The effects of aging and exercise training on endothelin-1 vasoconstrictor responses in rat skeletal muscle arterioles

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Abstract

Objectives: The incidence of cardiovascular disease increases with advancing age. Vascular dysfunction has been linked to cardiovascular disease and aging, although most research has focused on endothelium-dependent vasodilator dysfunction. Another possible mechanism for this vascular dysfunction with aging is enhanced vasoconstrictor responsiveness of the resistance vasculature, and in particular, reactivity of arterioles to endothelin-1 (ET-1). We hypothesized that vasoreactivity to ET-1 would be greater in skeletal muscle arterioles from old rats, and that endurance exercise training would abolish differences in ET-1 responsiveness between young and old animals.

Methods: Young sedentary (YS; 4 months; n=18), old sedentary (OS; 24 months; n=17), young trained (YT; n=9) and old trained (OT; n=7) male Fischer 344 rats were used. Training modality was treadmill exercise at 15 m/min up a 15° incline, 1 h/day, 5 days/week, for 12 weeks. Soleus and white gastrocnemius muscle first-order arterioles were isolated for in vitro experimentation. Intraluminal diameter was measured in response to increasing concentrations of ET-1 (10^{-11} to 10^{-8} M) or KCl (10–100 mM) in arterioles with an intact or denuded endothelium and with and without an ETA (BQ-123 [10^{-6} M]) or ETB (BQ-788 [10^{-8} M]) receptor antagonist present.

Results: There was an age-associated increase in gastrocnemius vasoconstrictor responsiveness and sensitivity to ET-1 in arterioles with intact endothelium (ET-1 EC_50: YS, 5.2 E^{-9} F 1.1 E^{-9} M; OS, 2.0 E^{-9} F 0.8 E^{-9} M); neither removal of the endothelium nor ETB blockade abolished this difference in arteriolar sensitivity to ET-1 between old and young rats. In contrast, ETA inhibition abolished the greater sensitivity (EC_50) of arterioles from old animals (ET-1 EC_50: YS, 10 E^{-9} F 0.7 E^{-9} M; OS, 10 E^{-9} F 1.5 E^{-9} M). Gastrocnemius muscle arteriolar responses to ET-1 and KCl were unaffected by aging. Additionally, exercise training had no effect on ET-1 vasoconstriction of soleus or gastrocnemius muscle arterioles.

Conclusions: Aging results in an augmented gastrocnemius muscle arteriolar vasoconstriction to ET-1 which is mediated through an enhanced ETA receptor signaling pathway and not through an ETB receptor mechanism associated with either the endothelium or vascular smooth muscle. These findings suggest that enhanced vascular ET-1 sensitivity in fast-twitch skeletal muscles may play a role in the vascular dysfunction that is often associated with old age.

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Keywords: Aging; Microcirculation; Vasoconstriction; Endothelins; Regional blood flow

1. Introduction

Aging is an independent risk factor for hypertension, atherosclerosis, and coronary heart disease. It has been hypothesized that vascular dysfunction precedes these pathological disease states and could contribute to the
progression of these diseases [1]. For example, aging is characterized by reduced vasodilation through endothelium-dependent mechanisms [2–4]. Alterations in resistance vessel responsiveness to endothelium-derived constricting factors may also contribute to the greater age-associated incidence of vascular disease. Endothelin-1 (ET-1) is a potent vasoconstrictor substance produced by endothelial cells which acts through vascular smooth muscle cell endothelin A (ET$_A$) and endothelin B (ET$_B$) receptors [5]. In addition, there is an inhibitory mechanism to ET-1 vasoconstriction via ET$_B$ receptors located on the endothelial cells, which produce endothelium-derived relaxing factors such as nitric oxide and prostacyclin that act to oppose the ET-1 mediated smooth muscle contraction [6,7].

Current research has documented an augmented ET-1 release in patients with hypertension [8], heart failure [9], atherosclerosis and obesity [10]. However, the effects of aging on ET-1 mediated contraction of the resistance vasculature in general, and skeletal muscle microvascular responsiveness to ET-1 in particular, remain largely unknown. Therefore, the primary purpose of this study was to determine the effects of aging on ET-1 vasoconstrictor responsiveness in skeletal muscle arterioles. First-order arterioles from the soleus, a predominantly slow-twitch oxidative muscle, and the superficial portion of the gastrocnemius, a fast-twitch glycolytic muscle, were investigated. Since aging is often associated with physical inactivity, a secondary purpose was to determine whether chronic increases in activity through exercise training would attenuate possible aging-induced alterations in ET-1 responsiveness. We hypothesized that aging would enhance ET-1 mediated vasoconstriction through an attenuation of the endothelial cell ET$_A$ receptor vasodilator mechanism [11], and that exercise training would attenuate the age-associated enhancement of the ET-1 vasoconstrictor response through an up-regulation of the endothelial ET$_B$ receptor vasodilator mechanism [12].

2. Materials and methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996, and all animal procedures were approved by the Texas A&M University Laboratory Animal Care Committee.

2.1. Animal characteristics

Young (4–6 months of age) and old (24–26 months) male Fischer 344 rats were obtained (Harlan) and housed in a temperature controlled (23±2°C) room with a 12:12-light–dark cycle. These ages represent young adulthood and senescence (~50% mortality rate), respectively. This animal model is supported by the National Institute on Aging to study the effects of old age on the cardiovascular system in the absence of overt cardiovascular disease.

2.2. Exercise training

All rats were habituated to treadmill exercise, during which time each rat walked on a motor-driven treadmill at 15 m/min (0° incline), 5 min/day for 3 days. At the end of the habituation period, young and old rats were assigned to a young sedentary (YS), old sedentary (OS), young exercise trained (YT) or old exercise trained (OT) group. Exercise trained rats performed treadmill running at 15 m/min on a 15° incline, 60 min/day, 5 days/week, for 10–12 weeks as previously described [12,13]. Vascular responses were determined 48 h after the last exercise bout in trained rats.

2.3. Microvessel preparation

Animals were anesthetized (60 mg/kg i.p., sodium pentobarbital) and euthanized via exsanguination. The gastrocnemius–plantaris–soleus muscle complex was excised from each leg and placed in cold (4°C) physiological saline solution (PSS) that contained 145.0 mM NaCl, 4.7 mM KCl, 2.0 mM CaCl$_2$, 1.17 mM MgSO$_4$, 1.2 mM Na$_2$HPO$_4$, 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 for isolation of gastrocnemius and soleus muscle first-order (1A) arterioles as previously described [4,14,15]. Arterioles were defined as the first arterial branch after the feed artery entered the muscle. One set of vessels was removed from the muscles and placed in Lucite chambers containing MOPS-buffered PSS equilibrated to room air. These arterioles were then cannulated on both ends with micropipettes and secured with 11-0 surgical nylon suture. After cannulation, the chambers were transferred to the stage of an inverted microscope for recording of luminal diameter and pressurized to the in vivo pressure with two independent hydrostatic pressure reservoirs. Intraluminal pressure was set according to the arteriolar internal diameter to coincide with the pressure measured in vivo [16,17], i.e., 60–125 μm—44 mmHg, 126–150 μm—49 mmHg, 151–175 μm—54 mmHg, 176–200 μm—60 mmHg, as used in previous skeletal muscle arteriole comparisons [18]. Leaks were detected by pressurizing the vessel and then closing the reservoirs to verify that diameter remained constant. Arterioles that did not maintain diameter were discarded. Arterioles were then warmed to 37°C and allowed to develop spontaneous tone during a 30–60-min equilibration period. Vessels that did not develop at least 20% baseline tone were excluded from study. A second set of isolated arterioles were frozen and stored for ET$_A$ and ET$_B$ receptor mRNA analysis.

2.4. Experimental design

2.4.1. Vasoreactivity to pharmacological agonists

In a series of three studies, concentration–response relations to the cumulative addition of ET-1 (1×10$^{-11}$ to 3×10$^{-8}$ M) were determined in arterioles with the endothelium intact and with the endothelium removed. Likewise,
[K⁺] was isotonically increased by varying concentrations of NaCl and KCl in the PSS buffer solution in order to achieve the desired molar concentration of K⁺ (10–100 mM) without increasing the bath solution osmolality.

2.4.1.1. Study 1. To determine whether ET-1 and KCl induced vasoconstriction of the skeletal muscle arterioles is altered by aging or exercise training, the diameter of soleus and gastrocnemius muscle arterioles was measured in response to ET-1 (1×10⁻¹¹ to 3×10⁻⁸ M) or KCl (10–100 mM). Arteriolar sensitivity was assessed by calculating the dose eliciting 50% of the maximal vasoconstrictor response (EC₅₀).

2.4.1.2. Study 2. Because differences in vascular reactivity to ET-1 and KCl were only found in gastrocnemius muscle arterioles with aging, a second series of studies were performed to determine whether the difference in ET-1 or KCl-induced vasoconstriction was mediated through the vascular endothelium. For these studies, the endothelium was denuded from gastrocnemius muscle arterioles by passing 3–5 ml of air through the lumen of the vessel. To ensure complete removal of the endothelium, arterioles were exposed to the endothelium-dependent vasodilator acetylcholine (3×10⁻⁵ M); any vessels that exhibited >5% vasodilation were excluded from the study. Following the acetylcholine test, the vessels were washed several times with PSS and allowed to reestablish spontaneous tone prior to the ET-1 or KCl dose response.

2.4.1.3. Study 3. In order to determine whether the vascular smooth muscle ETₐ or ETₐ receptors were responsible for the age-associated augmentation of gastrocnemius muscle arteriolar ET-1 responsiveness, the endothelial layer was removed as described above. One arteriole was then incubated with the ETₐ receptor antagonist BQ-123 (10⁻⁶ M) for 30 min prior to undergoing an ET-1 dose response. A second arteriole was incubated with the ETₐ receptor antagonist BQ-788 (10⁻⁸ M) for 30 min prior to undergoing an ET-1 dose response. These doses of ET receptor antagonists have been previously shown to inhibit ETₐ and ETₐ receptor vasoconstriction, respectively [19–21].

2.4.2. ETₐ and ETₐ receptor mRNA expression
Arterioles were snap frozen and stored at −80 °C in 0.5 ml microcentrifuge tubes. Arterioles were pulverized in lysate buffer and total RNA was extracted with the RNAqueous filter system (Ambion). Total RNA was used to perform real-time PCR with TaqMan® Pre-Developed Assay Reagents to quantitatively determine ETₐ (product number Rn00561137 m1) and ETₐ (product number Rn00569139 m1) receptor mRNA expression (ABI Prism 7700 Sequence Detection system) as previously described in detail [12].

2.4.3. Muscle oxidative enzyme activity
Sections of the soleus and gastrocnemius muscles from each animal were stored at −80 °C for determination of citrate synthase activity [22], a measure of muscle oxidative capacity, to determine the efficacy of the training regimen.

2.4.4. Solutions and stocks
Stock solutions of drugs were prepared in PSS and frozen. Fresh dilutions of these stocks were prepared daily. All drugs except for ET-1 were obtained from Sigma. ET-1 was purchased from Phoenix Pharmaceuticals.

2.4.5. Data presentation and statistical analysis
Vasoconstrictor responses were recorded as actual diameters and expressed as a percentage of possible constriction according to the formula:

\[ \text{Vasoconstriction (%Maximal Response)} = \left[ \frac{(D_B - D_S)}{D_B} \right] \times 100 \]

where \( D_S \) is the steady-state inner diameter recorded after each dose and \( D_B \) is the initial baseline inner diameter.

### Table 1: Characteristics of animals and skeletal muscle arterioles

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<td>Young</td>
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<td><strong>N</strong></td>
<td>18</td>
<td>17</td>
<td></td>
<td>9</td>
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<td>Body mass (g)</td>
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<td>425±7*</td>
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<td>348±8</td>
<td>408±12†</td>
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<td>LV/body mass ratio (mg/g)</td>
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<td>1.90±0.004</td>
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<td>2.00±0.003</td>
<td>2.17±0.009‡</td>
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<td>Soleus muscle/body mass ratio (mg/g)</td>
<td>0.45±0.002</td>
<td>0.35±0.001*</td>
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<td>0.45±0.001</td>
<td>0.43±0.001†</td>
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<tr>
<td>Gastrocnemius muscle/body mass ratio (mg/g)</td>
<td>4.98±0.007</td>
<td>3.81±0.007*</td>
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<td>4.94±0.007</td>
<td>4.43±0.009†</td>
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<tr>
<td>Soleus muscle arteriole lumen diameter (µm)</td>
<td>110±7</td>
<td>116±4</td>
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<td>106±6</td>
<td>126±8</td>
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<tr>
<td>Gastrocnemius muscle arteriole lumen diameter (µm)</td>
<td>147±5</td>
<td>154±4</td>
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<td>181±8‡</td>
<td>190±9‡</td>
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<td>Soleus muscle arteriole spontaneous tone (%)</td>
<td>35±5</td>
<td>43±7</td>
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<td>53±5†</td>
<td>43±6</td>
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<td>Intact Gastrocnemius Arteriole Spontaneous Tone (%)</td>
<td>31.0±4</td>
<td>30±7</td>
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<td>30±10</td>
<td>34±5</td>
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<td>Denuded Gastrocnemius Arteriole Spontaneous Tone (%)</td>
<td>35±4</td>
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* Indicates significant difference between young sedentary and old sedentary.
† Indicates significant difference between old sedentary and old trained.
‡ Indicates significant difference between young sedentary and young trained.
before the first addition of ET-1 or KCl. Spontaneous tone is expressed as a percentage of maximal intraluminal diameter. Repeated measures analysis of variance (ANOVA) was used to determine the significance of differences among young and old, sedentary and exercise trained groups. Planned contrasts utilizing the Fisher protected least significant difference post hoc test were used where appropriate. A one-way ANOVA was used to determine the significance of differences among groups in animal body mass, muscle mass and muscle citrate synthase activity. All data are presented as mean ± S.E.M. Significance was set at $P \leq 0.05$.

3. Results

3.1. Animal characteristics

Body mass was greater in old vs. young rats, and exercise training reduced body mass only in the old rats (Table 1). Left ventricle-to-body mass ratio was significantly higher in the trained rats compared to age-matched controls (Table 1). Gastrocnemius and soleus muscle mass-to-body mass ratio was reduced with aging and increased by exercise training (Table 1). Citrate synthase activity was significantly higher in
both muscles of young and old trained rats relative to the sedentary control groups (Soleus: YS 15 F, OS 11 F, YT 19 F, OT 14 F; Gastrocnemius: YS 16 F, OS 14 F, YT 19 F, OT 22 F), thus substantiating the efficacy of the exercise training regimen.

3.2. ET-1 vasoconstrictor studies

Aging did not affect ET-1-mediated vasoconstrictor responses or sensitivity (EC50) in soleus muscle arterioles (Fig. 1A). In contrast, aging shifted the ET-1 concentration–diameter relation and increased ET-1 sensitivity (EC50), but not maximal responsiveness, of arterioles from the superficial portion of the gastrocnemius muscle (Fig. 2A). Exercise training had no effect on ET-1 vasoconstriction in either young or old soleus (Fig. 1B and C) and gastrocnemius (Fig. 2B and C) muscle arterioles.

In the second series of studies, removal of the endothelial cell layer in gastrocnemius muscle arterioles did not abolish the age-related increase in ET-1 responsiveness or sensitivity (Fig. 3A), indicating that the increase in ET-1 vasoconstriction was not due to endothelial ET receptors and diminished endothelial vasodilator function in arterioles from aged rats.

Finally, in the presence of the ETB receptor antagonist BQ-788 the age-related increase in ET-1 responsiveness and sensitivity remained (Fig. 3B). In contrast, the ETA receptor antagonist BQ-123 eliminated the age-associated increase in ET-1 responsiveness (Fig. 3C). In fact, arteriolar vasoconstriction to ET-1 was less in old rats with ETA receptor inhibition. These blocking studies also indicate that ET-1...
sensitivity and maximal vasoconstriction were greater when mediated through the vascular smooth muscle ETA receptor compared to the ETB receptor in the skeletal muscle microcirculation, regardless of age.

3.3. ETA and ETB receptor mRNA expression

In gastrocnemius muscle arterioles, aging had no effect on ETA receptor mRNA expression (Fig. 4A), but ETB receptor expression tended to be lower (P < 0.1) in arterioles from old vs. young animals (Fig. 4B).

3.4. KCl vasoconstrictor studies

Aging had no effect on KCl-induced vasoconstrictor responses or sensitivity (EC50) in soleus muscle arterioles (data not shown). In contrast, aging diminished the vasoconstrictor response to KCl in gastrocnemius muscle arterioles, and exercise training decreased KCl responsiveness and maximal vasoconstriction in gastrocnemius arterioles from young and old rats (Fig. 5A). Removal of the endothelium abolished all aging and training associated alterations in the KCl response (Fig. 5B).

4. Discussion

Previous work has shown that systemic ET-1 infusion in old monkeys results in a greater elevation of systemic vascular resistance than that which occurs in young adult animals; this greater ET-1 mediated vasoconstriction appeared to occur through an age-related impairment of the endothelial ETB receptor-nitric oxide synthase signaling mechanism [11]. Because skeletal muscle makes up the largest portion of body mass of any single organ system and is a major contributor to systemic vascular resistance, and because impairment of endothelium-dependent vasodilation has been shown to occur with aging in skeletal muscle arterioles [4,12], we hypothesized that aging would increase ET-1 vasoconstrictor responses of skeletal muscle arterioles, and that this enhancement would occur through a diminished endothelial ETB receptor vasodilator mechanism. Furthermore, we hypothesized that exercise training would abolish the age-related enhancement of ET-1 vasoconstriction through an up-regulation of the endothelial ETB receptor-nitric oxide synthase signaling mechanism, since chronic exercise training has been shown to enhance endothelium-dependent vasodilation through the nitric oxide synthase pathway in skeletal muscle arterioles [12]. Consistent with our hypothesis, the results demonstrate from the current investigation an age-associated increase in ET-1 responsiveness (Fig. 2A) and sensitivity (EC50) in arterioles from the gastrocnemius muscle. However, contrary to our hypothesis, the augmented vasoconstriction to ET-1 in gastrocnemius muscle arterioles from old rats was not due to age-related changes in the endothelial or smooth muscle ETA receptor signaling mechanisms (Fig. 3A and C), but augmented smooth muscle ETA receptor mediated vasoconstriction (Fig. 3B). In addition, exercise training had no effect on ET-1 vasoconstriction of skeletal muscle arterioles from young or old rats.

4.1. Mechanism of age-related augmented ET-1 sensitivity

Arteriolar studies with and without an intact endothelial cell layer demonstrate that the old age-associated increase in ET-1 sensitivity of gastrocnemius muscle arterioles is not the result of or dependent on the vasodilator function of endothelial cell ET receptors. This confirms that augmented ET-1 sensitivity occurs through vascular smooth muscle ET receptors. Using specific inhibitors of ETA and ETB receptors on the vascular smooth muscle of denuded arterioles, the results demonstrate that ETA receptor-mediated vasoconstriction is greatly enhanced with aging (Fig. 3B), whereas ETB receptor-mediated vasoconstriction is slightly but significantly diminished with aging (Fig. 3C).
Since the receptor independent KCl-mediated contraction of denuded arterioles was similar between young and old (Fig. 5B), then changes in ET\textsubscript{A} and ET\textsubscript{B} receptor-mediated vasoconstriction are likely due to upstream alterations in the receptor-second messenger portion of the signaling pathway and not the result of alterations in the intracellular excitation–contraction mechanism or the contractile machinery of the arteriole. Although ET\textsubscript{A} and ET\textsubscript{B} receptor mRNA expression was not greater in gastrocnemius muscle arterioles from old animals (Fig. 4A and B), possible age-associated alterations in the ET receptor signaling mechanism could still be present due to differences in ET receptor protein stability or translation independent of transcription. Because the predominate mechanism through which ET-1 mediated vasoconstriction occurs in skeletal muscle arterioles is through the ET\textsubscript{A} receptor mechanism, the net effect of aging on the ET\textsubscript{A} and ET\textsubscript{B} receptor signaling mechanisms is an enhanced ET-1 vasoconstrictor response.

Although the results of the present study are consistent with the observation of Asai et al. [11] that ET-1 mediated vasoconstrictor responses are elevated with old age, the mechanisms through which this effect occurs differs between studies. Asai et al. found that the vasodilator component of the ET-1 vascular response was diminished with old age through an impaired endothelial ET\textsubscript{B} receptor-nitric oxide synthase signaling mechanism. In the present study, an enhanced smooth muscle ET\textsubscript{A} receptor signaling pathway mediates the greater ET-1 vasoconstriction with old age. In fact, the effect of aging on the endothelial component of the ET-1 vasoconstriction in gastrocnemius muscle arterioles appeared to be an enhancement of this vasorelaxation effect, i.e., age-associated differences in the ET-1 response appeared greater when the endothelium was removed (Fig. 3A) vs. when present (Fig. 2A). A similar pattern was also observed with the KCl response; with the endothelium present the KCl-induced vasoconstriction was lower in arterioles from old rats (Fig. 4A), and this difference was abolished with the removal of the endothelium (Fig. 4B). Thus, aging does not appear to adversely affect the endothelial ET receptor vasodilator component of the ET-1 response in the skeletal muscle resistance vasculature.

Several factors could account for the apparent discrepancies between the present study and that of Asai et al. [11]. First, the effects of aging on the resistance vasculature may differ between rats and monkeys. Second, Asai et al. used changes in systemic vascular resistance to assess vascular responsiveness to ET-1, which would reflect the net effect of aging on the vasculature of all organ systems in the body, whereas the present study was specific to skeletal muscle. Moreover, systemic infusion of vasoactive compounds can potentially evoke reflex responses when arterial pressure is altered, so differences in systemic vascular resistance could be due to age-associated changes in arterial baroreflex control of the circulation. In addition, infusion of a nonspecific nitric oxide synthase inhibitor can have multiple effects on the cardiovascular system, including alterations in the neural control of the circulation [23]. Therefore, differences in the mechanism of enhanced ET-1 vasoconstriction with aging reported by Asai et al. and the present study may reflect the different animal species used, organ systems studied, and experimental approaches employed.

The present study demonstrates that KCl-induced vasoconstriction is less in gastrocnemius muscle arterioles with intact endothelium from old animals (Fig. 5A). However, this is in contrast to a previous report from our laboratory indicating aging does not alter KCl-evoked constriction in gastrocnemius muscle arterioles [15]. The likely reason for this discrepancy is that in the present study [K\textsuperscript{+}] was incrementally increased isotonically by varying the concentrations of NaCl and KCl in the PSS buffer solution. In our previous study, [K\textsuperscript{+}] was incrementally increased with the cumulative addition of KCl, which elevates the osmolarity of the PSS buffer solution. Changes in osmolarity can affect vascular tone and, consequently, could account for the varying results in KCl-mediated vasoconstriction between studies.

### 4.2 Age-associated increases in ET-1 sensitivity: effects of muscle fiber type

The reported effects of aging on ET-1 mediated vasoconstriction are quite variable in the literature. Studies of arteries of varying sizes (e.g., conduit vs. resistance) and from various tissues show diminished [24–27], unchanged [26,28] and enhanced [25,27,29] contractile responses to ET-1 with aging. For example, the work of Lüscher et al. has shown diminished ET-1 sensitivity in small mesenteric arteries [24], unaltered responses in large conduit arteries [26], and augmented ET-1 vasoconstriction with aging in coronary arteries [29]. The present study likewise demonstrates the heterogeneity of aging effects on vascular responses between 1A resistance arterioles from different skeletal muscle types. Aging only enhanced ET-1 responsiveness and sensitivity of arterioles from the gastrocnemius muscle (Fig. 2). Because this muscle is composed primarily of fast-twitch glycolytic (type IIB) fibers, the fiber type that makes up approximately 70% of the total rat muscle mass [30], this response may represent the predominant vascular alteration induced by aging in skeletal muscle. In contrast, there was no aging effect on ET-1 sensitivity or maximal constriction of arterioles from the soleus muscle (Fig. 1), a muscle composed predominantly of slow-twitch oxidative (type I) fibers [30].

What remains unclear is which factor(s) contribute to the heterogeneity of ET-1 responses within muscle or among tissues with aging. Although no definitive answers currently exist, previous work with soleus and gastrocnemius muscle arterioles demonstrates that these vessels regulate vascular tone through distinct mechanisms. For example, soleus muscle arterioles rely principally upon the nitric oxide synthase mechanism to modulate basal tone and mediate endothelium-dependent vasodilation, while that in
gastrocnemius muscle arterioles occurs primarily through other endothelium-derived substances [12]. Since nitric oxide attenuates the local ET-1 system, we speculated that a diminished nitric oxide bioavailability, which is associated with old age in the soleus muscle arterioles, might make arterioles from this muscle type more sensitive to ET-1. Contrary to our hypothesis, old age did not influence ET-1 responsiveness or sensitivity in soleus muscle arterioles, despite aging-induced decrements in endothelium-dependent vasodilation through the nitric oxide synthase pathway in these vessels [4,12].

An alternative to the endothelial ETB receptor-nitric oxide synthase hypothesis is that the heterogeneity of age-associated changes in vascular responsiveness is related to alterations in sympathetic nerve activity. Previous work has shown that chronically high sympathetic outflow associated with mental stress augments the ETA receptor vasoconstriction [31]. Aging is likewise associated with elevations in sympathetic nerve activity [32]. Because the vasculature of fast-twitch glycolytic muscle is under greater adrenergic vasoconstrictor tone than that in highly oxidative muscle [33], old-age-associated elevations in sympathetic nerve activity may preferentially alter vascular responsiveness to ET-1 in muscles such as the gastrocnemius muscle. This hypothesis requires further investigation.

4.3. Exercise training effects on ET-1 sensitivity

Ten to twelve weeks of aerobic exercise training had no effect on ET-1 sensitivity or maximal responsiveness in skeletal muscle arterioles. Isolated endothelial cells exposed to shear stress have been shown to increase expression of endothelial ETB receptors [34]. These data, as well as other studies demonstrating improved endothelial function after chronic aerobic exercise training in young and old subjects [12,35,36], served as the basis for the hypothesis that chronic elevations in muscle blood flow during exercise would increase expression of endothelial ETB receptors and, consequently, attenuate ET-1 vasoconstriction in muscles from exercise-trained animals. Despite a previous report that exercise training augments endothelium-dependent nitric oxide-mediated vasodilation in soleus and gastrocnemius muscle arterioles [12], the in vitro data of the present study do not support a role for augmented endothelial ETB receptor vasodilator function with exercise training (Figs. 1 and 2).

4.4. Implications

Previously, it has been shown that skeletal muscle blood flow is lower and vascular resistance higher in older individuals [37,38]. Likewise, systemic vascular resistance [39] and blood pressure becomes elevated in many older subjects [40]. The findings of the present study demonstrate that ET-1 vasoconstrictor responsiveness is enhanced in the skeletal muscle microcirculation with aging. This finding, coupled with the observation that endothelial ET-1 release is augmented with aging [26], indicate that an enhanced ET-1 signal transduction mechanism may contribute to the elevations in skeletal muscle vascular resistance and, consequently, systemic vascular resistance that often occur in older individuals. Such changes in systemic vascular resistance with healthy aging could predispose older subjects to hypertension [41,42]. And finally, although exercise training did not affect responsiveness of skeletal muscle arterioles to ET-1 in young or old animals, the beneficial effect of exercise in restoring muscle blood flow capacity in the aged may occur through an alternate mechanism(s).

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