Effect of Diet and Cold Exposure on Norepinephrine Turnover in Brown Adipose Tissue of the Rat

JAMES B. YOUNG, ELIZABETH SAVILLE, NANCY J. ROTHWELL, MICHAEL J. STOCK, and LEWIS LANDSBERG, *Charles A. Dana Research Institute and Thorndike Laboratory, Department of Medicine, Beth Israel Hospital, Harvard Medical School, Boston, Massachusetts 02215; Department of Physiology, St. Georges Hospital Medical School, London SW 17, England*

ABSTRACT Brown adipose tissue (BAT) is an important site of adaptive changes in thermogenesis in the rat. The sympathetic nervous system, which richly supplies BAT, is thought to play an important role in the regulation of BAT thermogenesis because catecholamines stimulate and beta adrenergic blocking agents inhibit oxygen consumption in this tissue. The present studies were carried out to assess directly sympathetic activity in BAT in response to cold exposure and to changes in dietary intake, both of which alter heat production in the rat. Sympathetic activity was determined from the rate of norepinephrine (NE) turnover in interscapular brown adipose tissue (IBAT) after preliminary experiments validated the use of NE turnover techniques in IBAT. Acute exposure to 4°C increased NE turnover in IBAT 4- to 12-fold compared with ambient temperature controls, depending upon the interval over which the turnover measurement was made, while in the heart NE turnover doubled in response to the same cold stimulus. In animals exposed to cold continuously for 10 d before study, NE turnover measurements in IBAT and in the heart were elevated comparably to those obtained during acute exposure. Alterations in feeding were also associated with changes in NE turnover in IBAT. Fasting for 2 d decreased NE turnover in IBAT (−35% from 29.2±4.2 ng NE/h to 18.9±5.9) and in heart (−52%). In animals fed a "cafeteria" diet, a model of voluntary overfeeding in the rat, NE turnover was increased in both IBAT (+108% from 24.8±4.5 ng NE/h to 51.7±6.8) and heart (+66%). Because ganglionic blockade exerted a greater effect on NE turnover in IBAT in cafeteria-fed rats than in controls, the increase in NE turnover in IBAT with this overfeeding regimen reflects enhanced central sympathetic outflow. Thus NE turnover techniques can be satisfactorily applied to the assessment of sympathetic nervous system activity in IBAT.

The experiments reported here demonstrate changes in sympathetic activity in IBAT that parallel known adaptive changes in heat production in the rat. These studies, therefore, support the concept that the increased thermogenesis of chronic cold exposure and of cafeteria feeding occur by similar mechanisms and imply an important role for the sympathetic nervous system, mediated in part through BAT, in the regulation of energy balance in the rat.

INTRODUCTION

Brown adipose tissue (BAT)[1] is now recognized as a major site of metabolic heat generation (nonshivering thermogenesis) in cold-acclimated rats, despite the fact that it represents only 1–2% of body weight in these animals (1–3). This highly specialized organ is located in interscapular, axillary, and paraspinal regions and within the thoracic and abdominal cavities and is capable of markedly increasing heat production over short time intervals. Prolonged exposure to cold results in BAT hypertrophy and in a striking enhancement of its thermogenic capacity, changes that correspond to the development of the cold-acclimated state. Recent studies by Rothwell and Stock (4, 5) indicated that rats voluntarily overeating a "cafeteria" diet consumed more oxygen at rest and gained less weight in relation to food intake than animals eating a control diet; associated with this dietary alteration in energy balance were changes in BAT structure and function that resembled those of the cold-acclimated state. The increase in heat production and oxygen consumption, therefore, that occurs in response to both cold exposure

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[1] *Abbreviations used in this paper*: alpha-MPT, alpha-methyl-p-tyrosine; BAT, brown adipose tissue; 6-OHDA, 6-hydroxydopamine; IBAT, interscapular brown adipose tissue; k, fractional NE turnover rate; NE, norepinephrine.

(nonshivering thermogenesis) and excess caloric intake (diet-induced thermogenesis) in the rat may originate in BAT.

The sympathetic nervous system is thought to play an important role in the regulation of heat production by BAT. This tissue contains an extensive sympathetic innervation (6, 7) and increases oxygen consumption both in vivo and in vitro in response to norepinephrine (NE) administration or to sympathetic nerve stimulation (1–5, 8, 9). Furthermore, cold exposure and overfeeding activate the sympathetic nervous system in other tissues (10–12). The experiments described in this study were undertaken to assess sympathetic activity in BAT in different physiological situations associated with adaptive changes in thermogenesis in the rat.

Sympathetic activity in BAT was estimated from the rate of NE turnover within interscapular brown adipose tissue (IBAT), a readily accessible site of BAT comprising ~20–25% of total BAT in the rat. NE turnover was computed from the rate of disappearance of [^{3}H]NE after labeling of the endogenous NE stores with tracer quantities of [^{3}H]NE and from the rate of fall of endogenous NE after blockade of NE biosynthesis. Both techniques use a monoexponential model of NE kinetics (13, 14) in the calculation of a fractional NE turnover rate (k) and in the comparison of turnover rates in different groups of animals within the same experiment. Because physiological variation in NE turnover is principally dependent upon changes in sympathetic impulse traffic, higher NE turnover rates reflect increased sympathetic activity and lower rates diminished sympathetic activity. The results of these studies indicate that physiological changes in sympathetic nervous system activity in BAT occur in parallel with known alterations in thermogenesis in the rat. This association between sympathetic activity in BAT and thermogenesis emphasizes the importance of sympathetic nerves in the regulation of BAT function and supports the fundamental similarity between nonshivering thermogenesis and diet-induced thermogenesis.

METHODS

Male CD (Sprague-Dawley-derived) rats (100–125 g) obtained from Charles River Breeding Laboratories (Wilmington, MA) were housed two to three per single cage and five to six per double cage in a temperature-controlled animal room (20–22°C) and allowed free access to water and Charles River chow (R-M-H 3000; Agway-Country Food, Agway, Inc., Syracuse, NY), except where noted. While fasting, the animals were given a hypotonic saline solution containing 50 meq Na^+ per liter to drink and, during sucrose feeding experiments, a 10% sucrose solution to supplement the lab chow diet. During cafeteria feeding (15), animals were housed two per single cage and received four palatable food items daily (e.g., popcorn, canned meats, candy, crackers, etc.) in addition to chow, a voluntary feeding program known to increase caloric intake by 50–100% (4). During cold exposure, animals were housed two per single cage in a cold room (4°C) and were given free access to lab chow and water.

L-[N;7,8-^{3}H]NE (20–30 Ci/mmol, sp act; New England Nuclear, Boston, MA) was purified before use by column chromatography with alumina as described below. The [^{3}H]NE was diluted to an appropriate concentration with isotonic saline and injected into the tail veins of unanesthetized animals in a total volume of 1.0 ml. The dose of [^{3}H]NE used in these studies varied between 60 and 100 μCi/kg (0.4–0.8 μg NE/kg). The rats were killed at preselected times by cervical dislocation. For each time point in the studies of NE turnover four to seven animals were killed from each experimental group. The tissues were rapidly removed, frozen on dry ice, and stored at −20°C for later processing (usually within 2 wk). For NE analysis, the organs were weighed and homogenized in iced 0.4 N perchloric acid in a ground glass homogenizer (Kontes Co., Vineland, NJ) to extract the NE and precipitate the proteins. After volume adjustment the precipitated protein was removed by low-speed centrifugation.

Isolation of NE from the perchloric acid extract was by column chromatography with alumina as described (16). NE was adsorbed onto the alumina column at pH 8.6 and eluted with 0.2 N acetic acid. The alumina (neutral, Fisher Scientific Co., Pittsburgh, PA) has previously been purified according to the method of Anton and Sayre (17). Recovery of added NE was usually in excess of 70% with a variation between columns of <10%. Standards were run with all batches of samples and results were corrected for recovery. Assay of NE in the alumina eluate was by a modification of the spectrophotofluorometric method of Crout (18). Aliquots of the alumina eluates were counted for [^{3}H]NE by scintillation spectrometry in a Packard 460C liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, IL). Efficiency for ^{3}H in this system is 30–35%.

6-Hydroxydopamine (6-OHDA; Sigma Chemical Co., St. Louis, MO) was dissolved in isotonic saline containing 5 μl 2 N HCl and 1 mg ascorbic acid per milliliter and injected intravenously in a dose of 100 mg/kg 2 d before study. Chlorisondamine (Ciba-Geigy Corp., Pharmaceuticals Div., Summit, NJ) and tyramine (Sigma Chemical Co.) were dissolved in isotonic saline and injected intraperitoneally in doses of 5 and 40 mg/kg, respectively. DL-alpha-methyl-*p*-tyrosine methyl ester (alpha-MPT; Sigma Chemical Co.) was dissolved in isotonic saline and injected intraperitoneally in doses of 250 and 125 mg/kg.

Data are presented as means±SEM, unless otherwise noted. Statistical analyses were performed using analysis of variance and of covariance (19). In experiments requiring multiple comparisons, the presence of statistically significant variation was established among all groups before individual comparisons were made between any two groups; individual comparisons used either the Newman-Keuls test (19) or repeat analysis of covariance. In studies of NE turnover, the data were plotted semilogarithmically. The slope (fractional NE turnover rate, k) of the decline in NE specific activity over time after [^{3}H]NE administration or in endogenous NE after alpha MPT injection was calculated by the method of least squares. In all measurements of NE turnover using [^{3}H]NE, no significant variation in endogenous NE was observed over the 24 h of the experiment, unless otherwise noted. The statistical significance of each computed regression line was assessed by analysis of variance. Comparison of fractional turnover rates was made with analysis of covariance. NE turnover rates were calculated as the product

of the fractional turnover rate and the endogenous NE concentration (13, 14); in the experiments using alpha MPT the fractional turnover rates were multiplied by the endogenous NE concentration at the zero time point. 95% confidence intervals were determined for the NE turnover rates as described (20).

RESULTS

Pharmacological manipulation of NE stores in IBAT. The use of NE turnover techniques to assess sympathetic nerve activity in any organ is dependent upon the intraneuronal localization of NE within that tissue. Evidence in support of NE storage within sympathetic nerve endings in IBAT was obtained through the use of pharmacological agents with known effects upon NE stores in sympathetic nerves. Pretreatment with 6-OHDA, an agent that destroys sympathetic nerve endings, reduced the uptake of [^{3}H]NE in IBAT by 72% (from 7.25±0.52 to 2.04±0.30 nCi, $P < 0.0005$), and reduced the endogenous NE content by 91% (from 315.0±12.6 to 28.7±7.6 ng, $P < 0.0005$), findings of similar magnitude to those obtained from the heart (−94 and −74%, respectively). The administration of tyramine, an indirectly acting sympathomimetic amine that displaces NE from storage sites within sympathetic nerves, diminished [^{3}H]NE in IBAT by 74% (from 5.68±0.39 to 1.45±0.18 nCi, $P < 0.0005$) from rats previously injected with tracer [^{3}H]NE and endogenous NE levels by 50% (from 339.8±19.5 to 171.3±6.3 ng, $P < 0.0025$); hearts from the same animals, likewise, demonstrated reductions in [^{3}H]NE (−72%) and endogenous NE (−35%). A differential effect of tyramine on the displacement of [^{3}H]NE compared with that of endogenous NE has been seen previously in the heart (10, 12, 21) and is consistent with heterogeneity within the NE pool in sympathetic neurons of both IBAT and heart. As discussed below, ganglionic blockade increased retention of [^{3}H]NE in IBAT as a function of the reduction in postganglionic nerve impulse traffic, which controls neurotransmitter release. Thus, these pharmacological manipulations of NE in IBAT support the localization of ^{3}H and endogenous NE stores within the sympathetic nerve endings and therefore provide the basis for the measurement of NE turnover as an index of sympathetic activity in this tissue.

Effect of cold exposure on NE turnover in IBAT. Cold exposure is a well known stimulus of the sympathetic nervous system that has been shown to increase NE turnover in various organs (10–12). An initial attempt to measure NE turnover in IBAT using the [^{3}H]NE turnover method during acute exposure to 4°C was unsatisfactory due to a pronounced fall in endogenous NE level. After 24 h of cold exposure NE content in IBAT was only 52% of that in ambient temperature controls, as noted (22), thereby invalidating the assumption of steady-state conditions implicit in the use of the [^{3}H]NE turnover technique. The depletion of NE in IBAT of acutely cold-exposed rats, however, suggested intense sympathetic stimulation by cold in this tissue.

To evaluate further the sympathetic response to acute cold exposure, the synthesis inhibition technique was used in IBAT and heart. After biosynthesis is blocked with alpha MPT, endogenous NE levels fall at a rate that reflects the functional state of sympathetic nerves in that tissue (13). The results of this turnover experiment in control and cold-exposed rats are shown in Fig. 1. Over the 6 h of study endogenous NE levels in heart fell at a rate (k) of 8.6±1.6%/h in warm animals and at a rate of 18.1±3.5 ($P < 0.02$) in cold rats. Calculated NE turnover in the hearts of cold-exposed animals was twice that in control hearts, 68.1±16.3 ng NE/h (95% confidence intervals) and 32.6±7.6 ng NE/h, respectively. In IBAT, the cold-induced increase in NE turnover was more striking; k was 6.1±1.4%/h in control and 29.7±2.3%/h ($P < 0.0001$) in cold animals and calculated NE turnover was 20.0±5.3 ng NE/h in control animals (95% confidence intervals) and 97.0±11.3 ng NE/h in cold-exposed ones.

Although the endogenous NE levels in hearts from both groups and in IBAT of controls (Fig. 1) appeared to fit the monoexponential model, those in IBAT from

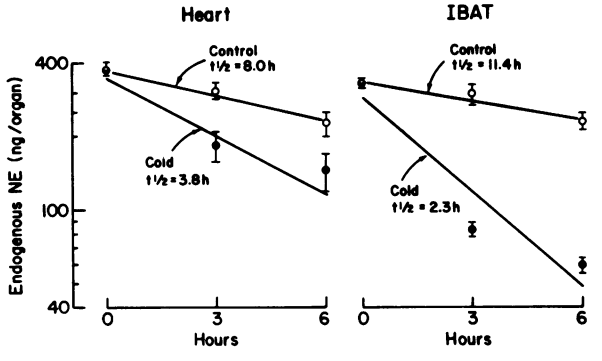

FIGURE 1 Effect of acute cold exposure on NE turnover in heart and IBAT. After injection of alpha-MPT (250 mg of the methyl ester/kg i.p. at t_0 and 125 mg/kg i.p. at t_{3h}), 14 animals were placed in a cold room (4°C) and 14 were kept at ambient temperature (22°C). 10 animals were not injected and served as the t_0 reference for both warm and cold groups. Half of the 14 animals were killed at 3 h and the other half were reinjected with alpha MPT and killed at 6 h. Data are plotted as mean±SEM for endogenous NE. Open circles represent warm animals and closed circles cold-exposed animals; statistical significance of each regression line was at least $P < 0.001$. Endogenous NE in heart was 372.7±18.4 ng and in IBAT was 325.3±12.2 ng. Cold exposure increased k and calculated NE turnover rates in both heart and IBAT.

the cold-exposed animals did not; the apparent rate of decrease in IBAT NE content was greater in the first 3 h at 4°C than during the second 3 h. A second experiment was performed to examine more closely the early changes in NE turnover in IBAT on exposure to cold and to compare this initial response with that associated with chronic cold exposure (10 d of continuous cold exposure). The results of this experiment are shown in Fig. 2. In heart NE turnover increased with both acute and chronic cold exposure, but due to the shorter time interval for observation and considerable interanimal variation, the changes were of marginal statistical significance. The rate of cardiac NE depletion (k) in control rats was 5.9±3.2%/h, in acutely cold-exposed animals 15.8±4.8%/h ($P < 0.1$ vs. control), and in chronically cold-exposed ones 16.7±5.5 ($P < 0.1$ vs. control). Calculated NE turnover was slightly greater in cold-exposed rats than in controls with turnover rates of 38.7±23.4 ng NE/h (95% confidence intervals) in control, 101±38.3 in acutely and 77.6±29.4 ng NE/h in chronically cold-exposed animals.

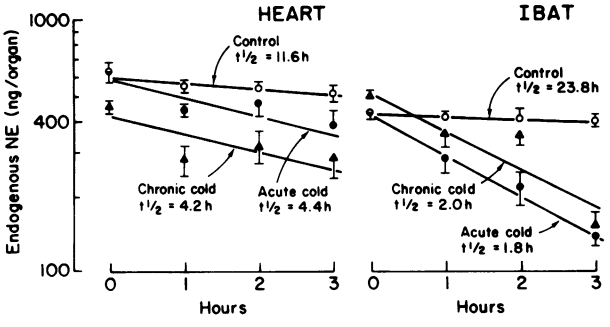

FIGURE 2 Effects of acute and chronic cold exposure on NE turnover in heart and IBAT. Chronically cold-exposed animals were maintained in a cold room (4°C) continuously for 10 d before and during the experiment. 32 warm and 12 cold-exposed animals received a single i.p. injection of alpha MPT (250 mg/kg) at t_0; half of the injected warm animals were placed in the cold, and four to six animals from each group were killed at hourly intervals over the ensuing 3 h. Eight animals from both warm and chronically cold-exposed groups were not injected and served as the t_0 reference for all three groups. Data are plotted as mean±SEM for endogenous NE. Open circles represent warm animals, closed circles the acutely cold-exposed rats, and closed triangles the chronically cold-exposed rats. Statistical significance of the regression lines was $P < 0.01$ in hearts and $P < 0.0001$ in IBAT of cold-exposed rats; because of the short time interval and low rate of NE turnover in control animals, neither regression line in heart or IBAT was statistically significant. Endogenous NE in heart was 622.2±55.0 ng in warm and 456.4±26.1 ng in chronically cold-exposed animals ($P < 0.025$) and in IBAT 432.7±27.6 ng in warm and 490.4±27.2 ng in chronically cold-exposed rats (not statistically different). The null hypothesis that all three regression lines could be represented by a common one was rejected for IBAT ($F_{2,62} = 23.96$, $P < 0.0001$), but not for heart ($F_{2,60} = 1.77$, $P = 0.18$).

As in the previous experiment (Fig. 1), the impact of cold exposure on NE turnover was considerably greater in IBAT. In both cold-exposed groups the rate of fall in NE content in IBAT was markedly increased compared with control; k was 2.9±2.6%/h in control, 38.0±4.4%/h in acute ($P < 0.0001$ vs. control) and 34.9±5.1%/h in chronic cold exposure ($P < 0.0001$ vs. control, not significantly different from acute cold). Calculated NE turnover was 11- to 12-fold higher in the cold-exposed groups than in control and was 13.4±12.3 ng NE/h (95% confidence intervals) in control, 166±29.5 ng NE/h in acutely, and 172±34.6 ng NE/h in chronically cold-exposed animals. In addition, endogenous NE levels in IBAT were not significantly different in rats maintained for 10 d in the cold compared with ambient temperature controls, consistent with induction of tyrosine hydroxylase during prolonged cold exposure (23, 24), in comparison with the acutely cold-exposed rats in which endogenous NE levels fell. Thus cold exposure, both acute and chronic, accelerates NE turnover in heart and to a greater extent in IBAT, but the optimum experimental design to demonstrate this increase is different in the two tissues.

Effects of fasting and short-term sucrose feeding on NE turnover in IBAT. Studies from this laboratory have demonstrated an effect of dietary intake on NE turnover in several sympathetically-innervated tissues. Fasting decreases and sucrose supplementation increases NE turnover and sympathetic activity in heart, pancreas, and liver (10). NE turnover in heart and IBAT was measured in three groups of rats: control animals feeding ad lib.; fasted animals that were without food for 48 h before and during the NE turnover study; and sucrose-fed animals that were given a 10% sucrose solution to drink for 3 d before and during the turnover measurement. The results of this experiment are shown in Fig. 3 and Table I. In hearts of control rats, k was 9.3±1.2%/h, in fasted animals 3.2±0.9%/h ($P < 0.001$ compared with control), and in sucrose-fed rats 11.6±1.2%/h ($P = 0.18$ vs. control; $P < 0.0001$ vs. fasted). Calculated NE turnover in heart was reduced 52% by fasting, from 37.1±7.3 ng NE/h (95% confidence intervals) in control rats to 17.7±5.4 ng NE/h in fasted animals and was increased only slightly (19%) by sucrose feeding to 44.2±5.8 ng NE/h. In IBAT the effects of dietary manipulation on NE turnover paralleled those seen in heart (Fig. 3). Control animals displayed a k in IBAT of 8.6±0.8%/h compared with 5.2±1.4 ($P < 0.05$) in fasted rats and to 10.7±0.9 ($P < 0.08$ vs. control and $P < 0.002$ vs. fasted) in sucrose-fed rats. Calculated NE turnover decreased 35% in IBAT with fasting, from 29.2±4.2 ng NE/h (95% confidence intervals) in control to 18.9±5.9 ng NE/h and increased marginally (18%) with sucrose feeding to 34.6±4.1 ng NE/h. Thus in IBAT (and

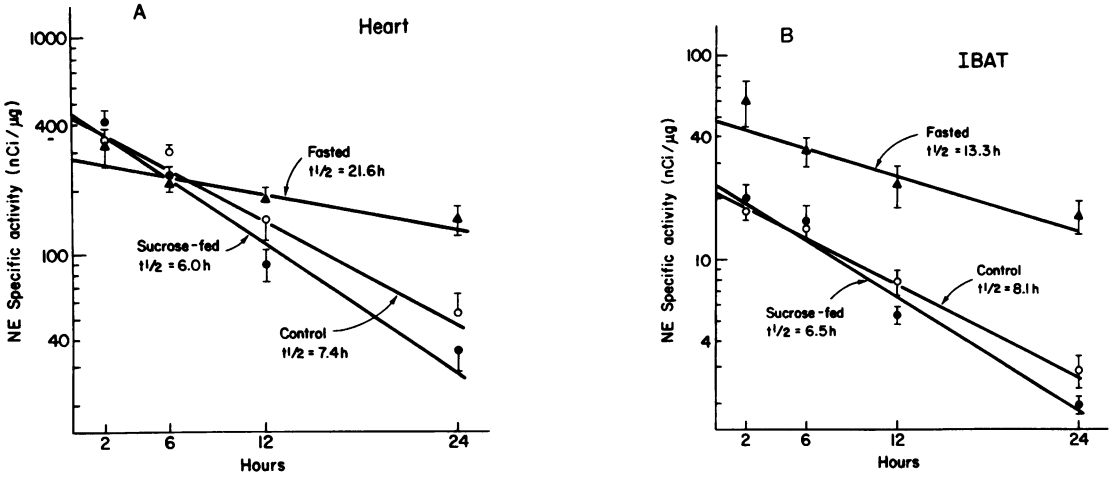

FIGURE 3 Effects of fasting and sucrose feeding on NE turnover in heart (A) and IBAT (B). Fasted animals were without food for 48 h before and during turnover measurement; sucrose-fed animals were given a 10% sucrose solution to drink for 3 d before and during the experiment. Data are plotted as mean±SEM for specific activity of NE in heart and IBAT from five to six animals in each group at each time point. Open circles represent control, ad lib.-fed animals, closed circles sucrose-fed animals, and closed triangles fasted animals. Statistical significance of each control and sucrose-fed regression line was $P < 0.001$, for hearts of fasted rats $P = 0.0015$, and for IBAT of fasted rats $P < 0.0012$. Endogenous NE levels in heart were 394.5±28.1 ng in control, 545.0±21.8 ng in fasted ($P < 0.001$ vs. control), and 379.9±11.5 ng in sucrose-fed rats (not statistically different from control; $P < 0.001$ vs. fasted). In IBAT endogenous NE in control animals (338.3±15.6 ng) was not different from that in either fasted (357.8±20.3) or sucrose-fed (321.5±12.5) animals. The null hypothesis that all three regression lines could be represented by a common one was rejected for both heart ($F_{5,26} = 15.56$, $P < 0.0001$) and IBAT ($F_{2,56} = 7.17$, $P = 0.0017$). k and calculated NE turnover rates were significantly slowed by fasting in both heart and IBAT and were slightly, but not significantly, increased by sucrose feeding. The lower specific activity of NE in hearts of fasted rats at t_0 probably reflects diminution of nerve activity, whereas the higher initial specific activity in IBAT from the same animals may be attributable to increased tissue blood flow because the initial uptake of tracer after intravenous injection depends upon both tissue blood flow and the functional state of the sympathetic nerves.

TABLE I

*Effects of Fasting and Sucrose Feeding on Body and Organ Weights**

		Heart		IBAT	
	Body weight	Weight	Body weight	Weight	Body weight
	g	g	%	g	%
Control (21)	160.8	0.5527	0.3482	0.2061	0.1286
SEM	3.0	0.0165	0.0174	0.0090	0.0054
Fasted (20)	116.6	0.4210	0.3607	0.1716	0.1471
SEM	1.3	0.0089	0.0056	0.0086	0.0072
P vs. Control	<0.001	<0.001	—	<0.025	<0.05
Sucrose-fed (21)	161.4	0.5393	0.3341	0.2687	0.1664
SEM	1.9	0.0123	0.0064	0.0113	0.0066
P vs. Control	NS	NS	—	<0.001	<0.001
P vs. Fasted	<0.001	<0.001	—	<0.001	<0.05
F ratio					
$F_{2,59}$	132.65	30.24	1.37	25.36	8.81
P	<0.0005	<0.0005	NS	<0.0005	<0.0005

* These data are from the experiment depicted in Fig. 3. The numbers in parentheses represent the number of animals in each group.

heart) short-term changes in dietary intake altered NE turnover although the suppressive effect of fasting was more clearly demonstrable than the stimulation by sucrose feeding.

The effects of these dietary regimens on body weight and heart and IBAT weight are presented in Table I. Body weight and heart and IBAT weights were lower in fasted animals. When expressed as percentage of body weight, heart weights in fasted rats were comparable to those in control, while IBAT weights were actually increased slightly ($P < 0.05$), indicating a greater loss of body weight than of IBAT. 3 d of sucrose feeding had no effect on body or heart weights, but did increase IBAT weight above control.

Effects of cafeteria feeding and chronic cold exposure on NE turnover in IBAT. NE turnover rates in heart and in IBAT after a 9-d schedule of either cafeteria feeding or exposure to 4°C with ad lib. access to lab chow are shown in Fig. 4. In hearts of control animals k was 7.2±0.5%/h, in cafeteria-fed animals 12.7±1.0%/h ($P < 0.001$ vs. control), and in chronically cold-exposed rats 19.6±2.0%/h ($P < 0.001$ vs. control and $P < 0.005$ vs. cafeteria-fed rats). Calculated NE turnover was increased 66% above control by cafeteria feeding, from 48.3±5.3 ng NE/h (95% confidence intervals) to 80.2±9.7 ng NE/h, and 105% above control by chronic cold exposure to 99.1±16.7 ng NE/h. In IBAT similar results were obtained. k in control animals was 5.8±0.8%/h, in cafeteria-fed rats 11.0±1.1%/h ($P < 0.001$ vs. control), and in chroni-

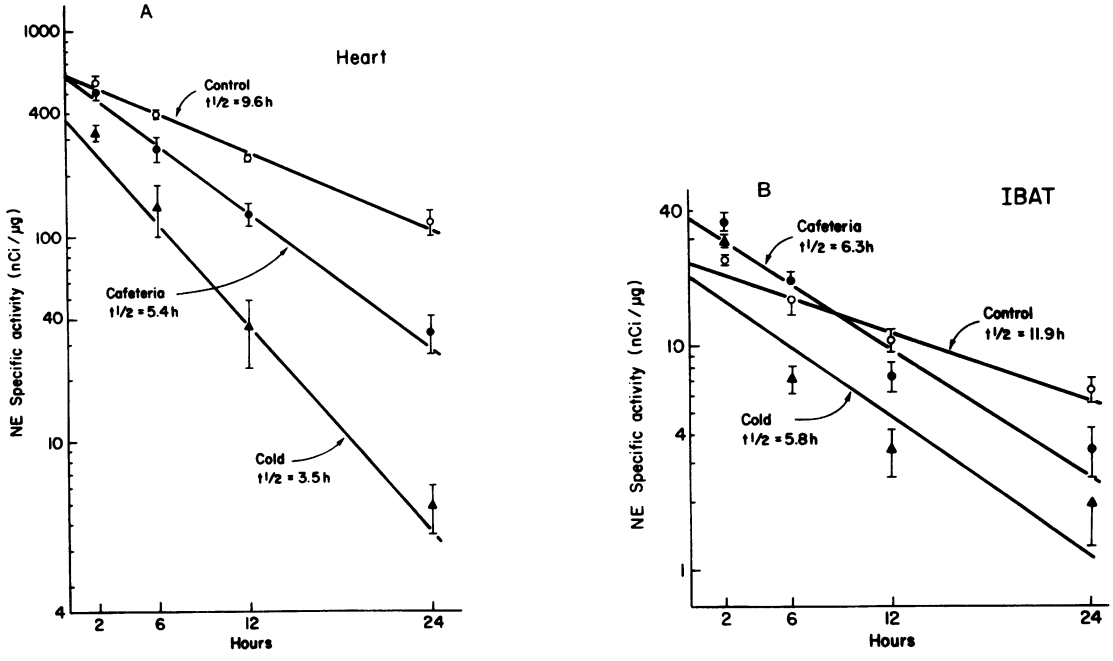

FIGURE 4 Effects of cafeteria feeding and chronic cold exposure on NE turnover in heart (A) and IBAT (B). Cafeteria-fed animals were given supplemental food items for 9 d before and during turnover study; chronically cold-exposed animals were kept in a cold room (4°C) during the same 9-d period. The cold-exposed animals were removed from the cold 1–2 h before i.v. injection of [^{3}H]NE and were returned to the cold immediately after administration of tracer. Data are plotted as mean±SEM for specific activity of NE in heart and IBAT from five to six animals in each group at each time point. Open circles represent control, ad lib.-fed animals, closed circles cafeteria-fed animals, and closed triangles cold-exposed animals. Statistical significance of each regression line was $P < 0.0001$. Endogenous NE levels in heart were 665.2±28.7 ng in control, 628.9±28.2 ng in cafeteria-fed, and 502.6±32.8 in cold-exposed animals ($P < 0.001$ vs. control) and in IBAT were 422.2±18.9 ng in control, 465.9±16.9 in cafeteria-fed, and 496.7±21.8 in cold-exposed animals ($P < 0.025$ vs. control). The null hypothesis that all three regression lines could be represented by a common one was rejected for both heart ($F_{2,58} = 23.00$, $P < 0.0001$) and IBAT ($F_{2,59} = 6.60$, $P = 0.0026$). k and calculated NE turnover rates were significantly increased by both cafeteria feeding and chronic cold exposure in heart and IBAT. The lower initial specific activity of NE in the hearts of chronically cold-exposed rats may reflect the effect of environmental warming that had taken place before injection; in IBAT the enhanced initial uptake of tracer in cafeteria-fed rats presumably is the result of increased nerve activity and of augmented local blood flow.

cally cold-exposed rats 11.9±1.7%/h ($P < 0.003$ vs. control; not significantly different from cafeteria-fed rats). Calculated NE turnover was elevated 108% above control with cafeteria feeding, from 24.8±4.5 ng NE/h (95% confidence intervals) to 51.7±6.8 ng NE/h; in chronically cold-exposed animals the apparent departure of the data points from the monoexponential model renders the calculated NE turnover rate of 59.5±11.0 ng NE/h only a rough approximation. The results of a second study (not shown) measuring NE turnover over the first 12 h after tracer injection demonstrated a similar lack of correspondence to the mathematical model, indicating that the selection of time points did not contribute to the observed results as it had in a previous experiment (Fig. 1). Nonetheless, in both heart and IBAT the data indicate acceleration of NE turnover by cafeteria feeding and chronic cold exposure.

The effects of cafeteria feeding and chronic cold exposure on body, heart, and IBAT weights are shown in Table II. Cold-exposed animals gained less weight than either of the other two groups, but hypertrophy of both heart and IBAT occurred as described (1, 5, 25, 26). Cafeteria feeding did not lead to greater weight gain than that seen in control animals, a consistent finding in rats of this age, but did stimulate IBAT growth (4). Hypertrophy of IBAT in cafeteria-fed rats served as an index of the efficacy of the supplemented feeding program. Cardiac hypertrophy, both in absolute weight and in percentage of body weight, developed in the cafeteria-fed animals as well.

Effect of ganglionic blockade on NE turnover in IBAT. Evidence that the increase in NE turnover in IBAT in cafeteria-fed rats originates from enhanced central sympathetic outflow is presented in Fig. 5. To the extent that NE turnover reflects changes in efferent neural impulse traffic, the imposition of ganglionic blockade produces a greater effect on NE turnover (increased retention of labeled [^{3}H]NE administered before the ganglionic blocking agent) in situations of increased sympathetic activity and a lesser effect under conditions of decreased sympathetic activity. The effect of ganglionic blockade with chlorisondamine on retention of [^{3}H]NE in control animals and in rats fed the cafeteria diet for 9 d is shown in Fig. 5. In IBAT from control animals [^{3}H]NE content was 34% greater in animals given chlorisondamine than in those given saline, an increase that was not statistically significant; in cafeteria-fed rats, however, [^{3}H]NE content in IBAT increased 84% ($P < 0.005$ vs. saline-treated, cafeteria-fed animals). Because the effects of ganglionic blockade are described in terms of the amount of tracer remaining 10 h after injection, the apparent increase in tracer content represents a lower rate of loss of tracer after injection. Moreover, initial tracer uptake is greater in IBAT of cafeteria-fed rats than in that of

TABLE II
*Effects of Cafeteria Feeding and Chronic Cold Exposure on Body and Organ Weights**

		Heart		IBAT	
	Body weight	Weight	Body weight	Weight	Body weight
	g	g	%	g	%
Control (22)	218.1	0.6711	0.3074	0.2200	0.1011
SEM	2.5	0.0103	0.0046	0.0047	0.0023
Cafeteria (22)	220.4	0.7287	0.3314	0.4368	0.1983
SEM	4.0	0.0143	0.0061	0.0188	0.0078
P vs. Control	NS	<0.025	<0.01	<0.001	<0.001
Chronic cold (22)	189.6	0.7272	0.3873	0.3572	0.1917
SEM	4.9	0.0172	0.0084	0.0166	0.0109
P vs. Control	<0.001	<0.025	<0.001	<0.001	<0.001
P vs. "Cafeteria"	<0.001	NS	<0.001	<0.001	NS
F ratio					
$F_{2,63}$	19.15	4.77	39.34	55.59	48.10
P	<0.0005	<0.025	<0.0005	<0.0005	<0.0005

* These data are from the experiment depicted in Fig. 4. The numbers in parentheses represent the number of animals in each group. Body weights at the beginning of the 9-d protocol were similar in the three groups: control animals weighed 143.8±1.6 g; cafeteria-fed animals, 143.9±1.8 g; and chronic cold-exposed animals, 144.1±1.4 g.

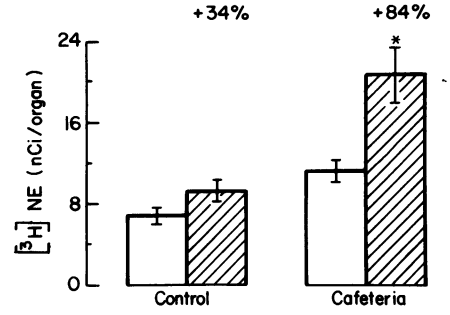

FIGURE 5 Effect of ganglionic blockade on retention of [^{3}H]NE in IBAT of control and cafeteria-fed rats. Cafeteria feeding had commenced 9 d before injection of [^{3}H]NE as in Fig. 4. After intravenous injection of [^{3}H]NE, chlorisondamine (ganglionic blocking agent), or saline was administered intraperitoneally at 15 min and 5 h. Animals were killed 10 h after initial injection. Data are presented as mean [^{3}H] content±SEM for groups of six to eight animals each. Open bars represent mean [^{3}H]NE content in hearts of saline-treated animals and closed bars, chlorisondamine-treated animals. Number over bar indicates percentage of increase over saline control. *$P < 0.005$ for comparison between ganglionic blockade and saline treatment. Endogenous NE levels in IBAT from control animals were 420.7±27.9 ng in saline-treated and 534.7±33.1 in chlorisondamine-treated rats ($P < 0.025$ vs. saline) and in cafeteria-fed animals were 475.6±26.7 ng in saline-treated and 539.6±47.2 in chlorisondamine-treated rats (not significantly different from saline-treated rats). □, saline; ▨, ganglionic blockade.

control (Fig. 4), but because of faster NE turnover, tracer content is of similar magnitude in the two saline-treated groups 10 h postinjection. Thus, the restraining effect of ganglionic blockade on NE turnover in IBAT was greater in animals fed the cafeteria diet than controls, a finding consistent with increased central sympathetic outflow to IBAT during cafeteria feeding. 9 d of cafeteria feeding in this group of rats also led to cardiac and IBAT hypertrophy as noted before in Table II.

DISCUSSION

Biochemical and physiological studies suggest an important role for sympathetic nerves in the regulation of BAT function. One of the goals of this investigation, therefore was to apply NE turnover techniques to the measurement of sympathetic activity in IBAT. Use of tracer [^{3}H]NE in the measurement of NE turnover requires that the tracer be taken up by the sympathetic nerve endings, be uniformly distributed throughout a single intraneuronal NE pool, and be released in response to sympathetic nerve impulses. These criteria have been examined in heart and other organs (10, 12), as well as in IBAT, and are adequately fulfilled to permit the use of the [^{3}H]NE technique as an estimate of NE turnover and of sympathetic activity in these tissues. The potential nonhomogeneity in the endogenous NE pool, as suggested by the results of the experiment with tyramine, argues against the acceptance of NE turnover as an absolute, descriptive measure of sympathetic activity, but not as a comparative index of nerve function.

Other factors operative in vivo may also limit the interpretation of turnover measurements in quantitative terms. Because the NE turnover rate represents the mean level of sympathetic activity over the interval in which it is measured, changes during the study period in tissue blood flow, in sympathetic activity itself, or in additional, unrecognized factors may adversely affect the NE turnover measurement. Whatever the explanation for NE turnover data that do not conform to the monoexponential model, one approach to the estimation of NE turnover in such circumstances is to reduce the interval over which the measurement is made in the hope of achieving more uniform physiological conditions as obtained in IBAT, for example, in Fig.1 in comparison with Fig. 2. NE turnover data that do not fit the monoexponential model affect the statistical comparisons among experimental groups, increasing the risk of false negative but not of false positive errors. Such occurrences (as in the chronic cold group in Fig. 4) emphasize the qualitative nature of NE turnover as an index of sympathetic activity. Thus, in order for NE turnover measurements in IBAT, or in any tissue, to be of quantitative value, sufficient time points (at least three and preferably four) must be included to evaluate the agreement between the experimental data and the theoretical, monoexponential model of NE turnover.

The experiments also demonstrate that IBAT contains almost as much NE as heart. If one assumes that endogenous NE levels (and thereby the density of sympathetic innervation) in BAT from other sites is similar to that in IBAT, then total BAT would contain four times as much NE as heart, or ~2 μg of NE in animals of the size studied here. Because this NE is stored within the immediate vicinity of cells exhibiting thermogenic responses to NE, the potential of BAT for sympathetically mediated heat production is fully consonant with measurements of oxygen consumption in this tissue in vivo during NE infusions[2] (3, 9). The concordance of changes in NE turnover in IBAT with the known effects of dietary manipulation and cold exposure on oxygen consumption in the rat further supports a prominent role for the sympathetic nervous

[2] Heterogeneous responses to infused NE have recently been noted among deposits of BAT at different sites (9); whether such variability occurs during cold exposure or with overfeeding is unknown.

system in the regulation of BAT function and heat production.

The stimulatory effect of cold exposure on the sympathetic nervous system and the crucial importance of sympathetic activation in the defense of body temperature in mammals are well recognized (27, 28); the nonhomogeneity of sympathetic outflow in this setting, however, is less widely appreciated. Acute cold exposure increases NE turnover in a variety of tissues in addition to heart and IBAT, including pancreas, lung, skeletal muscle, spleen, and kidney (10–12, 29); in other tissues, such as liver, intestine, and submandibular gland,[3] the changes in NE turnover with cold are minimal, if present at all (10, 11). Of all tissues studied only IBAT demonstrated (Figs. 1 and 2) a cold-induced rise in NE turnover substantially greater than that seen in heart. The dramatic acceleration of NE turnover in IBAT on acute exposure to cold emphasizes the potential importance of this tissue in the immediate thermogenic reaction to cold in the rat.

Rats fed the cafeteria diet exhibit changes in thermogenesis similar to those seen in animals chronically exposed to cold (5). Resting oxygen consumption is increased by a mechanism sensitive to beta adrenergic blockade; dependence upon shivering for heat production during acute cold exposure is markedly diminished; and stimulation of oxygen consumption by exogenous NE is greatly exaggerated. The impact of enhanced thermogenesis in the cafeteria-fed rats is illustrated by the failure of these animals to gain more weight than controls, despite a substantial increase in caloric intake. Although caloric intake was not measured in this investigation, earlier experience with cafeteria feeding demonstrated increases between 50 and 100% on this regimen (4). In our experiments IBAT hypertrophy served as the index of successful overfeeding. As seen in Fig. 4 NE turnover is elevated to a similar extent in cold-exposed and cafeteria-fed rats, an increase secondary to stimulation of central sympathetic outflow (Fig. 5). Previous studies have shown that dietary supplementation with sucrose for 3 d increased sympathetic activity in heart, pancreas, and liver (10). In a related experiment here (Fig. 3) the impact of supplemental sucrose on NE turnover in heart and IBAT was less than seen before (10), an observation that may reflect the differences in age and sex of the animals in the two studies. The elevation in sympathetic nervous system activity in heart and IBAT was clearly greater in cafeteria-fed rats (Fig. 4) than in control, indicating that overfeeding a mixture of nutrients, rather than sucrose alone, stimulates the sympathetic nervous system and, furthermore, that the increase in sympathetic activity is sustained over a 9-d period of overfeeding. Thus, one aspect of the beta adrenergically mediated increment in thermogenesis in cafeteria-fed, as well as in cold-exposed animals is enhanced sympathetic activity in BAT.

Fasting, on the other hand, reduces resting oxygen consumption slightly in the rat, ~7% below prefasting control values in normal animals within 3 d and 15% within 5 d (30). Because this fall in metabolic rate was observed in hypothyroid animals and was associated with diminished metabolic responsiveness to exogenous triiodothyronine, the hypometabolic effect of fasting in the rat cannot be secondary to fasting-induced changes in thyroid hormones. The decrease in NE turnover in IBAT in fasted rats (Fig. 3) provides evidence that withdrawal of sympathetic stimulation of BAT may underlie the decrease in metabolic rate seen in these animals. The alterations in IBAT sympathetic activity, therefore, in response to changes in dietary intake and to cold exposure are entirely consistent with a major role for the sympathetic nervous system in regulating BAT thermogenesis.

The importance of sympathetic nerves in the hypertrophy of BAT that occurs in cold acclimation and during cafeteria feeding is less certain. In the situations described here the association between increased sympathetic activity in IBAT and IBAT hypertrophy suggests involvement of the sympathetic nervous system in the increase in BAT mass. Evidence that catecholamines cause hypertrophy of various tissues when administered to experimental animals (31) and that the pattern of organ enlargement during chronic cold exposure roughly parallels the extent of sympathetic innervation (25), strengthens the connection between sympathetic nerve activity and tissue hypertrophy, particularly during cold exposure. Furthermore, the cardiac hypertrophy demonstrated here in cafeteria-fed rats is consistent with this hypothesis. Sympathetic nerves are thus likely to participate in the stimulation of BAT hypertrophy. On the other hand, enhanced sympathetic activity is not sufficient, in and of itself, to cause BAT hypertrophy because exposure to hypoxia (0.5 atmosphere) for 14 d increased IBAT sympathetic activity, but not tissue mass (32), and food restriction during cold exposure limited IBAT growth, but not sympathetic activation (33). Thyroid hormone, which can induce BAT hypertrophy in rats when administered in hyperthyroid doses (24), may not be an important growth factor for BAT in the situations studied here because IBAT hypertrophies in hypothyroid animals exposed to cold (34, 35) or fed the "cafeteria" diet[4]. Factors controlling the growth and hypertrophy

[3] Young, J. B., and L. Landsberg. Unpublished observations.

[4] Saville, M. E., and M. J. Stock. Unpublished observations.

of BAT under different physiological conditions warrant further investigation.

These studies, therefore, demonstrate that sympathetic outflow to IBAT in the rat changes markedly in situations known to affect thermogenesis. The findings are consistent with an important role for the sympathetic nervous system in the regulation of heat production by BAT and the overall control of thermogenesis in this animal. Although the potential significance of BAT for thermogenesis in other mammals, particularly larger, nonhibernating species, is controversial, Rothwell and Stock (4) have provided suggestive evidence for the functional existence of BAT in adult humans. Because diet-induced changes in sympathetic activity and in energy expenditure occur in man as well as in the rodent (36), the sympathetic nervous system may participate in the control of heat production in man, mediated in part through changes in BAT function. Accordingly, factors that influence sympathetic regulation of BAT thermogenesis may have important implications for the efficiency of fuel storage and, therefore, for the development of obesity in man.

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