Differential adenosine sensitivity of diaphragm and skeletal muscle arterioles

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Aaker, Aaron, and M. H. Laughlin. Differential adenosine sensitivity of diaphragm and skeletal muscle arterioles. J Appl Physiol 93: 848–856, 2002; 10.1152/japplphysiol.00032.2002.—The hyperemic response in exercising skeletal muscle is dependent on muscle fiber-type composition and fiber recruitment patterns, but the vascular control mechanisms producing exercise hyperemia in skeletal muscle remain poorly understood. The purpose of this study was to test the hypothesis that arterioles from white, low-oxidative skeletal muscle are less responsive to adenosine-induced dilation than are arterioles from diaphragm (Dia) and red, high-oxidative skeletal muscle. Second-order arterioles (2As) were isolated from the white portion of gastrocnemius muscle (WG; low-oxidative, fast-twitch muscle tissue) and two types of high-oxidative skeletal muscle (Dia and red portion of gastrocnemius muscle (RG)) of rats. Results reveal that 2As from all three types of muscle dilated in response to the endothelium-dependent dilator acetylcholine (WG: 48 ± 3%, Dia: 51 ± 3%, RG: 74 ± 3%). In contrast, adenosine dilated only 2As from WG (48 ± 4%) and Dia (46 ± 5%) but not those from RG (5 ± 5%). Thus adenosine-induced dilator responses differed among 2As of these different types of muscle tissue. However, the results do not support our hypothesis because 2As from Dia and WG dilated in response to adenosine, whereas 2As from RG did not. We conclude that the adenosine responsiveness of 2As from rat skeletal muscle cannot be predicted only by the fiber-type composition or oxidative capacity of the skeletal muscle tissue wherein the arteriole lies.

arteries; blood flow; endothelium; endothelial-derived factors; exercise

BLOOD FLOW IS LINEARLY RELATED to skeletal muscle oxygen consumption over a wide range of metabolic rates, lending support to the idea that local control of vascular resistance in skeletal muscle is driven by metabolic processes (23, 34). The metabolic control theory proposes that metabolic rate is coupled to blood flow by a number of vasoactive compounds (metabolites) released into the interstitium in proportion to metabolism. Adenosine is one of a number of metabolites believed to link blood flow to metabolic rate in muscle tissue and to contribute to exercise hyperemia in skeletal muscle (23, 30, 34). Three sets of observations portend an important role for adenosine in local control of blood flow and exercise hyperemia: 1) adenosine is a potent vasodilator in skeletal muscle vascular beds (23, 30, 34), 2) adenosine concentration increases in interstitium of skeletal muscle during exercise at a rate related to both the intensity of exercise and the amount of blood flow to the muscle (14), and 3) results of experiments that used adenosine-receptor antagonists and agents that modulate adenosine metabolism support a role for adenosine in exercise hyperemia (22, 30, 35).

Most, but not all, available evidence suggests that adenosine is more important in regulation of vascular resistance in highly oxidative muscle than in other types of skeletal muscle. For example, during treadmill exercise (70% of maximal oxygen consumption), treatment with dipryidamole, an adenosine uptake inhibitor, increased blood flow to highly oxidative muscles, including the diaphragm (Dia) and other respiratory muscles, but not in less oxidative muscle of miniature swine (22). Second, Schwartz and McKenzie (35) observed that administration of adenosine deaminase in cats attenuated the increase in blood flow during isometric contractions of the high-oxidative soleus muscle, whereas blood flow to the less oxidative gracilis muscle was unaltered by adenosine deaminase. They concluded: “These data indicate that the oxidative capacity of the muscle and the intensity of the metabolic stimulus influence the extent to which adenosine mediates the regulation of skeletal muscle blood flow during active hyperemia” (35). Schwartz and McKenzie propose that the cause of the differing level of importance of adenosine in active hyperemia in oxidative and glycolytic skeletal muscle is the muscle’s biochemical ability to produce adenosine. This interpretation assumes, by exclusion, that the sensitivity of arterioles in oxidative and glycolytic skeletal muscle is similar. In contrast, McCurdy et al. (26) recently reported that first-order arterioles (1As) from the white portion of the lateral head of the rat gastrocnemius muscle exhibited greater maximal vasodilation in response to adenosine and greater sensitivity to adenosine than did 1As from soleus muscle, suggesting that vasomotor responsiveness differs among resistance arteries and...
arterioles of vascular beds of muscles of different oxidative capacity and/or fiber type (26). It is important to determine whether adenosine is equipotent as a dilator in arterioles isolated from different types of skeletal muscle.

The purpose of this study was to test the hypothesis that arterioles from white, low-oxidative skeletal muscle [white portion of the medial head of gastrocnemius muscle (WG)] are less responsive to adenosine-induced dilation than arterioles from Dia and red, high-oxidative skeletal muscle [red portion of the medial head of gastrocnemius muscle (RG)]. Finally, when initial observations indicated that second-order arterioles (2As) from RG muscle did not dilate in response to adenosine, we examined the dose response of these arterioles to acetylcholine (ACh) and sodium nitroprusside (SNP) to establish that these 2As could vasodilate. Results indicate that 2As from RG exhibit even greater dilation in response to ACh than those of Dia and WG.

METHODS

Animals. Male Sprague-Dawley rats (weight 441 ± 7 g) were obtained from Harlan (n = 16) 1 wk before experimental use. Animals were housed in pairs in temperature (24°C)- and light (12:12-h light-dark cycle)-controlled 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.

Experimental design. We examined arterioles from the Dia in this study because our laboratory’s previous results indicated that dipyridamole produced increased blood flow in this study because our laboratory and Use Committee of the University of Missouri.

Preparation of arterioles. Rats were anesthetized with an injection of pentobarbital sodium (50 mg/kg ip). The triceps surae muscle group was dissected, and the medial head of the gastrocnemius transferred to a chamber containing cold PSS. 2As arterioles ~500–1,000 μm in length were identified and dissected from both muscles. Isolated arterioles were transferred to Lucite microvessel chambers containing cold PSS, where 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 and flushed with PSS-albumin (1 g/100 ml) to remove blood from the lumen. The other end of the arteriole was cannulated and secured with a micropipette having an outer diameter within 2 μm of the first pipette.

Microvessel chambers were mounted on an inverted microscope (Nikon Diaphot 200, ×20 and ×40 magnification, numerical aperture between 0.25 and 0.50) 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, allowing observation of the arteriole and measurement of arteriolar diameter. The pipettes were attached to independent fluid reservoirs containing PSS-albumin, allowing control of luminal pressure by adjusting the levels of each fluid reservoir to the same level where no flow occurred through the lumen of the arteriole (18). The microvessel chambers were warmed gradually to 37°C and maintained at this temperature for the duration of the experiment. The bathing solution in the chamber was replaced every 15 min during a 1-h equilibration period. 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 pressure was selected on the basis in vivo pressure measurements from rat skeletal muscle arterioles of similar size and branch order (3, 27, 43). 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. Arteriolar diameter and luminal pressure were monitored continuously for the duration of each experiment (sampling frequency 20/s).

After the 1-h equilibration period, endothelial and vascular smooth muscle function were assessed by challenging the arterioles with 10-4 M ACh and 80 mM KCl, respectively. 2As were considered to possess functional endothelium and vascular smooth muscle if they exhibited >20% dilation from baseline diameter in response to the ACh challenge and >30% constriction to an 80 mM KCl challenge. Vessels not meeting these criteria were discarded. After it was established that each 2A possessed functional endothelium and vascular smooth muscle, each 2A was allowed time to develop tone of 50% of maximal diameter. If they did not do so spontaneously, small doses of phenylephrine were administered to induce tone of this magnitude. Maximal diameter of each arteriole was determined by incubation in calcium-free PSS with 2 mM EDTA for at least 30 min. The calcium-free bathing solution was replaced every 15 min.

Adenosine experiments. After arterioles maintained ~50% constriction for 10 min, an adenosine dose-response curve was constructed by adding adenosine (10-9 to 10-4 M) to the bath in whole-log increments in concentration. After each dose of adenosine, the arteriole achieved a stable diameter before the next dose was administered. Typically, arterioles achieved stable diameter in <3 min.
SNP experiments. Because our results revealed that RG 2As did not dilate in response to adenosine, we did another set of experiments in which we examined dilation of 2A arterioles from RG and WG to SNP. After arterioles maintained ~50% constriction for 10 min, an SNP dose-response curve was constructed by adding SNP (10^{-9} to 10^{-4} M) to the bath in whole-log increments in concentration. After each dose of SNP, the arteriole achieved a stable diameter before the next dose was administered. Typically, arterioles achieved stable diameter in <3 min.

Endothelium-dependent responses. To determine the role of the endothelium in adenosine-induced dilation, responses of intact and denuded arterioles were compared. Arterioles were denuded by passing 3–5 ml of air through the lumen of the arteriole after one end of arteriole was cannulated as described by Sun et al. (38, 39). After denudation with air, the arteriole was flushed with PSS-albumin and the other end of the arteriole cannulated and secured to the second micropipette as described in Preparation of arterioles. Successful denudation was established if 10^{-4} M ACh produced <5% dilation while normal responses to KCl were maintained. Because results of the 10^{-4} M ACh experiments testing for functional endothelium indicated that 2As from RG exhibited greater ACh-induced dilation than did 2As from WG and Dia, we compared ACh reactivity of 2As isolated from RG, WG, and Dia in a dose-response manner (10^{-9} to 10^{-4} M).

Multiple dose-response curves. Control experiments were performed to determine the repeatability of adenosine dose-response curves by exposing each arteriole to three different adenosine dose-response relationships at 1-h intervals. Results of these experiments demonstrated that arterioles from Dia, but not arterioles from gastrocnemius, had repeatable dose-response curves for adenosine. Therefore, only a single adenosine dose-response curve was constructed for each RG and WG arteriole. One intact arteriole and one denuded arteriole was examined from each RG and WG muscle. In contrast, two dose-response curves were constructed for each Dia arteriole, the first with the arteriole intact and the second after denudation of the Dia arteriole. Dia arterioles received a maximum of two adenosine dose-response curves.

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.

All drug stocks were prepared in distilled, deionized water and frozen for later use. Albumin was obtained from United States Biochemical, and 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) percentage of the possible response between the baseline diameter before the first dose of the agent being utilized and maximal calcium-free diameter, and 3) percentage of the maximal response from baseline diameter.

A one-way ANOVA was utilized (SuperANOVA statistical software) when comparing maximal diameters, beginning tone, maximal responses, and the Emax data (Prism statistical software) among arterioles from different muscles (Dia vs. RG vs. WG). For comparing intact vs. denuded arterioles from the same muscle, comparisons were performed by utilizing the Student’s t-test.

Dose-response relationships were compared between groups (e.g., intact vs. denuded) by utilizing 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 statistical comparisons.

RESULTS

Characteristics of arterioles. The characteristics of intact and denuded 2As utilized in the adenosine experiments are presented in Table 1. Intact arterioles from RG had significantly greater maximal calcium-free diameters than did arterioles from Dia and WG. There were no differences between maximal calcium-free diameters or beginning tone for intact and denuded arterioles from Dia, RG, and WG.

Adenosine. The dose-response relationships of intact 2As from Dia, RG, and WG to adenosine are presented in Fig. 1. Adenosine produced dose-dependent dilation of arterioles from Dia and WG. However, diameters of RG arterioles were not significantly altered by adenosine, suggesting that 2As from RG do not dilate in response to adenosine.

Removal of the endothelium did not alter responses of arterioles from Dia or RG to adenosine. In contrast, denudation significantly attenuated the dilation produced by adenosine in arterioles from WG (Fig. 2). Denudation did not have a significant effect on the constriction produced by 80 mM KCl in any of the 2As examined. Before denudation, 2As from Dia, RG, and WG constricted 53 ± 5, 77 ± 4, and 80 ± 3%, respectively. After denudation, 2As from Dia, RG, and WG constricted 47 ± 5, 61 ± 9, and 77 ± 6% respectively. Beginning tone was similar among intact and denuded arterioles from Dia, RG, and WG (Table 1).

ACh responses. The change in diameter elicited by exposing intact and denuded arterioles used in the adenosine experiments (Figs. 1 and 2) to 10^{-4} M ACh is presented in Fig. 3. Intact arterioles from Dia, RG, and WG dilated in response to 10^{-4} M ACh (Fig. 3A), and ACh-mediated dilation was eliminated after denudation in all arterioles (Fig. 3B). Importantly, the same 2As from RG that did not significantly dilate in response to

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Table 1. Characteristics of second-order arterioles

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<td>Maximum</td>
<td>122 ± 3</td>
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Values are means ± SE. Number, total number of arterioles used for each muscle. Diameter of arterioles was measured in calcium-free buffer as described in the text. * Red gastrocnemius arterioles were significantly larger in diameter than were those of diaphragm and white gastrocnemius (P < 0.05). There were no differences between intact and denuded diameters or %tone.
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Indeed, ACh-induced dilation of the RG arterioles, expressed as percent possible dilation (top) and absolute diameter (bottom); n, no. of arterioles. B, baseline diameter before administration of the first dose of adenosine. EC\textsubscript{50} values could not be determined for the RG arterioles because of lack of response. Adenosine EC\textsubscript{50} values for the other second-order arterioles were 6.2 ± 4.3 × 10^{-6} M and WG = 1.3 ± 0.4 × 10^{-5}. Adenosine produced a significant increase in diameter (expressed both as μm and percent possible dilation) of the Dia and WG arterioles but not arterioles from RG muscle (P < 0.05).

Fig. 1. Adenosine-induced vasodilation of intact second-order arterioles from diaphragm (Dia) and red (RG) and white (WG) portions of the gastrocnemius muscle. Values are means ± SE expressed as percent possible dilation (top) and absolute diameter (bottom); n, no. of arterioles. B, baseline diameter before administration of the first dose of adenosine. EC\textsubscript{50} values could not be determined for the RG second-order arterioles because of lack of response. Adenosine EC\textsubscript{50} values for the other second-order arterioles were Dia = 6.2 ± 4.3 × 10^{-6} M and WG = 1.3 ± 0.4 × 10^{-5}. Adenosine produced a significant increase in diameter (expressed both as μm and percent possible dilation) of the Dia and WG arterioles but not arterioles from RG muscle (P < 0.05).

Discussion

The purpose of this study was to test the hypothesis that 2As from white, low-oxidative skeletal muscle (WG) are less responsive to adenosine-induced dilation than 2As from Dia and red, high-oxidative skeletal muscle (RG). This hypothesis was based on previous observations that, during moderate-intensity treadmill exercise, an adenosine uptake inhibitor (dipyridamole) produced increased blood flow to Dia and red, high-oxidative skeletal muscle but not low-oxidative skeletal muscle (22). Our hypothesis was that these differences in the role of adenosine in exercise hyperemia result from differences in the adenosine-induced vasodilator response of the arterioles in the different kinds of muscle. Although results of the present study indicate that vascular responses differed among 2As of these different types of muscle tissue (Fig. 1), these results do not support our hypothesis because 2As from Dia and WG dilated in response to adenosine, whereas 2As from RG did not. Consequently, on the basis of our results, we conclude that vasomotor responsiveness of 2As to adenosine cannot be predicted only by the fiber-type composition or oxidative capacity of the skeletal muscle tissue wherein the arteriole lies.

Before the role of fiber-type composition of skeletal muscle on arteriolar vascular reactivity is discussed, it is important to consider the basis of the adenosine hypothesis of metabolic control of skeletal muscle vascular resistance (23). Adenosine is one of a number of vasoactive compounds believed to link blood flow to metabolic rate and to contribute to exercise hyperemia in muscle tissues (23, 30, 34). The adenosine hypothesis holds that adenosine release into the interstitium is proportional to metabolic rate and that during exercise the amount of adenosine released into the interstitium increases and thus produces relaxation of vascular smooth muscle of resistance arteries, linking blood flow to metabolic rate (23). Consistent with the adenosine hypothesis of metabolic control of vascular resistance in muscle tissue, adenosine is a potent vasodilator in the coronary circulation of numerous species, including pig, horse, and dog (12, 24, 28, 32). Also, myocardial adenosine production increases during exercise and other conditions of increased myocardial metabolic demand (10, 40). Although the role of adenosine in regulating vascular resistance of skeletal muscle vascular beds is not as well established as it is in the coronary vascular bed (23), there is substantial evidence that adenosine is an important regulator of vascular resistance of skeletal muscle (14, 23, 30, 34).

As discussed above, our experimental design was based in part on evidence indicating that adenosine is more important in controlling vascular resistance in some types of skeletal muscle than others. For example, because dipyridamole inhibits adenosine transport into cells, it should result in increased interstitial adenosine accumulation in muscle tissue that releases adenosine into the interstitium. Dipyridamole treatment increased exercise blood flow to the Dia, other respiratory muscles, and slow-twitch skeletal muscles,
but it had no effect on the blood flow response to exercise in fast-twitch, low-oxidative skeletal muscle (22). Consistent with the notion that adenosine is more important as a dilator in slow-twitch skeletal muscle than fast, Schwartz and McKenzie (35) reported that treatment with adenosine deaminase reduced exercise hyperemia in highly oxidative, slow-twitch soleus muscle but had no effect on more fast-twitch gracilis muscle of cats. These previous results suggest either that adenosine is not released into the interstitium of fast-twitch muscle and/or that resistance arteries and/or arterioles in fast muscle are less sensitive to the effects of adenosine than arteries and/or arterioles in slow muscle (22, 35). The authors concluded that adenosine is released into the interstitium of SO muscle but that it is not released by FG muscle. Certainly, if adenosine is not released by fast-twitch muscle then blood flow should not be affected by blocking adenosine uptake into cells (dipyridamole) or by adenosine deaminase, which breaks down adenosine (22, 35).

The present experiments were designed to allow evaluation of the postulate that arterioles isolated from muscle composed of a large proportion of SO (type I) fibers are more responsive to adenosine than arterioles from FOG and FG (type II) skeletal muscle. We studied arterioles from RG and Dia muscle because these muscles consist of 51 and 44% type I fiber type, respectively, whereas WG has no type I muscle fibers (8). RG arterioles did not exhibit vasodilation in response to adenosine (Fig. 1), whereas arterioles from both Dia and WG muscle exhibited dose-dependent dilation in response to adenosine. These results indicate that a high percentage of type I fibers (51% in RG) composing skeletal muscle tissue does not necessarily mean that 2As from this muscle will exhibit increased adenosine sensitivity. Indeed, in the present study, arterioles from WG, which has negligible type I fibers, exhibited greater adenosine responsiveness than those from RG muscle. These results are consistent with those of McCurdy et al. (26), who reported that 1As from rat soleus muscle (90% SO fibers) exhibit lower adenosine responsiveness (maximal dilation and sensitivity) than 1As from the white portion of the lateral head of gastrocnemius muscle. Thus available evidence does not support the notion that arterioles from skeletal muscle composed of a large percentage of type I fibers have greater adenosine sensitivity than arterioles from other types of skeletal muscle.

It is also of importance to consider the notion that adenosine sensitivity of skeletal muscle arterioles is related to oxidative capacity of the muscle, independent of whether the muscle consists of FOG or SO fiber types. The fiber-type composition of Dia muscle is similar to that of RG muscle. That is, compared with RG muscle, Dia muscle has a similar percentage of type I fibers (Dia = 44% vs. RG = 51%) and similar proportion of type IID/X fibers (RG = 13% vs. Dia 18%), but the Dia muscle has a greater percentage of type IIB fibers (Dia = 32% vs. RG = 1%) (8). Importantly, Delp and Duan (8) report that RG and Dia muscle of rats

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**Fig. 2. Effects of denudation on adenosine-induced vasodilation of second-order arterioles from Dia (A), RG (B), and WG (C). Values are means ± SE expressed as absolute diameters (left) and percent possible dilation (right). n, no. of arterioles. B, baseline diameter before administration of the first dose of adenosine. *Dilation induced by adenosine in WG second-order arterioles is significantly less after denudation (P < 0.05). Denudation had no effect on the adenosine-induced responses of Dia or RG second-order arterioles.**
have similar oxidative capacities, as reflected in citrate synthase activity (RG = 36 μmol·min⁻¹·g⁻¹, Dia = 39 μmol·min⁻¹·g⁻¹). 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 2As from Dia and WG muscle exhibit dose-dependent dilation in response to adenosine, whereas 2As from RG muscle do not.

When these considerations are taken full circle, differences in adenosine sensitivity of the 2As in muscle are apparently not the reason that treatments which increase adenosine accumulation in the interstitium do not increase blood flow to active, fast-twitch skeletal muscle but do increase blood flow to Dia and/or skeletal muscle composed of a large percentage of type I fibers (22, 35). Rather, the present results demonstrating that 2As from fast-twitch muscle (WG) dilate in response to adenosine (Fig. 2) combined with these previous observations are most consistent with the interpretation that fast-twitch muscle does not release adenosine during moderate exercise. If fast-twitch, low oxidative muscle fibers do not release adenosine during moderate exercise, then treatments that block adenosine uptake into cells should have no effect on blood flow or vascular resistance, as reported (22, 35). Indeed, there is evidence that fast-twitch skeletal muscle does not release adenosine, because Arabadjis and coworkers (2) reported that active fast-twitch skeletal muscle releases inosine rather than adenosine. Furthermore, the observation that dipyridamole caused increased blood flow to Dia muscle indicates that the Dia releases adenosine (22). Consistent with this interpretation, the present results demonstrate that adenosine dilates Dia 2As. Laughlin et al. (22) also observed that dipyridamole increased vascular conductance in all muscles examined during exercise at or above intensities necessary to produce maximal oxygen consumption. They proposed that, during maximal exercise, fast-twitch muscle released adenosine because of hypoperfusion caused by limited cardiac output (16, 17, 22). Present results that WG 2As dilate in response to adenosine are also consistent with this notion.

In contrast, the observation that RG arterioles did not respond to adenosine, whereas dipyridamole in-
creased vascular conductance to all types of skeletal muscle, is intriguing. Indeed, we were surprised that RG arterioles did not dilate in response to adenosine. There are at least four reasonable explanations for the surprising observation that 2As from RG did not dilate in response to adenosine. First, it is possible that we selectively damaged RG 2As during isolation. The fact that these same arterioles exhibited the expected constriction in response to KCl and substantial dilation in response to ACh and SNP argues against this possibility. Also, similar RG arterioles in our hands show typical constrictor responses to norepinephrine, phenylephrine, and endothelin-1 (1). Furthermore, McCurdy et al. (26) reported in vitro results that 1As from the white portion of lateral gastrocnemius muscle of rats exhibit greater dilation (60%) in response to isoproterenol than rat soleus muscle 1As (42%). The maximal dilation of our RG 2As (75%) to isoproterenol (1) are similar to those for gastrocnemius 1As reported by McCurdy et al. Therefore, we do not believe the lack of response of RG 2As to adenosine reflects that our RG arterioles were damaged. A second possibility is that 2As from RG are less sensitive to adenosine than other (smaller) arterioles in RG muscle. Kuo et al. (19) reported that adenosine (and other metabolic by-products) produces the greatest dilatory effect in the smallest vessels of the vascular tree. Similar phenomena may exist in the vascular bed of RG muscle. Third, the lack of response of 2As from RG and modest dilation of arterioles from Dia and WG muscle in response to adenosine administration may be the result of isolation of the arterioles from the vascular network; that is, intact arterioles are more sensitive to adenosine than are isolated arterioles. It has been demonstrated, with in vivo microscopy, that adenosine activates capillaries and/or smaller branch-order arterioles, initiating dilation (31). In addition to initiating dilation of these small arterioles, it appears that signals are propagated upstream, leading to dilation of larger arterioles (including 2As) in skeletal muscle tissue (31). Rivers and Frame (31) reported that small increases in tissue adenosine concentration appeared to activate remote arteriolar dilation via vascular communications. These vascular network responses result in greater dilation of large arterioles than similar concentrations of adenosine applied directly to the large arterioles (31). Consistent with the notion that in vivo adenosine sensitivity exceeds that observed in isolated arterioles, Danialou et al. (7) demonstrated greater adenosine responses in Dia 2As in vivo than the responses observed in Dia 2As in this study. So it seems reasonable to propose that adenosine sensitivity in RG muscle arterioles is greater in vivo than that observed in the present study because of these phenomena of network vascular communication, propagated signals, and/or flow induced dilation as described in other types of skeletal muscle (31). A fourth possible explanation for the lack of response of RG arterioles to adenosine is that rat arterioles have been reported to be less sensitive to adenosine than arterioles from other species (5, 7, 12, 13). The mechanisms underlying these species differences were not evaluated in this study, but it is possible that RG 2As represent an extreme of these phenomena.

Our results also indicate that adenosine-induced dilation is partially endothelium dependent in arterioles from WG (Fig. 2). Multiple studies utilizing several vascular preparations have demonstrated a contribution of the endothelium to dilation in response to adenosine (4, 11–13, 25, 29, 37). Whereas the adenosine response of arterioles from WG appears partially endothelium dependent, it appears to be endothelium independent in arterioles from Dia, consistent with other reports that adenosine-mediated dilation is endothelium independent (6, 33), perhaps mediated by production of cAMP and opening of ATP-sensitive potassium channels on vascular smooth muscle (13). Danialou et co-workers (7) examined the adenosine response in rat Dia arterioles by utilizing in vivo microscopy and suggested that the adenosine response is mediated by adenosine A1 and A2a receptors through the production of nitric oxide. The reason that our Dia arterioles showed no evidence of an endothelial component to adenosine-induced dilation is not apparent at this time. As discussed above, it is possible that this is related to the removal of network responses by isolation of the arterioles and the fact that these network responses appear to be endothelium dependent (31).

Endothelium-dependent dilation was examined among arterioles from different muscles in the present study primarily to assess the effects of our isolation procedures on vasomotor reactivity. Results reveal that all three types of 2As exhibited dose-dependent dilations in response to ACh. Furthermore, endothelium-dependent dilation in response to ACh was significantly greater in 2As from RG muscle than in 2As from the other two muscles (Figs. 3 and 4). These results are consistent with in vivo results indicating that endothelial release of nitric oxide has greater relative importance in control of blood flow to

Fig. 5. Sodium nitroprusside (SNP)-induced vasodilation of intact second-order arterioles from RG and WG. Values are means ± SE expressed as percent possible dilation; n, no. of arterioles. B, baseline diameter before administration of the first dose of SNP. There were no significant differences between responses of RG and WG arterioles.
high-oxidative skeletal muscle (15). Also, these results are consistent with in vitro results of Wunsch et al. (42) indicating that rat soleus muscle 1As exhibit greater dilation (90%) in response to ACh than 1As from the white portion of gastrocnemius muscle (68%).

Finally, it is important to emphasize that it is tenuous to draw broad conclusions concerning the effects of muscle fiber-type composition on vascular responsiveness of arterioles only on the basis of the results of this study. There are two primary reasons for this caution. First, the potential importance of well-established differences among vasomotor properties of different branch orders within an arteriolar tree should be considered (19, 27). In the present study, vasomotor responses were only examined in one branch order of arteriole. Therefore, different results may be obtained in other branch orders within the arteriolar networks of these muscles. Also, previous studies that revealed the importance of relationships among muscle fiber-type composition, muscle fiber recruitment patterns, and vascular control mechanisms to blood flow distribution within and among muscles were only able to do so because they examined a large number of muscles with widely varying biochemical characteristics (15, 20–23).

In the present study, 2As from three different types of skeletal muscle were examined. Before general patterns of vascular responsiveness among muscle tissues with widely varying biochemical characteristics can be established, vascular responses need to be examined in arterioles from more muscles with differing fiber-type composition.

In summary, this study examined adenosine sensitivity of 2As from skeletal muscles with varying oxidative capacity and tested the hypothesis that arterioles from white, low-oxidative skeletal muscle (WG) are less responsive to adenosine-induced dilation than arterioles from Dia and red, high-oxidative skeletal muscle (RG) 2As. Results indicate that there are differences in the responses to adenosine in arterioles from different skeletal muscles. Arterioles from two different highly oxidative skeletal muscles, Dia and RG, did not have the same response to adenosine. Indeed, adenosine caused arterioles from Dia and WG to dilate in a dose-dependent manner, whereas arterioles from RG did not respond to adenosine. In contrast, 2As from RG exhibited greater dilation and sensitivity to ACh than did 2As from WG and Dia. We conclude that neither differences in fiber-type composition of the muscle nor differences in oxidative capacity of the muscles alone are the primary determinant of adenosine responsiveness of 2As.

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