AGING ALTERS REGULATION OF VISCERAL SYMPATHETIC NERVE RESPONSES TO ACUTE HYPOTHERMIA

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ABSTRACT

Hypothermia produced by acute cooling prominently alters sympathetic nerve outflow. Skin sympathoexcitatory responses to skin cooling are attenuated in aged compared with young subjects, suggesting that advancing age influences sympathetic nerve responsiveness to hypothermia. However, regulation of skin SND is only one component of the complex sympathetic nerve response profile to hypothermia. Whether aging alters the responsiveness of sympathetic nerves innervating other targets during acute cooling is not known. In the present study, utilizing multifiber recordings of splenic, renal, and adrenal sympathetic nerve activity, we tested the hypothesis that hypothermia-induced changes in visceral SND would be attenuated in middle-aged and aged compared with young F344 rats. Colonic temperature (Tc) was progressively reduced from 38°C to 31°C in young (3-6 month-old), middle-aged (12-month-old), and aged (24-month-old) baroreceptor-innervated and sinoaortic-denervated (SAD), urethane-chloralose anesthetized, Fischer 344 (F344 rats). The following observations were made. 1) Progressive hypothermia significantly (p<0.05) reduced splenic, renal, and adrenal SND in young baroreceptor-innervated F344 rats. 2) Reductions in splenic, renal, and adrenal SND to progressive hypothermia were less consistently observed, and when observed, were generally attenuated in baroreceptor-innervated middle-aged and aged compared with young F344 rats. 3) Differences in splenic, renal, and adrenal SND responses to reduced Tc were observed in SAD young, middle-aged, and aged F344 rats, suggesting that age-associated attenuations in SND responses to acute cooling are not the result of age-dependent modifications in arterial baroreflex regulation of SND. These findings demonstrate that advancing chronological age alters the regulation of visceral SND responses to progressive hypothermia, modifications that may contribute to the inability of aged individuals to adequately respond to acute bouts of hypothermia.
INTRODUCTION

Changing the level of activity in sympathetic nerves in response to acute environmental stress is an important strategy used by mammals to maintain physiological homeostasis. It is well established that acute cold stress prominently alters sympathetic nerve outflow. For example; spinal cord cooling decreases splanchnic and cardiac SND and increases cutaneous SND in decerebrate rabbits (16), whole-body hypothermia increases cervical SND in anesthetized rabbits (17) and increases lumbar SND but decreases renal SND in anesthetized, young Sprague-Dawley rats (20). Local skin and systemic cooling increase skin SND (32) whereas local cooling of the forehead, hand and mouth and cooling of the bronchial system via inhalation of cold air increase muscle SND (11) in human subjects.

Aging alters skin sympathetic nerve responses to hypothermia (8). Increases in skin SND in response to reduced skin temperature are significantly attenuated in aged compared with middle-aged and young human subjects (8). In contrast, skin SND responses to acoustically-elicited arousal are similar in young, middle-aged and aged human subjects (8), suggesting selective age-related impairment of skin SND responses to a thermal challenge. Regulation of skin SND, however, is only one component of the complex and highly differentiated sympathetic nerve response profile that is evident during progressive hypothermia. Although skin sympathoexcitatory responses are attenuated in aged compared with young subjects during reductions in skin temperature (8), the influence of age on the responsiveness of sympathetic nerves innervating other targets during decreased internal body temperature is not known.

The first aim of the present study was to determine the effect of progressive reductions in colonic temperature (Tc) on renal, splenic, and adrenal sympathetic nerve activity in baroreceptor-innervated young (3-6 month-old), middle-aged (12-month-old), and aged (24-month-old) Fischer 344 (F344 rats). We hypothesized, based on the diminished skin SND
responses to reduced skin temperature in aged human subjects, that hypothermia-induced changes in visceral SND would be attenuated in middle-aged and aged compared with young F344 rats.

As the results reveal, SND responses to hypothermia are markedly attenuated in senescent and middle-aged compared with young baroreceptor-innervated F344 rats. In addition, mean arterial blood pressure was significantly reduced during hypothermia in young and aged baroreceptor-innervated rats and tended to be reduced during acute cooling in middle-aged rats. Because it is known that baroreflex regulation of renal SND is impaired in aged rats (15) and because afferent baroreceptor mechanisms can modulate SND responses of central origin, a second aim of this study was to determine SND responses to hypothermia in sinoaortic denervated (SAD) young, middle-aged, and aged F344 rats.

METHODS

General Procedures. The Institutional Animal Care and Use Committee 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 (1). Experiments were performed on male 3-6 month old, (young 299+11 g, n=26), 12-month old (middle-aged 430+9 g, n=21), and 24-month old (aged 398+7 g, n=20) F344 rats. Rats were obtained from the National Institute of Aging colony maintained by Harlan Sprague Dawley and housed on 12:12 hour light/dark cycle in a 24°C room. Every 3 months sentinel rats housed in the same rooms were tested for specific organ disease and serotological abnormalities. All tests were negative. Rats were housed locally for a minimum of 2 weeks before being used in experimental protocols.

Rats were anesthetized with isoflurane (during surgical procedures only; 3% induction followed by 1.5%-2.5%), α-chloralose (initial dose 80 mg/kg ip, maintenance dose of 35-45 mg/kg/hr iv), and urethane (800 mg/kg ip). The trachea was cannulated with a polyethylene-240
catheter. Arterial pressure (femoral) was monitored using a pressure transducer connected to a blood pressure analyzer. The pulsatile arterial pressure output of the blood pressure analyzer was used to derive heart rate. A thermistor probe was inserted 5 – 6 cm into the colon and used to measure colonic temperature (Tc). During surgical procedures a homeothermic blanket was used to maintain Tc between 37.8°C and 38.0°C.

**Neural Recordings.** Renal, adrenal and splenic sympathetic nerves were isolated from a lateral approach. Activity was recorded biphasically (bandpass 30-3000 Hz) from the central end of cut or distally crushed nerves using a platinum bipolar electrode. Nerves were covered with a silicone gel and filtered neurograms were routed to an oscilloscope and a nerve traffic analyzer for monitoring during the experiment and for subsequent data analysis. Nerve potentials were full-wave rectified, integrated (time constant 10 ms) and quantified as volts x seconds (V·s) (18, 19, 21, 22). The level of SND was corrected for background noise after ganglionic blockade (trimethaphan camsylate, 10-15 mg/kg iv) or nerve crush.

**Sinoaortic Denervation.** Bilateral denervation of the aortic arch was completed in anesthetized rats by cutting the superior laryngeal nerve near its junction with the vagus nerve and removing the superior cervical ganglion (26). Bilateral carotid sinus denervation was completed by removing the adventitia from the carotid sinus bifurcation (26). Sinoaortic denervation was considered complete by demonstrating loss of coherence between the arterial pulse and SND (25). Experiments were completed in SAD rats to eliminate the influence of baroreceptor afferent feedback mechanisms that may alter SND responses of central origin.

**Experimental Protocols.** Rats were allowed to stabilize for at least 60 minutes before experimental cooling protocols were initiated. Following the control period, use of the homeothermic heating blanket was discontinued and ice packs were placed in close proximity to the dorsal and ventral surfaces of the rat. The rate of cooling was controlled by altering the
position of the ice packs to maintain a reduction in Tc of ~0.1°C/min from 38.0°C to 31°C. Mean arterial pressure (MAP), heart rate (HR) and SND were recorded continuously before and during progressive reductions in Tc. At the end of each experiment rats were euthanized by administration of an overdose (150 mg/kg iv) of methohexital sodium (Brevital).

**Data Analysis.** Values are means ± SE. SND data are expressed as percentage change from baseline. Values recorded at 38°C were considered as control. Results were analyzed using analysis of variance techniques with a repeated-measures-design followed by Bonferroni post-hoc tests. Significance for all tests was p<0.05.

**RESULTS**

The rate of decrease in Tc during cooling did not differ between baroreceptor-innervated young (9.3±0.2 min/°C), middle-aged (9.4±0.2 min/°C), and aged (9.1±0.3 min/°C) F344 rats. Control levels of MAP (young, 106±3 mmHg; middle-aged, 103±4 mmHg; aged, 83±4 mmHg) in baroreceptor-innervated rats were higher (p<0.05) in young and middle-aged rats compared with aged rats. Control levels of HR (young, 421±8 bpm; middle-aged, 371±5 bpm; aged, 337±7 bpm) were higher (p<0.05) in baroreceptor-innervated young compared with their middle-aged and aged counterparts. The rate of decrease in Tc during cooling did not differ between SAD young (9.4±0.4 min/°C), middle-aged (10.2±0.6 min/°C), and aged (9.6±0.5 min/°C) rats. Control levels of MAP (young, 102±3 mmHg; middle-aged, 99±6 mmHg; aged, 92±4 mmHg) did not differ among SAD rats of any age. Control levels of HR (young, 441±9 bpm; middle-aged, 378±18 bpm; aged, 355±7 bpm) were higher (p<0.05) in SAD young compared with SAD middle-aged and aged rats.

Figure 1 summarizes changes from control for MAP and HR during progressive decreases in Tc from 38°C to 31°C in baroreceptor-innervated young (n=16), middle-aged (n=12), and aged (n=10) F344 rats. MAP was significantly decreased from control during
hypothermia in young and aged (asterisks denote statistically significant reductions for MAP in both young and aged rats) but not in middle-aged rats. Significant ANOVA main effects were demonstrated for MAP responses to cooling between aged and middle-aged rats with nearly significant (p<0.07) main effects for MAP responses to cooling between young and middle-aged rats. HR was significantly decreased from control during hypothermia in each group of baroreceptor-innervated rats (asterisks denote statistically significant reductions for HR in young, middle-aged, and aged rats). Significant ANOVA main effects were demonstrated for HR responses to cooling between young and middle-aged rats and between middle-aged and aged rats.

Figure 2 summarizes percent changes from control for splenic, renal, and adrenal SND during hypothermia in baroreceptor-innervated young, middle-aged, and aged F344 rats. Splenic SND was significantly reduced from control during hypothermia in young (n=7) and middle-aged (n=8) but not in aged (n=6) rats. Splenic SND did not differ between middle-aged and aged rats during hypothermia, but was significantly reduced in young compared with middle-aged and aged rats at 32°C and 31°C. Renal SND was significantly reduced from control during progressive hypothermia in young (n=10) and aged (n=9) but not in middle-aged (n=7) rats. Renal SND was significantly reduced in young compared with middle-aged rats at 32°C and 31°C. Adrenal SND was significantly reduced from control during progressive hypothermia in young (n=6), middle-aged (n=6), and aged (n=9) rats. Adrenal SND was significantly reduced in young compared with middle-aged and aged rats at 31°C.

Figure 3 summarizes changes from control for MAP and HR during hypothermia in SAD young (n=10), middle-aged (n=9), and aged (n=10) F344 rats. MAP was significantly decreased from control during hypothermia in young and middle-aged but not in aged rats. Significant ANOVA main effects were demonstrated for MAP responses to cooling between young and middle-aged rats and between young and aged rats. HR was significantly decreased from
control during hypothermia in young, middle-aged, and aged rats (asterisks denote statistically significant reductions for HR in young, middle-aged, and aged rats). Significant ANOVA main effects were demonstrated for HR responses to cooling between young and middle-aged rats and between young and aged rats.

Figure 4 summarizes percent changes from control for splenic, renal, and adrenal SND during hypothermia in SAD young, middle-aged, and aged F344 rats. Splenic SND was significantly reduced from control during hypothermia in young (n=7) but not in middle-aged (n=7) and aged (n=7) rats. Splenic SND was significantly reduced in young compared with middle-aged rats at 31°C and in young compared with aged rats at 34°C. The splenic sympathoinhibitory response to hypothermia in young rats was characterized by a rapid onset as Tc was decreased from 38 to 34°C, however, with additional cooling (Tc decreased from 34 to 31°C), splenic SND demonstrated no additional inhibition. Renal SND was significantly reduced from control during hypothermia in young (n=7) and middle-aged (n=7) rats at 35°C, 34°C, and 33°C, but returned towards control levels at 32°C and 31°C. Renal SND was significantly reduced from control during hypothermia in aged (n=5) rats at 32°C and 31°C. Renal SND was significantly reduced in young and middle-aged compared with aged rats at 35°C and 34°C. Adrenal SND was significantly reduced from control during hypothermia in young (n=4) and middle-aged (n=7) but not in aged (n=5) rats. Adrenal SND was significantly reduced in young compared with middle-aged and aged rats at 32°C and 31°C.

Figure 5 summarizes changes from control for MAP, HR, and SND (splenic, renal, and adrenal) during hypothermia in baroreceptor-innervated and SAD young, middle-aged, and aged F344 rats. Significant ANOVA main effects were demonstrated for MAP and HR responses to hypothermia in SAD compared with baroreceptor-innervated young rats. MAP and HR responses to hypothermia did not differ in baroreceptor-innervated and SAD middle-aged or aged rats. Baroreceptor-innervated and SAD young rats demonstrated similar splenic and renal
SND responses during reductions in Tc from 38º to 33ºC, however, with additional cooling (Tc at 32ºC and 31ºC), splenic and renal SND were significantly lower in baroreceptor-innervated compared with SAD young rats. Adrenal sympathoinhibitory responses to hypothermia did not differ in baroreceptor-innervated and SAD young rats. Splenic, renal and adrenal SND responses to hypothermia did not differ in baroreceptor-innervated and SAD middle-aged rats, although renal SND responses to hypothermia tended to be lower in SAD middle-aged rats. Splenic and renal SND responses to hypothermia did not differ in baroreceptor-innervated and SAD aged rats, however, adrenal SND was significantly lower in baroreceptor-innervated compared with SAD aged rats at 31ºC.

DISCUSSION

We present three new findings concerning the effect of age on regulation of visceral SND during progressive hypothermia in anesthetized F344 rats. First, decreases in Tc from 38ºC to 31ºC significantly reduced splenic, renal, and adrenal SND in young baroreceptor-innervated F344 rats. Second, hypothermia-induced reductions in visceral SND were less consistently observed, and when observed, were often attenuated in baroreceptor-innervated middle-aged and aged compared with young F344 rats. Third, age-related differences in visceral SND responses to progressive hypothermia were evident in baroreceptor-denervated young, middle-aged, and aged F344 rats. These findings support the hypothesis that aging alters the regulation of visceral SND responses to progressive hypothermia.

Acute reductions in peripheral and internal body temperatures produce marked changes in the level of sympathetic nerve activity (8, 16, 20). Skin cooling increases skin SND in young human subjects (8), spinal cord cooling increases cutaneous SND but decreases splanchnic and cardiac SND in decerebrate rabbits (16), and whole-body cooling increases lumbar SND but decreases renal SND in young Sprague-Dawley rats (20). Increases in skin SND in
response to reduced skin temperature are attenuated in aged compared with young human subjects (8), demonstrating age-associated changes in skin sympathetic nerve responsiveness to peripheral cooling. Whether this effect is specific to skin SND or represents a more global age-associated alteration in sympathetic nerve regulation to hypothermia has not been established. In the current study we found that splenic, renal, and adrenal SND were progressively and significantly reduced during whole-body cooling in baroreceptor-innervated young F344 rats. With advancing age, however, SND responses to whole-body cooling were more heterogeneous and not as robust. Splenic and adrenal SND, but not renal SND, were significantly reduced during hypothermia in middle-aged rats, and the splenic and adrenal sympathoinhibitory responses to cooling were significantly attenuated in middle-aged compared with young rats. In aged rats, renal and adrenal SND, but not splenic SND, were significantly reduced during hypothermia, and the adrenal sympathoinhibitory response was significantly attenuated in aged compared with young rats. These results indicate that visceral SND responses to hypothermia are modified with advanced chronological age. In addition, reductions in heart rate to hypothermia were significantly attenuated in baroreceptor-innervated middle-aged compared with young F344 rats, and in SAD middle-aged and aged compared with young F344 rats, indicating that advanced chronological age influences the magnitude of hypothermia-induced bradycardic responses.

Aging impairs renal SND responses to unloading of the arterial baroreceptors in rats (15) and beagles (9, 10), whereas in human subjects the effect of age on SND responses to baroreceptor unloading remains somewhat controversial (3, 4, 35). Because in the current study mean arterial blood pressure was significantly reduced during hypothermia in young and aged baroreceptor-innervated rats, and because afferent baroreceptor mechanisms can influence SND responses of central origin, we determined sympathetic nerve responses to hypothermia in SAD young, middle-aged, and aged rats. Splenic, renal, and adrenal SND were
significantly reduced during whole-body cooling in SAD young F344 rats, whereas responses in SAD middle-aged and aged rats were more heterogeneous and less robust. Renal and adrenal but not splenic SND were significantly reduced during hypothermia in SAD middle-aged rats, and the adrenal sympathoinhibitory response to cooling was significantly attenuated in SAD middle-aged compared with SAD young rats. In SAD aged rats, renal SND, but not splenic or adrenal SND, was significantly reduced during hypothermia, and the renal sympathoinhibitory response was significantly attenuated in SAD aged compared with SAD young rats. Together, these data suggest that age-related differences in SND responses to hypothermia are not solely a function of age-dependent alterations in arterial baroreflex regulation of SND. As previously reported by Sabharwal et al. (31), acute hypothermia alters baroreflex regulation of heart rate and renal SND in anesthetized rats. Consistent with this observation, the current findings indicate that cardiovascular and SND responses to hypothermia are not identical in baroreceptor-innervated and SAD F344 rats, especially young rats. Hypothermia-induced reductions in mean arterial blood pressure and heart rate were augmented in SAD compared with baroreceptor-innervated young rats. In addition, although splenic and renal SND responses to cooling were similar in baroreceptor-innervated and SAD young rats until Tc reached 34°C, further reductions in Tc produced heterogeneous changes in splenic and renal SND in baroreceptor-innervated and SAD rats. These data indicate that the integrity of afferent baroreflex pathways affects cardiovascular and SND response profiles to hypothermia in young F344 rats.

Why study the effect of age on SND regulation to hypothermia? Undoubtedly, physiological responses to hypothermia are of great concern to aged and senescent individuals as these populations are characterized by reduced appetite (2, 14), attenuated brown adipose tissue thermogenesis (28, 29, 33), diminished metabolic rate (27), enhanced heat loss, and insensitivity to the cold (27, 29). In addition, aged individuals have a decreased ability to
maintain body temperature via the primary responses of shivering and vasoconstriction during acute hypothermia (39, 40), and demonstrate altered skin sympathetic nerve and vasomotor responses to reductions in peripheral and internal body temperatures. Specifically, reflex limb vasoconstrictor responses to reduced ambient temperature are attenuated in aged subjects (23) an effect that is partially related to decreased norepinephrine-mediated vasoconstriction (36, 37). In addition, a substantial component of the limb vasoconstrictor response in young subjects is mediated via sympathetic adrenergic cotransmitters, an effect that is absent in aged subjects (38). The current findings extend the current knowledge concerning the effect of age on physiological responses to hypothermia by demonstrating age-associated modifications in visceral SND responses to acute cooling; an effect that may contribute to the dysregulation of internal body temperature in aged subjects during acute cold stress.

There are at least four limitations to the present study. Anesthesia may influence SND responses to whole-body hypothermia. Although this cannot be entirely discounted, the present experiments were completed in chloralose-urethane anesthetized rats, an anesthetic regimen used widely in studies concerned with regulation of the sympathetic nervous and cardiovascular systems. Moreover, behavioral modifications can alter SND responses to thermal challenges; therefore, we studied SND regulation to hypothermia in anesthetized rats to eliminate this possibility. Differences in body weight between young, middle-aged, and aged rats may play a role in mediating age-related differences in SND responses to hypothermia; however, this seems unlikely because the rate of decrease in colonic temperature during acute cooling did not differ between baroreceptor-innervated young, middle-aged, and aged F344 rats or between baroreceptor-denervated young, middle-aged, and aged F344 rats. The sympathetic nervous system is capable of producing regionally-selective changes in efferent sympathetic nerve outflow (12, 16, 20, 30) therefore, the present findings are only applicable to splenic, renal and adrenal SND. The present study focused on the effect of age on hypothermia-induced changes
in the level of activity in regionally-selective sympathetic nerves, therefore, the current results must be considered separate from the well-established literature demonstrating that aging alters epinephrine release (5) and clearance (5-7), cold-induced physiological responsiveness (13, 23, 24, 34, 36, 37, 39), and co-transmitter-mediated vasoconstriction (34, 38).
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FIGURE LEGENDS

Figure 1. Mean arterial pressure (MAP) and heart rate (HR) responses during progressive hypothermia (colonic temperature, Tc, reduced from 38°C to 31°C) in young, middle-aged, and aged baroreceptor-innervated rats. Brackets indicate significant ANOVA main effects between middle-aged and aged rats for MAP, and between young and middle-aged and middle-aged and aged rats for HR. *MAP significantly different from 38°C at the designated Tc for young and aged rats at p<0.05. *HR significantly different from 38°C at the designated Tc for young, middle-aged, and aged rats at p<0.05.

Figure 2. Percent changes in splenic, renal, and adrenal sympathetic nerve discharge (SND) during progressive hypothermia (colonic temperature, Tc, reduced from 38°C to 31°C) in young, middle-aged, and aged baroreceptor-innervated rats. *Significantly different from 38°C at p<0.05. ‡Young significantly different from middle-aged and aged at p<0.05. †Young significantly different from middle-aged at p<0.05.

Figure 3. Mean arterial pressure (MAP) and heart rate (HR) responses during progressive hypothermia (colonic temperature, Tc, reduced from 38°C to 31°C) in young, middle-aged, and aged baroreceptor-denervated (SAD) rats. Brackets indicate significant ANOVA main effects between young vs middle-aged and young vs aged rats for MAP and HR. *MAP significantly different from 38°C at p<0.05. *HR significantly different from 38°C at the designated Tc for young, middle-aged, and aged rats at p<0.05.
Figure 4. Percent changes in splenic, renal, and adrenal sympathetic nerve discharge (SND) during progressive hypothermia (colonic temperature, Tc, reduced from 38°C to 31°C) in young, middle-aged, and aged baroreceptor-denervated rats. *Significantly different from 38°C. $Young significantly different from aged at p<0.05. †Young significantly different from middle-aged at p<0.05. #Young and middle-aged significantly different from aged at p<0.05. ‡Young significantly different from middle-aged and aged at p<0.05.

Figure 5. Mean arterial pressure (MAP), heart rate (HR), and splenic, renal and adrenal sympathetic nerve discharge (SND) responses during progressive hypothermia (colonic temperature, Tc, reduced from 38°C to 31°C) in baroreceptor-innervated and baroreceptor-denervated (SAD) rats. Brackets indicate significant ANOVA main effects between baroreceptor-innervated and SAD young rats for MAP and HR at p<0.05. Vertical lines indicate significance between baroreceptor-innervated and baroreceptor-denervated rats at the designated Tc at p<0.05.
Fig. 2

- Splenic SND (%Δ)
- Renal SND (%Δ)
- Adrenal SND (%Δ)

Tc (°C)

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Young
Middle Aged
Aged