Diaphragm arterioles are less responsive to $\alpha_1$-adrenergic constriction than gastrocnemius arterioles

AARON AAKER AND M. H. LAUGHLIN
Departments of Veterinary Biomedical Sciences and Medical Physiology, University of Missouri, Columbia, Missouri 65211

Received 21 November 2001; accepted in final form 8 January 2002

Aaker, Aaron, and M. H. Laughlin. Diaphragm arterioles are less responsive to $\alpha_1$-adrenergic constriction than gastrocnemius arterioles. J Appl Physiol 92: 1808–1816, 2002.—The sympathetic nervous system has greater influence on vascular resistance in low-oxidative, fast-twitch skeletal muscle than in high-oxidative skeletal muscle (17). The purpose of this study was to test the hypothesis that arterioles isolated from low-oxidative, fast-twitch skeletal muscle [the white portion of gastrocnemius (WG)] possess greater responsiveness to adrenergic constriction than arterioles isolated from high-oxidative skeletal muscle [red portion of the gastrocnemius muscle (RG) and diaphragm (Dia)]. Second-order arterioles (2As) were isolated from WG, RG, and Dia of rats and reactivity examined in vitro. Results reveal that Dia 2As constrict less to norepinephrine (NE) ($10^{-9}$ to $10^{-4}$ M) than 2As from RG and WG, which exhibited similar NE-induced constrictions. This difference was not endothelium dependent, because responses of denuded 2As were similar to those of intact arterioles. The blunted NE-induced constrictor response of Dia 2As appears to be the result of differences in $\alpha_1$-receptor effects because 1) arterioles from Dia also responded less to selective $\alpha_1$-receptor stimulation with phenylephrine than RG and WG arterioles; 2) arterioles from Dia, RG, and WG dilated similarly to isoproterenol ($10^{-9}$ to $10^{-4}$ M) and did not respond to selective $\alpha_2$-receptor stimulation with UK-14304; and 3) endothelin-1 produced similar constriction in 2As from Dia, RG, and WG. We conclude that differences in oxidative capacity and/or fiber type composition of muscle tissue do not explain different NE responsiveness of Dia 2As compared with 2As from gastrocnemius muscle. Differences in $\alpha_1$-adrenergic constrictor responsiveness among arterioles in skeletal muscle may contribute to nonuniform muscle blood flow responses observed during exercise and serve to maintain blood flow to Dia during exercise-induced increases in sympathetic nerve activity.

endothelium; endothelial-derived factors; norepinephrine; isoproterenol; phenylephrine; UK-14304; endothelin-1

BLOOD FLOW IS DISTRIBUTED nonuniformly within and among skeletal muscles both at rest and during exercise (19, 20). Blood flow is directed to high-oxidative skeletal muscle tissue under most conditions and only appears to be directed to low-oxidative skeletal muscle during high intensities of exercise (1, 18–20). Differences in sympathetic nervous system (SNS) control of vascular resistance may contribute to these differences in blood flow within and among skeletal muscles at rest and during exercise (18–20). Indeed, there is evidence that the SNS has greater influence in increasing vascular resistance (and therefore limiting blood flow) in low-oxidative, fast-twitch skeletal muscle than in high-oxidative skeletal muscle. For example, at rest, blockade of $\alpha$-adrenergic receptors with phentolamine produces increased blood flow in fast-twitch, low-oxidative muscle whereas blood flow to diaphragm (Dia) and high-oxidative muscle was not changed (17). Also, during exercise, $\alpha$-adrenergic receptor blockade produced increased blood flow to white, low-oxidative muscle whereas blood flow to high-oxidative skeletal muscle and Dia muscle was unchanged or decreased. These results suggest that adrenergic constriction is greater in low-oxidative, fast-twitch skeletal muscle than in high-oxidative muscle (including Dia muscle) both at rest and during exercise (17). Furthermore, Folkow and Halicka (7) and Hilton and colleagues (10) reported that activation of sympathetic nerves in cats produced a larger increase in resistance to blood flow in the fast-twitch gastrocnemius muscle than in the more highly oxidative, slow-twitch soleus muscle.

Increased sympathetic vasomotor tone in fast-twitch, low-oxidative skeletal muscle could be the result of at least two mechanisms. First, the increased vascular tone could result from greater sympathetic innervation in, and/or greater SNS activity directed to, low-oxidative skeletal muscle than to high-oxidative skeletal muscle. Second, it is possible that arterioles that control blood flow to low-oxidative skeletal muscle are more responsive to the constrictor effects of norepinephrine (NE) than arterioles in skeletal muscle with high oxidative capacity. The purpose of this study was to test the second hypothesis.

We examined intrinsic vasoreactivity of second-order arterioles isolated from low-oxidative, fast-twitch skeletal muscle [white portion of gastrocnemius muscle (WG)] and compared and contrasted these responses to those of arterioles isolated from high-oxidative, skele-
tal muscle [red portion of gastrocnemius muscle (RG) and Dia]. We used an isolated arteriolar preparation to allow comparison of vasoconstrictor responses among arterioles from different muscle tissue without the confounding effects of neurohumoral influences, metabolic effects, and mechanical forces present in vivo. Results presented below demonstrate that second-order arterioles from Dia constrict less in response to NE than arterioles from RG and WG. The attenuated response of Dia arterioles appears to result from differences in α₁-receptor effects as arterioles from Dia also responded less to phenylephrine (PE) than arterioles from RG and WG. These results support the conclusion that factors other than oxidative capacity and fiber type composition of the muscle tissue are responsible for the differences in NE responsiveness of arterioles isolated from Dia muscle and those isolated from gastrocnemius muscle.

**METHODS**

**Experimental Design**

Intrinsic vasoconstrictor responses were examined in second-order arterioles isolated from three different types of skeletal muscle tissue: WG [low-oxidative, 90% fast-twitch, glycolytic fibers (FG) (19)]; RG [high-oxidative, 34% slow-twitch oxidative (SO) and 60% fast-twitch, oxidative, glycolytic fibers (FOG) (19)], and Dia [high-oxidative, 44% SO fibers, 24% FOG fibers, and 32% FG fibers (5)] muscle of rats. Our rationale for selection of second-order arterioles included the following. 1) Second-order arterioles in these three muscle tissues have consistent anatomy and location within the arteriolar trees of these muscles. 2) Second-order arterioles in these three muscle tissues have similar diameters, lengths, and limited numbers of branches for in vitro experiments. 3) Second-order arterioles are a primary site of regulation of vascular resistance in skeletal muscle tissue (6).

We decided to study arterioles from the Dia because of the importance of this muscle in respiration and because previous results indicate that the SNS has limited effects on Dia vascular resistance (17). We compare and contrast responses of arterioles from the Dia to those from red and white portions of gastrocnemius muscle because the WG is 90% fast-twitch, glycolytic muscle whereas the RG is composed primarily of high-oxidative muscle fibers and because previous results indicated that SNS has limited effects on vascular resistance in the RG muscle (17).

We first examined NE-induced vasoconstrictor responses of arterioles from the three different types of muscle. Because we found that arterioles from Dia exhibited less constriction in response to NE than did arterioles from gastrocnemius muscle, we used a pharmacological approach to determine the relative importance of α- and β-adrenergic receptors in these different vasomotor responses. Vasomotor responses produced by the following selective agonists were examined: PE, a selective α₁-adrenergic receptor agonist; UK-14304, a selective α₂-adrenergic receptor agonist; and isoproterenol, a selective β-adrenergic receptor agonist. In addition, we evaluated the role of endothelium-dependent influences by comparing vasoconstrictor responses in intact arterioles to responses of arterioles after removal of the endothelium and after treatment with an arginine analog to inhibit production of nitric oxide via nitric oxide synthase. Finally, because Dia arterioles did not exhibit maximal constriction in response to any adrenergic agent studied, we examined responses of the arterioles to endothelin-1 (ET-1) to determine whether there was a structural limitation to maximal constriction in Dia arterioles.

**Experimental Animals**

Forty-nine male Sprague-Dawley rats (weight 444 ± 4 g) were obtained from Harlan (Indianapolis, IN) in groups of 12–16 at least 1 wk before experimental use. Animals were housed in pairs in temperature-controlled (24°C) and light-controlled (12:12-h light-dark cycle) rooms. Rat chow and water were available ad libitum. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Missouri.

**Preparation of Arterioles**

On the morning of the experiment, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). The medial head of the gastrocnemius muscle and Dia muscle were transferred to a chamber containing cold (4°C) MOPS-buffered physiological saline solution (PSS) containing (in mM) 145.0 NaCl, 4.7 KCl, 2.0 CaCl₂, 1.17 MgSO₄, 1.2 NaH₂PO₄, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 3.0 MOPS (pH 7.4).

Overlying muscle tissue was dissected away, revealing the vasculature of each muscle. The approach to this dissection in the medial head of the gastrocnemius muscle was similar to that described by Woodman et al. (35). Second-order arterioles ~500–1,000 μm in length were removed and transferred to a Lucite chamber containing cold MOPS-buffered PSS. One end of the arteriole was cannulated with a glass micropipette (45–55 μm OD) and tied securely to the micropipette with 11-0 ophthalmic suture. The arteriole was flushed with PSS-albumin (1 g/100 ml) to remove blood from the lumen before the other end of the vessel was cannulated and secured. Micropipette pairs were within 2 μm outer diameter of one another.

After cannulation of the arteriole, the microvessels chamber was transferred to an inverted microscope (Nikon Diaphot 200, ×20 and ×40 magnification, numerical aperture between 0.25 and 0.50). Each pipette was attached to an independent fluid reservoir containing PSS-albumin. By raising each fluid reservoir to the same level, luminal pressure was established where no flow occurred through the lumen of the arteriole (16). To view the cannulated arteriole, the inverted microscope was coupled to a camera (Javelin Electronics, Los Angeles, CA), video monitor (Sony), video micrometer (Microcirculation Research Institute, Texas A&M University, College Station, TX), and Macintosh/MacLab data acquisition system. Luminal diameter and pressure were monitored continuously for the duration of each experiment (sampling frequency 20 s⁻¹).

After transfer of the vessel chamber to the inverted microscope, the vessel chamber was warmed gradually to 37°C and maintained at this temperature for the duration of the experiment. Luminal pressure was set initially at 30 cmH₂O. After 20 min, the pressure was increased to an amount midway between 30 cmH₂O and the final pressure that would be established at 40 min. The final intraluminal pressure was selected based on in vivo measurements of pressure in rat skeletal muscle arterioles of similar size and branch order (2, 25, 36). Final pressure for arterioles with an initial diameter of 100–125 μm was 60 cmH₂O, and final pressure for arterioles with an initial diameter of 125–150 μm was 67 cmH₂O. The bathing solution was replaced every 15 min during the 1-h equilibration period.
After the 60-min equilibration period, endothelial and vascular smooth muscle function was assessed with 10−4 M acetylcholine (ACh) and 80 mM KCl, respectively. Arterioles were considered to possess functional endothelium and vascular smooth muscle if they exhibited >26% dilation from baseline diameter in response to the ACh challenge and greater than 30% constriction to an 80 mM KCl challenge. Vessels not meeting these criteria were discarded.

**Experimental Protocol**

After demonstration of functional endothelium and vascular smooth muscle, arterioles were allowed to achieve and maintain a stable diameter for at least 10 min before administration of a vasoactive agent. Cumulative dose-response curves for the agents of interest were constructed by adding the desired agonist to the bath in whole-log increments in concentration. Different groups of arterioles were used to examine responses of arterioles to the following adrenergic agonists (10−9 to 10−4 M): NE, PE, UK-14304, or isoproterenol. After each dose, arterioles were allowed 3 min before the next dose was administered.

**Endothelium-dependent responses.** To determine the role of the endothelium in vasomotor responses to adrenergic agents, arterioles were denuded to remove the endothelial layer as described by Sun and colleagues (31, 32). Briefly, after one end of arteriole was cannulated and 3–5 ml of air were passed through the lumen of the vessel, the arteriole was then flushed with PSS-albumin, and the other end of the arteriole was cannulated and secured to the other micropipette. Successful denudation was determined by challenging the arteriole with 10−4 M ACh. The arteriole was considered denuded if there was <5% dilation to 10−4 M ACh after the denudation procedure.

To determine the specific role of nitric oxide in vasomotor responses to adrenergic agents, arterioles were treated with the arginine analog N⁵-nitro-L-arginine (L-NNA) (1 mM, for 30 min before adrenergic agents) to inhibit production of nitric oxide by nitric oxide synthase. The efficacy of nitric oxide synthase inhibition was evaluated by exposure of the arterioles to 10−4 M ACh.

**Endothelin-induced vasoconstriction.** Because neither NE nor PE caused complete constriction in arterioles from Dia, the potent vasoconstrictor ET-1 was utilized to determine whether arterioles from Dia could exhibit complete constriction in response to this agent. After the ACh and 80 mM KCl challenges, arterioles were allowed to achieve a stable diameter before administration of ET-1 (10−12 to 10−7 M) was added to the bath in half-log increments.

**Multiple dose-response curves.** Experiments were performed to determine the repeatability of NE dose-response curves. Each arteriole had four NE dose-response relationships (constructed at 1, 2, 3, and 4 h after cannulation). Results revealed that only arterioles from WG exhibited repeatable responses (dose-response curves) to NE. Therefore, RG and Dia arterioles were exposed to only a single dose-response curve. Two arterioles were isolated from each Dia and RG muscle, and responses were compared between untreated and treated arterioles to examine the impact of denudation and/or L-NNA treatment. Because NE dose-response curves were repeatable for WG arterioles, in WG arterioles we used a control dose-response curve, followed by a dose-response curve for the experimental intervention (i.e., denudation, L-NNA, subtype-specific receptor agonist). WG arterioles received a maximum of two dose-response curves.

**Determination of Maximal Diameter**

To determine maximal diameter of each arteriole, arterioles were incubated in calcium-free PSS with 2 mM EDTA for at least 30 min. In some cases when the arteriole had not achieved a stable diameter, up to 1 h was allowed for incubation in calcium-free buffer. The calcium-free bathing solution was replaced every 15 min.

**Drugs and Solutions**

Warm PSS-albumin containing 10 g/l bovine serum albumin (fraction V, >98% pure, United States Biochemical, Cleveland, OH) was utilized within the arteriolar lumen and was pH 7.4 at 37°C. Warm PSS without albumin was utilized as superfusate for the isolated arteriole experiments and was pH 7.4 at 37°C. All PSS was prepared in advance, filtered through a 0.2-μm filter, and frozen for use on the day of experiment.

In the experiments with L-NNA treatment, warm PSS without albumin contained 25 mM MOPS because L-NNA was dissolved in 1 N HCl and this concentration of MOPS was required to maintain pH of 7.4 in the superfusate. Trials were conducted to determine that 25 mM MOPS was sufficient to maintain pH 7.4 after the addition of L-NNA.

Stock solutions of L-NNA were prepared in 1 N HCl and UK-14304 was prepared in 3.33% dimethyl sulfoxide. All other drug stocks were prepared in distilled, deionized water and frozen for later use. Albumin was obtained from United States Biochemical, and HCl, NaCl, KCl, and CaCl₂ were obtained from Fisher Scientific (Pittsburgh, PA). All other chemicals were obtained from Sigma Chemical (St. Louis, MO).

**Data Analysis**

Arteriolar diameter data were expressed as 1) absolute diameter (in μm), 2) the percentage of the possible response between the baseline diameter before the first dose of the agent being utilized and zero, and 3) the percentage of the maximal response from baseline diameter. The concentration of drug producing half-maximal effect (EC₅₀) was determined with Prism software as described previously (14). For comparing maximal diameters of arterioles, beginning tone, maximal responses, and EC₅₀ values among arterioles from different muscles (Dia vs. RG vs. WG), one-way ANOVA was utilized (SuperANOVA statistical software). Comparisons of control vs. treatment groups (intact vs. denuded, control vs. L-NNA treated, etc.) with arterioles from the same muscle were performed by utilizing the Student’s t-test.

Dose-response relationships between groups (e.g., intact vs. denuded) were compared by use of two-way repeated measures ANOVA (SuperANOVA statistical software). Differences between groups that were identified by ANOVA were located by utilizing Tukey’s multiple-comparisons post hoc test. Significance for all analyses performed was set at P < 0.05 for all comparisons.

**RESULTS**

**Characteristics of Arterioles**

The maximal diameters of second-order arterioles (obtained by incubating each arteriole in calcium-free PSS for a minimum of 30 min) from each muscle type for all arterioles from all groups of experiments are listed in Table 1. Maximal diameters of arterioles from RG were greater than those obtained for arterioles
from Dia and WG, and the diameter of WG and Dia second-order arterioles were not different. There were no differences in the amount of spontaneous tone among the three types of arterioles when arterioles from all groups are examined.

**Norepinephrine**

NE produced a dose-dependent constriction in arterioles from Dia, RG, and WG (Fig. 1). Importantly, NE produced significantly less constriction in arterioles from Dia compared with arterioles from RG and WG.

As shown in Fig. 2, denudation did not alter the NE dose-response relationship in arterioles from Dia and RG. In contrast, arterioles from WG were more sensitive to NE after denudation (Fig. 2) (EC50 of intact arterioles $-6.26 \pm 0.03$ log M vs. $-7.38 \pm 1.63$ log M for denuded arterioles). The maximal response of WG arterioles to NE was not changed by denudation, in that the arterioles still closed at $10^{-5}$ M NE.

**Contribution of Nitric Oxide to the NE Response**

Pretreatment with L-NNA significantly increased beginning tone (decreased diameter) in arterioles from Dia (control $105 \pm 2$ µm; L-NNA = 75 ± 1 µm), RG (control = 98 ± 7 µm; L-NNA = 56 ± 4 µm), and WG (control = 94 ± 8 µm; L-NNA = 78 ± 8 µm), suggesting that arterioles from all three types of muscle have basal release of nitric oxide and that second-order arterioles from RG have greater basal release of nitric oxide than those of WG and Dia. The results of the $10^{-4}$ M ACh test of the efficacy of nitric oxide synthase inhibition with L-NNA were interesting. L-NNA treatment nearly abolished ACh-induced dilation in Dia and WG arterioles, suggesting that nitric oxide release plays a key role in ACh-induced dilation of these arte-
rioles. Although the maximal ACh-induced diameter of RG second-order arterioles were decreased by 17% after L-NNA treatment, L-NNA treatment did not alter the magnitude of the increase in diameter produced by ACh or the ACh-induced percent dilation of RG arterioles. These results suggest that release of nitric oxide plays a greater role in ACh-induced dilation of second-order arterioles from WG and Dia than in ACh-induced dilation of second-order arterioles from RG.

Importantly, L-NNA treatment modestly, but significantly, increased NE sensitivity of second-order arterioles from RG and WG, shifting the NE dose-response relationships to the left (Fig. 3) (EC<sub>50</sub> −6.47 ± 0.15 log M and −7.05 ± 0.10 log M for intact and L-NNA-treated arterioles from WG). L-NNA treatment did not alter NE sensitivity of Dia arterioles (EC<sub>50</sub> −5.91 ± 0.20 log M and −6.02 ± 0.66 log M for intact and L-NNA-treated arterioles from Dia; Fig. 3).

**Adrenergic Receptor Subtypes**

**α-Adrenergic receptors.** Arterioles from each muscle constricted in a dose-dependent manner to PE (Fig. 4), and denudation did not significantly alter responses to PE. Thus both intact and denuded arterioles from Dia constricted significantly less in response to PE than arterioles from RG and WG. Although denudation did not alter the PE dose-response relationship in arterioles from RG and WG, it modestly increased PE sensitivity of Dia arterioles (EC<sub>50</sub> −5.63 ± 0.14 log M for intact arterioles vs. −5.18 ± 0.18 log M for denuded arterioles). Neither intact nor denuded arterioles from any of the three muscles exhibited a response to UK-14304 (data not shown).

**β-Adrenergic receptors.** Arterioles from Dia, RG, and WG demonstrated similar dose-dependent dilation in response to isoproterenol up to 10<sup>−5</sup> M (Fig. 5). Maximal dilation in response to isoproterenol was attenuated in arterioles from Dia after denudation and treatment with 1 mM L-NNA (Fig. 6A). Pretreatment with 1 mM L-NNA increased beginning tone in RG arterioles, but neither denudation nor pretreatment with L-NNA altered the dose-response relationship for isoproterenol in arterioles from RG (Fig. 6B). Finally, maximal dilation in response to isoproterenol was attenuated in arterioles from WG after denudation but was not significantly altered by pretreatment with L-NNA (Fig. 6C).

**ET-1**

Arterioles from Dia, RG, and WG constricted in response to ET-1 in a dose-dependent manner (Fig. 7).

---

Fig. 4. Phenylephrine-induced vasoconstriction of 2nd-order arterioles from Dia, RG, and WG. Values are means ± SE expressed as absolute diameters. B, baseline diameter before administration of 1st dose of phenylephrine. *Maximal vasoconstrictor response of Dia arterioles was significantly less than that of RG and WG arterioles (P < 0.05).
The sensitivity to ET-1 and the maximal constriction produced by ET-1 were similar among arterioles from Dia, RG, and WG.

DISCUSSION

The present study was stimulated in part by previous observations that blockade of \( \alpha \)-adrenergic receptors in conscious rats produced increased blood flow to white, low-oxidative muscle but not to high-oxidative skeletal muscle or Dia muscle (17). These results suggested that adrenergic constriction is greater in low-oxidative, fast-twitch skeletal muscle (WG) than in high-oxidative muscle (RG and Dia) both at rest and during exercise (17). The purpose of the present study was to test the hypothesis that second-order arterioles isolated from low-oxidative, WG skeletal muscle constrict more in response to NE than arterioles from highly oxidative skeletal muscle (RG and Dia). Consistent with this hypothesis, second-order arterioles from Dia responded significantly less to NE than arterioles from WG (Fig. 1). However, Dia arterioles also constricted less in response to NE than arterioles from RG. Indeed, there were no differences in the vasoconstrictor responses of arterioles from RG and WG even though RG muscle is much more oxidative than WG muscle (5, 19). Therefore, present results do not support our hypothesis that arterioles from low-oxidative skeletal muscle are more responsive to the vasoconstrictor NE than highly oxidative skeletal muscle. Furthermore, it appears that vasomotor responsiveness of second-order arterioles cannot be predicted simply from oxidative capacity or fiber type composition of the skeletal muscle in which the arteriole lies.

We have a longstanding interest in relationships between fiber type composition of skeletal muscle and control of blood flow to muscle (18, 20). In this regard, it is of interest to ascertain whether the different responses of second-order arterioles from Dia muscle vs. those of gastrocnemius muscle are related to differences in fiber type composition of the muscles or to other unique features of Dia muscle. Laughlin and Armstrong (17) proposed that the role of \( \alpha \)-adrenergic vasomotor tone was less in skeletal muscle with muscle fiber type composition of \( \geq 20\% \) SO muscle fibers because \( \alpha \)-adrenergic receptors had little or no effect on blood flow to rat skeletal muscles that had \( \geq 20\% \) of...
their muscle fibers of the SO type. The notion that resistance arteries perfusing SO skeletal muscle are less responsive to NE than those of fast-twitch skeletal muscle is also supported by results of experiments utilizing whole muscle preparations (7, 9, 10). Thus it is possible that Dia arterioles have a blunted response to NE because the Dia consists of >20% SO fiber type. However, because RG muscle consists of 34% SO fiber type and RG arterioles exhibit similar adrenergic responsiveness to arterioles from WG muscle, our results indicate that the percentage of SO fiber type composition alone cannot be used to predict adrenergic responsiveness. Consistent with this conclusion, Delp (4) reported that there was no difference between NE sensitivity or maximal NE-induced constriction of first-order arterioles from soleus (80% SO) and WG muscle of rats.

It is important to consider the notion that different responsiveness of arterioles from RG and Dia muscle result from other fiber type differences and/or oxidative capacity differences between these two types of muscle. Delp and Duan (5) report that Dia muscle has a greater percentage of FG fibers (type IIb) and similar percentage composition of SO (type I) fibers as RG muscle and that the proportion of FOG fibers consisting of type IID/X is similar in RG (13%) and Dia (18%) (5). Importantly, RG and Dia muscle of rats have similar oxidative capacities as reflected in citrate synthase activity [RG = 36 μmol·min⁻¹·g⁻¹, Dia = 39 μmol·min⁻¹·g⁻¹ (5)]. These data, combined with our results, lead us to the surprising conclusion that neither differences in fiber type composition of the muscle nor differences in oxidative capacity of the muscles can explain the finding that second-order arterioles from Dia exhibit blunted responses to NE, relative to arterioles from RG and WG. Clearly, second-order arterioles isolated from high-oxidative muscle (RG) consisting of between 34 (19) and 51% (5) SO fibers show vasoconstrictor responses to NE similar to those isolated from low-oxidative muscle (WG) (Fig. 1). It appears that factors other than fiber type composition and oxidative capacity of the muscle tissue are responsible for the different responsiveness of Dia arterioles to NE. The conclusion that fiber type composition and oxidative capacity of the muscle tissue are not responsible for the different responsiveness of Dia arterioles to NE does not mean that there are no differences in vascular control among muscles with differing fiber type composition. Indeed, there are such differences (20, 21). Also, it is important to emphasize that these results may be specific to second-order arterioles because we do not know whether we can apply these results to other arterioles in the arteriolar networks of these muscle tissues (14, 21).

We used selective adrenergic receptor subtype-specific agonists to evaluate the roles of α₁-, α₂-, and β-adrenergic receptors in differences in NE responsiveness between Dia arterioles and arterioles from RG and WG muscle. Responses of all three types of arterioles to PE were similar to NE responses in that Dia arterioles exhibited less constriction than did WG and RG arterioles (Fig. 4). UK-14304, a selective α₂-receptor agonist, did not produce constriction or dilation of second-order arterioles from any of the muscles examined, indicating that α₂-receptors have little or no contribution to the NE response in second-order arterioles from Dia, RG, or WG. These results confirm those of Ohyanagi and colleagues (27) and Nase and Boegehold (26), who reported that α₂-receptors are not present in proximal skeletal muscle arterioles. Our results do not allow us to evaluate the proposal that α₂-receptors mediate adrenergic responses of small, distal skeletal muscle arterioles whereas α₁-receptors mediate adrenergic responses in large, proximal arterioles (24, 27) because the distribution of α₁- and α₂-receptors throughout the vascular networks of Dia, RG, and WG muscle has not been established.

To evaluate the responsiveness of these arterioles to β-adrenergic receptors, we used isoproterenol, a β₁- and β₂-adrenergic receptor agonist. Isoproterenol produced similar vasodilation in arterioles from Dia, RG, and WG (Fig. 5), confirming that activation of β-receptors produces vasodilation in skeletal muscle resistance arterioles (3, 8, 13, 23). Our observation of similar reactivity of arterioles from these three types of muscle to isoproterenol is also consistent with previous observations demonstrating that β-receptor blockade with propranolol (β₁- and β₂-receptors) or butoxamine (selective for β₂-receptors) produce similar decreases in blood flow to all types of rodent skeletal muscle during exercise (17). Interestingly, McCurdy and colleagues (23) recently reported that maximal dilation and sensitivity of 1A arterioles from WG (lateral head) to isoproterenol are greater than in 1As from soleus muscle of rats. These results combined with present results suggest that slow-twitch muscle of limbs (soleus) have blunted isoproterenol responsiveness and/or that 1A and 2A arterioles may have significantly different sensitivities to isoproterenol.

The results of our present experiments, examining reactivity of arterioles to various adrenergic agonists,
support two conclusions. First, we conclude that NE-induced vasoconstriction in these second-order arterioles is primarily mediated by α₁-receptors. Second, we conclude that the blunted response of Dia second-order arterioles, relative to WG and RG arterioles, results from differences in α₁-mediated constriction. This decreased responsiveness of arterioles from Dia, compared with arterioles from RG and WG muscle, may be the result of fewer α₁-adrenergic receptors in smooth muscle of Dia arterioles than in arterioles from RG and WG muscle. Alternatively, differences in down-stream signaling pathways (receptor-G protein coupling, activation of phospholipase C, activation of protein kinase C, or calcium release from intracellular stores by inositol trisphosphate) in vascular smooth muscle of Dia arterioles may be responsible. Arterioles from Dia may be less responsive to NE and PE because of sustained activity of breathing in a manner similar to the effects of exercise on adrenergic vasomotor control in rabbits, for which a single bout of exercise is reported to diminish sensitivity to PE in hindlimb and thoracic aorta (11, 12), and/or when chronic exercise training decreases NE sensitivity of rat aorta (30).

Blunted α₁-receptor function, associated with exercise, has been proposed to result from either a reduction of receptor numbers or a reduction in phosphoinositol turnover in vascular smooth muscle (22, 34). Whether the blunted NE response in arterioles from Dia is the result of a similar exercise/muscle activity-related reduction in α₁-receptor content or changes in receptor/second messenger coupling cannot be established from our results. It is interesting that the blunted response to NE in arterioles from Dia in this study is similar to the blunted responses observed in isolated coronary arterioles, in that both demonstrate limited constrictor responses to NE and other adrenergic agents (15, 28, 33). The blunted adrenergic responsiveness of coronary and Dia arterioles may be beneficial in maintaining perfusion to these two highly important muscle tissues during exercise when sympathetic nerve activity and plasma NE concentrations increase.

The endothelium of Dia arterioles appeared to have less of a contribution to NE-induced constriction than did the endothelium of arterioles from WG and RG. The response of gastrocnemius arterioles to NE was blunted by the endothelium (Fig. 2) and by the production of nitric oxide by nitric oxide synthase (Fig. 3). In contrast, neither endothelial removal nor blockade of nitric oxide production appeared to influence NE responses of Dia arterioles. We are not aware of a mechanism for this surprising observation that the role of endothelium in NE responses is greater in arterioles from RG and WG compared with arterioles from Dia. However, the effects of removal of the endothelium in arterioles from RG and WG were modest.

In summary, this study tested the hypothesis that second-order arterioles from low-oxidative skeletal muscle are more responsive to NE than arterioles from high-oxidative skeletal muscle. Results demonstrate that second-order arterioles from Dia respond less to NE than arterioles from RG and WG. The blunted NE-induced constriction of Dia arterioles appears to be the result of differences in α₁-receptor effects because arterioles from Dia also responded less to PE than arterioles from RG and WG. Because ET-1 produced similar maximal constriction among arterioles from Dia, RG, and WG, it is clear that Dia arterioles have similar capacity for maximal constriction. Finally, on the basis of literature values for fiber type composition and measures of oxidative capacity of these muscle tissues, we conclude that factors other than fiber type composition and oxidative capacity of the muscle tissue are responsible for the different NE responsiveness of arterioles isolated from Dia muscle and those isolated from gastrocnemius muscle. These differences in adrenergically mediated vasoconstrctor responsiveness among arterioles in skeletal muscle may contribute to nonuniform muscle blood flow responses observed during exercise and serve to maintain blood flow to Dia during exercise-induced increases in sympathetic nerve activity. However, our results suggest that differences in adrenergically mediated vasoconstrctor responsiveness of arterioles do not explain the observation that the sympathetic nervous system has greater influence on vascular resistance in low-oxidative (WG) than in high-oxidative (RG) limb skeletal muscle at rest and during exercise (17). These differential effects of the sympathetic nervous system on vascular resistance must result from differential SNS innervation/SNS activity or from local, metabolic effects of muscle fiber activity on adrenergic control mechanisms (17, 20).

The authors thank Pam Thorne, Tammy Strawn, and Denise Holiman for excellent technical contributions to this work. This work was supported by National Heart, Lung, and Blood Institute Grant HL-36088 and by a Predoctoral Fellowship Grant from the American College of Sports Medicine to A. Aaker.

REFERENCES
ADRENERGIC RESPONSES IN STRIATED MUSCLE ARTERIOLES


