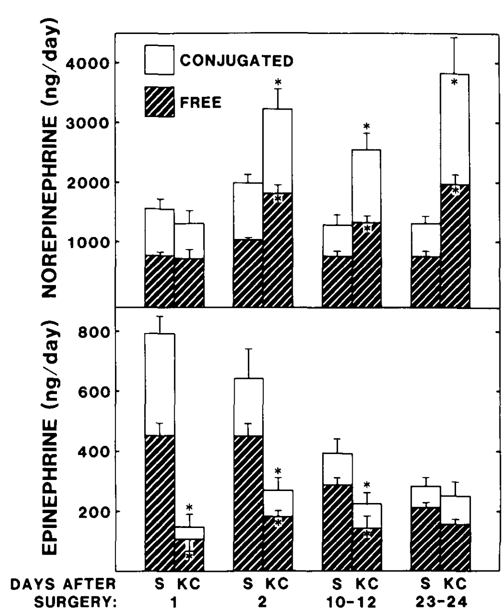

FIG. 2. Excretion of total and free norepinephrine and epinephrine in urine of sham-operated (S) and knife-cut rats (KC) on Days 1, 2, 10–12, and 23–24 after surgery. Each bar represents the mean ± SE for six to nine rats. Asterisks (*) indicate significant differences ($P < 0.05$) between sham-operated and knife-cut rats.

nephrine turnover (ng • organ^{-1} • hr^{-1}) was reduced in pancreas, IBAT, and WAT in knife-cut rats because the total norepinephrine content was reduced slightly in these tissues (Table II).

By 28 days after surgery knife-cut rats had considerably heavier hearts (25%) and their IBAT and WAT depots were at least three and five times heavier, respectively (Table III). Fractional turnover rates of norepinephrine were nearly doubled in heart and pancreas of knife-cut rats, but were unaffected by knife cuts in IBAT and WAT (Fig. 3). Thus, norepinephrine turnover (ng • organ^{-1} • hr^{-1}) was accelerated in heart (82%) and pancreas (63%) of knife-cut rats 28 days after surgery (Table III). In contrast, norepinephrine turnover rates in IBAT and WAT were reduced in obese knife-cut rats (27 and 28%, respectively) mainly as a result of reduced norepinephrine content in these tissues (Table III).

Discussion. Urinary excretion of norepinephrine was elevated within 2 days after rats received hypothalamic knife cuts demonstrating that gross obesity is not a prerequisite for enhanced norepinephrine excretion. It is likely that the enhanced norepinephrine excretion was a secondary consequence of hyperphagia rather than a primary response to the hypothalamic knife cuts because control of energy intake to levels similar to those of control rats also reduces norepinephrine excretion to control levels (5).

A limitation of using urinary norepinephrine excretion to assess sympathetic nervous system activity is that the origin of the norepinephrine cannot be identified. Direct examination of norepinephrine turnover in four organs revealed that the hypothalamic knife cuts

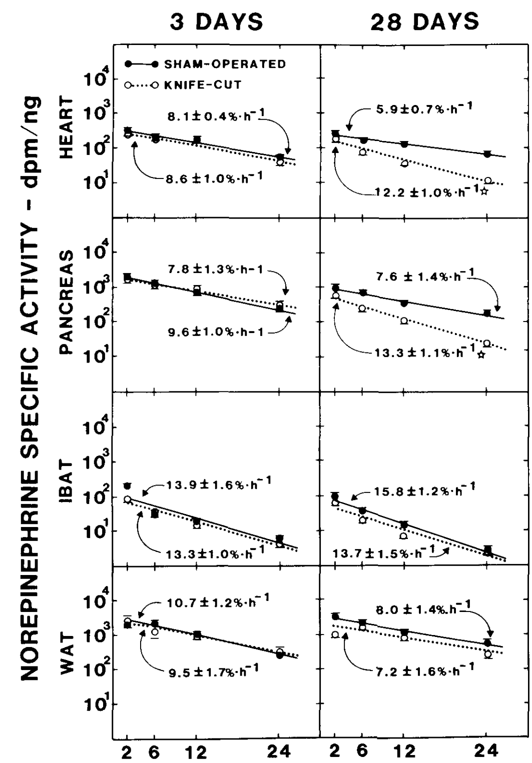

FIG. 3. ^{3}H-labeled norepinephrine turnover in heart, IBAT, WAT, and pancreas of sham-operated and knife-cut rats measured 3 days (left panels) or 28 days (right panels) after surgery. Data points represent means ± SE for four to six rats. Fractional turnover rates (k) ± SE calculated from the slopes (b) of the linear regressions are presented within each panel. Asterisks (*) indicate significant differences ($P < 0.05$) between sham-operated and knife-cut rats.

did not cause a generalized acceleration in sympathetic nervous system activity, and that the degree of obesity affected norepinephrine turnover in selected organs. The elevated rates of NE turnover observed in hearts of rats with hypothalamic knife cuts 4 weeks after surgery agree with previous reports (4, 5). This elevation may be a secondary consequence of developing obesity because it was not evident 3 days after surgery (Table II). Likewise, stimulation of NE turnover in the pancreas only occurred in grossly obese rats. The lungs are a major source of NE release to plasma (11) and may have contributed to the elevated NE excretion in rats with hypothalamic knife cuts.

Although the hypothalamic knife cuts failed to markedly suppress NE turnover in any of the organs examined as predicted by the autonomic hypothesis (1, 2), it must be recognized that the knife-cut rats consumed approximately 100% more energy than corresponding control rats (Table I). When normal rats were induced to overeat by only 10–30% (by presenting sucrose solution plus stock diet) for 3 days, rates of NE turnover in pancreas and brown adipose tissue were elevated by 25–68% (9). Thus, it is evident that the hypothalamic knife cuts disrupted dietary-induced sympathetic stimulation to the pancreas and brown adipose tissue.

Two lines of evidence point to functional consequences of disrupted sympathetic nervous system activity in rats with obesity-producing hypothalamic lesions. Pancreatic islets from rats with hypothalamic lesions exhibited altered sensitivity of insulin secretion to norepinephrine (12); similar alterations are present *in vivo* (13). These alterations coupled with suppressed adrenal medulla epinephrine outflow (Fig. 1) likely contribute to hyperinsulinemia, a major characteristic of rats with obesity-producing hypothalamic lesions (1). Impaired brown adipose tissue metabolism is another consequence of disrupted sympathetic nervous system activity in rats with obesity-producing hypothalamic damage (5, 14–18). This impairment would be expected to conserve energy. Low sympathetic nervous system activity in white adipose tissue, combined with low adrenal epinephrine outflow, would contribute to reduced mobilization of energy stores. Obesity in rats with hypothalamic knife cuts, thus, results from the combined impact of hyperphagia, energy conservation, and reduced mobilization of energy stores.

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