Dissociation between volume blood flow and laser-Doppler signal from rat muscle during changes in vascular tone

Larisa V. Kuznetsova, Nicole Tomasek, Gisli H. Sigurdsson, Andrej Banic, Dominique Erni, and Anthony M. Wheatley

Departments of  
1Visceral and Transplantation Surgery, 2Anesthesia and Intensive Care, and  
3Plastic Surgery, University of Berne, Inselspital, CH-3010 Berne, Switzerland

Kuznetsova, Larisa V., Nicole Tomasek, Gisli H. Sigurdsson, Andrej Banic, Dominique Erni, and Anthony M. Wheatley. Dissociation between volume blood flow and laser-Doppler signal from rat muscle during changes in vascular tone. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1248–H1254, 1998.—Although the laser-Doppler flowmetry (LDF) signal from skeletal muscle has been shown to provide a good measure of blood flow under some conditions, its behavior during administration of vasoactive substances has never been addressed. The aims of this study were to compare 1) changes in LDF signal with those in total muscle blood flow measured with radioactive microspheres after ganglionic blockade (chlorisondamine) and during administration of angiotensin II (ANG II), phenylephrine (PE), and isoproterenol (ISO) and 2) changes in vascular resistance estimated by the two techniques. The LDF signal from the biceps femoris muscle was investigated in anesthetized male Wistar rats. Ganglionic blockade led to a significant (P < 0.05) fall in mean arterial pressure (MAP) [medians (lower, upper quartiles): 78 (72, 83) vs. 127 (114, 138) mmHg under basal conditions], muscle blood flow (MBF, microsphere technique; 61%), and the LDF signal (29%). Muscle vascular resistance (MVR = MAP/MBF) was increased (64%, P < 0.05), but vascular resistance estimated as MAP/LDF signal (MVR_LDF) was unchanged. During ANG II and PE infusions, MAP rose (P < 0.05) to 178 (155, 194) and 127 (124, 142) mmHg, respectively; MBF did not change compared with the preinfusion (postganglionic blockade) level and remained significantly (P < 0.05) lower than baseline, whereas the LDF signal increased up to a level not different from baseline. MVR rose and was significantly (P < 0.05) higher than baseline, whereas MVR_LDF did not differ significantly from baseline. During ISO infusion, MAP fell [58 (56, 60) vs. 94 (92, 102) mmHg, P < 0.05], the LDF signal was reduced (49%, P < 0.05) despite a large increase in MBF (139%, P < 0.05), and MVR fell (74%, P < 0.05), whereas MVR_LDF did not change vs. preinfusion level. Our results suggest that 1) changes in the LDF signal from muscle may not correlate with changes in total muscle blood flow measured by the microsphere technique during infusion of vasoactive substances and 2) the use of LDF data for estimation of MVR during changes in vascular tone in rat skeletal muscle is probably not appropriate.

Laser-Doppler flowmetry (LDF) has in recent times been more frequently used as a method for the continuous measurement of blood flow in small, discrete areas of tissue and has also been used in the estimation of vascular resistance in different tissues (3, 7, 8, 10, 11, 13, 17, 20–22, 24, 32, 35). However, it must be appreciated that LDF is not specifically designed to measure volume flow, and given that volume flow is used in the calculation of vascular resistance, the application of LDF data in its estimation is open to question. In fact, the LDF signal is sensitive to changes in linear velocity of blood flow as reflected by red blood cell (RBC) velocity (V_{RBC}) and the number of RBCs passing through the sample volume per unit time (N_{RBC}), as can be demonstrated using both a simple in vitro model such as pumping dilute suspensions of RBCs through rigid tubing (1) and in vivo preparations (30). The product of V_{RBC} and N_{RBC} is indicative of the level of tissue perfusion (30). The use of LDF as a blood flow technique relies on the assumption that tissue perfusion and blood flow, defined as the volume of blood entering an organ per unit time, correlate so that the higher the flow, the higher the perfusion and vice versa. Indeed, a linear correlation between the LDF signal and blood flow measurements performed by other techniques has been found. In the skeletal muscle of the rat, LDF correlates well with such established techniques as radioactive microsphere distribution and electromagnetic flowmetry, which measure volume flow to an organ (27). However, this linear correlation may not necessarily apply if tissue perfusion estimated by LDF changes without any significant change in volume flow to an organ (31). In particular, if hematocrit is unchanged, constant volume flow suggests constant N_{RBC}. However, the same volume of blood may pass through an organ with different velocities depending on 1) the pressure gradient acting over the vascular bed and 2) vascular diameter. As a result, V_{RBC} and, hence, the LDF signal may change despite constant volume flow to an organ. Thus changes in the LDF signal may not correlate with those in volume flow, and changes in vascular resistance calculated using the laser-Doppler data [mean arterial pressure (MAP)/LDF signal; Refs. 3, 7, 8, 10, 11, 13, 17, 20–22, 24, 32, 35] may not reflect changes in vascular resistance calculated with blood flow values obtained by other techniques that measure volume flow to the whole tissue.

Changes in vasomotor tone induced by vasoconstrictors or vasodilators are believed under certain circumstances to induce changes in tissue perfusion without any significant change in volume blood flow to an organ. In the constant-flow perfused rat hindlimb preparation, a group of vasoconstrictors including angiotensin II (ANG II) and phenylephrine (PE) have been identified that enhance muscle O2 uptake (for review, see Ref. 4), which is indicative of alterations in the pattern of microvascular perfusion. Furthermore, O2 consumption can be reduced during vasodilation, even with an increase in volume blood flow to the skeletal muscle (for review, see Ref. 5).
Given that LDF is increasingly used as the technique of choice for the measurement of blood flow in different organs and tissues, it is perhaps pertinent to assess its ability to identify changes in volume blood flow and vascular resistance during administration of vasoactive drugs. The objectives of our study were thus 1) to study changes in the LDF signal and vascular resistance estimated using the LDF data during systemic administration of vasoactive substances and 2) to compare these changes in the LDF signal and vascular resistance to those in volume blood flow and vascular resistance estimated with radioactive microspheres.

MATERIALS AND METHODS

Animals

Male Wistar rats with initial body weights of 300–400 g (n = 27) were obtained from the Pathophysiology Institute (University of Berne) and housed in temperature- and humidity-controlled animal quarters under a 12:12-h light:dark cycle. The animals were maintained on a standard rat diet (Nafag, Gossau, Switzerland) with free access to tap water. All experiments were performed with the permission of the Animal Ethics Committee of Canton Berne.

Hemodynamic Studies

Microsphere technique. Cardiac output (CO) and volume organ blood flow were determined by the use of radioactive microspheres and the reference sample method (18, 19). The rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and cannulas were passed into the left ventricle via the right carotid artery for microsphere injection, into the abdominal aorta via the right femoral artery for blood pressure recording and reference sample withdrawal, and into the right femoral vein for drug administration.

The femoral artery cannula was connected via a pressure transducer (Statham P23 XL) to a Dynograph 611A pen recorder (Sensor Medics, Anaheim, CA) for measurement of arterial pressure. Heart rate (HR, beats/min) was determined using a tachometer (Sensor Medics). For CO measurement, once a constant reference sample flow (0.5 ml/min) was established, a precounted, sonicated aliquot of −100,000 113Sn-, 125I, or 57Co-labeled microspheres (15 µm, NEN-TRAC, DuPont De Nemours, Regensdorf, Switzerland) was injected slowly over ~30 s into the left ventricle and arterial withdrawal was continued for an additional 60 s. The microspheres were flushed from the cannula with 0.6 ml of 0.9% NaCl solution.

The rats were killed with a lethal dose of pentobarbital sodium, and organs and tissues were removed and weighed. The radioactivity [counts per minute (cpm)] of the reference sample and each organ was determined. Renal radioactivity was determined to activity [counts per minute (cpm)] of the reference sample and each organ was determined. Renal radioactivity was determined to activity [counts per minute (cpm)] of the reference sample and each organ was determined.

Experimental Protocols

Protocol 1. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and placed under a heating lamp controlled with a rectal thermoprobe to maintain body temperature at 37°C. The catheters and the LDF probe were positioned as described in Hemodynamic Studies, and the preparation was allowed to stabilize for 30 min. After the stabilization period, a baseline CO/C0 distribution measurement was performed (n = 21 rats). Twenty minutes later, chlorisondamine chloride, an autonomic blocking agent that blocks nicotinic ganglionic transmission, was administered intravenously (5 mg/kg). When arterial pressure, HR, and LDF signal had stabilized, a second CO/C0 distribution measurement was performed (n = 21). Twenty minutes later, either ANG II (0.3 mg·kg⁻¹·min⁻¹, infusion rate 0.025 ml/min; n = 7) or PE (0.5 mg·kg⁻¹·min⁻¹, infusion rate 0.01 ml/min; n = 7) was infused via the venous catheter. Arterial pressure, HR, and LDF signal were allowed to stabilize, and then a third CO/C0 distribution measurement was performed. The infusion was stopped, the rat was killed with an overdose of pentobarbital, and the LDF signal was again recorded to assess the “biological zero,” which was subtracted from the measured LDF signal to yield the actual LDF signal (2).

Protocol 2. Rats were anesthetized and prepared as in protocol 1 and then injected with the ganglionic blocker chlorisondamine (5 mg/kg iv) and the β₁-blocker atenolol (5 mg/kg iv) to prevent β₁-mediated cardiac stimulation during isoproterenol (Iso, combined β₁/β₂-agonist) infusion. When arterial pressure, HR, and LDF signal had stabilized, a first CO/C0 distribution measurement was performed (n = 6 rats). Twenty minutes later, Iso (75 mg·kg⁻¹·min⁻¹, infusion rate 0.025 ml/min) was infused via the venous catheter. Arterial pressure, HR, and LDF signal were allowed to stabilize, and then a second CO/C0 distribution measurement was performed (n = 6). The infusion was stopped, the rat was killed with an overdose of pentobarbital, and the LDF biological zero was again measured.

Statistics

Data are presented as medians (lower, upper quartiles) if not otherwise indicated and were analyzed using the Kruskal-Wallis nonparametric analysis of variance test followed by Dunn’s multiple-comparison test, the Mann-Whitney nonparametric test, and the Wilcoxon nonparametric test as appropriate. P < 0.05 was considered statistically significant.
Table 1. Systemic hemodynamics and muscle blood flow and vascular resistance measured by microsphere technique before and during vasoconstriction

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 21)</th>
<th>After Ganglionic Blockade (n = 21)</th>
<th>ANG II Infusion (n = 7)</th>
<th>PE Infusion (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>127 (114, 138)</td>
<td>78* (72, 83)</td>
<td>178† (155, 194)</td>
<td>127† (124, 142)</td>
</tr>
<tr>
<td>Total peripheral resistance, mmHg-mL⁻¹-min⁻¹·100 g body wt</td>
<td>5.24 (4.21, 6.21)</td>
<td>4.06* (3.32, 4.84)</td>
<td>8.11† (6.88, 8.81)</td>
<td>6.86† (6.61, 7.99)</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>390 (360, 420)</td>
<td>283* (260, 300)</td>
<td>345 (310, 360)</td>
<td>285* (245, 320)</td>
</tr>
<tr>
<td>Muscle blood flow, mL·min⁻¹·g muscle wt⁻¹</td>
<td>0.19 (0.10, 0.24)</td>
<td>0.07* (0.05, 0.08)</td>
<td>0.09* (0.05, 0.14)</td>
<td>0.08* (0.06, 0.11)</td>
</tr>
<tr>
<td>Muscle vascular resistance, mmHg-mL⁻¹·min⁻¹·g muscle wt</td>
<td>638 (567, 1,100)</td>
<td>1,167* (929, 1,340)</td>
<td>1,611* (1,085, 4,415)</td>
<td>1,538* (1,338, 2,109)</td>
</tr>
</tbody>
</table>

Data are medians (lower, upper quartiles). PE, phenylephrine; ANG II, angiotensin II. *P < 0.05 vs. baseline; †P < 0.05 vs. postganglionic blockade level (Kruskal-Wallis nonparametric analysis of variance).

RESULTS

Effect of Ganglionic Blockade

Basal and postganglionic blockade hemodynamic values are shown in Table 1 and Figs. 1 and 2. Ganglionic blockade caused an immediate fall in MAP (39%, P < 0.05), HR (27%, P < 0.05), and TPR (23%, P < 0.05) (Table 1). MBF measured with the microsphere technique was reduced (61%, P < 0.05), whereas MVR was increased (69%, P < 0.05). MBF and MVR expressed as a percentage of baseline are presented in Figs. 1 and 2.

Because of the high variability of baseline LDF signal (223 ± 163 PU [mean ± SD]), the LDF signal and MVR_LDF were expressed as a percentage of baseline. Ganglionic blockade led to a significant fall in the LDF signal from the muscle (29%, P < 0.05; Fig. 1). MVR_LDF was essentially equal to baseline (Fig. 2).

Effect of Vasoconstriction Induced by ANG II and PE

Infusion of both ANG II and PE caused an increase in MAP (128 and 63%, respectively, P < 0.05) and TPR (100 and 67%, respectively, P < 0.05) compared with the preinfusion (postganglionic blockade) level (Table 1). HR was unaltered. MBF measured with the microsphere technique did not change compared with the preinfusion (postganglionic blockade) level and remained significantly (P < 0.05) lower than baseline (Table 1, Fig. 1). MVR rose and was significantly (P < 0.05) higher than baseline (Table 1, Fig. 2).

During both ANG II and PE infusions, the LDF signal rose up to a level different from baseline (Fig. 1). MVR_LDF did not change significantly compared with both preinfusion (postganglionic blockade) and basal levels (Fig. 2).

Effect of Vasodilation Induced by Iso

The rats were pretreated with ganglionic blocker and atenolol (to prevent β₁-mediated increase in CI) and then infused with Iso. During Iso administration, MAP was significantly increased [58 (56, 60) mmHg] compared with the preinfusion level [94 (92, 102) mmHg; P < 0.05, Mann-Whitney nonparametric test]. Percent changes in MBF and MVR are presented in Fig. 3. MBF was increased (139%, P < 0.05), whereas MVR was decreased (74%, P < 0.05).

Iso infusion led to a significant fall in the LDF signal (49%, P < 0.05). MVR_LDF did not change significantly compared with the preinfusion level (Fig. 3).

DISCUSSION

In this study we have used an in vivo approach for combined macro- and microcirculatory studies on skeletal muscle in the intact anesthetized rat, combining the use of the microsphere technique for the measure-
Vasoconstriction Induced by ANG II and PE

Vasoconstriction induced by both ANG II and PE infusions resulted in a significant increase in MAP (Table 1). MBF as measured by the microsphere technique did not change compared with the preinfusion (postganglionic blockade) level and remained significantly lower than baseline (Table 1, Fig. 1). The LDF signal, however, was elevated by both vasoconstrictors up to a level not different from baseline (Fig. 1). At first sight, the increase in the LDF signal during vasoconstriction may appear somewhat surprising. There is, however, evidence to suggest that some vasoconstrictors (e.g., low-dose norepinephrine, ANG II, PE) can alter the microvascular perfusion pattern and increase O2 uptake in perfused rat hindlimb during constant-flow perfusion (for review, see Ref. 4). The mechanism for the constriction-associated increase in basal O2 uptake is not fully understood; however, one possible explanation is that these vasoconstrictors act at specific sites on terminal arterioles, resulting in perfusion of regions of hindlimb that were previously underper-
fused. Indeed, with the use of a vascular corrosion casting technique Newman and co-workers (23) found that low-dose norepinephrine induced a greater number of smaller vessels in the muscle to be perfused, whereas the diameters of the larger vessels appeared to be reduced. In our experiments, MBF did not change in the face of the elevated perfusion pressure manifested by the increase in MAP. Under such conditions the vasoconstrictor-induced rise in the LDF signal from muscle could be caused by an increase in the number of perfused capillaries. On the other hand, the same volume of blood may pass through the constricted vessels with higher linear velocity, resulting in an increase in $V_{RBC}$ and in the LDF signal.

MVR was elevated during both ANG II and PE infusions. However, the rise in MVR was not matched by a rise in $MVR_{LDF}$ (Fig. 2).

Vasodilation Induced by Iso

Infusion of the combined $\beta_2$-agonist Iso led to a significant decrease in MAP. The LDF signal was significantly decreased despite a marked increase in MBF (Fig. 3).

During Iso infusion, MBF rose by 139%. It is perhaps reasonable to expect that such a large rise in whole organ blood flow would be mirrored at least in part by a rise in the LDF signal. This proved not to be the case, because the LDF signal fell by 49% with Iso. The best explanation for the fall in the LDF signal is an Iso-induced fall in $V_{RBC}$ or $N_{RBC}$ or both. Indeed, an Iso-induced fall in $V_{RBC}$, up to and including complete stasis, has been observed by intravital microscopy in the peripheral microvessels in mammalian skeletal muscle (33). Furthermore, it has been shown that in the mesenteric arterioles of rats the major change during systemic administration of a vasodilator is a decrease in $V_{RBC}$ (28). In addition, a decrease in the number of perfused capillaries has also been observed during intra-arterial infusion of Iso in an isolated, autoperfused preparation of the abdominal muscles of the rat (34). Thus it would appear that a fall in muscle perfusion, and thus the LDF signal, in peripheral regions of the skeletal muscle during systemic administration of Iso can occur despite an increase in bulk flow to the muscle. The fall in muscle perfusion in the peripheral regions is perhaps best explained by a decrease in the pressure gradient acting over the vascular bed as a result of hypotension (manifested by the decrease in MAP) and transmission of MAP further downstream due to arteriolar dilation. Indeed, a $>50\%$ reduction in the arteriolar-venular pressure gradient in hamster striated muscle vessels has been observed during sodium nitroprusside-induced hypotension, resulting in reduced functional capillary density and tissue hypoxia (6). Decreased capillary exchange and tissue hypoxia during hypotensive vasodilation seems to be a common effect (for review, see Ref. 5).

As expected, a very marked fall in MVR (74%) was observed during Iso infusion because of the $\beta_2$-mediated vasodilatory effect of Iso (Fig. 3). No significant change in estimated $MVR_{LDF}$ was found, however, because the changes in the LDF signal in no way correlated with those in MBF measured by the microsphere technique.

Thus, in the present study, a marked dissociation between volume flow to the skeletal muscle of the rat measured with the microsphere technique and muscle perfusion assessed by LDF has been found during systemic administration of vasoactive drugs. The reason for this dissociation is not clear, but likely explanations are that 1) changes in linear velocity of blood flow in the microcirculation may not correlate with changes in volume blood flow to the organ under certain conditions and 2) redistribution of blood flow into (vasoconstriction) and out of the LDF measurement volume (vasodilation) may occur during changes in vascular tone irrespective of what happens to volume flow to the organ. Systemic infusion of both ANG II and PE was associated with an increase in MVR as a result of the vasoconstriction although volume flow to the muscle (MBF) was preserved because of an increase in the perfusion pressure (manifested by elevated MAP). It is not unreasonable to suggest that under such circumstances the same volume of blood passed through the constricted vessels with a higher linear velocity, resulting in an increase in $V_{RBC}$. Furthermore, others have shown that even a decreased volume flow to an organ can be accompanied by increased $V_{RBC}$. In particular, Le Noble and co-workers (15) have reported that during intravenous application of norepinephrine or other vasopressor substances, an increase in MAP and a decrease in volume flow to the rat mesenteric vascular bed were associated with a dose-dependent increase in $V_{RBC}$. It is important to note that if the hematocrit is constant, $N_{RBC}$ is a function of volume flow. In the current study, given that volume flow to the muscle was unchanged during vasoconstriction, it is thus unlikely that $N_{RBC}$ changed during the increase in vascular tone. As a result, the rise in LDF signal during vasoconstriction is best explained by an increase in $V_{RBC}$. In addition, given that an increase in $V_{RBC}$ is associated with a rise in perfused capillary density (29), one can conclude that tissue perfusion and hence the LDF signal from the muscle rises without any increase in volume blood flow. The converse would occur during vasodilation with a fall in the LDF signal in the face of an elevated volume blood flow. On the other hand, the LDF measurement volume is very small, with laser penetration into the tissue being $<1$ mm. The possibility exists that changes in vascular tone and perfusion pressure gave rise to subtle redistribution of blood flow in the muscle such that blood flow to the periphery of the muscle rose during vasoconstriction and fell during vasodilatation, leading to the observed changes in LDF signal. On balance, however, current evidence suggests that this is not the case and that the changes in LDF signal during changes in vascular tone are best explained by changes in $V_{RBC}$. Nonetheless, heterogeneity of muscle perfusion as a cause for the dissociation between volume flow and the LDF signal seen during
administration of vasoactive drugs cannot as yet be ruled out.

There has been a trend towards the use of LDF data in the calculation of vascular resistance not only in the skeletal muscle of the rat (8, 17) but also in a variety of other tissues such as rat brain (3, 11, 22, 24, 32) and gastric mucosa and kidney (3, 10), and rabbit brain (7) and kidney joint (20, 21). In addition, the use of LDF data has been applied in human studies to estimate skin vascular resistance under different physiological and pathophysiological conditions (13, 35). Presumably, this has been done in the belief that a direct correlation between volume blood flow and the LDF signal always applies. Given that during systemic administration of vasoactive drugs changes in the LDF signal from skeletal muscle may not correlate with alterations in vasoactive drugs changes in the LDF signal from skeletal muscle, the use of LDF data for estimation of MVR is probably not appropriate, at least in the case of rat skeletal muscle. In addition, until the appropriate experiments have been performed in which LDF is compared with other flow techniques during the administration of vasoactive drugs, we would further caution against its application in any tissue.

This study was supported by the Swiss National Foundation for Scientific Research (grant nos. 32–40761.94 and 3200–042555), by the Stanley Thomas Johnson Foundation, and by a grant from the Scientific Research (grant nos. 32–40761.94 and 3200–042555), by

REFERENCES


30. Tyml, K., and C. G. Ellis. Simultaneous assessment of red cell perfusion in skeletal muscle by laser Doppler flowmetry


