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Research Article

Increased energy intake activates the sympathetic nervous system (SNS) in animals and man. While dietary carbohydrate and fat stimulate, the impact of dietary protein on the SNS is not well defined. The present studies examine the effect of protein ingestion on sympathetic function based upon the measurement of [3H]norepinephrine (NE) turnover in heart and interscapular brown adipose tissue (IBAT) as the index of SNS activity. In these experiments, animals were pair-fed mixtures of laboratory chow and refined preparations of casein, sucrose, and lard to permit comparisons among nutrients with total energy intake held constant or with additional energy provided in the form of a single nutrient. After 5 d of eating a 2:1 mixture of chow and either casein or sucrose cardiac, [3H]NE turnover was less (P less than 0.005) in casein-fed rats (6.4%/h and 28.9 ng NE/h) than in animals given sucrose (11.2%/h and 46.5 ng NE/h). Similar results were obtained in IBAT and in experiments using 1:1 mixtures of chow and casein/sucrose. Casein-fed animals also displayed slower rates of NE turnover than lard-fed rats in both heart (7.8%/h vs. 13.2, P less than 0.001) and IBAT (7.0%/h vs. 12.8, P less than 0.01). Addition of casein (50% increase in energy intake) to a fixed chow ration raised NE turnover slightly, but not significantly, in heart (an average increase […]

Effect of Protein on Sympathetic Nervous System Activity in the Rat

Evidence for Nutrient-specific Responses

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Abstract

Increased energy intake activates the sympathetic nervous system (SNS) in animals and man. While dietary carbohydrate and fat stimulate, the impact of dietary protein on the SNS is not well defined. The present studies examine the effect of protein ingestion on sympathetic function based upon the measurement of [^{3}H]norepinephrine (NE) turnover in heart and interscapular brown adipose tissue (IBAT) as the index of SNS activity. In these experiments, animals were pair-fed mixtures of laboratory chow and refined preparations of casein, sucrose, and lard to permit comparisons among nutrients with total energy intake held constant or with additional energy provided in the form of a single nutrient. After 5 d of eating a 2:1 mixture of chow and either casein or sucrose cardiac, [^{3}H]NE turnover was less ($P < 0.005$) in casein-fed rats (6.4%/h and 28.9 ng NE/h) than in animals given sucrose (11.2%/h and 46.5 ng NE/h). Similar results were obtained in IBAT and in experiments using 1:1 mixtures of chow and casein/sucrose. Casein-fed animals also displayed slower rates of NE turnover than lard-fed rats in both heart (7.8%/h vs. 13.2, $P < 0.001$) and IBAT (7.0%/h vs. 12.8, $P < 0.01$). Addition of casein (50% increase in energy intake) to a fixed chow ration raised NE turnover slightly, but not significantly, in heart (an average increase of 15% in six experiments). Thus, in distinction to SNS activation seen with dietary carbohydrate or fat, the SNS response to dietary protein is minimal in both heart and IBAT, indicating that the effect of increased energy intake on the SNS is dependent upon diet composition.

Introduction

Alterations in nutrient intake affect sympathetic nervous system (SNS)[1] activity. Fasting suppresses (1), while overfeeding a mixed diet stimulates SNS activity (2). Furthermore, carbohydrate (sucrose) and fat (lard) both activate the SNS even when total caloric intake is not increased (3, 4). The effects of protein on the SNS, in comparison, are not well characterized. Recent studies demonstrating enhanced SNS activity in rats fed a low protein diet (formulated by isocaloric substitution of sucrose or glucose for casein) suggested that carbohydrate stimulates the SNS to a greater extent than protein (5, 6).

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1. *Abbreviations used in this paper:* IBAT, interscapular brown adipose tissue; NE, norepinephrine; SNS, sympathetic nervous system.

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These studies, however, left unanswered whether the decrease in casein or the increase in sucrose was more responsible for SNS stimulation by low protein feeding. Favoring the increase in sucrose was the previous report from this laboratory that described SNS activation by sucrose in the presence of a constant protein intake (4). Pointing toward the reduction in casein were the greater relative change in casein than in sucrose by this dietary substitution (in one study protein content was reduced from 22 to 7% while sucrose was raised from 42 to 58% [6]) and the neurotransmitter precursor hypothesis, which claimed that dietary intake of tyrosine, the amino acid precursor for catecholamine biosynthesis, normally exerted an inhibitory effect over peripheral SNS function (7).

Since the design of the earlier studies could not distinguish between the effects of adding one nutrient with those of removing another (5, 6), the following studies were undertaken to characterize the SNS response to protein, and to compare directly the effects of protein, carbohydrate, and fat on the SNS using a different feeding regimen. Sympathetic activity was estimated in heart and interscapular brown adipose tissue (IBAT) by the [^{3}H]norepinephrine (NE) turnover technique (2, 8). The results indicate that the SNS response to dietary casein is minimal and substantially less in heart and IBAT than the SNS activation observed with either sucrose or lard.

Methods

Animals. Female CD (Sprague-Dawley derived) rats (110–130 g; Charles River Breeding Laboratories, Wilmington, MA) were housed 2–3/cage in a constant temperature animal room (23°C) and allowed free access to water. Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHHS publication No. (National Institutes of Health) 78-23, revised 1978).

Diets. The various dietary mixtures were prepared from the following ingredients: standard laboratory chow (Prolab R-M-H 3200; Agway, Inc., Syracuse, NY), sucrose, lard (ICN Nutritional Biochemicals, Cleveland, OH), and vitamin-free casein (ICN Nutritional Biochemicals). Formulation of dietary mixtures was based upon energy content of the various ingredients according to the following factors: chow, 3.7 kcal/g (manufacturer's estimate of "digestible" energy); sucrose, 4.0 kcal/g; lard, 9.0 kcal/g; and casein, 3.6 kcal/g.

Within each experiment, all animals were pair-fed the same amount of lab chow (~5–6 g/100 g body weight). Various groups in each experiment also received sucrose, lard, and/or casein added to the chow. The energy content of the resulting mixtures was two parts chow to one part added nutrient (50% increase over chow alone) or one part chow to one part nutrient (100% increase). These proportions remained constant over a given experiment despite adjustments in the daily ration required to maintain pair feeding of chow energy. Animals were fed the different diets beginning 5 d before the start of and during the 24 h of the NE turnover measurement.

Turnover procedure. Levo-[ring-2,5,6-^{3}H]NE (40–60 Ci/mmol sp act; Dupont NEN Research Products, Boston, MA) was purified before use by column chromatography with alumina that had previously been prepared according to the method of Anton and Sayre (9); the labeled NE was adsorbed onto alumina at pH 8.6 and eluted in 0.2 N acetic acid. For each experiment, the [^{3}H]NE was diluted to an appropriate concentration with 0.9% NaCl and injected intravenously 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 25 and 100 μCi/kg ($\sim$0.1–0.4 μg NE/kg). The rats were killed at preselected times by cervical dislocation. For each time point in the studies of NE turnover, 4–8 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).

Extraction and isolation of catecholamines. For NE analysis, the organs were weighed and homogenized in iced 0.2 N perchloric acid in a ground glass homogenizer (Duall-Kontes Glass Co., Vineland, NJ) to extract the catecholamines and precipitate the proteins. After volume adjustment, the precipitated protein was removed by low-speed centrifugation. After addition of the internal standard, 3,4-dihydroxybenzylamine (DHBA; Aldrich Chemical Co., Milwaukee, WI), catecholamines were isolated from the perchloric acid extract by adsorption onto alumina (Woelm neutral, ICN Nutritional Biochemicals) in the presence of 2 M Tris(hydroxymethyl)aminomethane buffer (pH 8.7; Sigma Chemical Co., St. Louis, MO) containing 2% EDTA. Catecholamines were eluted from the alumina with 0.2 N perchloric acid. After removal of alumina fines by filtration (microfilter; Bioanalytical Systems, West Lafayette, IN), aliquots of the alumina eluate were injected onto a liquid chromatographic system for catecholamine analysis. Unless otherwise specified, all chemicals were obtained from Fisher Scientific Co., Fair Lawn, NJ.

Measurement of [^{3}H]NE. Aliquots of the alumina eluates were counted for [^{3}H]NE by scintillation spectrometry in a Packard 460C liquid scintillation counter (Packard Instrument Co., Downers Grove, IL). Efficiency for ^{3}H in this system is 30–35%.

Determination of endogenous NE levels. Analysis of catecholamines in the alumina eluates was performed by a slight modification of the method of Eriksson and Persson (10). The chromatographic system was composed of a pump (M45; Millipore Corp., Milford, MA), an automatic sample injector (Waters Intelligent Sample Processor, WISP; Millipore Corp.), a reverse-phase column (250 × 4 mm, Bio-Sil ODS-5S; Bio-Rad Laboratories, Richmond, CA) preceded by a 30 × 4.6-mm precolumn of a similar material (Brownlee Labs Inc., Santa Clara, CA), and a glassy carbon amperometric detector (LC-4A/17; Bioanalytical Systems). The mobile phase was an acetate-citrate buffer composed of sodium acetate (100 mM), sodium hydroxide (60 mM), and citric acid (40 mM, all three from Mallinckrodt, Inc., Paris, KY) at pH 5.3 containing 10% methanol and 1.0 mM sodium octyl sulfonate (Aldrich Chemical Co. or Eastman Kodak Co., Rochester, NY) flowing at a rate of 1.0 ml/min. The detector potential was set at +0.65 V vs. Ag/AgCl reference electrode. Detector response was quantitated by peak height using an integrating recorder (3390A, Hewlett-Packard Co., Avondale Div., Avondale, PA). Intraassay coefficients of variation for replicate determinations of NE specific activity in the same tissue sample are routinely 1–2%.

Data analysis. Data are presented as means±SEM. Statistical analyses were performed using analysis of variance and covariance (11). 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; posthoc comparisons were based upon the Newman-Keuls test (11). 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 was calculated by the method of least squares (8). 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. 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 (8). Comparison of NE turnover rates in different experiments used the nonparametric, Wilcoxon paired-sample test (11).[2]

Table I. Comparative Effects of Sucrose and Casein Added to Chow on [^{3}H]NE Turnover in Heart

Supplement	Body weight	Tissue weight	Fractional NE turnover	Endogenous NE	Calculated NE turnover rate
n	*g*	*g*	*%/h*	*ng*	*ng/h*
Sucrose (16)	133	0.458	11.2	415	46.5
SEM	1.4	0.007	0.9	19	
Casein (18)	139	0.477	6.4	452	28.9
SEM	2.1	0.011	1.2	16	
Pooled SE	1.8	0.010	1.0	18	
F ratio	6.07	1.9	9.36	2.2	
P	<0.02	NS	<0.005	NS	

The data in this table are from the same experiment as in Fig. 1. Animals were pair-fed 2:1 mixtures of chow and either casein or sucrose for 5 d before and during measurement of NE turnover.

Results

Effects of casein on [^{3}H]NE turnover in heart and IBAT: comparison with sucrose. Since previous studies demonstrated that adding sucrose to lab chow or substituting sucrose for casein in a synthetic diet accelerated NE turnover in a variety of tissues in the rat (4, 6), [^{3}H]NE turnover was compared in animals receiving mixtures of casein and chow with those receiving energetically equivalent amounts of sucrose and chow. The results of an experiment comparing 2:1 mixtures of chow with either casein or sucrose (in which energy intake was 33.3 kcal/100 g body weight/d) are presented in Table I and Fig. 1. After consuming their respective diets for 5 d, animals receiving casein exhibited slower fractional NE turnover in heart (6.4%/h vs. 11.2, $P < 0.005$) and 38% lower overall rates for cardiac NE turnover (28.9 ng NE/h vs. 46.5). A second experiment (data not shown) repeating the same feeding protocol yielded an identical result; fractional and overall rates for NE turnover in heart were less in rats given the casein-chow mixture than in those given sucrose-chow (3.4%/h vs. 6.5, $P < 0.03$; 18.4 ng NE/h vs. 33.0).

Similar results were also obtained when the ratio of chow to nutrient energy was changed from 2:1 to 1:1 (associated with an increase in total energy intake to $\sim$39.3 kcal/100 g per d), as illustrated in Table II and Fig. 2. Fractional NE turnover was slower in casein-fed rats than in sucrose-fed animals both in heart (7.6%/h vs. 13.2, $P < 0.001$) and IBAT (16.2%/h vs. 8.8, $P < 0.01$); calculated rates of NE turnover were also less in the casein-fed animals (33.1 ng/h vs. 48.6 in heart and 28.1 vs. 55.6 in IBAT). Thus, when provided to rats as isoenergetic supple-

2. The use of a nonparametric test in this circumstance reflects our view that NE turnover rates are ordinal, not numerical data. While intergroup comparisons of the temporal decline in tracer NE within a single experiment can use regression analysis based upon the monoexponential kinetic model for NE turnover data, comparisons between experiments cannot. This view derives from the recognition that the NE turnover rate, though proven useful as an index of SNS activity, is not a physiological entity, like oxygen consumption or glucose uptake, since several of the theoretical assumptions central to the mathematical model are known to be false.

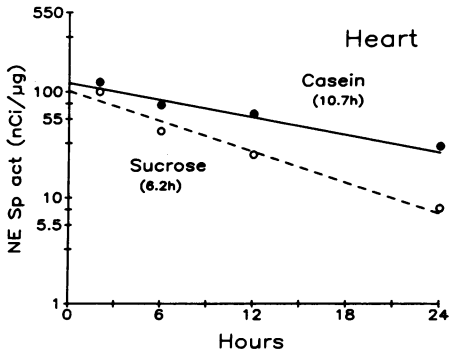

Figure 1. Effects of casein and sucrose on [^{3}H]NE turnover in heart. Animals were pair-fed 2:1 mixtures of chow and either casein or sucrose for 5 d before study. At the start of the turnover measurement, all rats received an intravenous injection of [^{3}H]NE (26 µCi/kg) and were killed at various times over the ensuing 24 h. Data from this experiment are presented in Table I. In the figure, data are plotted as means for specific activity of NE in heart from 4 to 6 animals in each group at each time point. Open circles (o) and the broken line (- - -) represent rats given chow + sucrose; closed circles and the solid line represent rats given chow + casein. The numbers in parentheses refer to the half-time of disappearance of tracer ($t_{1/2}$). Statistical significance for each regression line was $P < 0.001$. Cardiac NE turnover in animals fed the casein-containing mixture was less than in those given the sucrose-supplemented formula.

ments to a standard lab chow diet, casein accelerated [^{3}H]NE turnover to a lesser extent than sucrose in both heart and IBAT.

Effect of casein on [^{3}H]NE turnover in heart and IBAT: comparison with lard. In a manner similar to that employed with sucrose as described above, [^{3}H]NE turnover was compared in groups of rats fed chow-nutrient mixtures containing two parts

Table II. Comparative Effects of Sucrose and Casein Added to Chow on [^{3}H]NE Turnover in Heart and IBAT

Supplement	Body weight	Tissue weight	Fractional NE turnover	Endogenous NE	Calculated NE turnover rate
n	*g*	*g*	*%/h*	*ng*	*ng/h*
Heart					
Sucrose					
(19)	125	0.450	13.2	368	48.6
SEM	2.4	0.011	1.2	16	
Casein					
(22)	137	0.473	7.6	435	33.1
SEM	1.8	0.009	0.8	19	
Pooled SE	2.1	0.010	1.0	18	
F ratio	14.9	2.76	15.8	6.73	
P	<0.001	NS	<0.001	<0.025	
IBAT					
Sucrose					
(19)		0.184	16.2	343	55.6
SEM		0.010	2.3	16	
Casein					
(22)		0.154	8.8	319	28.1
SEM		0.005	1.3	11	
Pooled SE		0.008	1.8	14	
F ratio		7.17	8.58	1.46	
P		<0.02	<0.01	NS	

The data in this table are from the same experiment as in Fig. 2. Animals were pair-fed 1:1 mixtures of chow and either casein or sucrose for 5 d before and during measurement of NE turnover.

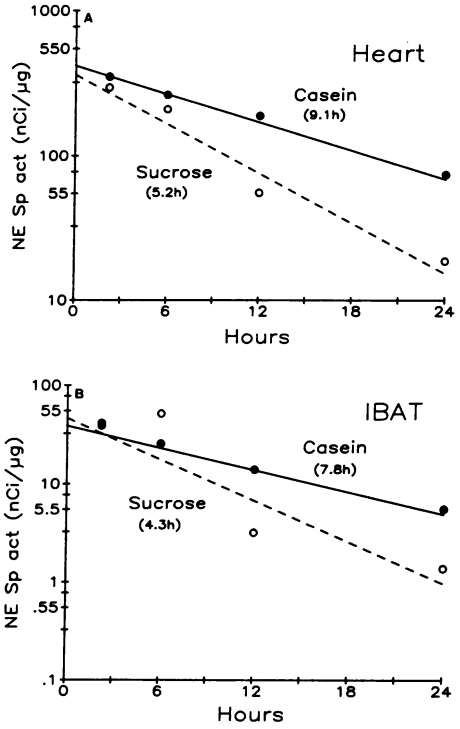

Figure 2. Effects of casein and sucrose on [^{3}H]NE turnover in heart (*A*) and IBAT (*B*). Animals were pair-fed 1:1 mixtures of chow and either casein or sucrose for 5 d before study. At the start of the turnover measurement, all rats received an intravenous injection of [^{3}H]NE (48 µCi/kg) and were killed at various times over the ensuing 24 h. Data from this experiment are presented in Table II. In the figure, data are plotted as means for specific activity of NE in heart and IBAT from 4 to 6 animals in each group at each time point. Open circles (o) and the broken line (- - -) represent rats given chow + sucrose; closed circles and the solid line represent rats given chow + casein. The numbers in parentheses refer to the half-time of disappearance of tracer ($t_{1/2}$). Statistical significance for each regression line was $P < 0.001$. NE turnover in both heart and IBAT was less in animals fed the casein-containing mixture than in those given the sucrose-supplemented formula.

chow energy and one part either casein or lard. Energy intake was ~33.3 kcal/100 g per d in both diet groups. The results of this experiment are presented in Table III and Fig. 3. Animals receiving the casein supplement displayed lower fractional NE turnover in both heart and IBAT (7.8%/h vs. 13.2, $P < 0.001$, in heart; 7.0%/h vs. 12.8, $P < 0.01$, in IBAT) and slower overall rates for NE turnover (34.7 ng NE/h vs. 52.5 in heart and 23.8 vs. 38.3 in IBAT). Thus, casein added to the basic lab chow ration provided less stimulation of [^{3}H]NE turnover than an isoenergetic supplement of lard.

Effect of supplemental casein on [^{3}H]NE turnover in heart and IBAT of chow-fed rats. In the studies described above, all experimental groups were fed equivalent amounts of chow and isoenergetic supplements of various nutrients, thereby permitting direct comparison between the effect of casein on [^{3}H]NE turnover with that of either sucrose or lard. Since the addition of sucrose or lard calories to a chow ration increased NE turnover in various tissues of the rat (4) and, as shown above, the effect of casein on [^{3}H]NE turnover was less than that of either sucrose or lard, the addition of casein to chow might reduce, increase slightly, or exert no effect on [^{3}H]NE turnover when compared

Table III. Comparative Effects of Lard and Casein Added to Chow on [^3H]NE Turnover in Heart and IBAT

Supplement	Body weight	Tissue weight	Fractional NE turnover	Endogenous NE	Calculated NE turnover rate
n	*g*	*g*	*%/h*	*ng*	*ng/h*
Heart					
Lard (22)	137	0.475	13.2	398	52.5
SEM	2.2	0.012	1.3	20	
Casein (20)	143	0.509	7.8	445	34.7
SEM	1.9	0.011	0.7	22	
Pooled SE	2.1	0.012	1.1	21	
F ratio	4.52	4.26	10.2	2.62	
P	<0.05	<0.05	<0.001	NS	
IBAT					
Lard (22)		0.195	12.8	299	38.3
SEM		0.008	1.4	14	
Casein (20)		0.165	7.0	340	23.8
SEM		0.006	1.1	14	
Pooled SE		0.008	1.3	14	
F ratio		7.55	3.76	4.12	
P		<0.01	<0.01	<0.05	

The data in this table are from the same experiment as in Fig. 3. Animals were pair-fed 2:1 mixtures of chow and either casein or lard for 5 d before and during measurement of NE turnover.

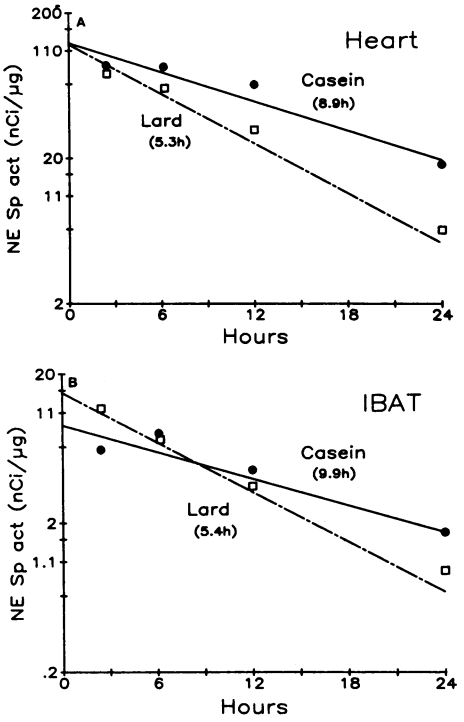

Figure 3. Effects of casein and lard on [^{3}H]NE turnover in heart (*A*) and IBAT (*B*). Animals were pair-fed 2:1 mixtures of chow and either casein or lard for 5 d before study. At the start of the turnover measurement, all rats received an intravenous injection of [^{3}H]NE (48 µCi/kg) and were killed at various times over the ensuing 24 h. Data from this experiment are presented in Table III. In the figure, data are plotted as means for specific activity of NE in heart and IBAT from 4 to 6 animals in each group at each time point. Open squares (□) and the broken line (-·-·) represent rats given chow + lard; closed circles and the solid line represent rats given chow + casein. The numbers in parentheses refer to the half-time of disappearance of tracer ($t_{1/2}$). Statistical significance for each regression line was $P < 0.001$. NE turnover in both heart and IBAT was less in animals fed the casein-containing mixture than in those given the lard-supplemented formula.

with chow alone. Several experiments were performed to examine these various possibilities.

In one experiment, [^{3}H]NE turnover was measured simultaneously in three groups of rats. Two groups were fed as described in the first experiment (Table I, Fig. 1) with 2:1 mixtures of chow with either sucrose or casein; a third group was given chow alone, in an amount equal to the chow content of the two mixtures. This control group thus received an energy intake (22.2 kcal/100 g/d) below its ad lib. consumption of chow. The results of this experiment are presented in Table IV and Fig. 4. Fractional and calculated NE turnover rates differed significantly among the three groups in both heart ($P < 0.001$) and IBAT ($P < 0.01$). In heart posthoc, pair-wise comparisons revealed that fractional NE turnover was greater in sucrose-fed rats than in either chow-fed ($P < 0.001$) or casein-fed animals ($P < 0.005$), confirming results of previous experiments (4) and Table I, Fig. 1. Fractional NE turnover was slightly, though not significantly, greater in the hearts of rats fed the casein-chow mixture than in hearts of animals fed just chow alone. Calculated rates of NE turnover in heart displayed a similar ranking among the groups.

In IBAT posthoc, pair-wise comparisons indicated that fractional NE turnover was significantly greater in sucrose-fed rats than in casein-fed animals ($P < 0.01$), a finding similar to that noted above (Table II, Fig. 2 *B*). Although the fractional NE turnover rate was faster in sucrose-fed rats than in animals fed chow alone, this difference was not statistically significant. In addition, despite the 50% greater energy intake in the casein-fed rats, fractional NE turnover was slightly slower (not significantly) in casein-fed animals compared with those given only chow. Calculated rates of NE turnover followed the same rank order among the three diet groups.

Since, in our experience, small differences in NE turnover that are not statistically significant in a single experiment may nonetheless be reproducibly present over a series of studies (12, 13), several additional experiments were performed comparing [^{3}H]NE turnover in animals fed chow alone with those given the 2:1 chow-casein mixture. The results of these experiments (including the experiment described in the preceding paragraphs) are presented in Table V, and one of these experiments (exp. 3) is also shown in Fig. 5. In five of six experiments performed under identical feeding conditions, NE turnover in heart was slightly faster in the casein-fed animals than in those given only chow; in one study (exp. 5), the acceleration of fractional NE turnover with casein supplementation was statistically significant ($P < 0.05$). In IBAT, the effect of added casein on [^{3}H]NE turnover was negligible. In two experiments, NE turnover in IBAT was slightly, though not significantly, less in casein-supplemented rats than chow-fed controls ($P < 0.06$ in one of these—exp. 3), while in the other, three NE turnover was marginally faster with added casein. Although these studies raise the possibility that ingestion of casein accelerates [^{3}H]NE turnover in heart, but not IBAT, the effect is small, an average rise in cardiac NE turnover of 15% for a 50% increase in total energy intake as casein.

Effect of supplemental casein on [^{3}H]NE turnover in heart of sucrose- and lard-fed rats. Since slowing of NE turnover by casein might be more apparent if the animals were ingesting a

Table IV. Comparative Effects of Sucrose and Casein Added to Chow on [^{3}H]NE Turnover in Heart and IBAT

Supplement	Body weight	Tissue weight	Fractional NE turnover	Endogenous NE	Calculated NE turnover rate
n	*g*	*g*	*%/h*	*ng*	*ng/h*
Heart					
Chow alone					
(20)	117	0.417	4.0	542	21.7
SEM	1.8	0.006	0.7	26	
Sucrose					
(22)	125	0.466	8.4	483	40.6
SEM	2.0	0.009	0.6	18	
Casein					
(20)	132	0.497	5.4	501	27.0
SEM	1.9	0.010	0.8	19	
Pooled SE	1.9	0.008	0.7	21	
F ratio	14.6	21.8	10.2	2.01	
P	<0.001	<0.001	<0.001	NS	
IBAT					
Chow alone					
(20)		0.102	9.9	248	24.6
SEM		0.004	1.6	9	
Sucrose					
(22)		0.173	12.0	291	34.9
SEM		0.010	1.0	10	
Casein					
(20)		0.148	7.6	262	19.9
SEM		0.009	0.9	10	
Pooled SE		0.008	1.2	10	
F ratio		20.5	3.76	5.00	
P		<0.001	<0.01	<0.01	

The data in this table are from the same experiment as in Fig. 4. Animals were pair-fed 2:1 mixtures of chow and either casein or sucrose or chow alone for 5 d before and during measurement of NE turnover.

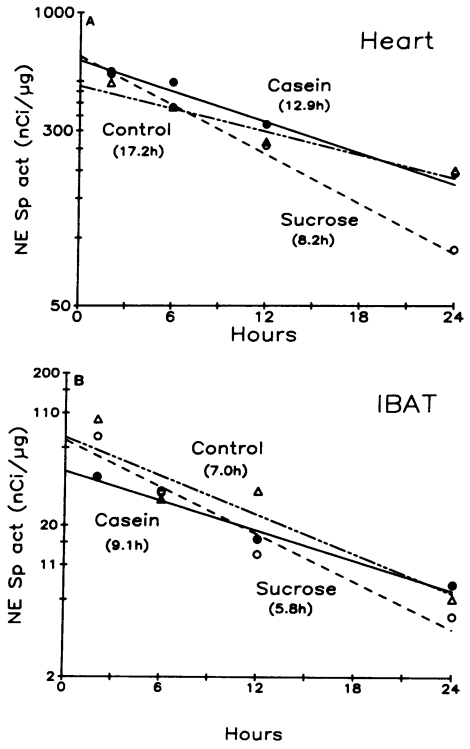

Figure 4. Effects of casein and sucrose on [^{3}H]NE turnover in heart (*A*) and IBAT (*B*). Animals were pair-fed 2:1 mixtures of chow and either casein or sucrose (as in Fig. 1) or chow alone for 5 d before study. At the start of the turnover measurement, all rats received an intravenous injection of [^{3}H]NE (83 µCi/kg) and were killed at various times over the ensuing 24 h. Data from this experiment are presented in Table IV. In the figure, data are plotted as means for specific activity of NE in heart and IBAT from 4 to 6 animals in each group at each time point. Open circles (o) and the broken line (- - -) represent rats given chow + sucrose; closed circles and the solid line represent rats given chow + casein; and open triangles (△) and the broken line (-·-·-) represent rats given chow alone. The numbers in parentheses refer to the half-time of disappearance of tracer ($t_{1/2}$). Statistical significance for each regression line was $P < 0.001$. NE turnover in both heart and IBAT was less in animals fed the casein-containing mixture and in those given chow alone than in those given the sucrose-supplemented formula.

diet more stimulatory of SNS activity than chow alone (as in the experiments listed in Table V), the effect of added casein was examined in animals receiving 2:1 mixtures of chow with either sucrose or lard. The casein-supplemented formula was 2:1:1 (chow/sucrose-lard/casein), representing a 33% increase in energy intake in the animals given the casein-containing mixture. Energy intake in the groups fed the noncasein supplemented mixtures was ~33 kcal/100 g per d in these two studies. The data from both experiments are presented in Table VI. Addition of casein energy did not alter cardiac [^{3}H]NE turnover in either study.

Discussion

These experiments thus demonstrate that the effect of casein on sympathetic nervous system activity, as measured by [^{3}H]NE turnover, differs markedly from that of sucrose or lard. Comparative studies repeatedly documented lower rates of NE turnover in heart and IBAT in animals receiving casein than in those given isoenergetic supplements of either sucrose or lard. In addition, the nature of the SNS response to added casein in the diet was specifically examined. The results of these experiments (Tables V and VI) indicated that casein ingestion did not appreciably affect SNS activity.

These findings are important in several respects. First, the results reported here add to the accumulating evidence that SNS responses to nutrient ingestion are specific for each nutrient group. In various experiments (Tables I–IV and Figs. 1–4), the effect of protein clearly differed from that of either carbohydrate or fat. Data reported elsewhere indicated that sympathetic stimulation induced by sucrose or lard occurs by mechanisms unrelated to protein ingestion and sensitive to specific treatments that block intestinal absorption of these nutrients (4, 14). Diet-induced changes in SNS activity may thus reflect the sum of sympathetic reactions to the individual nutrients ingested.

Second, these data do not support the notion that protein intake inhibits SNS activity. When the impact of increasing casein intake on [^{3}H]NE turnover was examined (Figs. 4 and 5, Tables IV–VI), no single experiment and no pattern from replicate measurements (Table V) demonstrated a significant reduction of NE turnover by added casein in either heart or IBAT. Although caution is required in the interpretation of statistically nonsignificant results from [^{3}H]NE turnover experiments, since theoretical considerations suggest that this method may underestimate changes in SNS activity (15), the lack of suppression

Table V. Effect of Casein Added to Chow on [^{3}H]NE Turnover in Heart and IBAT

Diet	Heart			IBAT		
	Fractional NE turnover	Endogenous NE	Calculated NE turnover rate	Fractional NE turnover	Endogenous NE	Calculated NE turnover rate
n	*%/h*	*ng*	*ng/h*	*%/h*	*ng*	*ng/h*
Experiment 1						
Chow alone (20)	3.8	478	18.2	—	—	—
SEM	0.6	21				
Chow + casein (21)	4.3	460	19.8	—	—	—
SEM	0.7	21				
Pooled SE	0.7	21				
F ratio	0.4	0.4				
P	NS	NS				
Experiment 2 (extracted from Table IV)						
Chow alone (20)	4.0	542	21.7	9.9	248	24.6
SEM	0.7	26		1.6	9	
Chow + casein (20)	5.4	501	27.0	7.6	262	19.9
SEM	0.8	19		0.9	10	
Pooled SE	0.8	23		1.3	10	
F ratio	1.5	1.6		1.6	1.1	
P	NS	NS		NS	NS	
Experiment 3						
Chow alone (20)	5.0	556	27.8	9.2	280	25.8
SEM	0.6	27		1.4	16	
Chow + casein (22)	5.7	530	30.2	5.7	260	14.8
SEM	0.5	14		1.1	14	
Pooled SE	0.6	21		1.3	15	
F ratio	0.8	0.7		3.86	0.8	
P	NS	NS		<0.06	NS	
Experiment 4						
Chow alone (20)	3.9	491	19.1	5.1	323	16.5
SEM	0.6	19		1.5	13	
Chow + casein (22)	5.1	464	23.7	5.2	326	17.0
SEM	0.7	17		0.8	11	
Pooled SE	0.7	18		1.1	12	
F ratio	1.7	1.1		0.01	0.02	
P	NS	NS		NS	NS	
Experiment 5						
Chow alone (20)	4.6	500	23.0	6.4	332	21.2
SEM	0.6	17		1.0	12	
Chow + casein (22)	6.7	467	31.3	6.6	337	22.2
SEM	0.8	22		0.9	10	
Pooled SE	0.7	20		1.0	11	
F ratio	4.19	1.4		0.02	0.1	
P	<0.05	NS		NS	NS	
Experiment 6						
Chow alone (20)	5.7	474	27.0	5.0	269	13.4
SEM	0.8	18		0.8	11	
Chow + casein (22)	5.2	494	25.7	6.1	289	17.6
SEM	0.5	15		1.2	11	
Pooled SE	0.7	16		1.1	11	
F ratio	0.3	0.7		0.5	1.6	
P	NS	NS		NS	NS	

The data in this table are from six separate experiments presented in the chronological order in which they were performed. Animals were fed either a 2:1 mixture of chow and casein or the same amount of chow without casein for 5 d before and during the measurement of NE turnover. Exp. 2 is also presented in Table IV and Fig. 4, and in exp. 4 in Fig. 5.

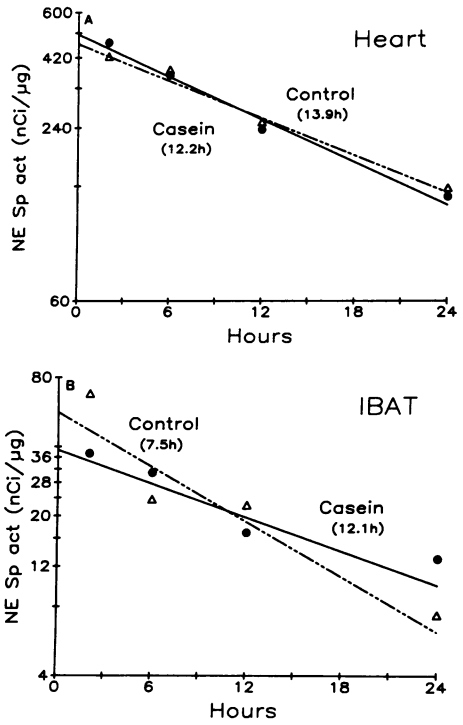

Figure 5. Effects of casein on [^{3}H]NE turnover in heart (*A*) and IBAT (*B*). Animals were fed either a 2:1 mixture of chow and casein or chow alone for 5 d before study. At the start of the turnover measurement, all rats received an intravenous injection of [^{3}H]NE (100 μCi/kg) and were killed at various times over the ensuing 24 h. Data from this experiment are presented in Table V. In the figure, data are plotted as means for specific activity of NE in heart and IBAT from 4 to 6 animals in each group at each time point. Closed circles (●) and the solid line (——) represent rats given chow + casein; open triangles (△) and the broken line (-··) represent animals given chow alone. The numbers in parentheses refer to the half-time of disappearance of tracer ($t_{1/2}$). Statistical significance for each regression line was $P < 0.001$. Though not statistically significant, NE turnover in heart of casein-fed rats was slightly greater than in those fed only chow, while in IBAT, NE turnover was slightly less in the casein-fed animals.

Table VI. Effect of Casein Added to 2:1 Chow-nutrient Mixture on [^{3}H]NE Turnover in Heart

Supplement	Body weight	Tissue weight	Fractional NE turnover	Endogenous NE	Calculated NE turnover rate
	g	*g*	*%/h*	*ng*	*ng/h*
Sucrose					
Sucrose (20)	138	0.448	7.6	661	50.2
SEM	2.4	0.009	1.0	32	
Sucrose + casein (21)	153	0.531	6.0	679	40.7
SEM	4.3	0.018	0.4	17	
Pooled SE	3.5	0.014	0.7	25	
F ratio	8.8	17.1	2.4	0.2	
P	<0.01	<0.001	NS	NS	
Lard					
Lard (20)	137	0.465	8.6	400	34.4
SEM	2.1	0.008	0.6	23	
Lard + casein (21)	144	0.501	9.8	381	37.3
SEM	1.7	0.007	1.0	18	
Pooled SE	1.9	0.007	0.8	21	
F ratio	7.0	12.2	1.0	0.5	
P	<0.02	<0.001	NS	NS	

The data in this table are from two separate experiments. Animals were fed either a 2:1 mixture of chow and sucrose/lard or a 2:1:1 mixture of chow, sucrose/lard, and casein for 5 d before and during measurement of NE turnover.

by casein probably does not represent a false negative inference, since the only effect of casein to approach statistical significance in repeated trials was the small stimulatory response in heart shown in Table V. The major implication of this minimal SNS response to casein for the previous studies of protein restriction is that the principal dietary change leading to SNS stimulation is not limitation of protein, but rather increased provision of carbohydrate or fat, known SNS stimulants as demonstrated in the current report (Fig. 4 and Table IV) and elsewhere (4).

Our contention that protein does not suppress SNS activity may be controversial. If one were to assume that SNS activation by sucrose or lard supplementation represented an SNS response to caloric intake per se, then one might view the lack of SNS reaction to the ingestion of casein as evidence of protein inhibition of the SNS response to increased caloric intake. This caloric hypothesis for dietary regulation of SNS activity, however, leaves open the question of how the brain regions that mediate diet-induced changes in the SNS perceive the increase in caloric intake. Since stimulation of the SNS by administration of glucose and insulin in combination is presumably analogous to the effect of carbohydrate feeding (16), the link between SNS function and the intake of various nutrients seems likely to involve feeding-related changes in hormone secretion and in the flux of substrates, individual fatty acids and/or amino acids. If so, then the SNS response to an alteration in nutrient intake may ultimately depend upon the constituents of the various nutrients rather than upon their caloric value. Since present information is insufficient in this regard, resolution of this question must await the results of future studies.

Third, the data do not support the hypothesis that dietary tyrosine regulates sympathetic responses to dietary manipulation (7). Adding casein to the basic lab chow ration increased tyrosine intake from ~48 mg/100 g body weight per d to nearly 210, yet this protein supplement affected SNS function minimally. When coupled with the previous observation that addition of tyrosine alone failed to slow the acceleration in NE turnover associated with protein restriction (6), the present findings indicate that the precursor hypothesis simply does not apply to dietary regulation of the SNS. Whether tyrosine plays a role in regulating responses of other catecholamine systems, such as the adrenal medulla or the renal dopaminergic system (6, 17), to dietary manipulation, remains to be determined.

The finding that protein is less potent a sympathetic nervous system stimulant than either carbohydrate or fat has at least one potential implication for mammalian physiology. Alterations in diet, both in the level of energy intake and in the composition of ingested foodstuffs, affect metabolic rate (so-called diet-induced thermogenesis). Since similar dietary manipulations influence sympathetic function, it has been proposed that dietary alterations in sympathetic activity (especially brown adipose tissue of small mammals) contribute to the regulation of diet-induced thermogenesis, an hypothesis for which there is increasing

experimental support (reviewed in reference 18). The absence of sympathetic stimulation in IBAT by casein implies that protein feeding may activate the sympathetic component of diet-induced thermogenesis less than isoenergetic amounts of either carbohydrate or fat. If so, weight gain in animals or man may be positively related to the protein content of the diet, a potential benefit for animals on a low protein diet in that consumption of a greater quantity of protein-poor food would lead to less gain in body fat. Evidence in support of this notion was reported previously (19), and in the current studies, higher body weights were noted with casein feeding in seven of eight experiments in which the experimental groups were fed isoenergetic amounts of casein-chow or either sucrose-chow or lard-chow. While these data are consistent with (but do not establish) a positive connection between protein intake and weight gain, they do underscore the need for further investigation in this area.

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