Senescence alters blood flow responses to acute heat stress

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Kenney, Michael J., and Timothy I. Musch. Senescence alters blood flow responses to acute heat stress. Am J Physiol Heart Circ Physiol 286: H1480–H1485, 2004. First published December 11, 2003; 10.1152/ajpheart.00857.2003.—Renal and splanchnic sympathetic nerve discharge (SND) responses to heating are significantly reduced in senescent compared with young Fischer-344 (F344) rats (Kenney MJ and Fels RJ. Am J Physiol Regul Integr Comp Physiol 283: R513–R520, 2002). However, the functional significance of this finding is not known. We tested the hypothesis that blood flow distribution profiles to heating are altered in senescent (24 mo old) compared with mature (12 mo old) and young (3 mo old) F344 rats. Visceral organ, skeletal muscle, and tail blood flows were determined with the radionuclide-tagged microsphere technique before (control, 38°C) and during heating that increased body temperature to 41°C in anesthetized F344 rats. Vascular conductance in the kidney, stomach, large intestine, pancreas, spleen, and tail was significantly reduced during control before heating in senescent compared with young F344 rats. Heating significantly decreased kidney, stomach, small and large intestine, and pancreas vascular conductance in young and mature but not senescent F344 rats. Vascular conductance at 41°C in the kidney and small intestine was significantly lower and in the stomach tended to be lower in young compared with senescent rats. Splenic conductance increased during heating in young and senescent rats but was highest in young rats. Tail conductance during heating was significantly increased in young rats but remained unchanged in mature and senescent rats. These results demonstrate a marked attenuation in heating-induced vascular conductance changes in senescent rats, suggesting an important functional consequence for the attenuated SND responses to heating in aged rats.

Fischer-344 rats; microspheres; vascular conductance

ACUTE HEAT STRESS in young rats provokes a potent stimulus to visceral sympathetic nerve discharge (SND) as evidenced by hyperthermia-induced increases in renal, splenic, and splanchnic sympathetic nerve activity and changes in the SND bursting pattern (9, 11–15, 17). Aging alters sympathetic nerve responses to acute heat stress (13, 14). For example, renal and splanchnic SND responses to heating are significantly reduced in senescent compared with young Fischer-344 (F344) rats and increased internal body temperature (Tc) transforms the SND bursting pattern from cardiac-related to low-frequency bursts in young but not senescent F344 rats (13). These results demonstrate that the responsiveness of sympathetic neural circuits to hyperthermia is reduced in senescent F344 rats, but the functional implication of this finding is not known.

Because activation of the sympathetic nervous system in young rats plays an important role in mediating cardiovascular responses to hyperthermia (12, 16, 18) and because senescent rats demonstrate attenuated SND responses to increased Tc (13, 14), it may be that the blood flow distribution profile to hyperthermia is altered in senescent rats. Consistent with this notion, Cox et al. (5) reported increased internal and decreased tail temperature responses to acute heat stress in senescent compared with young rats, providing indirect evidence for attenuated tail vasodilatory responses to acute heating in aged rats. In addition, Stauss et al. (28) reported that renal blood flow is reduced during heating in 12- but not 24-mo-old F344 rats; however, renal blood flow data from young F344 rats were not reported. Although the results of these two studies support the idea that aging alters blood flow responses to heating, they are limited with respect to information about blood flow to multiple organs and tissues. To our knowledge, no previous study has thoroughly examined visceral and peripheral blood flow distribution profiles to heating in senescent, mature, and young F344 rats.

The purpose of the present study was to determine, using the radionuclide-tagged microsphere technique, visceral organ, skeletal muscle, and tail blood flows before (control, 38°C) and during acute heating that increased Tc to 41°C in young, mature, and senescent F344 rats. We tested the hypothesis that during heating senescent rats would redistribute less blood flow away from visceral organs and skeletal muscle beds and would demonstrate reduced perfusion of the tail compared with mature and young F344 rats. Findings supporting the hypothesis would suggest an important functional consequence of the attenuated renal and splanchnic SND responses to heating in senescent rats (13).

METHODS

General procedures. The Institutional Animal Care and Use Committee of Kansas State University approved the experimental procedures and protocols used in the present study, and all procedures were performed in accordance with the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings” (1). Blood flow experiments were performed on male 3- to 6-mo-old (young, 281 ± 4 g; n = 9), 12-mo-old (mature, 453 ± 9 g; n = 9), and 24-mo-old (senescent, 416 ± 9 g; n = 8) F344 rats. Ganglionic blockade experiments were completed in young (261 ± 16, n = 5) and senescent (414 ± 2, n = 4) F344 rats. Anesthesia was induced (3%) and surgical procedures were completed (1.5–2.5%) with isoflurane. A catheter (polyethylene-10 connected to polyethylene-240 catheter). Colonic temperature was maintained between 37.8 and 38.0°C during surgical procedures and protocols used in the present study, and all procedures were performed in accordance with the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings” (1). Blood flow experiments were performed on male 3- to 6-mo-old (young, 281 ± 4 g; n = 9), 12-mo-old (mature, 453 ± 9 g; n = 9), and 24-mo-old (senescent, 416 ± 9 g; n = 8) F344 rats. Ganglionic blockade experiments were completed in young (261 ± 16, n = 5) and senescent (414 ± 2, n = 4) F344 rats. Anesthesia was induced (3%) and surgical procedures were completed (1.5–2.5%) with isoflurane. A catheter (polyethylene-10 connected to polyethylene-50) was placed in the femoral vein for the administration of α-chloralose (initial dose 50 mg/kg, maintenance dose 35–50 mg/kg kg·h) during the heating intervention (11–13). The trachea was cannulated with a polyethylene-240 catheter. Colonic temperature was maintained between 37.8 and 38.0°C during surgical procedures by a temperature-controlled table.

Tissue blood flow determination. Catheters were placed in the right carotid artery and the medial caudal or femoral artery. The right carotid artery catheter was advanced toward the heart and secured in position just inside the aortic arch. The medial caudal or femoral

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artery catheter was advanced toward the descending aorta and secured in place. The carotid catheter was connected to a pressure transducer, and the medial caudal or femoral artery catheter was connected to a 1-ml syringe placed in a Harvard withdrawal pump. For each blood flow determination, blood withdrawal from the medial caudal or femoral artery catheter was initiated at a rate of 0.25 ml/min. At the same time, arterial blood pressure was recorded from the carotid artery catheter. Heart rate (HR) was derived from the pulsatile arterial pressure output of the blood pressure analyzer. After 30 s of blood withdrawal, the carotid artery catheter was disconnected from the pressure transducer and radioactive microspheres (~6–7 × 10^5 in number) were injected into the aortic arch. Labeled microspheres were 15 ± 3 μm in diameter. The microspheres were suspended in normal saline containing 0.01% Tween 80 with a specific activity ranging from 7 to 15 mCi/g. Before each injection, the microspheres were thoroughly mixed and agitated by sonication to prevent clumping. Microspheres were injected into the ascending aorta in a volume of ~0.10 ml, and the different radioactive labels (46 Sc, 85 Sr, 113 Sn, and 141 Ce) were used in random order. At the end of each experiment, the rat was killed with an overdose of methohexital sodium (150 mg/kg iv). The placement of each catheter was verified by anatomic dissection. The kidneys, organs of the splanchnic region, total hindlimb musculature (individual muscles and muscle parts), and tail were removed, blotted, weighed, and placed immediately into counting vials. The radioactivity of tissue samples was determined on a Packard Cobra II Auto-Gamma Spectrometer set to record the peak energy activity of each isotope for 5 min and were analyzed by computer, taking into account the cross-talk fraction between the different isotopes.

Tissue blood flow was calculated by the reference sample method (10) and expressed in milliliters per minute per 100 g of tissue. Adequate mixing of the microspheres was verified for each injection by demonstrating a <15% difference in blood flows to the right and left kidneys. Blood flow results were normalized to mean arterial pressure (MAP) and expressed as conductance (ml·min^{-1}·100 g^{-1}·mmHg^{-1}). Lautt (19) has argued that conductance better reflects changes in vascular tone in animal preparations where the experimental paradigm produces significant changes in blood vessel diameter that lead to changes in blood flow. In contrast, it appears that vascular resistance (resistance = 1/conductance) better reflects changes in vascular tone in constant-flow preparations where changes in vessel diameter lead to changes in the perfusion pressure gradient (19).

**Experimental protocols.** After completion of the initial surgical procedures (e.g., arterial and venous cannulations), the anesthetized rats were allowed to stabilize for 30 min. Tc was maintained at 38°C during the stabilization period. At the end of this period, baseline hemodynamic parameters (MAP and HR) and tissue blood flows were determined. Tc was then increased at a rate of 0.1°C/min from 38 to 41.0°C with a heat lamp (11–15). Hemodynamic parameters and tissue blood flows were determined for each animal during heating at Tc values of 40 and 41°C. The effect of ganglionic blockade on the level of MAP was determined by the intravenous administration of 10 mg/kg trimethaphan camsylate after Tc was elevated to 41°C in young (n = 5) and senescent (n = 4) F344 rats.

**Data analysis.** All values are means ± SE. Results were analyzed with analysis of variance techniques with a repeated-measures design (ANOVA-R) followed by statistical simple effects procedures. In addition, a priori comparisons of means between any two experimental groups were determined with the least significant difference test. P < 0.05 indicated statistical significance. Tail conductance was similar in rats in which the reference blood flow catheter was placed in either the medial caudal artery or the femoral artery. Therefore, results from these groups were combined for statistical analysis.

**RESULTS**

**MAP and HR responses to heating.** MAP measured at Tc of 38°C was significantly higher in mature compared with young and senescent rats (young 111 ± 5, mature 121 ± 7, senescent 109 ± 5 mmHg; Fig. 1A). MAP was significantly increased during heating in young and mature but not in senescent rats. MAP responses to heating at 40 and 41°C were reduced in senescent compared with mature and young rats (P < 0.05). Ganglionic blockade at 41°C reduced MAP from 166 ± 15 to 39 ± 5 mmHg in young rats (n = 5) and from 128 ± 13 to 34 ± 9 mmHg in senescent rats (n = 4).

HR measured at Tc of 38°C was significantly higher in young and mature compared with senescent rats (young 390 ± 8, mature 379 ± 9, senescent 348 ± 8 beats/min; Fig. 1B). HR was significantly increased during heating in young, mature, and senescent rats; however, HR responses to heating at 41°C were reduced in senescent compared with mature and young rats (P < 0.05) and in mature compared with young rats (P < 0.05).

**Heating-induced changes in tissue conductance.** Renal conductance measured at Tc of 38°C was significantly higher in young and mature compared with senescent rats (Fig. 2). Renal conductance during heating was decreased (P < 0.05) in young

![Fig. 1. Mean arterial pressure (A) and heart rate (B) measured before (38°C) and during progressive increases (40 and 41°C) in internal body temperature (Tc) in young, mature, and senescent Fischer-344 (F344) rats. Values at 38°C were considered as control. *Significantly different from 38°C; †significantly different from senescent rats; ‡significantly different from mature rats.](http://ajpheart.physiology.org/)
and mature rats but remained unchanged in senescent rats. At 41°C, renal conductance was significantly lower in young compared with senescent rats.

Stomach, large intestine, and pancreas conductance measured at Tc of 38°C were significantly higher in young and mature compared with senescent rats (Fig. 3). Small intestine conductance measured at Tc of 38°C was significantly higher in mature compared with senescent rats. Heating significantly reduced stomach, small intestine, large intestine, and pancreas conductance in young and mature rats but had no effect on the conductance measured for these organs in senescent rats (Fig. 3). At 41°C, small intestine conductance was significantly lower and stomach conductance tended to be lower (P < 0.07) in young compared with senescent rats.

Significant group differences in splenic conductance existed at Tc of 38°C and were maintained during heating (Fig. 4). Splenic conductance was increased (P < 0.05) during heating in young and senescent rats but was highest in young rats and lowest in senescent rats (Fig. 4).

Tail conductance measured at Tc of 38°C was significantly higher (P < 0.05) in young and senescent compared with mature rats and was higher in young compared with senescent rats (Fig. 5). Tail conductance during heating was significantly increased in young rats but remained unchanged in mature and senescent rats (P < 0.05). At 40 and 41°C, tail conductance was significantly higher in young compared with mature and senescent rats.

Total hindlimb muscle conductance measured at Tc of 38°C was similar in young, mature, and senescent rats (Table 1). Total hindlimb muscle conductance remained unchanged from control during heating in young, mature, and senescent rats (Table 1). Because the hindlimb muscles of the rat are heterogeneous in their fiber type composition and function (3), individual muscles or muscle parts of the posterior portion of the leg were examined. Vascular conductance in muscles...
containing a majority of fast-twitch glycolytic along with fast-twitch oxidative glycolytic (FOG) types of fibers (i.e., plantaris and the mixed and white portions of the gastrocnemius muscle) remained unchanged from control during heating in young, mature, and senescent rats (Table 1). In contrast, muscles containing a majority of slow-twitch oxidative and FOG types of fibers (i.e., soleus and the red portion of the gastrocnemius muscle) demonstrated a significant decrease in conductance during heating in young but not in mature or senescent F344 rats (Fig. 6).

**DISCUSSION**

This study determined visceral organ, skeletal muscle, and tail blood flow responses to acute heating in anesthetized young, mature, and senescent F344 rats. The current results provide experimental support for five observations supporting the hypothesis that aging alters blood flow distribution profiles to acute heat stress. First, heating significantly decreased visceral (kidney, stomach, small and large intestine, and pancreas) vascular conductance in young and mature but not senescent F344 rats. Second, conductance in the renal and small intestine vasculature was significantly lower and stomach conductance tended to be lower at 41°C in young compared with senescent rats. Third, tail conductance increased during heating in young but not mature or senescent rats. Fourth, splenic conductance increased during heating in young and senescent rats but was highest in young rats. Finally, conductance in the soleus and red portion of the gastrocnemius muscle was reduced during heating in young but not mature or senescent F344 rats, despite the fact that total hindlimb muscle conductance remained unchanged during heating.

The current results demonstrate a marked attenuation in the responsiveness of the visceral vasculature to acute heating in senescent compared with young rats. What factors may be responsible for the diminished responses? Three ideas are considered. First, the basal level of vascular conductance in senescent rats may represent the physiological minimum, that is, decreases in visceral vascular conductance to acute heating in aged rats may be limited by a basement effect. This is likely not the case, however, because conductance at 41°C in the small intestine and kidney was significantly lower and in the stomach tended to be lower (P < 0.07) in young compared with senescent rats. Second, anesthesia may selectively influence blood flow responses to heating in senescent rats. This does not appear to be the case because renal blood flow (conductance) remains unchanged during heating in conscious (28) and anesthetized (current study) senescent F344 rats. Finally, because renal and splanchnic SND responses to progressive increases in Tc are attenuated in senescent rats (13, 14), we speculate that the blunted visceral organ blood flow responses to acute heating in aged rats may be secondary, at least in part, to the reduced sympathetically mediated vasoconstriction. The current results do not exclude the possibility that visceral vascular conductance during heating in aged rats might

![Fig. 4. Spleenic conductance measured before (38°C) and during progressive increases (40 and 41°C) in Tc in young, mature, and senescent F344 rats. Values at 38°C were considered as control. *Significantly different from 38°C; †significantly different from senescent rats; ‡significantly different from mature rats.](http://ajpheart.physiology.org/)

**Table 1. Skeletal muscle conductance measured in young, mature, and senescent rats during total body heating**

<table>
<thead>
<tr>
<th>Muscle Type</th>
<th>38°C</th>
<th>40°C</th>
<th>41°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hindlimb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>0.098±0.018</td>
<td>0.085±0.012</td>
<td>0.098±0.014</td>
</tr>
<tr>
<td>Mature</td>
<td>0.084±0.016</td>
<td>0.073±0.015</td>
<td>0.075±0.014</td>
</tr>
<tr>
<td>Senescent</td>
<td>0.084±0.010</td>
<td>0.089±0.013</td>
<td>0.081±0.013</td>
</tr>
<tr>
<td>Plantaris</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>0.111±0.015</td>
<td>0.098±0.011</td>
<td>0.107±0.006</td>
</tr>
<tr>
<td>Mature</td>
<td>0.121±0.026</td>
<td>0.111±0.018</td>
<td>0.115±0.023</td>
</tr>
<tr>
<td>Senescent</td>
<td>0.128±0.028</td>
<td>0.133±0.026</td>
<td>0.108±0.030</td>
</tr>
<tr>
<td>Gastrocnemius, mixed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>0.097±0.013</td>
<td>0.103±0.009</td>
<td>0.105±0.010</td>
</tr>
<tr>
<td>Mature</td>
<td>0.085±0.014</td>
<td>0.087±0.013</td>
<td>0.087±0.014</td>
</tr>
<tr>
<td>Senescent</td>
<td>0.130±0.015</td>
<td>0.126±0.017</td>
<td>0.104±0.017</td>
</tr>
<tr>
<td>Gastrocnemius, white</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>0.110±0.011</td>
<td>0.086±0.006</td>
<td>0.094±0.013</td>
</tr>
<tr>
<td>Mature</td>
<td>0.091±0.014</td>
<td>0.086±0.015</td>
<td>0.095±0.015</td>
</tr>
<tr>
<td>Senescent</td>
<td>0.125±0.020</td>
<td>0.140±0.060</td>
<td>0.114±0.023</td>
</tr>
</tbody>
</table>

Values (in ml·min⁻¹·100g⁻¹·mmHg⁻¹) are means ± SE.

![Fig. 5. Tail conductance measured before (38°C) and during progressive increases (40 and 41°C) in Tc in young, mature, and senescent F344 rats. Values at 38°C were considered as control. *Significantly different from 38°C; †significantly different from senescent rats; ‡significantly different from mature rats.](http://ajpheart.physiology.org/)
findings. Considering that previous studies have shown that there is less redistribution of blood flow away from the renal and splanchnic circulations during heating in aged men (21), the present results suggest that the senescent rat may be a useful model for studying the mechanisms that contribute to altered cardiovascular regulation during heating in aged humans. It is known that vasodilation of the tail plays an important thermoregulatory role in the rat (26, 31). In the present investigation, tail conductance during heating was reduced in senescent compared with young rats, consistent with the observation that older men direct less blood flow to the skin during heating than do their young counterparts (21). Of course, the use of any animal model to study questions related to human physiology has numerous limitations. For example, it is known that sweating is a primary thermoregulatory effector in humans (8) but not in rats (26, 31); however, this does not negate the similarities in blood flow distribution profiles to heating found in these species.

Acute heat stress in young rats increases the level of splenic SND and changes the pattern of SND bursts (12), indicating prominent heating-induced splenic sympathoexcitation. Contrary to what was expected, splenic conductance increased during heating in young as well as senescent F344 rats. In contrast to other visceral organs, little information is known about the role of the sympathetic nervous system in regulation of splenic blood flow in the rat (27); therefore, we can provide little insight into mechanisms modulating splenic blood flow alterations during heating.

The present results demonstrate that conductance in the soleus and red portion of the gastrocnemius (B) muscles before (38°C) and during progressive increases (40 and 41°C) in Tc in young, mature, and senescent F344 rats. Values at 38°C were considered as control. *Significantly different from 38°C; †significantly different from senescent rats; ‡significantly different from mature rats.

be maintained in part by other vasoconstrictors and/or the reduced efficacy of endogenous vasodilators. However, the fact that pharmacological blockade of ganglionic neurotransmission at a Tc of 41°C produced an immediate and significant reduction in MAP to values in the 30- to 40-mmHg range demonstrates a primary role for the sympathetic nervous system in arterial blood pressure regulation during hyperthermia in young and senescent rats.

Aging alters cardiovascular responses to heating in human subjects (2, 21). Compared with young subjects, aged men demonstrate reduced cardiac output and stroke volume, attenuated cardiac inotropic function, reduced skin blood flow, and less redistribution of blood flow from the splanchnic and renal circulations in response to direct passive heating (21). These findings suggest a reduction in sympathetic nerve responsiveness to heating in aged humans; however, direct recordings of SND during heating in human subjects have not been completed. As stated above, Kenney and Fels (13, 14) reported reduced responsiveness of sympathetic neural circuits to heating in senescent compared with young F344 rats, but these investigators did not measure visceral organ blood flow responses to heating, limiting the functional implication of their findings.
to anesthesia. Because sympathetic and hemodynamic responses to heating demonstrate some degree of similarity in conscious and anesthetized F344 rats and because anesthetized rats were used in previous studies examining SND responses to heating in young, mature, and senescent F344 rats (13, 14), we chose to use anesthetized animals in the present investigation. In addition, physiological responses to increased Tc can be altered by behavioral modifications (4); therefore, we chose to study blood flow responses to heating in anesthetized rats to eliminate this influence. Finally, further understanding of mechanisms mediating altered sympathetic and hemodynamic responses to heating in senescent rats will require the completion of experiments examining central regulation of SND, studies that will likely involve the use of an anesthetized preparation. Concerning this point, Kenney and Fels (14) recently used anesthetized, decerebrate rats to demonstrate that aging alters the functional organization of pathways regulating SND and MAP responses to acute heating.

Much controversy exists concerning the effect of age on resting tissue blood flow (or conductance). For example, similar levels of renal blood flow have been observed in conscious young, mature, and senescent rats (7, 20, 29, 30). In contrast, in the present study resting renal conductance was significantly reduced in senescent compared with young anesthetized rats, consistent with previously published results of Musch et al. (24) in conscious rats and Tuma et al. (30) in anesthetized rats. With regard to splanchic blood flow, several studies showed that splanchic blood flow remains relatively constant during the aging process (7, 20, 30). Consistent with these findings, in the present study resting vascular conductance in the small intestine was similar in senescent and young F344 rats, although resting vascular conductance in the stomach, large intestine, and pancreas was lower in senescent compared with young rats. Unfortunately, the current results do not definitively allay any of the inconsistencies found in the literature.

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