Norepinephrine release in brown adipose tissue remains robust in cold-exposed senescent Fischer 344 rats

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Gabaldón, Annette M., David A. Gavel, Jock S. Hamilton, Roger B. McDonald, and Barbara A. Horwitz. Norepinephrine release in brown adipose tissue remains robust in cold-exposed senescent Fischer 344 rats. Am J Physiol Regul Integr Comp Physiol 285: R91–R98, 2003.—Near the end of life, old F344 rats undergo a transition, marked by spontaneous and rapidly declining function. Food intake and body weight decrease, and these rats, which we call senescent, develop severe hypothermia in the cold due in part to blunted brown fat [brown adipose tissue (BAT)] thermogenesis. We tested the hypothesis that this attenuation may involve diminished sympathetic signaling by measuring cold-induced BAT norepinephrine release in freely moving rats using linear microdialysis probes surgically implanted into interscapular BAT 24 and 48 h previously. In response to 2 h at 15°C, senescent rats increased BAT norepinephrine release 6–10-fold but did not maintain homeothermy. This increase was comparable to that of old presenescent (weight stable) rats that did maintain homeothermy during even greater cold exposure (2 h at 15°C followed by 1.5 h at 8°C). Tail temperatures, an index of vasoconstrictor responsive-ness to cold, exhibited similar cooling curves in presenescent and senescent rats. Thus cold-induced sympathetic signaling to BAT and tail vasoconstrictor responsive-ness remain robust in senescent rats and cannot explain their cold-induced hypothermia.

aging; brown fat; vasoconstriction; sympathetic signaling; hypothermia; weight loss

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ture brown adipocytes isolated from senescent vs. presenescent rats generated similar levels of cAMP in the presence of norepinephrine and showed similar increases in lipolytic activity as measured by glycerol release.

Although our findings above indicate that senescent brown adipocytes remain responsive to norepinephrine in vitro (i.e., cAMP levels, lipolytic activity, and oxygen consumption all increase in its presence), the degree to which cold exposure stimulates brown fat thermogenesis in senescent rats in vivo remains to be determined. While reduced brown fat thermogenic capacity contributes to the senescent rats’ inability to maintain homeothermy during cold exposure, it is also possible that blunted sympathetic signaling to the tissue is a contributing factor.

In the present study, we tested the hypothesis that cold-induced activation of the sympathetic nervous system is attenuated in senescent F344 rats. For this, we assessed brown fat sympathetic activity in vivo by implanting tissue microdialysis probes into the interscapular brown fat depot and measuring norepinephrine levels in freely moving presenescent and senescent rats exposed to cold. We also assessed tail vasoconstrictor responsiveness to cold (a sympathetic-mediated process) by measuring cold-induced changes in tail temperature (Ttail).

MATERIALS AND METHODS

Animals and Animal Care

Male F344 rats, aged 24–25 mo, were obtained from the National Institute on Aging colony maintained by Harlan Sprague Dawley Laboratory (Indianapolis, IN). All procedures were performed in accordance with the American Physiological Society’s “Guidelines for Research Involving Animals and Human Beings” (1) and were approved by the University of California Davis Animal Care and Use Committee. On arrival, animals were placed into a laminar flow unit (DuoFlo; Lab Products, Maywood, NJ) that provided clean air via circulation through high-efficiency particle filters. Animals were housed individually in hanging wire-bottom cages (20 × 25 × 18 cm) and maintained at 25–26°C and 50% humidity on a 12:12-h light-dark cycle (lights on at 0600, off at 1800). NIH-31 laboratory chow (Teklad Research Diets, Indianapolis, IN) and distilled water were provided ad libitum. Body weight of each animal was measured every 1–2 days throughout the experimental period. Necropsy and microbiological examinations were not performed, but animals were routinely inspected for external symptoms of disease (i.e., labored breathing, presence of mucus around the nose, tumors, swelling of appendages). Rats with overt signs of disease were not used in the experiments. Included was one rat that developed a swollen body and exhibited very poor cold tolerance despite no rapid loss of body weight. Also excluded from analysis were two senescent rats that did not recover well from the surgery in experiment 2. Each animal was used in two different experiments. The experimental protocol and details of the surgery and experiments are described below.

Surgery

Two weeks after arrival, radiotelemetry units (Mini-Mitter, Sunriver, OR) used for remote monitoring of body temperature were implanted into the peritoneal cavity through a small incision made in the midline of the abdominal wall. Surgeries were performed under halothane anesthesia (~3%) using sterile techniques; surgery time was typically 15 min. At the beginning of surgery, rats were given an intramuscular injection (0.3 ml) of Aquacillin (Vedeo, NDC 50869–170–12-penicillnin G procaine, 300,000 U/ml). At the end of surgery, a powdered wound antibiotic (Nitrofurazone, 2.6% Ken Vet KV3555) was applied over the skin incision, and the rat was returned to its home cage. A recovery period of 2–3 wk was allowed before the start of biweekly acute cold exposure tests (experiment 1). For experiment 2, a linear microdialysis probe (LM-5; BAS, Bioanalytical Systems, West Lafayette, IN) with a 5-mm dialyzable membrane was implanted into the interscapular brown fat depot. Rats were anesthetized with halothane and prophylactically given an antibiotic as described above. In addition, rats were given 18 ml lactated Ringer solution (Baxter, Deerfield, IL) administered subcutaneously in 6-ml volumes throughout the surgery to ensure good hydration postsurgery. The skin in the interscapular area was shaved and cleansed with 70% ethanol, and a small incision was made along the midline of the interscapular depot of white fat and the underlying interscapular brown fat. A 25-gauge hypodermic needle was inserted lengthwise through one of the bilateral interscapular brown fat lobes, and the microdialysis probe was inserted through the lumen. The needle was then removed, and the probe was positioned in place. The ends of the probe were sutured to the underlying muscle, looped under the skin, and exteriorized through the midscapular skin incision. The skin incision was sutured closed, and antibiotic wound powder was applied. Rats were fitted with a Lycra/spandex jacket (Harvard Apparatus, model AE-62–0058) that is part of the tether system used in microdialysis studies on awake animals. The jacket prevented excessive lateral turning that could dislodge the probe and protected the exteriorized probe lines from being bitten. Rats had good overall mobility in the jackets and were mainly limited in their ability to groom their head and neck areas. Rats were allowed to recover in plastic cages with wood shavings, and they had access to a heated pad. Food and water were provided ad libitum. A recovery period of 24 h was allowed before rats were used in experiment 2. The survival rate of senescent rats from this surgery was 71%; that of the presenescent rats was 100%.

Experimental Protocol

Experiment 1: biweekly acute cold exposure tests and Ttail. Acute cold exposure tests were performed once every 2 wk to evaluate body temperature and tail temperature responsiveness. A total of three to five cold tests was performed on each rat while it was weight stable (i.e., presenescent). The final cold test for the group of rats that became senescent was performed 2–4 days around the onset of weight loss. The final cold test for the group of rats that remained presenescent occurred at chronological ages matching those of the senescent rats. For each test, rats were placed individually into a glass animal bowl fitted with a plastic floor and open at the top. The ambient temperature of the chamber (Tair) was maintained between 25 and 26°C, which is within the thermoneutral zone for these animals. Each experimental test consisted of 60 min noncold exposure (NCE; Tair = 25–26°C), followed by 90 min cold exposure (CE; Tair = 6–7°C), and a return to noncold exposure for 60 min (NCE; R; Tair = 25–26°C). For cold exposure, the animal bowl was surrounded with cold water and ice; for rewarming, the bowl was transferred to a warm water bath. Rates of cooling and rewarming...
of the chamber air were reproducible between experiments. Colonic temperature ($T_c$) was measured at the beginning of the experiment with a thermistor probe (YSI 400 series) inserted 5–6 cm into the colon. The probe was held in position for ~30 s. Two additional measurements of $T_c$ were made at the end of the cold exposure and the rewarming periods. $T_{tail}$ was measured as an index of tail vasomotor tone. A surface disk thermistor of 4-mm diameter (YSI) was taped to the dorsal surface of the tail, ~1 cm from the base, using a fabric bandage that also insulated the thermistor from the environment. Intrapерitoneal temperature ($T_{ip}$), $T_{tail}$, and $T_{air}$ were recorded at 2-s intervals and averaged every 20 s via a computer data-acquisition system (Mini-Mitter, Sunriver, OR).

**Experiment 2: cold-induced norepinephrine release in brown adipose tissue.** Two to three days after the final cold test in experiment 1, a linear microdialysis probe was surgically implanted into the interscapular brown fat as described above (see Surgery). Senescent rats had a mean weight loss of 7.4 ± 1.0%, with a range of 4.4–12.8%, at the time of surgery. Rats were allowed to recover for 24 h before the first microdialysis experiment. A second identical microdialysis experiment was performed on each rat 48 h postsurgery. At 0800–0900 on the day of each experiment, rats were placed into a plastic animal bowl with a few wood shavings, and a hook on the jacket was connected to a tether line. The tether was attached to a counterbalance, and the microdialysis probe was attached to a liquid swivel. The swivel was then connected to a BeeHive microperfusion pump (BAS) and perfused with sterile Ringer solution [(in mM) 140 NaCl, 4.0 KCl, 3.4 CaCl$_2$, 1.0 MgCl$_2$] at a flow rate of 8–10 μl/min for 10 min. (In in vitro tests, this procedure was found to dislodge any air bubbles that might have collected in the membrane.) The flow was then reduced to 1 μl/min for experiments. Dialysates were collected every 20 min (20–μl samples) into 0.6-ml microcentrifuge tubes kept at 22°C and shielded from light. In pilot studies, we found no significant difference in norepinephrine levels in dialysates collected on ice vs. those collected at 22°C in the dark. After an equilibrium period of 1 h, dialysates were collected for 2–3 h at $T_{air} = 28°C$, followed by collections at $T_{air} = 25–26°C$ (2 h), during cold exposure (senescent rats: 2 h at 15°C; presenescent rats: 2 h at 15°C followed by 80 min at 8°C), and during rewarming (3 h at $T_{air} = 28°C$). $T_{air}$ was controlled by varying the temperature of a circulating water bath outside of the animal bowl. Measurements of $T_{ip}$ were used to monitor the thermal state of the animal. To avoid any handling stress that might influence the norepinephrine release, $T_c$ was not measured. Rats were removed from the cold prematurely if $T_{ip}$ fell to ≤33°C; this occurred in one of the senescent rats and in none of the presenescent rats. At the end of each experiment, rats were returned to their home cage with ad libitum access to food and water. After the final experiment, 48 h postsurgery, rats were killed by decapitation and microdialysis probe placement was verified by visual inspection. The probes were found to be intact and well placed within the interscapular brown fat in all rats.

**Microdialysis Probe Effectiveness**

In vitro norepinephrine recovery was determined for each probe 1–2 days before surgical implant. These values, ranging from 45 to 65%, were used to correct for variation between individual probes. For this determination, microdialysis probes were placed into vials containing 1,000 pg/ml norepinephrine in Ringer solution and allowed to equilibrate at room temperature for 1 h while perfused with Ringer at a flow rate of 1 μl/min. Samples were collected every 20 min for the next 3 h into 0.6-ml microcentrifuge tubes kept on ice and shielded from light. The relative probe recovery was calculated as the concentration of norepinephrine in the dialysate divided by the concentration of norepinephrine in the surrounding medium.

**Analysis of Brown Fat Dialysate Norepinephrine Levels**

Norepinephrine levels in brown fat dialysates were measured immediately after collection via HPLC-EC. A C$_{18}$ hydrophobic microbore column (1 × 150 mm; 3 μm particle size; ESA) was used with a mobile phase consisting of 75 mM NaH$_2$PO$_4$, 2.8 mM octanesulfonic acid, 0.01 mM EDTA, and 5% acetonitrile brought to pH 3.1 with H$_3$PO$_4$. The flow rate was 60 μl/min; quantification was by electrochemical detection (ESA Coulochrome II, Bedford, MA) using a glassy carbon electrode set at 200 mV. Area under the norepinephrine peak for the samples was compared with norepinephrine standards. These standards were prepared daily by diluting stocks maintained at ~70°C. Each dialysate had five to seven standards, ranging from 1 to 20 fmol norepinephrine in volumes of 1 to 5 μl. All standard curves appeared linear and had correlation coefficients of 0.98 or better. Unknown samples were assigned values from the standard curve regression line only when they were within the range of the standards. If the unknowns fell outside the range, they were remeasured using an adjusted range of standards. One femtomole was the limit of resolution (i.e., signal > $3\times$ noise).

**Data Calculations and Statistical Analysis**

The heat loss index (HLI) was calculated for experiment 1 according to the equation HLI = ($T_{tail}$ – $T_{air}$)/(2$T_{ip}$ – $T_{air}$) (15). Normally, the HLI changes between 0 (maximal heat conservation due to skin vasoconstriction) and 1 (maximal heat loss due to skin vasodilation); it is a useful index under steady-state $T_{air}$ conditions. Average HLI values were calculated over the last 10 min of noncold exposure and at one 10-min interval during cold exposure ($t = 50–60$ min), after $T_{air}$ had stabilized at ~6°C after an exponential decrease during rapid cooling. Changes in $T_{air}$ during cold exposure and rewarming were also calculated. Data are presented as means ± SE. In experiment 1, comparisons between the two groups of presenescent rats [i.e., those that remained presenescent throughout the experiment (Pre) and those that eventually became senescent (Sen-Prior)] were made using a two-way ANOVA (group and exposure as main effects). In both experiments, a two-way ANOVA with one repeated factor was used to determine possible differences between the senescent rats before (Sen-Prior) and after (Sen-Post) they entered senescence, and means were compared using Fisher least-significance post hoc test. $P ≤ 0.05$ was considered to be statistically significant.

**RESULTS**

**Body Weight**

Spontaneous rapid weight loss began at different ages in different rats (ranging from 24.4 to 28.3 mo). The average weight of senescent rats before the onset of their weight loss (i.e., while they were still presenescent) was 416.3 ± 8.0 g. These values did not differ significantly from those of old rats that remained weight-stable/presenescent during the study (417.0 ± 13.0 g). The average rate of weight loss (%baseline body wt/day) during senescence ranged from 2.0 to
Table 1. $T_{co}$ responses to acute cold exposure in presenescent, senescent, and 12-mo-old rats

<table>
<thead>
<tr>
<th></th>
<th>Pre (n = 9)</th>
<th>Sen-Prior (n = 9)</th>
<th>Sen-Post (n = 9)</th>
<th>Young (12 mo) (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{co}$ (°C)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NCE</td>
<td>37.96 ± 0.08</td>
<td>37.79 ± 0.12</td>
<td>37.62 ± 0.12</td>
<td>37.20 ± 0.08</td>
</tr>
<tr>
<td>CE</td>
<td>36.03 ± 0.18</td>
<td>35.83 ± 0.25</td>
<td>34.37 ± 0.17</td>
<td>37.65 ± 0.36</td>
</tr>
<tr>
<td>NCE-R</td>
<td>37.33 ± 0.09</td>
<td>36.99 ± 0.16</td>
<td>36.36 ± 0.21</td>
<td>37.85 ± 0.23</td>
</tr>
<tr>
<td>$\Delta T_{co}$ (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>-1.94 ± 0.21</td>
<td>-1.97 ± 0.21</td>
<td>-3.25 ± 0.18</td>
<td>0.44 ± 0.31†</td>
</tr>
<tr>
<td>NCE-R</td>
<td>1.30 ± 0.23</td>
<td>1.17 ± 0.20</td>
<td>1.99 ± 0.14</td>
<td>0.20 ± 0.59†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. NCE, noncold exposure (1 h at 25–26°C); CE, cold exposure (≥90 min at 6–7°C); and NCE-R, rewarmed (1 h at 25–26°C). Cold exposure times were 90 min for the 9-old rats that remained presenescent during the length of the experiment (Pre), for the presenescent rats that became senescent during the experiment (Sen-Prior, i.e., before they entered senesence), and for the 3 young (12-mo-old) rats. Of the 9 rats that entered senesence (Sen-Post), 4 were cold exposed for 90 min while 5 were cold exposed for 45–80 min. The values for the Pre group represent the final biweekly cold test before experiment 2; the values for the Sen-Prior group represent the last cold test before transition to senescence (i.e., while they were still presenescent); and the values for the Sen-Post rats represent the final cold test after transition to senescence (just before experiment 2). Young (12-mo-old) rats underwent a single cold exposure test. $\Delta T_{co}$ CE is the difference in colonic temperature ($T_{co}$) between NCE and CE values; $\Delta T_{co}$ NCE-R is the difference in $T_{co}$ between NCE-R and CE values. *Significantly different from Sen-Prior value ($P < 0.05$); †significantly different from Pre, Sen-Prior, and Sen-Post values ($P < 0.05$).

6.2%, with a mean of 3.6 ± 0.6%. The total percentage of weight loss from the day of onset to the day of the microdialysis surgery ranged from 4.4 to 12.8%, with a mean of 7.4 ± 1.0%.

**Experiment 1: Biweekly Acute Cold Exposure Tests and Temperature Measurements**

$T_{co}$. The $T_{co}$s during NCE, CE, and NCE-R are shown in Table 1. Repeated exposure to cold did not significantly affect $T_{co}$, nor did the NCE values of $T_{co}$ differ significantly from the first to the final cold test while rats remained weight stable. Thus we have presented only the $T_{co}$ values for the final biweekly cold test in presenescent rats (Pre) and for the final pretransition (Sen-Prior) and posttransition (Sen-Post) periods in the group of rats that became senescent. There were no significant differences in NCE values of $T_{co}$ between Pre and Sen-Prior rats, nor did NCE values of $T_{co}$ change significantly after transition to senescence. During CE (90 min at 6–7°C), $T_{co}$ decreased moderately and to the same degree in Pre (−1.94 ± 0.21°C) and in Sen-Prior (−1.97 ± 0.21°C) rats. Significantly greater cold-induced decreases in $T_{co}$ occurred in rats after their transition to senescence (−3.25 ± 0.18°C, Sen-Post). Because some of the senescent rats were removed from the cold prematurely due to very rapid declines in $T_{ip}$ (6 of the 9 rats were cold exposed for only 45–80 min), the average decrease in $T_{co}$ in Sen-Post rats (Table 1) is less than it would have been had all rats been kept in the cold for the full 90 min. $T_{co}$ increased significantly during the rewarmin period in both Pre and Sen rats, with a greater increase observed in the Sen-Post (1.99 ± 0.14°C) vs. Sen-Prior (1.17 ± 0.20°C) states. In the 12-mo-old male F344 rats subjected to the same cold exposure test, $T_{co}$ did not decrease and was significantly higher after CE than in Pre and Sen rats (Table 1).

The relationship between body weight, onset of rapid weight loss, and magnitude of the change (decrease) in $T_{co}$ after cold exposure is illustrated in Fig. 1. Representative graphs are shown for one rat that remained presenescent during the length of the study (Fig. 1A) and for three rats that underwent transition to senescence (Fig. 1, B–D). As seen in Fig. 1, B–D, the thermoregulatory response to cold remained relatively unchanged while the rats were weight stable but deteriorated.
orated markedly within 1–3 days of the onset of rapid weight loss.

$T_{tail}$- Average values of $T_{ip}$ and $T_{tail}$ responses to CE before and after transition to senescence are summarized in Fig. 2. During CE, $T_{tail}$ declined exponentially with time in parallel with $T_{air}$, the $T_{tail}$ cooling curves being similar during pre- and posttransition tests. Absolute $T_{tail}$ values, as well as the cold-exposed HLI values calculated after $T_{air}$ had stabilized at ~6°C, also did not differ significantly between the presenescence and senescent rats (Table 2). In contrast, the rate of decline in $T_{ip}$ during cold exposure increased after transition.

**Experiment 2: Effects of Cold on Norepinephrine Release in Brown Adipose Tissue**

To assess the level of sympathetic activity in brown adipose tissue in freely moving rats, we used tissue microdialysis to measure extracellular norepinephrine levels in the interscapular depot over 20-min intervals. In both presenescence and senescent rats, there were no differences in day 1 (24 h postsurgery) vs. day 2 (48 h postsurgery) brown fat dialysate norepinephrine levels during NCE or CE (Fig. 3, A and B), nor did the degree of cold-induced hypothermia differ significantly between the 2 days.

At the starting temperature of 28°C, presenescence and senescent rats exhibited similar $T_{ip}$ values and brown fat dialysate norepinephrine levels. When $T_{air}$ was reduced from 28 to 26°C, dialysate norepinephrine levels increased by ~2.5-fold in both presenescence and senescent rats, and $T_{ip}$ remained relatively unchanged. When the chamber temperature was lowered to the first level of cold (2 h at 15°C), dialysate norepinephrine levels rapidly increased in both presenescence and senescent rats, peaking at about 6- to 10-fold above the resting levels within the day 2 (24 h postsurgery) vs. day 1 h). Presenescent rats not exhibiting any significant decrease in $T_{ip}$ at 15°C exhibited a 2.3°C drop in $T_{ip}$ after 2 h at 15°C (day 1, and a ~1.9°C drop on day 2.) Presenescence rats not exhibiting any significant decrease in $T_{ip}$ at 15°C were subjected to a second level of cold exposure (80 min at 8°C) to ensure full activation of the sympathetic outflow to brown fat. (This included all but the one rat that dropped body temperature at 15°C.)

![Fig. 2. Changes in tail temperature (Tail) and intraperitoneal (IP) temperature during cold exposure in presenescence (Pre) and senescent rats (Sen-Prior = last pretransition biweekly cold test; Sen-Post = posttransition cold test). Changes in tail temperature are differences from the average over the 20-min time interval before cold exposure. Values are means ± SE; where not shown, SE is smaller than the size of the symbol. The average absolute temperatures during noncold exposure (NCE), cold exposure (CE), and re-warming (NCE-R) are in Table 2.](image)

### Table 2. $T_{ip}$, $T_{tail}$, and heat loss index during acute cold exposure of presenescence and senescent rats

<table>
<thead>
<tr>
<th></th>
<th>Pre (n = 9)</th>
<th>Sen-Prior (n = 9)</th>
<th>Sen-Post (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{ip}$ °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCE</td>
<td>37.03 ± 0.18</td>
<td>36.66 ± 0.22</td>
<td>36.47 ± 0.27</td>
</tr>
<tr>
<td>CE</td>
<td>35.32 ± 0.23</td>
<td>34.98 ± 0.25</td>
<td>33.89 ± 0.45</td>
</tr>
<tr>
<td>Tair °C</td>
<td>36.40 ± 0.20</td>
<td>35.59 ± 0.22</td>
<td>34.72 ± 0.28</td>
</tr>
<tr>
<td>$T_{tail}$ °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCE</td>
<td>27.58 ± 0.58</td>
<td>25.62 ± 0.74</td>
<td>26.70 ± 0.53</td>
</tr>
<tr>
<td>CE</td>
<td>8.20 ± 0.33</td>
<td>6.60 ± 0.43</td>
<td>7.08 ± 0.18</td>
</tr>
<tr>
<td>NCE-R</td>
<td>26.30 ± 0.35</td>
<td>24.48 ± 0.28</td>
<td>24.91 ± 0.25</td>
</tr>
<tr>
<td>Heat loss index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCE</td>
<td>0.21 ± 0.03</td>
<td>0.13 ± 0.02</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td>CE</td>
<td>0.08 ± 0.01</td>
<td>0.02 ± 0.02</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>NCE-R</td>
<td>0.22 ± 0.04</td>
<td>0.16 ± 0.02</td>
<td>0.14 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE for the same rats shown in Table 1. Values shown represent 10-min averages over time 50–60 min at each exposure. Animal groups and treatments are the same as described in Table 1. Pre and Sen-Prior cold exposure times were 90 min; 4 Sen-Post rats were cold exposed for 90 min, 3 were cold exposed for 60–80 min, and 2 were cold exposed for 45 min (the latter 2 were not included in the analysis). Pre, presenescence group, final biweekly cold test before experiment 2; Sen-Prior, last cold test before transition (i.e., while the rats were still weight stable/presenescence); Sen-Post, cold test after transition to senescence, just before experiment 2. $T_{ip}$, intraperitoneal temperature. aSignificantly different from Pre and from Sen-Prior values ($P < 0.05$). Cold-induced changes in tail temperature ($T_{tail}$) and heat loss index did not differ significantly between the different groups ($P > 0.05$).
hour of rewarming. The net changes in brown fat dialysate norepinephrine levels (i.e., compared with the values at 26°C) are summarized in Fig. 4.

DISCUSSION

The present investigation tested the hypothesis that cold-induced activation of the sympathetic nervous system is blunted in senescent F344 rats. We evaluated two target tissues for cold-induced norepinephrine action: brown adipose tissue for metabolic heat production and the tail for heat conservation. In the first study, old male F344 rats (aged 24–28 mo) were cold exposed biweekly to characterize changes in body temperature and vasoconstrictor responses occurring before and after transition to senescence. In the second study, in vivo measurements of cold-induced norepinephrine release in interscapular brown fat were made via microdialysis in the same presenescence and senescent rats as in experiment 1. Our major findings are that senescent rats do not exhibit blunted cold-induced norepinephrine release in their interscapular brown adipose tissue nor do they exhibit dysregulation of tail vasoconstriction. Brown fat dialysate norepinephrine levels were as markedly elevated in cold-exposed senescent rats as in cold-exposed presenescence rats, despite the greater hypothermia in the senescent animals. Moreover, Ttail cooling curves were similar in the rats before and after they entered senescence, suggesting comparable heat retention at this major peripheral site of heat exchange. Thus senescent rats do not become insensitive to cold (i.e., the pathways for heat production and heat retention appear to be activated in a manner similar to that of the presenescence rats). In contrast, reduced brown fat thermogenic capacity does

Fig. 3. Brown fat dialysate norepinephrine (NE) levels and intraperitoneal temperatures for presenescence (A) and senescence (B) rats on day 1 (24 h postsurgery) and day 2 (48 h postsurgery). Dialysates were collected at 20-min intervals during NCE, CE, and NCE-R. NE values (designated by square symbols) were corrected for relative recovery of the individual probes as measured in vitro. Intraperitoneal temperatures (designated by circles) were recorded via radiotelemetry; points shown are temperatures recorded at the time of dialysate collection. Values are means ± SE.
appear to play a major role in the early development of severe cold-induced hypothermia in the senescent F344 rat (5, 11). In the biweekly acute cold exposure tests, body temperature declined on average by about 0.5–2°C in rats while they were weight stable/presenescent. The degree of cold-induced hypothermia was specific to the individual rat, and it remained constant between the first and final cold test performed 6–10 wk later as long as body weight remained relatively constant. In 12-mo-old F344 male rats that were subjected to the same cold exposure treatment, there was no significant decrease in Tco (Table 1). These findings are consistent with our previous studies on old presenescent vs. younger F344 male rats (10) and demonstrate that with chronological age, there is generally only a moderate loss of the ability to maintain homeothermy during cold exposure, an effect of age that is also observed in mice and other rat strains (8). As expected, all rats that exhibited rapid weight loss during this study also developed severe cold-induced hypothermia. Because the cold tests in experiment 1 were performed biweekly, it is not possible to know the exact day that thermoregulation became severely impaired in the senescent rats. However, for many of them, it appears to have occurred shortly before the onset of their rapid weight loss. We have previously shown that the onset of rapid weight loss and onset of severely impaired thermoregulation are closely coupled temporally despite the fact that they are independent physiological events (11). That is, although they may share a common trigger for their occurrence, each develops without major influence from the other, at least in the early stages of senescence.

One question that we had not previously addressed in the senescent rat model was if physiological regulation of heat conservation is impaired. In rodents, the surface area of the furless tail accounts for a significant percentage of the total body surface area and is highly vascularized for regulation of blood flow and heat exchange (6). It is the major site for active regulation of heat exchange in rats and mice, and it serves an important role in maintaining homeothermy in thermoneutral as well as at extreme temperature environments. Cold-induced vasoconstriction of the tail is under tight sympathetic control, leading us to hypothesize that this regulation might be disrupted in senescent rats and contribute to their cold-induced hypothermia. Measurement of Ttail and calculation of the HLI are commonly used to evaluate vasomotor function. Using these methods, we found no evidence to suggest that tail vasoconstrictor responsiveness to cold exposure changed significantly with the transition to senescence. Ttail declined exponentially in parallel with the change in Tair and in a manner predicted for a tail with little or no surface blood perfusion. Moreover, the cooling curves resembled those generated in the rats before entry into senescence. These data indicate that norepinephrine-mediated vasoconstriction of the tail is intact in senescent rats. Thus, if an increased rate of heat loss contributes to the greater cold-induced hypothermia of senescent vs. presenescent rats, it occurs from other highly vascularized appendages such as the feet and ears, and/or across the dorsal body surface, which becomes more sparsely furred in senescence.

We have previously shown that the brown fat of senescent rats is reduced in mass (11) as well as in thermogenic capacity (5). The latter is evidenced by significantly lower levels of UCP-1 per brown adipocyte as well as significantly lower norepinephrine- and β3-adrenergic stimulation of brown adipocyte oxygen consumption (5). In contrast, β3-adrenergic stimulation of cAMP or of lipolysis is not altered in brown adipocytes isolated from senescent rats (5). These findings indicate that senescent brown adipocytes remain responsive to norepinephrine in vitro. The current study extends our previous conclusion to include in vivo signaling, demonstrating that cold-induced release of norepinephrine from sympathetic nerves innervating brown fat remains robust in the senescent rat.

One confounding issue in all studies of aging is “selective” mortality. We have considered the possibility that the lack of impaired norepinephrine release observed in the surviving senescent rats may not have occurred in the senescent rats that did not recover from the surgery required to implant the microdialysis fibers (i.e., that the nonsurvivors had impaired sympathetic activity). While we cannot eliminate this possibility, we note that the nonsurvivors of the microdialysis surgery had all been tested in experiment 1, where vasoconstrictor response was measured as a functional index of the cold-induced sympathetic release. Notably, none of the senescent rats that did not survive the microdialysis surgery had poorer cold tolerance (based on their responses to the cold tests before the surgery) than did the surviving senescent rats. This suggests that the rats used for measuring brown fat sympathetic activity were not more cold tolerant than those that did not survive the surgery.
Because cold exposure stimulates blood flow to brown fat, it is possible that some fraction of the measured tissue norepinephrine was derived from the circulation, either from adrenal-medullary release or from sympathetic neural stimulation of other tissues. Our previous studies and those of other investigators do show significant cold-induced elevations in circulating catecholamine levels in old rats as well as in young rats (2, 4, 9). Nevertheless, locally released norepinephrine is likely to be much higher than that in the circulation during cold exposure, favoring spillover from brown fat into the blood, rather than transfer from blood to brown adipocytes. Supporting this view is the work of Gronlund et al. (7), who used microdialysis to measure norepinephrine release in forearm white adipose tissue during cold exposure of the limb. They concluded that norepinephrine in the microdialysate samples largely reflected local neuronal release with significantly less contribution from the general circulation. With these considerations, we believe that our microdialysis measurements of brown fat norepinephrine levels provide an accurate picture of the level of sympathetic activity to the tissue.

This study extends our knowledge about the physiological mechanisms underlying the poor cold tolerance of senescent rats and is a step in the exploration of how central and peripheral nervous system function might be altered with transition to senescence. Our studies have largely focused on brown fat thermogenesis as a model for studying the effects of age on neural and hormonal influences on peripheral tissue integrity and function and for identifying alterations in regulatory systems that might underlie senescence. In rodents, brown fat is highly adaptive, with neural/hormonal input modifying its thermogenic capacity and activity in response to the energy and thermal requirements of the animal. The marked decrease in brown fat thermogenic capacity, specifically the decreased amount of UCP-1 per adipocyte, that occurs in senescent rats is unexpected for an animal rapidly losing body weight and becoming more sensitive to cold. Transcriptional regulation of mitochondrial UCP-1 in mature brown adipocytes is highly influenced by norepinephrine derived from sympathetic innervation, although other factors contribute as well. While our present study shows that sympathetic activity to brown fat can be acutely activated by cold exposure in senescent rats, we do not know if there are any alterations in the circadian patterns/levels of brown fat sympathetic activity in the days before transition. Our microdialysis technique developed for this study would be a useful tool in answering this question.

In summary, our findings indicate that severe cold-induced hypothermia in senescent rats exhibiting rapid weight loss cannot be explained by blunted release of norepinephrine in brown adipose tissue or by blunted tail vasoconstrictor responsiveness to cold. Both the afferent and efferent components of the neural pathways connecting the periphery to the brain for detection of cold and activation of target tissues remain intact and functional in senescent rats.

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