Aging impairs endothelium-dependent vasodilation in rat skeletal muscle arterioles

JUDY M. MULLER-DELP,1 SCOTT A. SPIER,1 MICHAEL W. RAMSEY,1 AND MICHAEL D. DELP1,2
Departments of 1Health and Kinesiology and 2Medical Physiology, Texas A&M University, College Station, Texas 77843

Received 18 February 2002; accepted in final form 18 June 2002

Muller-Delp, Judy M., Scott A. Spier, Michael W. Ramsey, and Michael D. Delp. Aging impairs endothelium-dependent vasodilation in rat skeletal muscle arterioles. Am J Physiol Heart Circ Physiol 283: H1662–H1672, 2002.—Blood flow capacity in skeletal muscle declines with age. Reduced blood flow capacity may be related to decline in the maximal vasodilatory capacity of the resistance vasculature. This study tested the hypothesis that aging results in impaired vasodilatory capacity of first-order (1A) arterioles isolated from rat-hindlimb locomotory muscle: 1A arterioles (90–220 μm) from gastrocnemius and soleus muscles of young (4 mo) and aged (24 mo) Fischer-144 rats were isolated, cannulated, and pressurized via hydrostatic reservoirs. Vasodilatory responses to increasing concentrations of ACh (10−9 to 10−4 M), adenosine (ADO, 10−9 to 10−4 M), and sodium nitroprusside (SNP, 10−9 to 10−4 M) were evaluated at a constant intraluminal pressure of 60 cmH2O in the absence of flow. Flow-induced vasodilation was also evaluated in the absence of pressure changes. Responses to ADO and SNP were not altered by age. Endothelium-dependent vasodilation induced by flow was significantly reduced in arterioles from both gastrocnemius and soleus muscles. In contrast, endothelium-dependent vasodilation to ACh was reduced only in soleus muscle arterioles. These results indicate that aging impairs vasodilatory responses mediated through the endothelium of resistance arterioles from locomotory muscle, whereas smooth muscle vasodilatory responses remain intact with aging. Additionally, ACh-induced vasodilation was altered by age only in soleus muscle arterioles, suggesting that the mechanism of age-related endothelial impairment differs in arterioles from soleus and gastrocnemius muscles.

acetylcholine; adenosine; nitric oxide; soleus; gastrocnemius; N\textsuperscript{6}-nitro-l-arginine methyl ester; indomethacin

EXERCISE CAPACITY AND MAXIMAL oxygen consumption decline with age (17, 40). This age-related decline in exercise capacity is due in part to a decrease in the ability of the cardiovascular system to provide oxygen to working muscles. In particular, a portion of the loss in the functional capacity of senescent individuals is known to be due to a diminished ability of the heart to elevate cardiac output during exercise, which results from aging-associated decreases in both maximum heart rate and stroke volume (15, 17, 29, 40).

Although changes in central cardiovascular control mechanisms clearly affect functional exercise capacity, it is also possible that a reduction in skeletal muscle blood flow capacity could limit exercise performance in the elderly. However, relatively little is known about the effects of aging on skeletal muscle blood flow capacity and/or age-related changes in vascular control mechanisms of skeletal muscle. Previous work in conscious dog and rat models has shown that muscle blood flow at rest is not changed in senescent animals (10, 19, 20, 24); however, blood flow to actively contracting skeletal muscle was found to be lower in old rats (24). In humans, Wahren et al. (47) showed that the rise in leg blood flow during exercise was decreased in older male subjects (52–59 yr) compared with the values measured in young male subjects (25–30 yr) in an earlier study (26). Proctor et al. (41) have also shown that leg blood flow and vascular conductance during submaximal cycling exercise at a given level of whole body oxygen consumption are substantially reduced in older men compared with their younger counterparts. Thus age-associated decrements in functional exercise capacity appear to be related in part to alterations in control of skeletal muscle blood flow.

The effects of age on local vasodilatory control mechanisms within the skeletal muscle resistance vasculature remain to be determined. Impaired endothelium-dependent vasodilation is one potential local control mechanism that may contribute to a reduction in skeletal muscle blood flow with advancing age. Although this possibility has not been explored in resistance arterioles from skeletal muscle, aging-induced impairment of endothelial function has been documented in conduit and resistance arteries from other vascular beds (7, 16, 18, 23, 27, 34, 46). The purpose of this study was to determine whether aging reduces vasodilatory responses of first-order (1A) arterioles isolated from the soleus muscle, which is composed predominantly of slow-twitch fibers (9), and the superficial portion of the gastrocnemius muscle, which is composed predominan-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: J. M. Muller-Delp, Dept. of Health and Kinesiology, Texas A&M Univ., College Station, TX 77843 (E-mail: jmd@hil.k.tamu.edu).
nantly of fast-twitch fibers (9). We specifically investigated responsiveness to both endothelium-dependent and -independent agents to determine whether aging alters vasodilatory mechanisms within the endothelium and/or the vascular smooth muscle. In addition, the effects of age on the contribution of nitric oxide (NO) and prostaglandins to endothelium-mediated vasodilation were determined.

**METHODS**

**Animals.** All procedures performed in this study were approved by the Texas A&M University Laboratory Animal Care Committee. All methods conformed to the National Research Council *Guide for the Care and Use of Laboratory Animals* (Washington, DC: National Academy Press, Revised 1996).

Male Fischer-344 rats [68 young (4 mo old), 59 aged (24 mo old)] were obtained from Harlan (Indianapolis, IN). The animals were housed in a temperature-controlled (23 ± 2°C) room with a 12:12-h light-dark cycle. Water and rat chow were provided ad libitum.

**Microvessel preparation.** The rats were anesthetized with pentobarbital sodium (60 mg/kg ip). The gastrocnemius-plan-teraris-soles muscle group from each hindlimb was carefully dissected free and placed in cold (4°C) physiological saline solution (PSS) that contained 145.0 mM NaCl, 4.7 mM KCl, 2.0 mM CaCl2, 1.17 mM MgSO4, 1.2 mM NaH2PO4, 5.0 mM glucose, 2.0 mM pyruvate, 0.02 mM EDTA, 3.0 mM MOPS buffer, and 1 g/100 ml BSA, pH 7.4. With the aid of a dissecting microscope (Olympus SVH10), 1A arterioles from the soleus muscle and the white portion of the gastrocnemius muscle were isolated and removed from the surrounding muscle tissue as described previously (35, 38). In soleus muscle, 1A arterioles were defined as the first branch that occurred after the feed artery had entered the muscle tissue. In gastrocnemius muscle, 1A arterioles were defined as the first branch off the feed artery that runs over the superficial portion of the muscle. The arterioles (length, 0.5–1.0 mm; inner diameter, 90–220 μm) were transferred to a Lucite chamber that contained PSS equilibrated with room air. Each end of the arteriole was cannulated with a micropipette and secured with nylon suture. After cannulation, the microvessel chamber was transferred to the stage of an inverted microscope (Olympus IX70) equipped with a videocamera (Panasonic BP310), video caliper (Microcirculation Research Institute), and data acquisition system (MacLab/Macintosh) for on-line recording of intraluminal diameter. Arterioles were initially pressurized to 60 cmH2O with two independent hydrostatic pressure reservoirs. Leaks were detected by pressurizing the vessel and then closing the valves to the reservoirs and verifying that intraluminal pressure remained constant. Arterioles that exhibited leaks were discarded. Arterioles that were free from leaks were warmed to 37°C and allowed to develop initial spontaneous tone during a 30- to 60-min equilibration period.

**Experimental design.** To determine whether aging altered the vasodilator responsiveness of the endothelium and/or vascular smooth muscle and the signaling mechanisms involved in altered responsiveness, two series of experiments were performed. In the first, vasodilatory responses to intraluminal flow, ACh, adenosine, and sodium nitroprusside (SNP) were evaluated. In the second series of experiments, vasodilatory responses to intraluminal flow and ACh were evaluated in the presence of NO synthase and cyclooxygenase inhibitors.

**Evaluation of vasodilatory responses to intraluminal flow.** After a steady level of spontaneous tone was displayed, arterioles were exposed to graded increases in intraluminal flow in the absence of changes in intraluminal pressure. This was accomplished by altering the heights of independent pressure reservoirs in equal and opposite directions so that the pressure difference was created across the vessel without alteration of the mean intraluminal pressure. Diameter measurements were determined in response to incremental pressure differences of 4, 10, 20, 40, and 60 cmH2O. In a subset of vessels, red blood cell velocity (V_fl) was measured at these pressure gradients. The vessels were cannulated on pipettes of similar inner tip diameter and resistance as those used in subsequent flow experiments. Volumetric flow (Q) was then calculated from mean V_fl and inner diameter (D) of these vessels according to the equation

\[ Q = \pi(V_{fl}/1.6)(D/2)^2 \]

Corresponding shear stress (τ) was then calculated from Q according to the equation

\[ \tau = 4\eta Q/R^3 \]

where η is viscosity (0.8 cP) and R is vessel radius.

**Evaluation of vasodilatory responses to pharmacological agents.** Concentration-response relationships to cumulative addition of ACh (10^-9 to 10^-4 M), SNP (10^-10 to 10^-4 M), and adenosine (10^-10 to 10^-4 M) were determined. These vasodilators were selected because they produce vasodilation through release of endothelial NO, direct activation of smooth muscle guanylate cyclase (donation of NO), and activation of smooth muscle adenylyl cyclase, respectively. The vessels were allowed to equilibrate between successive determinations of vasodilatory responses and were not included in analyses unless at least 20% baseline tone was present before beginning the addition of vasodilatory agents.

No more than three vasodilatory responses (including flow-induced vasodilation) were assessed in an individual vessel. After completion of the final concentration-response relationship, the vessel was washed twice in calcium-free PSS. This solution was similar to PSS-albumin solution except that it contained 2 mM EDTA and CaCl2 was replaced with 2.0 mM NaCl.

**Evaluation of inhibitory effects of NO^2^-nitro-l-arginine methyl ester and indomethacin.** Arterioles were cannulated and allowed to develop spontaneous tone. Vasodilatory responses to intraluminal flow or ACh were evaluated after a 20-min incubation with one of the following: 1) control PSS, 2) 10^-5 M NO^2^-nitro-l-arginine methyl ester (l-NAME), 3) 10^-5 M indomethacin, or 4) 10^-5 M l-NAME plus 10^-5 M indomethacin.

**Solutions and drugs.** Stock solutions of drugs were prepared in distilled water and frozen. Fresh dilutions of these stock solutions were prepared daily. All drugs were purchased from Sigma Chemical (St. Louis, MO).

**Data analysis.** Two-way ANOVA was used to determine differences in body weight, developed spontaneous tone, and maximal diameter between young and old groups and between soleus and gastrocnemius muscles. Vasodilatory responses were recorded as actual diameters and subsequently expressed as a percentage of maximal relaxation according to the formula

\[ \text{relaxation} \% = (D_s - D_h)/(D_m - D_h) \]

where D_m is the maximal inner diameter recorded at 60 cmH2O in calcium-free PSS, D_s is the steady-state inner diameter recorded after each addition of the drug or increase
in flow, and $D_b$ is the initial baseline inner diameter recorded immediately before the first addition of the vasodilatory agent or initiation of flow. Two-way repeated-measures ANOVA was used to detect differences between (young vs. old) and within (drug concentration or flow rate) factors. Post hoc analyses were performed with Bonferroni’s test for pairwise comparisons where appropriate. All data are presented as means ± SE. In all statistical analyses, $n$ indicates the number of animals in each group. Significance was defined as $P \leq 0.05$.

**RESULTS**

**Animals.** Average age of young rats at the time of death was 5 mo (range, 4–7 mo). Average age of old rats at death was 25 mo (range, 24–26 mo). Body weight increased significantly with age: young rats weighed $357 ± 6$ g and aged rats weighed $410 ± 6$ g. In young rats, the weights of soleus and gastrocnemius muscles were $0.161 ± 0.004$ and $1.88 ± 0.04$ g, respectively. In aged rats, soleus muscle weight did not change ($0.161 ± 0.005$ g), but gastrocnemius muscle weight was lower ($1.58 ± 0.05$ g) compared with that of young rats. Muscle mass-to-body mass ratio did not change for soleus muscle but was lower for gastrocnemius muscle.

**Characteristics of isolated vessels.** Vessel characteristics are reported in Table 1. Maximal inner diameter measurements of arterioles from the superficial gastrocnemius muscle of young rats ranged 102–206 μm and in aged rats 130–222 μm; maximum diameter measurements of gastrocnemius muscle arterioles from old animals were larger than those of the young group. Maximal inner diameter measurements of arterioles from the soleus muscle ranged 81–200 μm in young and 85–193 μm in aged animals. There was no difference between maximum diameter values for soleus muscle arterioles from young and old rats. Although the 1A arterioles were isolated from both muscle types, the average diameter of soleus muscle arterioles was significantly smaller than that of gastrocnemius arterioles in both age groups.

The level of spontaneous tone present before each intervention is shown in Table 1. No significant differences in spontaneous tone were found between young and old groups; however, these data were drawn only from arterioles that developed at least 20% tone. Consistent with previous findings, the proportion of vessels that did not develop sufficient tone was greater in the aged animals (38). Treatment with L-NAME increased tone in soleus muscle arterioles from young and aged rats. Treatment with indomethacin did not alter tone in soleus muscle arterioles from young rats but significantly increased tone in soleus muscle arterioles from aged rats. In gastrocnemius muscle arterioles from young rats, neither L-NAME nor indomethacin changed spontaneous tone. In contrast, treatment with either L-NAME or indomethacin increased tone in gastrocnemius muscle arterioles from old rats. Combined treatment with L-NAME and indomethacin increased spontaneous tone in arterioles from soleus and gastrocnemius muscles of young and old rats.

**Vasodilatory responses to flow.** Vasodilation to flow was significantly reduced in arterioles from both soleus and gastrocnemius muscles of aged animals (Fig. 1). In soleus muscle arterioles from young and old animals (Fig. 1A), maximal dilation to flow (45 and 11%, respectively) occurred when flow was initiated at a rate of 5 nl/s. Arterioles from gastrocnemius muscle (Fig. 1B) displayed more vigorous and sustained dilation in response to flow than arterioles from soleus muscle in both young and old animals. In gastrocnemius muscle arterioles from young animals, 67% vasodilation occurred in response to an average flow rate of 34 nl/s. In old animals, the response of gastrocnemius muscle arterioles to the same flow rate was only 24% dilation.

**Shear stress in arterioles from young and old rats.** Because shear stress at the vessel endothelium is dependent on vessel radius, we calculated shear stress in arterioles from young and old rats at each level of volumetric flow. Spontaneous tone and thus initial radii were similar in arterioles from young and old rats, which resulted in similar levels of shear stress in both groups at the onset of intraluminal flow. However, with exposure to flow, arterioles from young animals displayed greater dilation than arterioles from aged animals. As a result, shear stress remained relatively constant in arterioles from young animals as flow increased, whereas shear stress increased in arterioles from aged animals (Fig. 2). Thus vasodilation to known flow rates was reduced in arterioles from aged animals.

**Table 1. Characteristics of first-order arterioles from soleus and superficial portion of gastrocnemius muscles**

<table>
<thead>
<tr>
<th></th>
<th>Soleus Muscle</th>
<th>Gastrocnemius Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td>Maximal diameter, μm</td>
<td>131 ± 4*</td>
<td>129 ± 3*</td>
</tr>
<tr>
<td></td>
<td>(60)</td>
<td>(59)</td>
</tr>
<tr>
<td>Spontaneous tone, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow</td>
<td>41 ± 7</td>
<td>42 ± 4</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(9)</td>
</tr>
<tr>
<td>ACh</td>
<td>48 ± 5</td>
<td>46 ± 4</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(14)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>55 ± 8</td>
<td>56 ± 5</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(9)</td>
</tr>
<tr>
<td>Nitroprusside</td>
<td>48 ± 6</td>
<td>42 ± 4</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(13)</td>
</tr>
<tr>
<td>Pre-L-NAME</td>
<td>48 ± 4</td>
<td>51 ± 5</td>
</tr>
<tr>
<td></td>
<td>(12)</td>
<td>(7)</td>
</tr>
<tr>
<td>L-NAME</td>
<td>60 ± 6‡</td>
<td>50 ± 5‡</td>
</tr>
<tr>
<td></td>
<td>(12)</td>
<td>(7)</td>
</tr>
<tr>
<td>Preindomethacin</td>
<td>49 ± 6</td>
<td>60 ± 1</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(7)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>46 ± 6</td>
<td>64 ± 2‡</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(7)</td>
</tr>
<tr>
<td>Pre-L-NAME +</td>
<td>51 ± 6</td>
<td>57 ± 4</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>(9)</td>
<td>(11)</td>
</tr>
<tr>
<td>L-NAME +</td>
<td>64 ± 4‡</td>
<td>69 ± 5‡</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>(8)</td>
<td>(11)</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$ (in parentheses), no. of animals in each group. L-NAME, Nω-nitro-L-arginine methyl ester. *P < 0.05, soleus vs. gastrocnemius muscle arterioles; ‡P < 0.05, young vs. old groups; †P < 0.05, from pretreatment level of spontaneous tone.
despite the presence of similar or higher levels of shear stress.

Vasodilatory responses to ACh. ACh-induced vasodilation was reduced by age only in arterioles from the soleus muscle (Fig. 3A). Both sensitivity (IC$_{50}$) and the maximal response to ACh were reduced. Neither sensitivity nor maximal vasodilation in response to ACh was changed in arterioles from the gastrocnemius muscle of aged animals compared with young animals (Fig. 3B).

In young animals, no differences were detected in the vasodilatory responses to ACh between soleus and gastrocnemius muscle arterioles. However, in aged animals, ACh-induced vasodilation in soleus muscle arterioles was less than that in gastrocnemius muscle arterioles.

Vasodilatory responses to SNP and adenosine. Vasodilation to the direct NO donor SNP was unchanged in soleus and gastrocnemius muscle arterioles from aged animals (Fig. 4). In both young and old animals, arterioles from gastrocnemius muscle displayed greater vasodilation to SNP than arterioles from soleus muscle.

In young animals, adenosine-induced relaxation in gastrocnemius muscle arterioles was greater than the dilation in arterioles from soleus muscle. Relaxation in response to adenosine was not altered by age in either soleus or gastrocnemius muscle arterioles (Fig. 5). However, despite the lack of a significant age effect on vasodilation in arterioles from either muscle, the muscle-associated difference in responsiveness to adenosine was no longer present in arterioles from aged animals.

Effects of NO synthase inhibition. In a second cohort of animals, flow-induced vasodilation was again decreased in arterioles from both the soleus and gastrocnemius muscles of aged rats. Treatment with L-NAME significantly reduced flow-induced vasodilation ($P < 0.01$, control vs. L-NAME) in soleus muscle arterioles of young rats (Fig. 6A) but had a lesser inhibitory effect in soleus muscle arterioles of aged rats ($P = 0.09$). Treatment with L-NAME reduced the aging-related difference in the flow-induced vasodilation ($P = 0.48$, young vs. old rats after L-NAME treatment). In contrast to the...
results found in soleus muscle arterioles, L-NAME treatment did not significantly alter flow-induced vasodilation in gastrocnemius muscle arterioles from young rats (Fig. 7A). However, L-NAME produced a significant reduction in the flow-induced vasodilation in gastrocnemius muscle arterioles from aged rats ($P < 0.05$, control vs. L-NAME treatment), which indicates a greater dependence on NO signaling in gastrocnemius muscle arterioles from young and old rats. Furthermore, the aging-induced reduction in flow-induced vasodilation was not removed by treatment with L-NAME in gastrocnemius muscle arterioles.

In the second series of experiments, vasodilation in response to ACh was again reduced in arterioles from the soleus muscle but not the gastrocnemius muscle of aged rats. In soleus muscle arterioles from both young and aged rats, treatment with L-NAME significantly inhibited ACh-induced vasodilation, and this inhibitory effect was especially pronounced in arterioles from young animals (Fig. 8A). Importantly, treatment with L-NAME eliminated the difference in ACh-induced vasodilation between soleus muscle arterioles from young and aged rats. In gastrocnemius muscle arterioles, L-NAME inhibited vasodilation to low doses of ACh ($10^{-9}$ to $10^{-7}$ M) only in arterioles from aged rats (Fig. 9A); however, as found in the first group of animals (see Fig. 3), no differences were detected between young and old groups in either the presence or absence of L-NAME.

**Effects of cyclooxygenase inhibition.** In soleus muscle arterioles, indomethacin did not significantly alter flow-induced vasodilation in either young or aged rats (Fig. 6B). However, owing to nonsignificant but directionally opposite shifts in the flow-diameter relationships of arterioles from young and old rats, differences in the flow-induced vasodilatory responses of soleus muscle arterioles from young and aged rats were no longer detectable after treatment with indomethacin. In gastrocnemius muscle arterioles, indomethacin treatment did not have a detectable effect on flow-
induced vasodilation regardless of the age of the rats (see Fig. 7B); however, after treatment with indomethacin, differences in flow-induced vasodilation between young and old groups were no longer significant ($P = 0.23$, young vs. old after treatment with indomethacin).

Indomethacin treatment only inhibited ACh-induced vasodilation in soleus muscle arterioles from young animals (see Fig. 8B), and the difference in responses between young and old groups was eliminated in the presence of cyclooxygenase blockade. In arterioles from gastrocnemius muscle, indomethacin had no effect on dilation to ACh in either group of rats (Fig. 9B).

**Effects of combined NO synthase and cyclooxygenase inhibition.** Simultaneous treatment with L-NAME and indomethacin reduced flow-induced vasodilation in arterioles from the soleus muscle of young but not aged rats (see Fig. 6C). In gastrocnemius muscle of both

---

**Fig. 5.** Concentration-response relationship of soleus (A) and gastrocnemius (B) muscle arterioles from young and old rats to adenosine. Adenosine-induced vasodilation was not different between arterioles from young and old rats. Vasodilation to adenosine was greater ($P < 0.01$) in arterioles from the gastrocnemius muscles compared with the soleus muscle in young rats. In aged rats, no differences existed between the vasodilatory responses of arterioles from the soleus and gastrocnemius muscles ($P = 0.37$).

**Fig. 6.** Effects of indomethacin (Indo) and N^G^-nitro-L-arginine methyl ester (L-NAME) on flow-induced vasodilation in soleus muscle arterioles from young and old rats. A: L-NAME reduced flow-induced vasodilation in arterioles from young and old rats (after treatment with L-NAME, $P = 0.48$) B: indomethacin did not alter flow-induced vasodilation in arterioles from either young or old rats. C: combined treatment with L-NAME and indomethacin reduced responses to flow in young but not old rats. Combined treatment also abolished differences between young and old groups.
Fig. 7. Effects of indomethacin and L-NAME on flow-induced vasodilation in gastrocnemius muscle arterioles from young and old rats. A: L-NAME inhibited flow-induced vasodilation in arterioles from old but not young rats. In the presence of L-NAME, differences in responsiveness to flow persisted between young and old groups. B: indomethacin did not alter responses to flow in arterioles from either young or old rats. Differences in flow-induced vasodilation between young and old groups remained after blockade with indomethacin. C: combination of L-NAME and indomethacin produced inhibition of flow-induced vasodilation in arterioles from both young and old rats. Combined treatment eliminated aging-related differences in the flow response.

Fig. 8. Effects of indomethacin and L-NAME on responses to ACh in soleus muscle arterioles from young and old rats. A: L-NAME inhibited vasodilation to ACh in arterioles from young and old rats. L-NAME abolished the differences between young and old groups. B: indomethacin diminished responses to ACh in arterioles from young rats but not old rats. Differences in ACh-induced vasodilation between groups were eliminated after blockade with indomethacin. C: combination of L-NAME and indomethacin produced significant inhibition of the response to ACh in arterioles from both young and old rats. Combined treatment eliminated aging-related differences in the ACh response.
young and aged rats, flow-induced vasodilation was inhibited by the combination of L-NAME and indomethacin (see Fig. 7C). Application of both L-NAME and indomethacin reduced maximal vasodilation to ACh in soleus muscle arterioles from both young and aged rats (see Fig. 8C) but had no effect on the maximal dilatory response of gastrocnemius muscle arterioles from young and aged rats (Fig. 9C). However, the combined treatment diminished gastrocnemius muscle arteriolar sensitivity (IC50) to ACh in young and aged rats. After treatment with both L-NAME and indomethacin, no aging-related differences were detected in responses to flow or ACh in arterioles from either the soleus muscle or the gastrocnemius muscle.

DISCUSSION

The purpose of this study was to determine whether aging alters the intrinsic vasodilatory responsiveness of the endothelium and/or vascular smooth muscle of resistance arterioles isolated from locomotory muscles of different fiber composition. The primary finding of this study is that endothelium-dependent vasodilation to flow is reduced in 1A arterioles isolated from the soleus, which is a highly oxidative, postural muscle, and from the gastrocnemius, which is a highly glycolytic muscle recruited during high-intensity activity (9). Additionally, endothelium-dependent vasodilation to ACh was decreased with age, but only in arterioles from the soleus muscle. The aging-related differences in flow- and ACh-induced vasodilation of soleus muscle arterioles were eliminated following treatment with L-NAME. In gastrocnemius muscle, the aging-related difference in the flow-induced vasodilatory response was not altered by treatment with L-NAME but was slightly reduced by treatment with indomethacin and eliminated by combined blockade with indomethacin and L-NAME. Finally, smooth muscle vasodilation elicited through activation of either guanylate cyclase or adenylate cyclase mechanisms was not altered in arterioles from aged animals regardless of muscle fiber type.

Previous studies in the literature have also demonstrated that aging impairs endothelium-dependent relaxation in resistance vessels from other vascular beds. A number of studies have shown that aging specifically impairs endothelium-dependent function in conduit arteries and resistance vessels (7, 16, 18, 23, 27, 34, 46). Mayhan et al. (34) reported reduced vasodilation to the endothelium-dependent agents ACh and bradykinin in cerebral arterioles of aged rats. Similar findings have been reported in the aorta and carotid artery of aged rats (7, 23). In humans, forearm blood flow measured in response to ACh was reduced in aged individuals (14, 18, 45). The present data suggest that aging reduces endothelium-dependent vasodilatory responses in 1A arterioles from both fast- and slow-twitch locomotory skeletal muscles.

Unique to this study is the finding that ACh-induced vasodilation is reduced only in arterioles from soleus muscle and not in arterioles isolated from the superfi-

Fig. 9. Effects of indomethacin and L-NAME on responses to ACh in gastrocnemius muscle arterioles from young and old rats. A: L-NAME did not alter maximal vasodilatory responses to ACh in arterioles from either young or old rats, although L-NAME reduced arteriolar sensitivity (IC50) to ACh in old rats. B: indomethacin had no effect on responses to ACh in arterioles from either young or old rats. C: combination of L-NAME and indomethacin diminished vascular sensitivity (IC50) to ACh in arterioles from young and old rats but did not alter the maximal vasodilatory response.
cular portion of the gastrocnemius muscle. In contrast, endothelium-dependent vasodilation to flow was reduced in arterioles from both soleus and gastrocnemius muscles of aged animals. In soleus muscle arterioles from young rats, the inhibitory effect of L-NAME indicates a significant role for NO in mediating both flow- and ACh-induced vasodilations. In contrast, blockade of NO did not significantly affect either flow- or ACh-induced vasodilation in gastrocnemius muscle arterioles from young rats. Furthermore, in soleus muscle arterioles, elimination of the aging-induced differences in both the ACh and flow responses by NO blockade indicates that the impairment is related to a reduction in nitroxidergic signaling. In contrast, blockade of NO production did not alter the age-associated difference between flow-induced responses in gastrocnemius muscle arterioles. In gastrocnemius muscle arterioles, neither NO nor prostanooid vasodilators contributed significantly to ACh-induced vasodilation. It is possible that endothelium-derived hyperpolarizing factor serves as the predominant signaling mechanism for this response and that this mechanism is relatively insensitive to aging. These findings suggest that endothelial signaling pathways are specifically adapted to muscle-fiber type and function. Furthermore, the results of this study indicate that aging can produce endothelial impairment through alterations of distinct signaling pathways and that these aging-induced adaptations of the vascular endothelium are related to muscle-fiber composition and muscle function. Other studies have also indicated that vascular responses differ in muscles of varying fiber composition. NO synthase inhibition preferentially affects the exercise hyperemia in highly oxidative muscle (22). McCurdy et al. (35) have reported that sensitivity to adenosine and SNP is greater in arterioles from the gastrocnemius muscle than in arterioles from the soleus muscle, and we have previously found that myogenic responses to transmural pressure differ between arterioles from the soleus and gastrocnemius muscles (38).

The current results indicate that the reduction in vasodilation that occurs in response to flow and ACh in skeletal muscle arterioles from aged animals is not due to alterations in smooth muscle responsiveness to NO. Vasodilatory responses to SNP were not altered by age in arterioles from either the gastrocnemius or soleus muscles. Studies in other vascular beds have reported similar results. In rat cerebral resistance vessels, endothelium-dependent vasodilation to ACh and bradykinin was impaired, whereas responsiveness to the exogenous NO donor nitroglycerin was unchanged with age (34). Similarly, vasodilation to ACh was reduced, but relaxation to SNP was unchanged in aorta from aged Fischer-344 rats (7). Results from human studies are consistent with these findings in rats. Aging reduced ACh-induced increases in forearm blood flow, although nitroprusside-induced increases in forearm blood flow were not altered by old age (14, 18, 44). These results suggest that smooth muscle sensitivity to NO- and cGMP-mediated relaxation remain intact with age.

Adenosine-induced relaxation was also preserved in arterioles from both the soleus and gastrocnemius muscles. Adenosine binds with A$_2$ receptors on smooth muscle to increase cAMP production and subsequent relaxation (21, 32, 33). Thus it appears that smooth muscle relaxation mediated through cAMP is also preserved in skeletal muscle arterioles from aged rats. In contrast, decreased responsiveness to adenosine has been reported in mesenteric resistance vessels of aged rats (3). However, mesenteric resistance vessels showed a much more vigorous dilation to adenosine than those reported here for skeletal muscle arterioles. These disparate findings could be related to diverse aging-associated adaptations of arterioles from different vascular beds.

We examined arterioles from soleus (predominantly type I fibers) and the superficial portion of the gastrocnemius (predominantly type IIb fibers) muscles to test the possibility that aging would differentially affect the responses of resistance vessels from muscles of varying fiber composition. In addition to differences in the muscle-fiber composition and oxidative capacity (9), these muscles vary in recruitment order (1, 2) and blood flow patterns at rest and during exercise (1, 6). Any of these differential characteristics could directly or indirectly influence the vasodilatory responses of the resistance arteries within the muscle and therefore provide the stimulus for aging-induced deficits in endothelium-mediated vasodilation. For example, both increases and decreases in muscle activity have been shown to affect endothelial responsiveness in various arterial vascular beds. Chronic increases in blood flow associated with elevations in physical activity have been shown to increase endothelium-mediated vasodilation, vascular NO synthase mRNA, and protein expression (11, 13, 37, 42, 48), whereas chronic reductions in blood flow induced by hindlimb unloading (36) have been shown to diminish aortic endothelium-mediated vasodilation to ACh (7).

The skeletal muscle microvasculature appears to be similarly affected by activity-induced changes in blood flow. Chronic reductions in soleus muscle blood flow via hindlimb unloading (36) result in a reduced ACh-induced vasodilation and diminished NO synthase mRNA and protein expression (8, 25, 49). It is therefore possible that the aging-induced reduction in endothelium-mediated vasodilation of soleus muscle arterioles may be the result of decreases in physical activity and correspondingly diminished blood flow through the vessels. The unloading of soleus muscle, however, is a continuous and extreme form of physical deconditioning that results in significant muscle atrophy and reductions in maximal arteriolar diameter (5, 8, 25); this type of vascular remodeling is indicative of chronic decreases in blood flow and intravascular shear stress (30, 31). With aging, neither soleus muscle mass nor maximal diameter of soleus muscle arterioles are altered. In addition, skeletal muscle blood flow during the rats’ sleeping period of the daily light-dark cycle is not different between young and aged rats (10). Therefore, we speculate that cage activity and soleus muscle...
blood flow may only be lower in the old animals during the night portion of the light-dark cycle when young rats are normally active and skeletal muscle blood flow is highest (12). Compared with unloading, this milder form of muscular inactivity may only lower soleus muscle blood flow sufficiently to diminish endothelium-mediated vasodilation without inducing vascular remodeling.

The evidence from the present study that the old rats are in fact less active than their younger counterparts can be inferred from the atrophy of the gastrocnemius muscle. Previous reports of muscle atrophy and reductions in muscle oxidative capacity in old Fischer-344 rats further support this contention (43). Therefore, as proposed for the soleus muscle, one might expect that the relative inactivity of old rats and a corresponding absence of an exercise hyperemia in gastrocnemius muscle would also result in a diminished endothelium-dependent vasodilation. Although flow-induced vasodilation was diminished in gastrocnemius muscle arterioles, ACh-induced dilation was unchanged by age. One possibility for the lack of a change in this endothelium-dependent mechanism of vasodilation with old age could be that muscular activity of the superficial portion of gastrocnemius muscle remains unaltered by age. In other words, during normal activity, the motor units in this muscle portion are typically not recruited in young rats (2, 9), and the units presumably remain relatively inactive throughout the life of the rat. A second possibility could be that activity-induced alterations in endothelial responsiveness are primarily due to changes in nitroxidergic vasodilation, and the ACh-induced vasodilation of 1A gastrocnemius muscle arterioles does not appear to depend on a nitroxidergic mechanism (see Fig. 9A). Finally, a third possibility for the lack of an aging effect on ACh-induced vasodilation in gastrocnemius muscle arterioles could be that despite a putative decrease in total gastrocnemius muscle blood flow with old age, there is not a corresponding decrease in the rate of Q through individual arteriolar vessels. This situation could arise if there were fewer arteriolar vessels in the gastrocnemius muscle, and thus intravascular blood flow and shear stress would not decrease despite a reduction in total muscle flow. To estimate the likelihood of this possibility, we counted the number of feed arterioles leading to the gastrocnemius muscle in young and old rats. There were significantly fewer feed arterioles leading to the gastrocnemius muscles of old rats (young, 5.5 ± 0.2, n = 6; old, 4.6 ± 0.2, n = 9). These data support the notion that arteriolar rarefaction occurs in the gastrocnemius muscle with aging. Vascular rarefaction may also explain the increase in the maximal diameter and the greater dependence on NO signaling in the flow-induced (see Fig. 7A) and ACh-induced (see Fig. 9A) vasodilation of gastrocnemius muscle arterioles from aged rats. Both increases in vessel diameter (30) and upregulation of nitroxidergic vasodilation (39) are indicative of increased intravascular blood flow and shear stress. Therefore, despite a decrease in total muscle blood flow that would be predicted from muscle atrophy and inactivity, a reduction in arteriolar density could increase blood flow and shear stress in individual vessels and provide a stimulus for these adaptations. Clearly, more work is needed to delineate the complex relations between aging, physical inactivity, muscle blood flow, vascular remodeling, and endothelium-dependent vasodilation.

In conclusion, the results of this study indicate that aging impairs endothelium-dependent vasodilatory responses of resistance arterioles from locomotory skeletal muscles, whereas endothelium-independent vasodilatory responses are preserved. Vasodilatory responses to both pharmacological and mechanical stimuli differ between arterioles isolated from the soleus muscle, which is composed predominantly of slow-twitch fibers, and arterioles isolated from the superficial gastrocnemius muscle, which is composed predominantly of fast-twitch fibers. Aging-induced adaptations of vasodilatory responses also differed between arterioles from the soleus and gastrocnemius muscles. In soleus muscle arterioles, diminished endothelium-dependent responsiveness resulted from a decrease in NO-mediated vasodilation. In gastrocnemius muscle arterioles, adaptation of the endothelium occurs through an alternate pathway. These findings suggest that impaired endothelial function and vasodilatory capacity of resistance arterioles may contribute to aging-associated reductions in skeletal muscle blood flow during physical activity.

This work was supported by American Heart Association, Texas Affiliate, Grant 98BG801, National Institute on Aging Grant AG-19248-01, and a Sam Houston State University Institutional Award.

REFERENCES