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Reduced feeding response to muscimol and neuropeptide Y in senescent F344 rats

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Coppola, Jessica D., Barbara A. Horwitz, Jock Hamilton, James E. Blevins, and Roger B. McDonald. Reduced feeding response to muscimol and neuropeptide Y in senescent F344 rats. Am J Physiol Regul Integr Comp Physiol 288: R1492–R1498, 2005. First published February 24, 2005; doi:10.1152/ajpregu.00554.2004.—Many mammals experience spontaneous declines in their food intake and body weight near the end of life, a stage we refer to as senescence. We have previously demonstrated that senescent rats have blunted food intake responses to intracerebroventricular injections of neuropeptide Y (NPY). In the present study, we tested the hypothesis that responsiveness to GABA, a putative potentiator of NPY’s effect, is also diminished. Young and old male F344 rats received injections of NPY, muscimol, (MUS, a GABA-A receptor agonist), combinations of these two agents, and vehicle [artificial cerebrospinal fluid (aCSF)] into the hypothalamic paraventricular nucleus (PVN). Both young and old presenescent rats increased their food intake in response to NPY, MUS, and the combination of the two (in comparison to injections of aCSF). The combination treatment was generally more effective than either NPY or MUS alone. These data are consistent with suggestions that both NPY and GABA play a role in the regulation of feeding behavior. Senescent rats exhibited an attenuated NPY-induced food intake, no increase in response to MUS, and a response to NPY + MUS that was no larger than that of NPY alone. We conclude that PVN injections of GABA, as well as NPY, are less effective in stimulating feeding in senescent rats and suggest that alterations in their signaling pathways play a role in the involuntary feeding decrease seen near the end of life.

WE HAVE PREVIOUSLY SHOWN that old rats undergo rapid and spontaneous declines in food intake and body weight near the end of their lives (3, 4, 11). Reductions of food intake and body weight are two of several biochemical/physiological functional changes in these old rats. The combined alterations are part of a distinct stage of life that we, and others, define as senescent because the animals are no longer thriving, but rather, are progressing rapidly toward death (9, 16, 21). The factors underlying entry into senescence or the declines in physiological function have yet to be elucidated. In a series of previous studies, we focused on mechanisms that may be responsible for the inability of senescent animals to maintain their body weight. We found that the body weight loss of senescent male F344 rats involves a reduction in food intake (4), that this reduction is accompanied by diminished responsiveness to intracerebroventricular injections of neuropeptide Y (NPY), a strong stimulator of food intake (3), and that neither the expression of NPY Y1 nor Y5 receptors in the hypothalamic paraventricular nucleus (PVN), a major site of NPY stimulation of feeding, nor the number of PVN neurons containing Y1 protein differ in senescent vs. presenescent rats (7). One possible explanation for the blunted responsiveness of the senescent rats to NPY, despite no apparent reduction in receptors, is altered modulation of the NPY action by other neuropeptides and/or neurotransmitters. For example, GABA is colocalized with NPY (1, 6, 13), and it has been suggested that under some circumstances, they may be released together (15, 17). Previous investigations have shown that GABA and GABA agonists were only mild stimulants of food intake in the absence of NPY; however, administration of the GABA-A receptor agonist muscimol (MUS) with NPY yielded a more robust food intake response than either NPY or MUS alone when injected into the striatum (18) or PVN (25). It is possible, therefore, that the attenuated food intake response of senescent rats involves altered GABA, as well as altered NPY responsiveness.

In this investigation, we have begun to explore this possibility by testing the hypothesis that senescent rats do not increase their food intake as robustly as do presenescent animals when stimulated with a GABA-A agonist. For this, we measured food intake after injecting MUS, NPY, or a combination of NPY plus MUS into the PVN of young, old presenescent, and senescent male Fischer 344 (F344) rats. Our results confirm our previous findings that the NPY-induced increase in feeding in the senescent rats is significantly blunted in the senescent vs. old presenescent animals. They also show that although MUS enhanced feeding in the young and old presenescent rats, it did not do so in the senescent animals, and unlike the case in the young and old presenescent rats, the effect of MUS plus NPY injection was not greater than that of NPY itself. Thus senescence is accompanied by attenuated responsiveness to GABA, as well as to NPY, with respect to feeding behavior.

MATERIALS AND METHODS

Animals and animal care. Male F344 rats, 6 and 23 mo of age were obtained from the National Institute on Aging colony maintained by
Harlan Sprague-Dawley Laboratory (Indianapolis IN). On arrival, rats were housed individually in hanging wire-bottom cages (20 × 25 × 18 cm) and maintained at 25–26°C and 50% humidity on a reversed 12:12-h light-dark cycle (lights off at 0700, on at 1900). Rats were provided with NIH-31 laboratory chow (Teklad Research Diets, Indianapolis, IN) and distilled water ad libitum. All rats were maintained in our facility for a minimum of 2 wk before experimentation to acclimate them to the colony conditions (light cycle, food). At the time that the rats were killed, they were visually inspected for evidence of unexpected gross pathology (i.e., pathologies other than what would be expected to be seen in old F344 rats). Two sentinel rats, housed in the same facility, were also tested at two-mo intervals for specific organ disease and serological abnormalities. All tests were negative, and, therefore, no rats were excluded from the experiment.

All procedures were performed in accordance with the American Physiological Society’s guidelines for research involving animals and were approved by the University of California, Davis, Animal Care and Use Committee.

A subgroup of the young (7 mo of age; n = 8) and old presenescent (26 mo; n = 8) rats used in these experiments were fed -70% of their ad libitum food intake, or about 12 g per day, until they lost at least 10% of their body weight (between 10 and 15% over 7 to 10 days), to evaluate the effect of weight loss per se on GABA/NPY-induced food intake.

Experimental protocol. After the 2-wk acclimation period, rats were placed in polycarbonate metabolic cages designed for automated food intake measurement, as previously described (4). 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 on a spreadsheet (Microsoft Excel) using Software-Wedge (T.A.L. Technologies, Philadelphia, PA). Food and water were replenished, cage bedding was changed, and rats were weighed and recorded on a spreadsheet (Microsoft Excel) using Software-Wedge (T.A.L. Technologies, Philadelphia, PA). Food and water were replenished, cage bedding was changed, and rats were weighed and recorded on a spreadsheet (Microsoft Excel) using Software-Wedge (T.A.L. Technologies, Philadelphia, PA).

Fig. 1. Changes in body weight during transition from presenescence to senescence for two representative rats. A: The most common pattern of weight loss observed in Fischer 344 (F344) rats upon entry into senescence, -60%. B: The other commonly observed pattern of weight loss observed in F344 rats upon entry into senescence, -40%. Final measurements were taken before death.

Senescence in old rats was determined as described previously (3, 22). Briefly, presenescen rats appearing to be in a period of rapid and spontaneous weight loss over three consecutive days were determined to have entered senescence. The senescence period was confirmed by plotting the body weights throughout the experiment and having two individuals unfamiliar with the senescent state select a point at which they believed there had been a rapid change in the slope for body weight corresponding to the senescent state (see Fig. 1, A and B). The senescent-period injections were initiated only after the two independent reviewers confirmed the rapid and spontaneous decline in body weight.

Surgical procedures. After 7 days acclimation to the metabolic cages, 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) and stereotactically implanted with a stainless steel guide cannula (26 gauge; Small Parts, Miami Lakes, FL) targeted at the PVN. The order of PVN injections for young and old presenescent ad libitum (AL) and food-restricted (FR) rats was randomly selected for each animal.

Previous results (3) suggested that some of the senescent animals would not live long enough during their terminal weight loss phase to receive all nine injections. Therefore, the injection schedule for the senescent animals was predetermined so that the first injection was the dose eliciting the greatest food intake response in the young and old presenescen ad libitum (AL) and food-restricted (FR) rats was randomly selected for each animal.

Preparation/injection of neuropeptide Y, muscimol, norepinephrine, and aCSF. Porcine NPY (Peninsula Laboratories, San Carlos, CA), norepinephrine bitartrate (NE, Sigma Aldrich, Milwaukee, WI), and the GABA-A receptor agonist muscimol (MUS, Sigma Aldrich) were diluted to the appropriate concentrations with artificial cerebrospinal fluid (aCSF) containing (in mM): 128 NaCl, 2.5 KCl, 2.5 CaCl2, 1.0 MgCl2, and 1.2 Na2PO4, pH 7.4. Dilutions were stored in 7-μl aliquots at -70°C until use. Rats received PVN injections of aCSF, NE, MUS, NPY, and combinations of NPY plus MUS. The concentrations of agents in each of the injections are listed in Table 1. All agents were manually injected in a volume of 0.3 μl over 1 min using a 10 μl Hamilton syringe attached by PE-20 polyethylene tubing to a 33-gauge stainless steel injector 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 PVN. The order of PVN injections for young and old presenescen ad libitum (AL) and food-restricted (FR) rats was randomly selected for each animal.

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Food and water were removed 1 h before (0900) all injections in AL-fed rats. FR rats were allowed 10 min of access to 0.5 g of food
Within 72 h after completion of injections, rats were anesthetized with carbon dioxide and decapitated. Brains were then removed, placed on powdered dry ice, and sliced in 50-μm coronal sections with a cryostat. The brain sections were mounted onto slides, stained with 10% cresyl violet, and examined microscopically to determine cannula placement.

**Food intake analysis.** Food intake and latency to feed were analyzed using data collected during the 24-h postinjection period. Food intake was analyzed for data expressed as total intake (g food/unit time), as well as intake that was body mass independent (g food x kg body mass\(^{-0.67}\)/unit time (14). Because statistical differences were identical for both analyses, we are only presenting data expressed as total intake (g intake/unit time).

**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 or FR) and treatment [aCSF, NPY (both concentrations), MUS (both concentrations)] and combination doses on feeding variables. Food intake measurements were analyzed by repeated-measures ANOVA followed by Bonferroni/Dunn post hoc tests. Unpaired t-tests were used to determine differences in food intake between young and old rats for a given variable (see Tables 2 and 3). Differences were considered significant at \( P \leq 0.05 \). Values presented are means \( \pm \) SE. The food intake data are reported as 2-h cumulative values except where otherwise indicated. We selected the 2-h period because previous work (17), as well as the data from the present study, indicated that the effect of NPY and MUS on food intake was greatest during this time period (see Fig. 2).

**RESULTS**

**Body weight.** Although there was variation in the pattern of weight loss among the old rats, all demonstrated rapid and spontaneous declines in food intake and body weight near the end of their lives (Fig. 1, A and B). Old rats generally followed one of two patterns of weight loss. The more common pattern (~60% of the rats in this study) showed relatively stable body weight before entering senescence (Fig. 1A). The remainder of the animals (~40%) showed two rates of decline—a gradual period of weight loss, followed by a rapid decline upon entry into senescence (Fig. 1B).

Preoperative body weights for the young (\( n = 8 \); age \( = 7 \pm 1 \) mo) and the old presenescent (\( n = 8 \); age \( = 26 \pm 1 \) mo) AL rats were 350 \pm 45 and 431 \pm 25 g, respectively, and differed significantly. After PVN cannulation, body weight loss de-

### Table 1. Order of injection of neuropeptide Y, artificial cerebrospinal fluid, or muscimol into the paraventricular nucleus of senescent rats

<table>
<thead>
<tr>
<th>Injection Order</th>
<th>Treatment, nmol</th>
<th>Number of Rats Injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>NPY (<em>{0.06} + ) MUS (</em>{0.44})</td>
<td>10</td>
</tr>
<tr>
<td>2nd</td>
<td>aCSF</td>
<td>10</td>
</tr>
<tr>
<td>3rd</td>
<td>NPY (_{0.06})</td>
<td>10</td>
</tr>
<tr>
<td>4th</td>
<td>MUS (_{0.44})</td>
<td>9</td>
</tr>
<tr>
<td>5th</td>
<td>NPY (<em>{0.03} + ) MUS (</em>{0.11})</td>
<td>7</td>
</tr>
<tr>
<td>6th</td>
<td>NPY (_{0.03})</td>
<td>6</td>
</tr>
<tr>
<td>7th</td>
<td>MUS (_{0.11})</td>
<td>6</td>
</tr>
<tr>
<td>8th</td>
<td>NPY (<em>{0.03} + ) MUS (</em>{0.44})</td>
<td>6</td>
</tr>
<tr>
<td>9th</td>
<td>NPY (<em>{0.06} + ) MUS (</em>{0.11})</td>
<td>5</td>
</tr>
</tbody>
</table>

The order in which injections were given to the senescent rats and the number of these rats receiving each treatment is shown. All other rats were given treatments in random order. NPY, neuropeptide Y; MUS, muscimol; GABA A receptor agonist; aCSF, artificial cerebrospinal fluid; + denotes combinations of the two drugs.

70 min before injection to stimulate the feeding response. Injections were administered beginning at 1000 h and ended no later than 1200 h under low-intensity red light (i.e., during the dark period). The animals were then returned to their cages for data collection of food and water intakes.

Pilot studies determined that a 48-h period between injections prevented conditioning to the treatments and allowed for consistent maximal responses. Our data also indicate that injections during the presenescent period did not affect the timing of entry into senescence. That is, the average age at which spontaneous rapid weight loss began and the duration of senescence were similar to data reported previously (3, 4, 22, 23; see RESULTS). Moreover, the two senescent animals that did not receive presenescent injections entered senescence at different times and at chronological ages that were 2–3 mo shorter than three of the animals that had received presenescent injections.

Pilot studies also demonstrated that maximal responses to injected NPY were obtained at 0.06 nmol and to MUS at 0.44 nmol. Appropriate positioning of the PVN cannulas was determined before NPY/ GABA injection by the effectiveness of NE (40 nmol) in stimulating food intake (2, 5). Proper positioning was retrospectively verified via histological examination (unpublished observation; data available upon request). All animals used in the data analyses increased their food intake in response to NE and had proper cannula placement upon histological examination.

### Table 2. Differences in food intake between young and old rats

<table>
<thead>
<tr>
<th></th>
<th>Presescent Ad Libitum</th>
<th>Presescent Food-Restricted</th>
<th>Senescent Ad Libitum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aCSF</td>
<td>NPY (_{0.06})</td>
<td>aCSF</td>
</tr>
<tr>
<td>2-h intake, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>1.1±0.2(^a)</td>
<td>5.6±0.4(^b)</td>
<td>5.8±0.7(^b)</td>
</tr>
<tr>
<td>Old</td>
<td>1.1±0.3(^a)</td>
<td>4.3±0.4(^b)</td>
<td>9.3±1.4(^**)</td>
</tr>
<tr>
<td>24-h intake, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>14.9±1.7(^a)</td>
<td>17.5±1.6(^a)</td>
<td>19.4±1.5(^b)</td>
</tr>
<tr>
<td>Old</td>
<td>15.1±0.6(^a)</td>
<td>16.7±0.9(^a)</td>
<td>18.8±3.1(^b)</td>
</tr>
<tr>
<td>First Meal, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>1.3±0.2(^a)</td>
<td>4.1±0.9(^b)</td>
<td>6.6±2.0(^b)</td>
</tr>
<tr>
<td>Old</td>
<td>1.1±0.3(^a)</td>
<td>2.9±1.4(^**)</td>
<td>4.4±1.2(^**)</td>
</tr>
<tr>
<td>Latency to first meal, min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>18.9±23.3(^a)</td>
<td>2.6±0.7(^b)</td>
<td>0.5±0.1(^c)</td>
</tr>
<tr>
<td>Old</td>
<td>58.4±31.5(^**)</td>
<td>1.6±0.4(^b)</td>
<td>0.9±0.1(^c)</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE. Food intake of presenescent old (\( n = 8 \)) and young (\( n = 8 \)) rats and senescent rats (\( n = 10 \)) after injection of artificial cerebrospinal fluid (aCSF) or 0.06 nmol neuropeptide Y (NPY 0.06). Within a row, values sharing the same letter superscripts do not differ significantly (\( P \leq 0.05 \)). \(^*\)Value is significantly different from young rats (\( t\)-test; \( P \leq 0.05 \)).
increased an average of 14.0 ± 1.7% in the old rats and 11.3 ± 2.1% in the young rats, a drop that did not significantly differ. 

Body weight loss of the 10 senescent rats averaged 5 ± 2% of initial body weight at the first injection, and 17 ± 5% at the final injection. The average age at which spontaneous rapid weight loss began was 29 ± 2 mo (range = 26 to 30 mo). The average duration of senescence before death was 16 ± 7 days. The average age of the senescent rats at their final injection was 28 ± 2 mo (range = 26 to 29 mo).

Postoperative daily food intake before injections was significantly greater in the AL old presenescent (n = 8) than in the young (n = 8) rats, averaging 14.5 ± 0.3 vs. 12.2 ± 0.5 g/day, respectively. Comparisons of 24-h intakes between aCSF injection days and the 24-h periods separating injection days, showed no differences in any of the groups of animals.

Food intake response in senescent rats in response to NPY and MUS vs. aCSF. Of the 10 senescent rats used in this experiment, 5 received all 9 injections (Table 1), one received 8 injections, three received 4 injections, and one rat received only the first 3 injections.

Senescent rats significantly increased their 2-h cumulative food intake in response to NPY0.06 nmol, although this increase (1.4 g, 127%) was attenuated compared with that in the presenescent rats (3.3 g, 290% increase) (Fig. 2, Table 2). Moreover, unlike the young and old presenescent rats, the senescent animals did not significantly increase their 2-h cumulative food intake in response to PVN injections of NPY0.03 nmol + MUS0.11 nmol, or a combination of these three treatments (Table 3, Figs. 2 and 3). Senescent rats did not significantly increase food intake above aCSF values in response to the combination dose of NPY0.06 nmol and MUS0.11 nmol, which was the most efficacious treatment in the young and presenescent rats (Figs. 2 and 3).

NPY0.03 nmol with or without MUS0.44 nmol significantly reduced the latency to first meal in the young, as well as the old presenescent rats. In contrast, it had no significant effect on the senescent rats, which had much longer latencies under all treatments, including aCSF injection (Tables 2 and 3).

Young and presenescent responses. Young (n = 8) and old presenescent (n = 8) AL rats had robust 2-h responses to all injected treatments compared with aCSF injections (Tables 2 and 3; Fig. 3). Combinations of NPY and MUS were generally more effective in stimulating food intake than either NPY or MUS alone. The only exception to this was NPY0.03 nmol combined with MUS0.11 nmol. This combination did not result in a significant increase in feeding in senescent rats compared with either agent injected alone.

Young and presenescent food-restricted responses. Young FR (n = 8) and old presenescent FR rats (n = 8) exhibited robust eating at each time interval measured, in response to each treatment, including aCSF (Tables 2 and 3). For these animals, there was no significant difference in food intake with any of the agonists when compared with the very high food intake with aCSF alone, suggesting that the food intake pathway(s) in these rats were already maximally stimulated.

**DISCUSSION**

The two major findings in this study are that GABA-mediated, as well as NPY-mediated, increases in food intakes...
are severely compromised after transition from presenescence into senescence and that the PVN and/or projections from the PVN are associated with this compromise. That is, young and old presenescence rats increased their food intake dramatically in response to PVN injection of NPY, MUS, or NPY plus MUS compared with injections of aCSF (Fig. 3). However, senescent rats displayed little or no stimulation of food intake above that elicited by aCSF for all but the injection of NPY, 0.06 nmol, and even the latter increase was blunted significantly compared with the responses in the young and old presenescence animals. In addition, latency to the first meal eaten after PVN injection of NPY or MUS was not significantly lower in the senescent rat from that following injection of aCSF—in contrast to the reduced postinjection latency seen in the young and old presenescence rats. These results are consistent with our previous findings of greatly attenuated food intake in senescent vs. presenescence rats following ICV injections of NPY. Moreover, the fact that the PVN injection of the GABA-A receptor agonist muscimol failed to stimulate food intake in the senescent rat suggests alteration(s) in the GABA pathway in the senescent rats.

Where such alterations might be occurring, however, is not clear. One possibility involves NPY modulation of GABA availability. If NPY acts in the young and old presenescence rats to inhibit GABA reuptake in the PVN as it appears to do in the striatum (18), the reduced responsiveness of the senescent rats to exogenous NPY could reflect failure of such inhibition and, as a result, less GABA being available to stimulate food intake. This possibility would not, however, explain the lack of responsiveness of the senescent rats to MUS. An alternative mechanism for the interaction of NPY and GABA stems from the observations of Pronchuk et al. (24) who found that NPY could inhibit postsynaptic inhibitory currents in the PVN mapped from hypothalamic slices isolated from 3 to 5-wk-old Sprague-Dawley rats. These results suggest that NPY inhibits GABA release. If this is the case, then it is possible that in the senescent rats, inhibitory effects of NPY on GABA release/transmission are more effective, resulting in less GABA release. However, the fact that Pronchuk et al. (24) found that NPY had no effect on the action of MUS implies that the blunted response of the senescent rats to MUS seen in the present study reflects changes independent of GABA release. Thus, even if there are modulatory effects of NPY on GABA availability, the GABA and the NPY pathways appear to be able to stimulate feeding independently [as indicated by responses of young and old presenescence rats to injection of NPY or MUS (Tables 2 and 3)]; and the failure of the senescent rats to respond to MUS either with or without NPY suggests alterations in some portion of the GABA pathway independent of the NPY pathway.

The mechanisms underlying the food-intake stimulatory effects of GABA and NPY are complex and not well understood. Our data show that these agents can have considerably different effects on food intake if injected separately or simultaneously. That is, we find that when MUS and NPY are administered together into the PVN of young and old presenescence rats, food intake is greater, for the most part, than when either agent is injected alone. However, the increase in food intake after simultaneous injections is less than that predicted from the food intake values when the agents are injected independently (see Fig. 3). These results are consistent with data of Pu et al. (25) who also found that coadministration of NPY and MUS injected into the PVN of 2- to 3-mo-old rats increased feeding response over that stimulated by NPY or MUS injected independently. Similar to our data, the increase in food intake after coadministration of NPY and MUS was less than the additive value of each agent injected separately. These authors suggest a dual role for GABA. That is, GABA, which is coreleased with NPY into the synaptic cleft, may inhibit the actions of NPY, while independently stimulating food intake. That GABA has an inhibitory effect on NPY is consistent with our data in the senescent rat that shows an increase in food intake above aCSF values after injection of NPY, 0.06, but no increase in food intake when NPY is given with MUS. Pu et al. further speculate that the independent effects of each agent represent a “safety net” for food intake if either pathway becomes dysfunctional. Clearly, further research is necessary to better define the independent and synergistic actions NPY and GABA on food intake.

With respect to alterations in the NPY pathway itself, results from our previous work exclude several NPY-mediated processes as possible mechanisms for the blunted food intake of the senescent rats. For example, we have found that serum leptin concentrations in these animals are significantly lower than those in presenescence rats despite the fact that the senescent rats have significantly reduced food intake (4). Although the low serum leptin concentration is consistent with the declining body and fat weight in the senescent animals, it would be expected to signal increased, rather than decreased, food intake. It is also unlikely that insufficient endogenous levels of NPY within the PVN account for the reduced food intake of the senescent rats because injections of NPY, directly into the PVN of senescent rats, elicited severely blunted food
intake responses. Moreover, we have recently shown that neither the relative amounts of NPY Y1 nor Y5 receptor mRNA nor the number of neurons that contain Y1 protein differ between presenescence and senescent rats (7). Together, these data suggest that the blunted NPY-induced food intake of the senescent vs. presenescence rats reflects events occurring beyond the NPY-receptor.

One potential mechanism that could underlie the reduction in NPY-induced food intake is an alteration in the G protein/adenyl cyclase cascade. Previous reports of age-related alterations in this signal transduction pathway are inconsistent and appear to be tissue and G-protein-type dependent. For example, recent data describe significant alterations in the Gα and Gα/alpha subunit complex of the adrenergic pathways in aged cardiac tissue and brains from Alzheimer-type dementia, but such alterations do not appear in brains without specific pathology (12, 19, 20). Although we are unaware of data documenting age-related alterations to the Gi protein-dependant pathway of signal transduction within neurons from the PVN, some reports suggest disruption in this pathway in aged cells isolated from liver, kidney, and bladder (8, 10). On the other hand, Gabaldon et al. (11) found no attenuation of norepinephrine-induced cAMP formation in brown adipocytes from senescent vs. presenescence F344 rats. Clearly, additional research that focuses specifically on possible age- and senescent-related alteration to G protein-dependent signal transduction within neurons of the PVN is warranted.

Hypothalamic regulation of food intake involves several neural areas of which the ventromedial hypothalamic nucleus (VMH), the PVN, and the arcuate nucleus (ARC) have had considerable attention (17). We have focused on the PVN because dysregulation of several homeostatic systems in senescence (e.g., thermoregulation and food intake) share the PVN as a specific site of neuronal control. Results based on lateral ventricular injections of NPY and our understanding of accepted physiological mechanisms led us to conclude that the attenuation of food intake observed in the senescent rats is accompanied by alterations in neural signaling within the PVN. Our conclusion was based, in part, on circumstantial evidence, as injections into the lateral ventricle can diffuse into the VMH, PVN, and ARC. Indeed, histological analysis from previous investigations show that ink injected into the lateral ventricle disperses throughout the brain. In this study, we positioned our injection cannula directly over the PVN and found that the pattern of food intake following NPY injection was almost identical to that seen after injection into the lateral ventricle. Although we cannot rule out contributions from other hypothalamic areas to the attenuated NPY-induced food intake of the senescent rats, it is clear that the PVN and/or PVN pathways are involved in this attenuation.

In conclusion, our results suggest that the ineffective regulation of food intake in the senescent vs. presenescence rat reflects, in part, altered neural transmission within the PVN and involved altered responses to both NPY and to GABA. Whether these alterations occur at a site common to both pathways has yet to be determined.

**Perspectives**

The data presented here are part of more comprehensive and ongoing investigations that focus on possible neural mechanisms that could explain the rapid and simultaneous senescence-related alterations in several hypothalamic-mediated physiological systems. Previous investigations have demonstrated that, within the same time period, senescent animals exhibit disruption in several independent hypothalamic-related functions, including food intake, body weight regulation, cold-induced thermogenesis, and circadian rhythm of body temperature (3, 4, 7, 22). This study suggests that the blunted food intake of the senescent rat likely involves alterations in the pathways of GABA and NPY, neural pathways that also have significant roles in the regulation of several other hypothalamic-mediated systems. Although we cannot rule out the possibility of independent alterations specific to each pathway and/or system, the extensive nature of the GABA and NPY networks within the hypothalamus suggests the possibility of alterations in cellular event(s) common to all the affected systems. Although it is far too early to suggest what such alterations might be, we believe that one approach to identifying the mechanism(s) triggering the initiation of senescence involves investigations focused on the commonalities of age- and senescent-related dysfunction.

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