Is tonic sympathetic vasoconstriction increased in the skeletal muscle vasculature of aged canines?

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DeLorey DS, Buckwalter JB, Mittelstadt SW, Anton MM, Kluess HA, Clifford PS. Is tonic sympathetic vasoconstriction increased in the skeletal muscle vasculature of aged canines? Am J Physiol Regul Integr Comp Physiol 299: R1342–R1349, 2010. First published August 11, 2010; doi:10.1152/ajpregu.00194.2010.—We tested the hypothesis that tonic adrenergic and nonadrenergic receptor-mediated sympathetic vasoconstriction would increase at rest and during exercise with advancing age. Young (n = 6; 22 ± 1 mo; means ± SE) and old (n = 6; 118 ± 9 mo) beagles were studied. Selective antagonists for alpha-1, alpha-2, neuropeptide Y (NPY), and purinergic (P2x) receptors were infused at rest and during treadmill running at 2.5 mph and 4 mph with 2.5% grade. Prazosin caused similar increases in vascular conductance in young and old beagles at rest (Young: 158 ± 34%; Old: 98 ± 19%) and during exercise at 2.5 mph (Young: 80 ± 10%; Old: 58 ± 12%) and 4 mph and 2.5% grade (Young: 57 ± 5%; Old: 26 ± 4%). Rauwolscine caused similar (P > 0.05) increases in vascular conductance in old compared with young dogs at rest (Young: 119 ± 25%; Old: 64 ± 22%) and at 2.5 mph (Young: 86 ± 13%; Old: 60 ± 7%) and 4 mph with 2.5% grade (Young: 61 ± 5%; Old: 43 ± 7%). N2-(diphenylacetyl)-N-[4-hydroxyphenyl)methyl]-d-arginine amide (BIBP) caused a smaller increase (P < 0.05) in vascular conductance in old compared with young dogs at rest (Young: 179 ± 44%; Old: 91 ± 22%), whereas similar increases (P > 0.05) of experimental limb vascular conductance in young and old dogs occurred following BIBP during exercise at 2.5 mph (Young: 56 ± 16%; Old: 50 ± 12%) and 4 mph and 2.5% grade (Young: 45 ± 10%; Old: 25 ± 7%). Pyridoxal-phosphate-6-azephyrin-2’-4’-disulfonic acid infusion produced a larger increase in vascular conductance in old compared with young beagles at rest (Young: 88 ± 14%; Old: 191 ± 58%), whereas similar increases were observed at 2.5 mph (Young: 47 ± 18%; Old: 31 ± 11%) and 4 mph with 2.5% grade (Young: 26 ± 13%; Old: −18 ± 8%). At rest, NPY receptor-mediated restraint of skeletal muscle blood flow was reduced with advancing age, whereas P2x receptor-mediated restraint of skeletal muscle blood flow was increased. During exercise, the magnitude of adrenergic and nonadrenergic sympathetic vasoconstriction was not different between young and old dogs. Overall, these data demonstrate that adrenergic receptor-mediated vasoconstriction was not elevated at rest, but nonadrenergic sympathetic vasoconstriction was altered under basal conditions in aged beagles.

aging; sympathetic vasoconstriction; skeletal muscle; exercise

AGING IS ASSOCIATED WITH A DECLINE IN AERobic CAPACITY (12–14). An inability to augment cardiac output during exercise has been functionally linked to the reduced exercise tolerance with aging (1, 23, 36, 53). A blunted skeletal muscle blood flow response to dynamic exercise has also been reported in older humans and animals (1, 33, 37, 47, 48, 50). However, other studies have documented preserved skeletal muscle blood flow during exercise in aged humans (38, 40, 44, 49). Thus, the effect of aging on the control of skeletal muscle blood flow appears to be an unresolved issue in the scientific literature.

The observation of elevated efferent muscle sympathetic nerve activity in older adults at rest (16, 43) has led to the notion that augmented sympathetic vasoconstriction may blunt the blood flow response to exercise with aging. It should be recognized, though, that no direct measurements of sympathetic nerve activity to exercising muscle have been made in old animals or humans, and indirect evidence from norepinephrine (NE) spillover measurements is inconclusive. During moderate-intensity (50–60% of Vo2peak) cycling exercise, whole-body (39) and leg NE spillover (49) did not differ between young and old men. In another study, leg NE spillover was similar during mild- and moderate-intensity cycling, but it was increased at near-maximal intensity in older compared with younger endurance-trained men (50). These exercise data appear to be consistent with other studies that have documented similar increases in sympathetic outflow in old relative to young adults in response to physiological stressors such as hypoxia, thermal stress (cold-pressor test), and ischemic hand-grip (11, 42). Thus, the accumulated scientific evidence suggests that the sympathetic response to physiological stressors, such as exercise, is not increased in old compared with young adults.

The functional consequence of sympathetic efferent nerve activity in the skeletal muscle vascular bed is vasoconstriction. The literature on the effect of aging on tonic sympathetic vasoconstriction in the skeletal muscle vascular bed is limited and contradictory. In the human forearm, nonselective α-adrenergic receptor blockade produced a smaller increase in resting blood flow in old compared with young adults (15), suggesting that tonic α-adrenergic receptor-mediated vasoconstriction was reduced with age. In contrast, in the leg, nonselective α-adrenergic receptor blockade elicited greater increases in resting blood flow and vascular conductance in older adults, suggesting that tonic α-adrenergic receptor-mediated vasoconstriction was elevated with age (18). Not only is the effect of aging on resting α-adrenergic vasoconstriction unsettled, but previous studies have investigated the effect of aging on the magnitude of tonic α-adrenergic vasoconstriction during dynamic exercise.

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Traditionally, it was believed that sympathetic vasoconstriction was mediated entirely by NE binding to postsynaptic α1-adrenergic receptors. However, it is now recognized that NE also binds to postsynaptic α2-receptors and that the nonadrenergic neurotransmitters ATP and NPY contribute to sympathetic vasoconstriction through binding with purinergic (P2x) and neuropeptide Y (NPY) receptors on vascular smooth muscle (2, 4–7, 10, 20–22, 34, 35, 46, 51, 52, 54, 55). The type, pattern, and quantity of neurotransmitter released are sensitive to the frequency of neuron firing (2, 20, 22, 34, 35, 46, 54, 55). In particular, low-discharge frequencies favor ATP release followed by NE, whereas midrange discharge frequencies produce both ATP and NE release, and high discharge frequencies favor NPY release (21, 34, 46, 51). Our laboratory has previously documented (4–7) the presence of P2x and NPY-Y1 receptor-mediated tonic vasoconstriction in the skeletal muscle vascular bed of young dogs at rest and during exercise. A greater magnitude and frequency of basal muscle sympathetic activity in the old compared with the young may increase sympathetic cotransmitter release in the old, which would provide a proportionally greater role for nonadrenergic receptor-mediated sympathetic vasoconstriction in aged skeletal muscle. Thus, a thorough analysis of the effect of aging on tonic sympathetic vasoconstriction must take into account the effects of both adrenergic and nonadrenergic receptors, and the effect of aging on nonadrenergic receptor-mediated sympathetic vasoconstriction has yet to be investigated.

Therefore, the purpose of the present study was to investigate the effect of aging on adrenergic and nonadrenergic receptor-mediated sympathetic vasoconstriction in resting and contracting skeletal muscles. We hypothesized that aging would be associated with increased adrenergic and nonadrenergic receptor-mediated vasoconstriction at rest and during exercise.

METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the American Physiological Society’s Guiding Principles in the Care and Use of Animals. Young (n = 6; 22 ± 1) and old (n = 6; 118 ± 9 mo) beagles were selected for their willingness to run on a motorized treadmill and were chronically instrumented in a series of three sterile surgeries. For each surgery, anesthesia was induced with thiopental sodium (25 mg/kg; Genedia Pharmaceuticals, Irvine, CA). Animals were then intubated with a cuffed endotracheal tube, and a surgical level of anesthesia was maintained through mechanical ventilation with 1.5% isoflurane (Halocarbon Laboratories, River Edge, NJ), and 98.5% O2. Postoperatively, animals were given an analgesic for pain management (burowonoprine hydrochloride; 0.3 mg; Reckitt and Coleman, Kingston-upon-Hull, UK) and received antibiotics for 10 days, (cefazolin sodium, 500 mg twice a day; Apothecon, Prince- and Coleman, Kingston-upon-Hull, UK) and received antibiotics for 10 days, (cefazolin sodium, 500 mg twice a day; Apothecon, Prince- and Coleman, Kingston-upon-Hull, UK) and received antibiotics for 10 days, (cefazolin sodium, 500 mg twice a day; Apothecon, Prince-

To minimize changes in body temperature during the exercise sessions, laboratory temperature was maintained below 20°C for all experiments. For each experiment, the dog was brought to the laboratory and rested in a sling, while the flow probes were connected to a flowmeter (Transonic Systems) and a 20-gauge intravascular catheter (Insyte, Becton-Dickinson, Sandy, UT) was inserted retrogradely into the lumen of one carotid artery and attached to a solid-state pressure transducer (Abbott, North Chicago, IL) for measurement of arterial pressure.

To investigate the effect of aging on endothelium-dependent vasodilation ACh (0.1 µg bolus) was infused into the experimental hindlimb, while the animals rested quietly.

To investigate the effect of aging on tonic adrenergic and nonadrenergic receptor-mediated sympathetic vasoconstriction, four series of investigations (series 1–4) were completed.

Adrenergic, series 1 and 2. To investigate the effect of aging on tonic alpha-1 and alpha-2 adrenergic receptor-mediated vasoconstriction in skeletal muscle, selective alpha-1 (prazosin; 0.25 µg·ml⁻¹·min⁻¹; series 1) and alpha-2 (rauwolscine; 1.25 µg·ml⁻¹·min⁻¹; series 2) receptor antagonists were infused into the experimental hindlimb, while the animals rested quietly and while the dogs ran on a motorized treadmill at moderate (2.5 mph) and heavy (4 mph and 2.5% grade) exercise intensities. We determined the effectiveness and selectivity of prazosin and rauwolscine antagonism by infusing the selective alpha-1 receptor agonist (PE 0.1 µg·ml⁻¹·min⁻¹) and alpha-2 receptor agonist (clonidine; 1 µg·ml⁻¹·min⁻¹) prior to and 1 min after observation of the peak increase in limb blood flow with receptor antagonism.

Nonadrenergic, series 3 and 4. To investigate the effect of aging on tonic NPY-Y1 and P2x receptor-mediated vasoconstriction in skeletal muscle, selective NPY-Y1 (BIBP; 2.5 mg bolus; series 3) and P2x [pyridoxal-phosphate-6-azophenyl-2'-4'-disulfonic acid (PPADS); 40 mg bolus, series 4] receptor antagonists were infused into the experimental hindlimb, while the animals rested quietly and while they ran on a motorized treadmill at moderate (2.5 mph) and heavy (4 mph and 2.5% grade) exercise intensities. The effectiveness and selectivity of BIBP and PPADS antagonism were determined by infusions of the selective NPY Y1 receptor agonist [Leu³-Pro⁴] NPY (1 µg·ml⁻¹·min⁻¹) and P2x receptor agonist (α,β-methyleneadenosine 5'-triphosphate lithium salt; α,β-methylene ATP; 1 µg·ml⁻¹·min⁻¹) prior to and 1 min after observation of the peak increase in limb blood flow with receptor antagonism.

Pharmacological infusions. Each antagonist was infused at rest and at each exercise intensity on a separate day in random order; thus, each animal was brought to the laboratory on six occasions to complete series 1 and 2 and six additional occasions to complete series 3 and 4. Infusions during exercise took place after ~5 min of treadmill running. Small volumes of each drug (<1 ml) were infused, followed by a 3-ml saline flush. Each infusion was ~3–5 s in duration. The effect of the infusion vehicle (prazosin: 3 parts sterile water; 1 part polyethylene glycol; rauwolscine: saline; PPADS: saline; BIBP: saline) was determined in each animal at rest and during exercise. Vehicle infusions had no effect on hindlimb blood flow or systemic hemodynamics.

Data analysis. Arterial blood pressure and external iliac blood flow were recorded at 100 Hz directly to a computer with a Powerlab data acquisition system (ADInstruments, Castle Hill, Australia). Data were analyzed off-line to calculate the absolute and relative change in mean arterial pressure (MAP), experimental and contralateral (control) limb iliac blood flow, and iliac vascular conductance (iliac blood flow/mean arterial pressure) in response to intra-arterial infusions. For each infusion of antagonist, measurements were averaged over 30 s before the antagonist infusion (“Pre” values in tables). After the antagonist infusion, all variables were averaged over 5-s intervals, and the
highest 5-s average was chosen as the peak response. The percent change in vascular conductance was calculated as \( \frac{\text{Post} - \text{Pre}}{\text{Pre}} \times 100 \). Data for each drug and receptor type were analyzed separately. All data are presented as means ± SE. A P value of <0.05 was considered statistically significant.

**RESULTS**

As shown in Table 1, all of the antagonists markedly inhibited the reduction in blood flow elicited by the respective agonist, and there were no age-related differences in the effectiveness of the antagonists in the canine hindlimb. Baseline hemodynamics and responses to intra-arterial infusions of antagonists are presented in Table 2 (prazosin), Table 3 (rauwolscine), Table 4 (BIBP), and Table 5 (PPADS). For all trials, control and experimental limb blood flow and vascular conductance increased from rest \( (P<0.05) \) in an exercise intensity-dependent manner. Control and experimental limb blood flow and vascular conductance were not different between young and old dogs at rest or during exercise prior to drug infusion. The response to infusion of ACh was significantly attenuated in old compared with young dogs (Fig. 1).

**Series 1: alpha-1-adrenergic receptor-mediated restraint of skeletal muscle blood flow in young and old dogs.** Intra-arterial infusions of prazosin caused similar increases \( (P > 0.05) \) in experimental limb vascular conductance in young compared with the respective agonist before (pre) and after (post) administration of the corresponding receptor antagonist.

**Table 2. Baseline hemodynamics and vascular response to intra-arterial infusion of alpha-1 receptor antagonist**

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mmHg</th>
<th>Control Limb Blood Flow, ml/min</th>
<th>Experimental Limb Blood Flow, ml/min</th>
<th>Control Limb Conductance, ml·min⁻¹·mmHg⁻¹</th>
<th>Experimental Limb Conductance, ml·min⁻¹·mmHg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>117±4</td>
<td>112±1</td>
<td>56±8</td>
<td>61±5</td>
<td>154±27*</td>
</tr>
<tr>
<td>2.5 miles/h</td>
<td>129±3</td>
<td>124±3</td>
<td>232±29</td>
<td>209±29</td>
<td>352±39*</td>
</tr>
<tr>
<td>4 miles/h 2.5% grade</td>
<td>136±3</td>
<td>134±3</td>
<td>258±42</td>
<td>248±35</td>
<td>378±42*</td>
</tr>
<tr>
<td>Old</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>123±12</td>
<td>126±11</td>
<td>73±23</td>
<td>57±9</td>
<td>116±20*</td>
</tr>
<tr>
<td>2.5 miles/h</td>
<td>137±8</td>
<td>131±11</td>
<td>230±20</td>
<td>190±15</td>
<td>282±24*</td>
</tr>
<tr>
<td>4 miles/h 2.5% grade</td>
<td>153±10</td>
<td>156±10</td>
<td>279±15</td>
<td>242±23</td>
<td>308±26*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 indicates a significant difference from Pre value.
a small increase in vascular conductance was observed in young dogs. Despite these directionally different responses, the change in vascular conductance in response to PPADS infusion was not different (P > 0.05) between young and old beagles at 4 mph and 2.5% grade (Fig. 5). The amount of tonic vasoconstriction during infusion of PPADS was reduced (i.e., sympatholysis) during exercise at 2.5 mph and at 4 mph and 2.5% grade compared with rest (main effect of exercise intensity; P < 0.001).

**DISCUSSION**

The purpose of this study was to investigate the effect of aging on adrenergic and nonadrenergic receptor-mediated sympathetic vasoconstriction in resting and contracting skeletal muscle. The main findings of this study were that 1) skeletal muscle blood flow and MAP were similar between young and old dogs at rest and during exercise; 2) the magnitude of alpha-1 and alpha-2 adrenergic receptor-mediated restraint of skeletal muscle blood flow was not different in old compared with young beagles at rest or during dynamic exercise; 3) the magnitude of NPY-Y1 receptor-mediated restraint of skeletal muscle blood flow was reduced in old beagles at rest but similar in young and old dogs during dynamic exercise; 4) the magnitude of P3α receptor-mediated restraint of skeletal muscle blood flow was increased in old dogs at rest but not different in old compared with young beagles during dynamic exercise.

Skeletal muscle blood flow was similar between young and old beagles at rest and during treadmill exercise at 2.5 mph and 4 mph and 2.5% grade. Previous studies of the effects of aging on skeletal muscle blood flow in humans and animals have produced conflicting results. Consistent with the present findings, several other studies have reported no decline in skeletal muscle blood flow with aging (38, 40, 44, 49), whereas others have reported reduced blood flow to skeletal muscles at rest (16–18) and during exercise (1, 33, 37, 47, 48, 50). Whether the maintained skeletal muscle blood flow with aging in the present study reflects an inherent difference between experimental models of aging or lifestyle factors that affect vascular function cannot be discerned.

The decreased skeletal muscle blood flow with aging in other studies has been attributed to increased efferent sympathetic outflow (16) and elevated postsynaptic receptor responsiveness (19). Although there is good evidence of an age-related increase of sympathetic efferent nerve activity to resting muscle (16, 43), there is little experimental evidence of elevated efferent muscle sympathetic outflow during exercise in aged animals or humans (39, 49, 50). Aging-related changes in canine muscle sympathetic nerve activity have not yet been characterized. The only published data show that renal sympathetic nerve activity at rest was elevated in old compared with young dogs at similar carotid sinus pressures (32). It seems likely that there would be a similar age-related increase in sympathetic outflow to the canine skeletal muscle vascular bed; however, this has not been established experimentally, and the effect of exercise on the magnitude and pattern of sympathetic outflow in aged animals remains unknown.

Since infusion of selective adrenergic antagonists produced similar increases in vascular conductance at rest, we conclude that the magnitude of tonic alpha-1 and alpha-2 adrenergic receptor-mediated restraint of skeletal muscle blood flow was similar in old compared with young beagles at rest. Previous studies in humans have produced conflicting results. Nonselective \( \alpha \)-adrenergic receptor blockade produced a smaller increase in forearm blood flow in old compared with young adults, (15), suggesting that tonic \( \alpha \)-adrenergic-receptor-mediated vasoconstriction was reduced in older adults. In contrast,

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**Table 3. Baseline hemodynamics and vascular response to intra-arterial infusion of alpha-2 receptor antagonist**

<table>
<thead>
<tr>
<th>Group</th>
<th>Intensity</th>
<th>MAP, mmHg</th>
<th>Control Limb Blood Flow, ml/min</th>
<th>Experimental Limb Blood Flow, ml/min</th>
<th>Control Limb Conductance, ml·min⁻¹·mmHg⁻¹</th>
<th>Experimental Limb Conductance, ml·min⁻¹·mmHg⁻¹</th>
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<tbody>
<tr>
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<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Young</td>
<td>Rest</td>
<td>112±3</td>
<td>113±3</td>
<td>62±8</td>
<td>67±13</td>
<td>65±10</td>
</tr>
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<td></td>
<td>2.5 miles/h</td>
<td>121±5</td>
<td>117±3</td>
<td>195±31</td>
<td>198±23</td>
<td>171±25</td>
</tr>
<tr>
<td></td>
<td>4 miles/h 2.5% grade</td>
<td>130±5</td>
<td>125±6</td>
<td>252±36</td>
<td>266±37</td>
<td>227±26</td>
</tr>
<tr>
<td>Old</td>
<td>Rest</td>
<td>127±13</td>
<td>130±12</td>
<td>84±20</td>
<td>88±17</td>
<td>61±11</td>
</tr>
<tr>
<td></td>
<td>2.5 miles/h</td>
<td>132±7</td>
<td>132±7</td>
<td>225±33</td>
<td>255±33</td>
<td>171±14</td>
</tr>
<tr>
<td></td>
<td>4 miles/h 2.5% grade</td>
<td>137±6</td>
<td>135±6</td>
<td>320±37</td>
<td>338±49</td>
<td>232±32</td>
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</table>

Values are expressed as means ± SE. *P < 0.05 indicates a significant difference from Pre value.

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**Table 4. Baseline hemodynamics and vascular response to intra-arterial infusion of NPY-Y1 receptor antagonist**

<table>
<thead>
<tr>
<th>Group</th>
<th>Intensity</th>
<th>MAP, mmHg</th>
<th>Control Limb Blood Flow, ml/min</th>
<th>Experimental Limb Blood Flow, ml/min</th>
<th>Control Limb Conductance, ml·min⁻¹·mmHg⁻¹</th>
<th>Experimental Limb Conductance, ml·min⁻¹·mmHg⁻¹</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Young</td>
<td>Rest</td>
<td>120±2</td>
<td>116±7</td>
<td>74±12</td>
<td>87±17</td>
<td>61±7</td>
</tr>
<tr>
<td></td>
<td>2.5 miles/h</td>
<td>138±5</td>
<td>130±12</td>
<td>166±14</td>
<td>188±28</td>
<td>176±17</td>
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<td></td>
<td>4 miles/h 2.5% grade</td>
<td>143±5</td>
<td>134±9</td>
<td>212±20</td>
<td>230±22</td>
<td>222±24</td>
</tr>
<tr>
<td>Old</td>
<td>Rest</td>
<td>118±6</td>
<td>118±19</td>
<td>65±13</td>
<td>78±16</td>
<td>59±6</td>
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<td></td>
<td>2.5 miles/h</td>
<td>155±11</td>
<td>143±27</td>
<td>194±25</td>
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<td>4 miles/h 2.5% grade</td>
<td>155±9</td>
<td>156±25</td>
<td>311±32</td>
<td>305±37</td>
<td>222±34</td>
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</table>

Values are expressed as means ± SE. *P < 0.05 indicates a significant difference from Pre value.
Table 5. Baseline hemodynamics and vascular response to intra-arterial infusion of P2x receptor antagonist

<table>
<thead>
<tr>
<th>Group</th>
<th>Intensity</th>
<th>MAP, mmHg</th>
<th>Control Limb Blood Flow, ml/min</th>
<th>Experimental Limb Blood Flow, ml/min</th>
<th>Control Limb Conductance, ml·min⁻¹·mmHg⁻¹</th>
<th>Experimental Limb Conductance, ml·min⁻¹·mmHg⁻¹</th>
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<tr>
<td>Young</td>
<td>Rest</td>
<td>117 ± 4</td>
<td>74 ± 8</td>
<td>112 ± 9</td>
<td>0.64 ± 0.20</td>
<td>0.96 ± 0.07</td>
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<tr>
<td></td>
<td>2.5 miles/h</td>
<td>127 ± 3</td>
<td>184 ± 31</td>
<td>211 ± 29</td>
<td>1.48 ± 0.25</td>
<td>1.68 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>4 miles/h 2.5% grade</td>
<td>139 ± 7</td>
<td>267 ± 30</td>
<td>256 ± 41</td>
<td>1.92 ± 0.18</td>
<td>1.81 ± 0.24</td>
</tr>
<tr>
<td>Old</td>
<td>Rest</td>
<td>120 ± 9</td>
<td>80 ± 22</td>
<td>64 ± 20</td>
<td>0.69 ± 0.21</td>
<td>0.54 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>2.5 miles/h</td>
<td>141 ± 6</td>
<td>243 ± 20</td>
<td>186 ± 24</td>
<td>1.77 ± 0.16</td>
<td>1.32 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>4 miles/h 2.5% grade</td>
<td>156 ± 9</td>
<td>345 ± 54</td>
<td>232 ± 30</td>
<td>2.40 ± 0.50</td>
<td>1.48 ± 0.14</td>
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</table>

Values are expressed as means ± SE. *P < 0.05, indicates a significant difference from Pre value.

However, basal leg blood flow and vascular conductance were lower in old compared with young adults and nonspecific α-adrenergic blockade abolished the group differences between old and young (18). The present study demonstrated, for the first time, that there is tonic P2x and NPY-Y1 receptor-mediated vasoconstriction in skeletal muscle vasculature of old dogs. The magnitude of tonic NPY-Y1 receptor-mediated vasoconstriction was lower in old compared with young beagles at rest, suggesting that in older dogs, NPY makes a smaller contribution to the overall control of skeletal muscle vascular tone. P2x receptor-mediated vasoconstriction was increased in old compared with young dogs at rest. Overall, these data demonstrate that adrenergic receptor-mediated vasoconstriction was not elevated at rest, but nonadrenergic sympathetic vasoconstriction was lower in old compared with young beagles. The magnitude of tonic NPY-Y1 receptor-mediated vasconstriction was increased in old compared with young dogs at rest. Overall, these data demonstrate that adrenergic receptor-mediated vasoconstriction was not elevated at rest, but nonadrenergic sympathetic vasoconstriction was altered under basal conditions in aged beagles. NPY-Y1 receptor-mediated vasoconstriction was reduced in old beagles, whereas P2x receptor-mediated vasoconstriction was increased in old dogs. Potentially, age-related changes in the frequency of sympathetic nerve discharge may be responsible for the altered contribution of the nonadrenergic neurotransmitters ATP and NPY to the overall regulation of skeletal muscle vascular tone at rest in aged animals. Changes in the responsiveness of postsynaptic NPY and P2x receptors with aging may also be involved in the altered contribution of these neurotransmitters to resting vascular tone. Collectively, the similar magnitude of adrenergic receptor-mediated restraint of skeletal muscle blood flow in old and young dogs and the directionally opposite age-related changes in the contribution of NPY and P2x to tonic vasoconstriction are consistent with the maintenance of basal skeletal muscle vascular conductance with aging in the present study.

To our knowledge, the present study is the first to investigate the effect of aging on the magnitude of adrenergic and nonadrenergic receptor-mediated vasoconstriction in the skeletal muscle vascular bed during exercise. The results demonstrate a similar magnitude of alpha-1 and alpha-2 adrenergic receptor-mediated restraint of skeletal muscle blood flow in old and young dogs during dynamic exercise. Moreover, P2x and NPY-Y1 receptor-mediated vasoconstriction was not different between young and old dogs during exercise. These findings suggest that there is not an obligatory increase in adrenergic and/or nonadrenergic receptor-mediated sympathetic vasoconstriction during exercise as a function of aging. Interestingly, the age-related differences in nonadrenergic receptor-mediated vascular control under basal conditions were eliminated during exercise. This finding implies that the neural regulation of the circulation may differ at rest compared with exercise with aging. Advancing age may alter the pattern of sympathetic outflow and the relative contributions of different neurotransmitters.

Fig. 1. Percent change in experimental limb iliac vascular conductance in response to intra-arterial infusion of ACh (0.1 μg·bolus) at rest in young (open bars) and old (solid bars) beagles. Values are expressed as means ± SE. **P < 0.001 indicates a significant difference between young and old.

Fig. 2. Percent change in experimental limb iliac vascular conductance in response to intra-arterial infusion of the selective α1-adrenergic receptor antagonist prazosin (0.25 μg·ml⁻¹·min⁻¹) at rest and during treadmill running at 2.5 mph and 4 mph with 2.5% grade in young (open bars) and old (black bars) beagles. Absolute values for all hemodynamic variables prior to and following antagonist infusion are presented in Table 2. Values are expressed as means ± SE. †P < 0.001 indicates a main effect of exercise intensity.
mitters to overall sympathetic vasoconstriction under basal conditions; however, in response to physiological stress, such as exercise, where sympathetic outflow is increased several-fold above resting levels, the pattern and magnitude of sympathetic outflow appear to be similar in young and older dogs.

Experimental considerations. A major strength of the present experimental approach is the ability to study basic physiological mechanisms of vascular control in conscious dynamically exercising animals by delivering selective agonists and antagonists to a discrete vascular bed without altering blood pressure or blood flow in other vascular beds. The findings of the present study in young and old dogs were similar in several respects to previous studies from our laboratory in young mongrel dogs (5, 6, 8, 9). Consistent with those studies, local and systemic hemodynamic variables increased in a manner dependent on exercise intensity and evidence of tonic adrenergic and nonadrenergic sympathetic vasoconstriction was observed at rest and during exercise. The magnitude of sympathetic vasoconstriction has been shown to vary as a function of exercise intensity (3). A limited number of exercise intensities were used in the present study, and it is conceivable that the effect of aging on sympathetic vasoconstriction may be different at other intensities. It should also be recognized that infusion of pharmacological agents into the entire hindlimb does not enable determination of the relative contribution of different segments of the vascular tree to the vascular response. The findings of Musch et al. (40), who reported similar hindlimb blood flows in old and young rats running at the same absolute intensity, but an altered distribution of hindlimb limb flow toward type II muscles in aged animals, highlight the potential for differential vascular control in different vascular beds and segments of the vascular tree that could not be investigated with the present experimental approach.

The canine model has been used previously to investigate the effect of aging on the cardiovascular system (24–32). The mean lifespan of beagles is 12.6 yr (41). The aged dogs in the present study had a mean age of 9.8 yr and were, therefore, on average in the last quarter of their expected lifespan. Studies of the comparative longevity of dogs and humans indicate that a chronological age of ~10 years in beagles corresponds to a physiological age of ~66 years in humans (45). The vascular response to the infusion of ACh was also blunted in the aged dogs in the present study. Thus, the dogs used in the present study appear to be "old" in both chronological and physiological terms.

Because of the long lifespan of this experimental model, the experimental design was necessarily cross-sectional. The animals used in this study were raised in a temperature-controlled, enriched environment in which they were provided balanced

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**Fig. 3.** Percent change in experimental limb iliac vascular conductance in response to intra-arterial infusion of the α2-adrenergic receptor antagonist rauwolscine (1.25 μg·ml⁻¹·min⁻¹) at rest and during treadmill running at 2.5 mph and 4 mph with 2.5% grade in young (open bars) and old (black bars) beagles. Absolute values for all hemodynamic variables prior to and following antagonist infusion are presented in Table 3. Values are expressed as means ± SE.

**Fig. 4.** Percent change in experimental limb iliac vascular conductance in response to intra-arterial infusion of the selective NPY-Y1 receptor antagonist BIBP (2.5 mg bolus) at rest and during treadmill running at 2.5 mph and 4 mph with 2.5% grade in young (open bars) and old (black bars) beagles. Absolute values for all hemodynamic variables prior to and following antagonist infusion are presented in Table 4. Values are expressed as means ± SE. *P < 0.05 indicates a significant difference between young and old at rest. ‡P < 0.001 indicates a main effect of exercise intensity.

**Fig. 5.** Percent change in experimental limb iliac vascular conductance in response to intra-arterial infusion of the purinergic receptor antagonist PPADS (40 mg bolus) at rest and during treadmill running at 2.5 mph and 4 mph with 2.5% grade in young (open bars) and old (black bars) beagles. Absolute values for all hemodynamic variables prior to and following antagonist infusion are presented in Table 5. Values are expressed as means ± SE. *P < 0.05 indicates a significant difference between young and old. ‡P < 0.001, indicates a main effect of exercise intensity.
nutrition and regular veterinary supervision. Presumably, this lifestyle was associated with minimal behavioral stress and a decreased likelihood of disease related to negative lifestyle choices available to humans, and this may have had a protective effect of vascular function in the present study. Parker et al. (44) recently reported that leg vascular conductance was significantly higher during incremental exercise in older men with a \( V_{\text{O2max}} \) equal to or greater than 60% of age predicted compared with older men with a \( V_{\text{O2max}} \) below the 30th percentile for age group norms. These findings illustrate the potential positive impact of lifestyle choices that maintain functional capacity and vascular function.

In conclusion, this study demonstrated that basal and contractile vascular muscle blood flow does not inexorably decline as a function of age in beagles. Alpha-1 and alpha-2 adrenergic receptor-mediated restraint of resting skeletal muscle blood flow did not differ between young and old beagles. NPY-Y1 receptor-mediated vasoconstriction in resting skeletal muscle blood flow did not increase with aging, suggesting a decreased contribution of NPY and an increased contribution of ATP to the control of vascular tone in resting skeletal muscle of old dogs. During exercise, the magnitude of adrenergic and nonadrenergic receptor-mediated restraint of hindlimb skeletal muscle blood flow was not different in young and old beagles.

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