Reduced feeding response to neuropeptide Y in senescent Fischer 344 rats

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1Department of Nutrition, Section of Neurobiology, Physiology, and Behavior, 2Division of Biological Sciences, Veterinary Medicine: Anatomy, Physiology, and Cell Biology, and 3Food Intake Laboratory, University of California, Davis, California 95616

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Blanton, Cynthia A., Barbara A. Horwitz, James E. Blevins, Jock S. Hamilton, Eduardo J. Hernandez, and Roger B. McDonald. Reduced feeding response to neuropeptide Y in senescent Fischer 344 rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R1052–R1060, 2001.—The anorexia of aging syndrome in humans is characterized by spontaneous body weight loss reflecting diminished food intake. We reported previously that old rats undergoing a similar phenomenon of progressive weight loss (i.e., senescent rats) also display altered feeding behavior, including reduced meal size and duration. Here, we tested the hypothesis that blunted responsiveness to neuropeptide Y (NPY), a feeding stimulant, occurs concurrently with senescent-associated anorexia/hypophagia. Young (8 mo old, n = 9) and old (24–30 mo old, n = 11) male Fischer 344 rats received intracerebroventricular NPY or artificial cerebrospinal fluid injections. In response to a maximum effective NPY dose (10 μg), the net increase in size of the first meal after injection was similar in old weight-stable (presenescent) and young rats (10.85 ± 1.73 and 12.63 ± 2.52 g/kg body wt0.67, respectively). In contrast, senescent rats that had spontaneously lost ~10% of body weight had significantly lower net increases at their first post-NPY meal (1.33 ± 0.33 g/kg body wt0.67) than before they began losing weight. Thus altered feeding responses to NPY occur in aging rats concomitantly with spontaneous decrements in food intake and body weight near the end of life.

NEUROPEPTIDE Y (NPY) is recognized as an important component of the energy homeostasis system. Because of its powerful effects to initiate and sustain eating (5), NPY has been investigated to elucidate normal and abnormal regulation of food intake and body weight. One example of altered energy regulation is the “anorexia of aging,” a syndrome seen in the elderly and characterized by spontaneous decrease in food intake and body weight. Efforts to identify the etiology of age-related anorexia/hypophagia include studies testing NPY function in aged animals. For example, Pich et al. (15) showed that old (24 mo old) rats responded less robustly to the feeding-stimulatory action of intrahypothalamic NPY injection than did young (3 mo old) rats. Gruenewald et al. (7) demonstrated lower fasting-induced increases in hypothalamic arcuate nucleus NPY mRNA with age in 3-, 12-, and 24-mo-old rats. Additionally, the old rats were slower to recover food intake and body weight after refeeding than the young animals. From these results, a causal relationship was suggested between reductions in activation of the NPY pathway and decreased feeding during aging.

We previously studied the feeding behavior of an animal model for age-related anorexia/hypophagia that was characterized by transition to a physiological state in which there was a rapid spontaneous decline in food intake and body weight (2). To this end, we monitored food intake continuously in 24-mo-old male Fischer 344 (F344) rats as they passed from their period of weight stability (presenescence) into spontaneous rapid weight loss (senescence). Regardless of age at which senescence began, all rats ate significantly smaller meals of shorter duration during their early stages of senescence than during presenescence. These findings, combined with the fact that meal frequency did not differ between presenescence and senescence, clearly demonstrated altered feeding behavior as one cause of age-related anorexia. The consumption of smaller, smaller meals, despite progressively declining body weight (which averaged 5% at the end of the experiment), implicated dysfunction in short- and long-term regulation of energy balance. Moreover, the senescent male F344 rat exhibits significant functional deterioration in a number of systems regulated by the hypothalamus, e.g., food intake, body weight, temperature homeostasis, and circadian rhythmicity of body temperature (2, 11, 12). These findings, together with data suggesting that paraventricular hypothalamic concentrations of NPY do not differ significantly between presenescent and senescent animals, led us to postulate that attenuated hypothalamic NPY responsiveness may contribute to the altered feeding behavior of the senescent rat.

The specific aim of this investigation was to test the hypothesis that the decline in food intake observed in

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senescent male F344 rats near the end of their lives is accompanied by a significant reduction in their feeding response to NPY. To test this hypothesis, NPY was injected into the lateral ventricle in old (24 mo old) rats before and after the onset of senescence. Feeding behavior was monitored continuously for 24 h after injections, and comparisons were made between the presenescent and senescent periods. In addition, we measured serum levels of leptin and corticosterone, hormones influencing food intake and body weight (4, 14).

MATERIALS AND METHODS

Animals and animal care. Male F344 rats aged 24 mo (n = 11) and 8 mo (n = 9) were obtained from the National Institute on Aging colony maintained by Harlan Sprague Dawley Laboratory (Indianapolis, IN). Animals were housed individually and maintained at 25–26°C and 50% humidity on a reverse 12:12-h light-dark cycle (lights on at 1900, lights off at 0700). Rats were provided with ground NIH-31 laboratory chow (Teklad Research Diets, Indianapolis, IN) and distilled ad libitum, except when indicated otherwise.

Experimental protocol. On arrival at our facility, rats were placed in polycarbonate metabolic cages designed for automated food intake measurement, as previously described (2). Briefly, digital scale measurements of the food cup and food spillage were transmitted to a computer every 15 s, and changes of 0.1 g were time stamped and recorded onto a spreadsheet (Microsoft Excel) using SoftwareWedge (T. A. L. Technologies, Philadelphia, PA). Food and water were replenished, cage bedding was changed, and rats were weighed and examined daily between 0800 and 1000 under low-intensity red light (15–20 lux).

After 7 days of environmental acclimation to our facility, when food intake and body weight had stabilized, each rat was anesthetized intraperitoneally with a mixture of ketamine hydrochloride (60–80 mg/kg) and xylazine (7.5–10 mg/kg), each separated by a 48-h period. Physiological positioning of the cannula was initially verified to be properly cannulated and were subsequently given NPY. Solutions were injected in volumes of 5 μl over 5 min via a microliter injector pump (Bioanalytical Systems, West Lafayette, IN) using a 10-μl Hamilton syringe attached to a 33-gauge stainless steel injection needle that extended 1 mm beyond the guide cannula tip. The needle was left in place for 60 s after injection to allow for diffusion of remaining solution into the lateral ventricle.

The protocol for injections is displayed in Table 1. Young (8-mo-old) and old (24 to 28 mo old) rats received all injections ad libitum-fed (AL) and food-restricted (FR; see below) conditions. In addition, injections were administered to old rats during their period of terminal spontaneous rapid weight loss (i.e., during senescence). Senescent rats received as many injections as possible before death was imminent, and the animal was euthanized. The 8-μg dose of NPY was administered first in old animals to ensure that they received an NPY concentration normally able to elicit a marked feeding response. In pilot studies using young and old rats, we found the 8-μg dose of NPY to reliably stimulate food intake. For each old rat, the randomized order of injection used during its presenescent AL state was the same as that used during its presenescent FR and senescent states. The possible effect of NPY injection order on feeding response was tested in young presenescent AL animals by dividing them into two groups and administering NPY in increasing concentrations or in a randomized order; no significant difference between the two types of sequences was found (data not shown).

Food and water were removed 1 h before all injections. Rats were wrapped in cotton mesh, and injections were administered between 0800 and 1000 under low-intensity red light (i.e., during their night period). The animals were returned to their cages immediately after testing and observed for onset of eating and drinking. Water intake was measured every 5 min for a duration of 20 min after injection. Time 0 was defined as the time when the injection needle was removed from the guide cannula.

After young FR rats and senescent rats were tested, they were anesthetized with halothane, and 5 μl of India ink were injected via the guide cannula. Rats were killed by cardiac puncture, and blood was collected in untreated tubes. Sera were frozen at −70°C until analysis. Brains were removed, frozen, and sliced in 100-μm coronal sections by means of a cryostat. The brain sections were mounted onto slides, stained with cresyl violet, and examined microscopically to verify cannula placement.
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NEUROPEPTIDE Y-INDUCED FEEDING IN SENESCENT RATS

Table 1. Injection protocol for old and young rats

<table>
<thead>
<tr>
<th>Order of injections</th>
<th>Old Presenescent</th>
<th>Senescent</th>
<th>Young Presenescent</th>
<th>Food restricted (divided into 2 groups: n = 3, n = 6)</th>
<th>Food restricted (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum fed</td>
<td>(n = 11)</td>
<td>(n = 11)</td>
<td>Ad libitum fed</td>
<td>(n = 9)</td>
<td></td>
</tr>
<tr>
<td>NPY (8 μg)</td>
<td>Randomized: aCSF, ANG II, NPY (4 μg), NPY (10 μg), NPY (12 μg)</td>
<td>NPY (8 μg)</td>
<td>Randomized: ANG II, NPY (2 μg), NPY (4 μg), NPY (10 μg), NPY (12 μg)</td>
<td>NPY (2 μg)</td>
<td>Randomized: aCSF, ANG II, NPY (2 μg), NPY (4 μg), NPY (8 μg), NPY (10 μg), NPY (12 μg)</td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>

Old rats were 24–30 mo old; young rats were 8 mo old. NPY, neuropeptide Y. Artificial cerebrospinal fluid (aCSF) contained (in mM) 128 NaCl, 2.5 KCl, 2.5 CaCl₂, 1.0 MgCl₂, and 1.2 Na₂HPO₄, pH 7.4. Dose of ANG II was 150 ng.

The serum samples from the 8-mo-old rats were destroyed in a freezer malfunction. Thus blood was collected in the manner described above from separate groups of nonsurgically treated presenescent AL older (28 ± 1 mo, n = 6) and younger (7 mo, n = 6) rats. Animals were handled daily and housed under conditions identical to those described for the NPY-injected rats. Sera were used in leptin and corticosterone analyses.

Food restriction. To determine the effect of weight loss per se on NPY-induced feeding, presenescent rats were tested with NPY after weight loss due to food restriction. Rats were given a daily amount of food equal to two-thirds of their ad libitum consumption until a weight loss of ~10% of their prerestricion weight was observed (6–7 days). The 24-h allotment of food was divided into four equal parts that were sequentially dispensed into the food cup at 6-h intervals by an adapted aquarium feeder set to a timer (Rainbow Lifegard Aquarium Products, El Monte, CA). The purpose of distributing the food four times over 24 h was to minimize the possible effects on endogenous levels of NPY caused by rats eating their food at one time (19). Seventy minutes before testing, rats were allowed access to 0.5 g of food for 10 min to simulate the active feeding status that rats displayed at this time during ad libitum feeding. Sixty minutes before injection, all food and water were removed. The injection protocol was the same as that for AL experiments, except animals were food restricted during the 24- to 48-h period after each injection.

Food intake analysis. Feeding responses were analyzed using data collected during the 24-h postinjection period. Feeding variables included size and duration of the first meal after injection, latency to feed, and meal frequency. Meals were defined as the consumption of ≥0.2 g within 15 min, separated from other periods of feeding by ≥15 min. Quantitative feeding variables are presented as body mass-independent values (g intake/kg0.67) (8) to account for differences in body weight among the animal groups. The patterns of feeding responses among and within animal groups were similar for the different NPY doses. For ease of presentation, feeding measures in Table 2 and Figs. 2 and 3 are displayed only for the maximally effective dose of NPY (10 μg).

Serum analysis. Serum corticosterone was measured by RIA using a diagnostic kit from ICN Pharmaceuticals (Orangeburg, NY). Serum leptin was measured by RIA using a diagnostic kit from Linco Research (St. Charles, MO).

Statistical analysis. ANOVA and post hoc Fisher’s protected least significant difference test were used to evaluate the effects of age (young, old presenescent, and old senescent), feeding state (AL and FR), and treatment (aCSF and NPY) on feeding variables. Hourly food intake measurements were analyzed by repeated-measures ANOVA followed by Bonferroni/Dunn post hoc tests. Differences were considered significant at P ≤ 0.05. Values are means ±SE.

RESULTS

Body weight. Average preoperative body weights for the old and young rats were 409.8 ± 8.7 and 375.5 ± 0.4 g, respectively (P < 0.0001). Postoperative body weight loss averaged 11.3 ± 0.8% (old rats) and 11.1 ± 0.3% (young rats) and did not differ significantly. Neither group returned to preoperative weights. In the old senescent rats, mean body weight loss was 9.8 ± 1.1% at the first injection (8 μg of NPY) and 18.0 ± 1.5% at the final injection. The age at which spontaneous rapid weight loss began in the senescent rats varied from 809 to 917 days, and average duration of senescence before euthanasia was 19 ± 2 days. Temporal patterns of body weight and food intake for three representative old rats (of a total of 11) are presented in Fig. 1. Although there was variation in the shape of the curves for each animal, all exhibited simultaneous declines in food intake and body weight. These spontaneous losses occurred in senescent rats regardless of age (note differences in the experimental day at which the decline occurred in Fig. 1).

Food intake analysis. Postoperative daily food intake before injections was significantly greater in old presenescent than in young rats: 27.91 ± 0.31 vs. 23.50 ± 0.44 g·day⁻¹·kg⁻₀.₆₇. Twenty-four-hour food intake was significantly reduced on aCSF injection days compared with prior injection days in the old presenescent and senescent, but not young, rats: 17.50 ± 1.1% vs. 21.05 ± 2.85, and 23.50 ± 2.85 vs. 23.50 ± 2.85, respectively. Comparisons of daily intakes between aCSF injection days and interinjection days (the 24-h periods separating injection treatments) showed no differences within animal groups (P = 0.24).

Response to NPY in young and old presenescent and senescent rats. As shown in Fig. 2, the ability of NPY to stimulate food intake at the first meal after injection in
Table 2. Feeding patterns of old and young rats during ad libitum feeding and food restriction

<table>
<thead>
<tr>
<th>Feeding Variable</th>
<th>Presenescence Ad Libitum</th>
<th>Presenescence Food Restricted</th>
<th>Senescent Ad Libitum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aCSF NPY 10</td>
<td>aCSF NPY 10</td>
<td>aCSF NPY 10</td>
</tr>
<tr>
<td>1st meal intake, g/kg(^{0.67})</td>
<td>Old</td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td>1.26 ± 0.19(^a)</td>
<td>12.11 ± 2.22(^b)</td>
<td>12.52 ± 2.12(^b)</td>
<td>19.93 ± 3.09(^c)</td>
</tr>
<tr>
<td>3.32 ± 0.73(^a)</td>
<td>15.94 ± 3.06(^b)</td>
<td>12.63 ± 2.13(^b)</td>
<td>21.62 ± 2.28(^e)</td>
</tr>
<tr>
<td>24-h intake, g/kg(^{0.67})</td>
<td>Old</td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td>17.90 ± 2.87(^a)</td>
<td>25.51 ± 2.04(^b)</td>
<td>39.64 ± 3.85(^c)</td>
<td>43.78 ± 3.38(^d)</td>
</tr>
<tr>
<td>20.99 ± 4.08(^a)</td>
<td>32.40 ± 2.08(^b)</td>
<td>42.05 ± 3.46(^b)</td>
<td>48.32 ± 2.01(^e)</td>
</tr>
<tr>
<td>1st meal duration, min</td>
<td>Old</td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td>12.22 ± 2.60(^a)</td>
<td>100.25 ± 17.21(^b,c)</td>
<td>90.05 ± 14.49(^b)</td>
<td>126.56 ± 11.97(^a)</td>
</tr>
<tr>
<td>23.78 ± 6.45(^a)</td>
<td>115.36 ± 26.78(^b)</td>
<td>79.58 ± 13.65(^c)</td>
<td>126.81 ± 7.83(^b)</td>
</tr>
</tbody>
</table>

Values are means ± SE of feeding variables of old presenescence and senescent (24 to 30 mo old, n = 11) and young (8 mo old, n = 9) male Fischer 344 rats during ad libitum feeding and food restriction. Feeding variables: 1st meal, 1st meal after injection; net change, g increase in size of 1st meal compared with aCSF; values expressed as net change and % of aCSF are 0 and 100%, respectively, for aCSF treatment. Treatments: NPY 10, 10 μg of NPY. a–e Within a row, values sharing letters do not differ significantly. f Values between old and young rats within the same feeding state and treatment are significantly different. g 24-h intake % of aCSF value for NPY 10 for senescent rats is higher than that for other doses; the main effect of NPY treatment on 24-h intake % of aCSF did not differ between ad libitum-fed old presenescence and senescent rats.

Young and old presenescence rats was significantly attenuated after the onset of senescence. Increased duration and net change of the first meal after NPY injection were also blunted in senescent vs. presenescence AL and FR rats (P < 0.0001; Table 2). When size and duration of the first meal after injection were expressed as percentages of aCSF values, senescent and presenescence FR rats showed smaller increases than presenescence AL rats (P < 0.0001). In response to NPY, 24-h food intake increased and latency to feed decreased in senescent and presenescence AL, but not presenescence FR, rats. Although differences were not found among any of the older groups in eating rate (g intake/min), senescent rats consumed more meals during aCSF and NPY treatments than did old presenescence and young groups (P = 0.002).

Repeated-measures analyses of postinjection feeding for each hour (g intake/kg\(^{0.67}\)) demonstrated significant differences among the old rats. Feeding after NPY injection was less in senescent than in presenescence AL rats at hours 1, 2, and 3 (Fig. 3) and less than in presenescence FR rats for hours 1–10. During presenescence, food intake was significantly greater in FR than in AL animals for the first 11 h after NPY injection. A main effect of NPY on feeding (g/kg\(^{0.67}\)) was seen in senescent rats during postinjection hour 1 (P = 0.03) and in presenescence AL and FR rats during hours 1 and 2 (P = 0.0006).

Response to NPY in young and old presenescence rats. A main effect of age was found on baseline (i.e., response to aCSF injection) feeding (g/kg\(^{0.67}\)) at the first postinjection meal that became less pronounced, yet remained significant, after treatment with NPY (Table 2). During ad libitum feeding and food restriction, young rats ate more (g/kg\(^{0.67}\)) than did old presenescence rats at the first meal (P < 0.008) and 24 h (P < 0.001) after injection. Rates of eating were faster in young than in old animals in both feeding states (P < 0.0001). No differences between age groups in NPY-induced feeding responses (calculated relative to aCSF values) were found, except for a greater decrease in latency to feed in young AL rats than in their old counterparts (P < 0.014). A main effect of NPY was seen on most measurements of feeding in young and old presenescence animals during ad libitum and restricted feeding. Repeated-measures analysis revealed that, within both feeding states, old rats ate significantly less than did young rats during postinjection hour 1 (P < 0.0001); after this time, hourly intakes were similar between the age groups.
Water intake. ANG II elicited significant increases compared with aCSF and NPY in 20-min and 24-h postinjection water intake in old presenescent and senescent rats. As shown in Fig. 4, the ability of ANG II to stimulate immediate drinking was significantly different among old rats: 2.4 ± 1.9, 14.5 ± 1.0, and 8.0 ± 2.5 ml in senescent, presenescent AL, and presenescent FR rats, respectively. Differences were also found among old rats in 24-h water intake: 27.66 ± 4.28, 42.83 ± 4.89, and 49.12 ± 7.89 ml in senescent, presenescent AL, and presenescent FR rats, respectively. NPY caused an increase in 24-h postinjection drinking in presenescent AL, but not in senescent or presenescent FR, rats.

In comparisons between young and old presenescent rats, age and feeding state had main effects on water consumption. After injection of ANG II, young and old presenescent rats displayed similar 20-min drinking responses within feeding states. However, for 24-h postinjection measures, water intake was stimulated by ANG II to a greater degree in old presenescent than in young rats (P < 0.0001). Young rats did not demonstrate the main effect of NPY on drinking that was seen in old rats. There was no effect of NPY dose on water consumption in any group.

Serum leptin. As shown in Table 3, senescent rats had significantly lower serum leptin levels than did old presenescent AL rats (P = 0.005). The 72% reduction in
serum leptin was seen in association with an average body weight loss of 18% in senescent rats. No differences were found between the old presenescent and young animals (P = 0.839).

**Serum corticosterone.** Serum corticosterone concentrations did not differ between old presenescent and senescent rats (Table 3). During presenescence, age had a significant effect on corticosterone concentrations; levels were lower in old than in young rats (P = 0.009).

**DISCUSSION**

We report here that, after transition from their weight-stable (presenescent) to rapid-weight-loss (senescent) period, old rats were markedly less sensitive to NPY’s action to stimulate feeding at the first postinjection meal. The increases in first postinjection meal size and duration during senescence were only 25 and 33%, respectively, of their presenescent levels. Differences between presenescent and senescent rats in NPY-stimulated feeding were still apparent at hour 2, i.e., when NPY’s effects are normally most potent (1, 10, 15). In addition to confirming our previous finding of altered feeding behavior of the rapid-weight-loss animals, this investigation supports our hypothesis of an alteration in NPY’s action to acutely induce eating in senescent rats.

The mechanisms underlying the attenuation of the NPY-induced acute feeding response in senescent rats are unknown, and this investigation was not designed to fully elucidate any particular possibility. Given that NPY was injected directly into the brain, it is logical to assume that the defect occurs along the NPY pathway. Many elements are involved in the signaling cascade mediating the final effects of NPY, including NPY-receptor number and affinity, cAMP and calcium sec-

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**Fig. 2.** Food intake at the 1st meal after injection in young (n = 8) and old (24–30 mo, n = 11) presenescent rats during ad libitum feeding, old presenescent rats during food restriction, and senescent rats during ad libitum feeding. Animals were treated with intracerebroventricular injection of artificial cerebrospinal fluid (aCSF) or NPY (2, 4, 8, 10, and 12 μg). * Significantly different from senescent ad libitum fed. ** Significantly different from senescent and presenescent ad libitum fed. ‡ Significantly different from old presenescent ad libitum fed and food restricted and senescent ad libitum fed. ‡‡ Significantly different from old presenescent and senescent ad libitum fed.

**Fig. 3.** Average hourly food intake after intracerebroventricular injection of aCSF and NPY (10 μg) in old (24–30 mo, n = 11) rats during presenescent ad libitum feeding and senescent ad libitum feeding.
of exogenous peptide (15, 22). The possibility that the blunted response to NPY administration in senescent rats is the result of elevated endogenous hypothalamic NPY levels is unlikely in light of our finding that there is no significant difference in paraventricular nucleus NPY content in senescent vs. AL presenescence. Measurements of serum corticosterone showed no difference in levels between old presenescence and senescent rats. Nonetheless, presenescence and senescent rats (that were comparably handled on a daily basis) had significantly different responses to NPY. Thus it is unlikely that alternate leptin or corticosterone levels can account for the blunted NPY-induced feeding.

Young animals had higher serum corticosterone values than did old presenescence rats. These results differ from those of Sapolsky (20), who showed age-related increases in plasma corticosterone in rats. However, disagreement exists in the literature on glucocorticoids and aging (see Ref. 20 for review). In most investigations, as in ours, measurements were made at only one time of day and thus do not assess the effect of age on the diurnal pattern of corticosterone. Also, differences in sampling procedures and species contribute to the lack of consensus among reports.

Table 3. Serum concentrations of leptin and corticosterone in senescent, old presenescence, and young rats

<table>
<thead>
<tr>
<th></th>
<th>Senescent (n = 11)</th>
<th>Old Presenescence (n = 6)</th>
<th>Young Presenescence (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin, ng/ml</td>
<td>1.79 ± 0.54*</td>
<td>6.45 ± 2.12†</td>
<td>6.10 ± 0.44†</td>
</tr>
<tr>
<td>Corticosterone, ng/ml</td>
<td>206.54 ± 50.26*</td>
<td>181.28 ± 63.81*</td>
<td>423.00 ± 31.02†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. Senescent rats were 26–30 mo old and exhibited spontaneous losses in food intake and body weight; old presenescence rats were 27–29 mo old and maintained stable body weight; young rats were 7 mo old. All rats were fed ad libitum. Within a row, means sharing a common symbol (*) and † are not significantly different.

In reaching our conclusions, we considered other factors that might contribute to the reduced NPY-induced acute feeding response observed in senescent rats. Among these are altered levels of endogenous NPY, serum hormone abnormalities, and incomplete delivery of injected NPY. Endogenous levels of hypothalamic NPY are relatively low in AL rats, and NPY administration elicits a strong feeding response in these animals (18, 19). In contrast, food restriction causes endogenous hypothalamic NPY levels to rise and saturate binding sites, thereby blunting the effects
A third potential reason for reduced responsiveness to exogenous NPY in senescent animals is incomplete delivery of NPY due to cannula obstruction, resulting primarily from cell adhesion at the base of the guide cannula, especially in the old rats, in which the cannula was in place for up to 6 mo. However, several lines of evidence argue against such cannula obstruction: 1) on the basis of previous reports alerting us to this possibility (9), we designed our injector needles to extend 1 mm beyond the tip of the permanent guide cannula, thus ensuring penetration of any ependymal growth and entry into the ventricle. 2) Results presented here for old presenescent and young rats, as well as data from pilot studies, show that injection of ANG II elicited a strong drinking response, even after repeated (up to 13) treatment injections or in animals with the cannula in place for 160 days (see MATERIALS AND METHODS). 3) Histology showed extensive ventricular diffusion of ink injected at the time of eu- thanasiasis induction (11), and other surgical procedures lasting longer than those used here (13) did not result in permanent postoperative losses in body weight. Thus the procedures associated with brain cannulation, rather than abdominal surgery and/or the effects of anesthesia, may be the cause of this failure of the young and old rats to recover their body weight. Moreover, FR rats, which were forced to lose ~10% of their postoperative body weight, returned to their postoperative, rather than preoperative, weight after refeding; this is consistent with the establishment of a new energy set point. The basis for this does not appear to be due to age and warrants further investigation.

In conclusion, we find that the decline in food intake in senescent rats is associated with a significantly reduced response to intracerebroventricular injection of NPY. The suppressed stimulation of first postinjection meal size and duration in senescent rats is consistent with impairment in NPY’s normal action to increase food intake. These data provide strong evidence implicating altered NPY responsiveness in the mechanism(s) underlying senescence-associated anorexia.

**Perspectives**

Our previous investigations describe a rapid, spontaneous, and apparently concurrent decline in food intake (2, 11), body weight (2, 13), cold-induced thermoregulation (11), and circadian rhythm of body temperature (12) in senescent vs. presenescent rats. We now add attenuated hypothalamic NPY-induced feeding and ANG II-induced drinking to the list of dysfunctions occurring in the senescent rat. From our perspective, the fact that regulation of all these functions is centered more or less within the hypothalamus is intriguing. More important, however, is the diversity of hypothalamic anatomic locations, physiological/biochemical pathways, and peripheral feedback loops associated with these various alterations. For example, endogenous circadian rhythm of body temperature is mediated by the supraopticchiasmatic nucleus with virtually no peripheral feedback input. Conversely, NPY-stimulated food intake and ANG II-induced drinking involve the paraventricular and supraoptic nuclei, respectively, and several peripheral feedback mechanisms. These observations of senescence-related hypothalamic dysregulation are increasingly supportive of the concept that multiple mechanisms and/or the interaction among the mechanisms contribute to dysregulation of the senescent hypothalamus. We suggest that investigators consider this in their approach to hypothesis development and testing involving aged animals.
We thank Kristina Mirizzi, Rachael Raspino, Sheila Shaﬁee, Tamara Parker, Jessica Job, and Tyler Pierce for technical assistance and insightful comments.

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