Blood flow and glucose uptake in denervated, insulin-resistant muscles

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BUSE AND BUSE (8) were first to show that interruption of nerve supply to skeletal muscle results in the development of insulin resistance in the affected muscle. This insulin resistance is characterized by a decreased ability or inability of insulin to stimulate transport of sugars (6, 8, 9, 16, 24, 27, 28), glycogen synthesis (6, 24), and amino acid transport (16, 27, 28) in the denervated muscles. The signs of insulin resistance in the denervated rat muscles in vivo can be observed as early as 3 h after sectioning the nerve (the earliest time tested) (27). We have previously investigated individual hindlimb muscles of the rat 3 h to 17 days after sectioning the sciatic nerve and have observed that the manifestations of insulin resistance were influenced by the fiber population of the muscles (27). Although both slow-twitch and fast-twitch muscles exhibited a progressive lowering of insulin-induced glucose uptake in vivo during the first 24 h after sectioning the nerve, they differed in the manifestations of insulin resistance at 3–17 days after denervation (27). During the latter period the slow-twitch muscles were completely unresponsive to stimulation with insulin, whereas the fast-twitch muscles showed a normal glucose uptake when stimulated by insulin; however, the insulin-induced increment in glucose uptake was reduced because it was superimposed on already elevated basal glucose uptake (27). The mechanism(s) of denervation-induced insulin resistance remains elusive. Binding of insulin to its receptor is not significantly altered by muscle denervation (14). Studies at 1 day after denervation, when glucose uptake in response to insulin in vivo is decreased by up to 80% (27) show no decrease in the ability of insulin to stimulate tyrosine kinase activity of the insulin receptor (7, 14) and activity of phosphatidylinositol-3-kinase (11, 14), an enzyme that is believed to transduce the stimulatory action of insulin on translocation of GLUT-4 transporters to the plasma membrane (10, 13). At 1 day after denervation there is also no change in the abundance of GLUT-1 and GLUT-4 transporters in skeletal muscle (14).

The purpose of the present study was to evaluate the effect of denervation on muscle blood flow to assess whether changes in blood flow could contribute to the measured insulin resistance in the denervated muscles. The experiments were performed on rats in which muscles of the right hindlimb were denervated by sectioning the sciatic nerve. In these animals, muscles of the contralateral sham hindlimb are used as an internal control, because they show the same growth rates and the same response to insulin as muscles of age-matched nonoperated rats. Because muscles with different muscle fiber composition exhibit different capillary-to-fiber ratio (21), different magnitude of the insulin-stimulated glucose uptake (27, 28), and different responses to denervation (27), the present study includes three hindlimb muscles of the rat: 1) soleus muscle, which consists predominantly (≥80%) of slow-twitch oxidative fibers; 2) plantaris muscle, which is composed of 53% fast-twitch oxidative-glycolytic fibers, 41% fast-twitch glycolytic fibers, and 6% slow-twitch oxidative fibers; and 3) gastrocnemius muscle, which consists of 37% fast-twitch oxidative-glycolytic fibers, 58% fast-twitch glycolytic fibers, and 5% slow-twitch oxidative fibers (1). Blood flow through individual muscles was measured with 51Cr-labeled microspheres. Basal and insulin-stimulated glucose uptake was assessed with a 3H-labeled, nonmetabolizable glucose analog, 2-deoxy-D-glucose.

METHODS

Animals and materials. Adult male Sprague-Dawley rats with an initial weight of 230–240 g were purchased from Taconic Farms (Germantown, NY). "Guiding Principles in the Care and Use of Animals" approved by the Council of the American Physiological Society was strictly adhered to. The
experimental procedures were approved by the Institutional Animal Care and Use Committee and the institutional veterinarian. \([1,2^-\text{H}(N)]2\text{-deoxy-D-glucose and microspheres (15 \mu m in diameter) labeled with } \text{^{51}Cr were from New England Nuclear. } [U-\text{\textsuperscript{14}C}]\text{sucrose was from ICN Pharmaceuticals. All other chemicals were from Sigma.}

Single hindlimb denervation. The right hindlimb of each rat was denervated under ether anesthesia as previously described (14, 27, 28). Briefly, the thigh muscles were bluntly separated from the lateral side, and a 5-mm segment of the sciatic nerve was excised. On the contralateral (sham) hindlimb, the sciatic nerve was visualized in the same way, but not touched. The skin wounds were closed with surgical clips and coated with disinfectant (Betadine). No bleeding was associated with the surgery and no infection was observed during the subsequent recovery period. The denervation had no effect on daily food intake during the experimental period. The rats were studied at 1 and 3 days after surgery. At these test times, muscles from the denervated hindlimb display minimal, if any, changes in muscle mass or extracellular fluid volume (27). All animals were fasted overnight (18 h) before measurements were performed.

Cellular uptake of 2-deoxy-D-glucose Uptake of 2-deoxy-D-glucose by individual hindlimb muscles in vivo was evaluated as described previously (14, 27, 28). The rats were anesthetized with 50 mg pentobarbital sodium/kg body wt and then injected via the dorsal vein of the penis with 10 \(\mu\)Ci of \([1,2^-\text{H}(N)]2\text{-deoxy-D-glucose with or without 0.1 U bovine insulin in 0.4 ml of 0.1% defatted bovine serum albumin/rat.

All experiments were terminated 25 min after the injection of labeled 2-deoxy-D-glucose. Rats were killed by rapid exsanguination from a deep transverse cut through the chest. Blood was collected, and soleus muscles, plantaris muscles, and transverse sections of gastrocnemius muscles were excised from both hindlimbs. Tissue samples and serum were digested separately in Solvable tissue solubilizer (New England Nuclear) and the radioactivity was determined, after addition of Formula-989 (New England Nuclear). 

Radioactivity from both hindlimbs. Tissue samples and serum were digested separately in Solvable tissue solubilizer (New England Nuclear) and the radioactivity was determined, after addition of Formula-989 (New England Nuclear). By liquid scintillation counting, Cellular uptake of 2-deoxy-D-glucose was calculated as the difference between the total tissue radioactivity (disintegrations/min) and the amount of radioactivity present in the tissue extracellular (sucrose) space. Cellular disintegrations per minute were then expressed per milligram muscle weight.

Muscle blood flow. Blood flow through individual hindlimb muscles in vivo was measured with \textsuperscript{51}Cr-labeled microspheres (15 \(\mu\)m in diameter) suspended in 0.9% NaCl with 0.01% Tween 80 as previously described (29). Each rat was anesthetized with 60–70 mg pentobarbital sodium/kg body wt ip, and the left carotid artery was cannulated. Total radioactivity of a 1-ml aliquot of the suspension of microspheres was determined in the gamma counter, and the aliquot was injected via the carotid artery cannula over 30 s, followed by injection of 0.5 ml of 0.9% NaCl. Total radioactivity in the syringe and cannula was determined and subtracted from the total activity. At 3 min after injection of the microspheres, the animal was killed and the \textsuperscript{51}Cr activity present in the heart, kidneys, soleus muscles, plantaris muscles, and a transverse section of gastrocnemius muscles from both hindlimbs was determined. The data are expressed as the percentage of injected dose of activity (total − residual) per gram wet tissue weight (% ID/g).

Tissue blood flow was also measured under the same conditions used to assess the insulin-stimulated 2-deoxy-D-glucose uptake by muscles. Pentobarbital sodium-anesthetized rats were injected with 0.1 U insulin in 0.4 ml of 0.1% defatted bovine albumin via the dorsal vein of the penis. The carnulation of the left carotid artery began at 15 min after insulin injection, microspheres were injected at 22 min, and the animal was killed and tissues were excised at 25 min after insulin injection.

To study the effect of physiological hyperinsulinemia on blood flow through individual hindlimb muscles, pentobarbital sodium-anesthetized rats were injected with 1 g glucose/kg body wt as 40% dextrose via the dorsal vein of the penis. Cannulation of the left carotid artery began at 30 min after glucose injection, microspheres were injected at 37 min, and each rat was killed and tissues were excised at 40 min after glucose administration.

In all experiments we found very low \textsuperscript{51}Cr activity in the heart (0.032 ± 0.007 %ID/g), indicating that few of the microspheres reached the heart during the injection. Radioactivity in the kidneys was symmetrically distributed, demonstrating adequate mixing of microspheres in the aortic blood. Data evaluation. The results are expressed as means ± SE and were analyzed by analysis of variance followed by the Newman-Keuls test.

RESULTS

Uptake of 2-deoxy-D-glucose by muscles in vivo. Figure 1 depicts basal and insulin-stimulated uptakes by hindlimb muscles of the rats at 1 day (Fig. 1A) and 3 days (Fig. 1B) after a single hindlimb denervation. The data on individual muscles of the sham (control) hindlimb show that both basal and insulin-induced uptakes of 2-deoxy-D-glucose are influenced by the fiber population of the muscles. Basal 2-deoxy-D-glucose uptake by sham plantaris and gastrocnemius muscles was, on the average, 32 and 60%, respectively, lower than that of sham soleus muscle (P < 0.02). In addition, basal uptake by sham gastrocnemius muscle was 40% (P < 0.05) lower than that of the sham plantaris muscle. Insulin-induced 2-deoxy-D-glucose uptake by sham plantaris and gastrocnemius muscles was, on the average, 46 and 66%, respectively, lower than that of the sham soleus muscle (P < 0.01). Also, insulin-stimulated uptake by sham gastrocnemius muscle was 36% lower than that of the sham plantaris muscle (P < 0.02).

At 1 day after denervation (Fig. 1A), the denervated soleus muscle exhibited a 58% decrease in basal uptake (P < 0.002) and an 80% decrease in insulin-stimulated 2-deoxy-D-glucose uptake (P < 0.001) compared with the contralateral sham soleus muscle. At 1 day, denervation had no effect on basal 2-deoxy-D-glucose uptake by plantaris muscle but decreased the insulin-stimulated uptake by 64% (P < 0.001) compared with the contralateral sham counterpart. At 1 day, denervation also had no effect on basal 2-deoxy-D-glucose uptake by gastrocnemius muscle. However, it resulted in a 42% (P < 0.01) reduction in insulin-induced 2-deoxy-D-glucose uptake by the denervated gastrocnemius muscle compared with the corresponding contralateral sham muscle.

At 3 days after denervation (Fig. 1B), the denervated soleus muscle exhibited a 60% lower basal 2-deoxy-D-glucose uptake compared with the contralateral sham soleus muscle (P < 0.02) and was completely unresponsive to stimulation with exogenous insulin. At the 3-day postdenervation interval, the denervated plantaris muscle showed a 262% elevation of basal uptake com-
pared with the contralateral sham plantaris muscle (P < 0.001). The absolute level of insulin-stimulated 2-deoxy-D-glucose uptake by sham and denervated plantaris muscles did not differ. However, the insulin-induced increment in 2-deoxy-D-glucose uptake by the denervated plantaris muscle, although statistically significant (P < 0.03), was reduced by 68% (P < 0.01) compared with that in the sham counterpart. A similar pattern of changes was also observed in the gastrocnemius muscle. At 3 days after denervation, gastrocnemius muscle exhibited a 105% higher basal 2-deoxy-D-glucose uptake compared with that in the sham counterpart. A similar pattern of changes was also observed in the gastrocnemius muscle. At 3 days after denervation, gastrocnemius muscles exhibited a complete unresponsiveness to insulin. In contrast, plantaris and gastrocnemius muscles, which consist mainly of fast-twitch fibers, show a normal 2-deoxy-D-glucose uptake when stimulated with exogenous insulin; however, the insulin-induced increment in 2-deoxy-D-glucose uptake is reduced because it is superimposed on already elevated basal 2-deoxy-D-glucose uptake.

Muscle blood flow. Figure 2 depicts blood flow through individual hindlimb muscles of the rat at 1 day (Fig. 2, A and B) and 3 days (Fig. 2, C and D) after a single hindlimb denervation. At both intervals, blood flow through sham muscles was affected by the fiber population of the muscles.

As shown in Fig. 2, A and C, basal blood flow through sham plantaris and gastrocnemius muscles, both fast-twitch muscles, was on the average 79 and 81%, respectively, lower compared with that of the sham soleus muscle, a slow-twitch muscle (P < 0.01). There was no difference in blood flow between sham plantaris and sham gastrocnemius muscles. At 1 day after denervation, basal blood flow through denervated soleus, plantaris, and gastrocnemius muscles was increased 63 (P < 0.01), 323 (P < 0.006), and 304% (P < 0.05), respectively, compared with the corresponding muscles in the contralateral sham hindlimb. In contrast, a different pattern of blood flow through denervated muscles was observed at 3 days after denervation (Fig. 2C). At this interval, blood flow through denervated soleus muscle was decreased by 86% (P < 0.001), and blood flow through denervated plantaris and gastrocnemius muscles was unchanged compared with corresponding contralateral sham muscles.

Blood flow was also investigated at 25 min after intravenous injection of 0.1 U insulin/rat because insulin-stimulated 2-deoxy-D-glucose uptake had been measured under such conditions. As shown in Fig. 2, B and D, sham and denervated muscles exhibited the same pattern of changes and statistical differences as under basal conditions depicted in Fig. 2, A and C. However, blood flow through denervated soleus, plantaris, and gastrocnemius muscles at 1 day was 50, 83, and 96%, respectively, higher (P < 0.04) and that of sham soleus muscle at 3 days was 44% lower (P < 0.001) than blood flow in the same muscles under basal conditions (Fig. 2, A and C).

Additional studies were also performed in rats during physiological hyperinsulinemia induced by intravenous administration of 1 g glucose/kg body wt. Administration of this dose of glucose increases the plasma glucose concentration of our rats from 136 ± 21 to 439 ± 29, 342 ± 13, and 180 ± 8 mg/100 ml at 5, 20, and 60 min after glucose injection, respectively (25). Simultaneously, plasma insulin concentration increases from initial 0.41 ± 0.04 to 4.15 ± 0.25, 3.29 ± 0.46, and 2.06 ± 0.15 ng/ml at 5, 20, and 60 min after glucose administration, respectively (25). Blood flow measurements were performed at 40 min after glucose adminis-
As depicted in Fig. 3, sham and denervated muscles exhibited the same pattern of changes as under basal conditions shown in Fig. 2, A and C. There was no difference in blood flow through individual muscles between glucose-treated rats and rats studied under basal conditions (Fig. 2, A and C), except for blood flow through sham soleus muscle at 3 days, which was lower by 57% (P < 0.001) compared with the corresponding muscle under basal conditions.

**DISCUSSION**

The present study demonstrates that basal and insulin-stimulated glucose uptake by muscle as well as muscle blood flow are influenced by the fiber population of the muscle. As summarized in Table 1, all measured parameters were lower in plantaris and gastrocnemius muscles, which are comprised mostly of fast-twitch fibers, than in the soleus muscle, which consists predominantly of slow-twitch fibers. These findings are consistent with reports that the capillary-to-fiber ratio in plantaris and gastrocnemius muscles is lower than that in the soleus muscle (21).

In contrast, blood flow in denervated muscles did not change in parallel with alterations in either basal or insulin-stimulated glucose uptake (Table 2). At 1 day after denervation, all three muscles exhibited a pronounced increase in blood flow at a time where the same muscles showed a 42–80% decrease in insulin-stimulated glucose uptake. The higher blood flow at this interval is consistent with increased arteriolar diameter and increased number of perfused capillaries in the rat cremaster muscle after denervation (23, 31). At 3 days after denervation, blood flow through plantaris and gastrocnemius muscles was unchanged, whereas the muscles exhibited a pronounced increase in basal glucose uptake. Only in 3-day postdenervation soleus muscle did we observe a parallel decrease in basal and insulin-stimulated 2-deoxy-D-glucose uptake and in blood flow. Taken together, these findings suggest that blood flow changes do not contribute to the denervation-induced changes in basal and insulin-stimulated 2-deoxy-D-glucose uptake by muscles and do not contribute to the insulin resistance in denervated muscles.

The present study also demonstrates that the pattern of blood flow through sham and denervated muscles was not systematically affected by either short-term action of a pharmacological dose of insulin or by 40 min of physiological hyperinsulinemia induced by glucose administration. It has been proposed that insulin stimu-
lates muscle glucose uptake not only by causing translocation of GLUT-4 transporters to the plasma membrane, but also as a result of a delayed increase in muscle blood flow (2, 5). Although the ability of physiological hyperinsulinemia to stimulate muscle blood flow is controversial (2, 30), prolonged high-dose insulin infusions markedly increase blood flow across the leg or a forearm (3, 4, 5, 17, 18). Available evidence indicates that vasodilatory effects of insulin are mediated by nitric oxide release (22, 26). Relevant to the present study are the findings that the dose-response curve for insulin’s vasodilatory effect across the leg is shifted to the right in human obesity (17) and type II diabetes mellitus (18). Impairment of insulin-induced vasodilation was also reported in hypertension (3, 15). These observations led to suggestions that impairment of insulin-induced vasodilation contributes to the state of insulin resistance seen in these conditions (2). It should be noted, however, that the above studies on patients with type II diabetes were done under euglycemic clamp conditions (18). In other studies in which plasma glucose levels of patients with type II diabetes were maintained at their regular fasting hyperglycemic levels, no impairment in insulin-induced vasodilation was observed compared with healthy euglycemic age- and weight-matched control subjects (12).

It has also been reported that augmentation of muscle blood flow two- to threefold with methacholine results in increased rates of leg glucose uptake above those already achieved by maximally effective insulin concentrations (2, 5). This suggested that perfusion per se is an independent determinant of glucose uptake by skeletal muscle. In other studies, infusion of adenosine to the forearm of overweight patients with essential hypertension has been observed to increase both the forearm blood flow and glucose uptake (19). However, when such patients were studied during euglycemic hyperinsulinemic clamp, a superimposed 100% increase in one forearm blood flow with adenosine did not augment glucose uptake by the infused forearm compared with the contralateral control forearm (19). Our observations on denervated muscles at 1 day after interrupton of nerve supply do not support the concept that blood flow per se regulates glucose uptake by skeletal muscle. Although the denervated soleus, plantaris, and gastrocnemius muscles are perfused with identical blood as their counterparts in the contralateral sham hindlimb and although the denervated muscles exhibit the same abundance of GLUT-1 and GLUT-4 transporters as the sham muscles (14), an

Table 1. Comparison of basal and insulin-stimulated 2-DG uptake and blood flow in sham soleus, plantaris, and gastrocnemius muscles

<table>
<thead>
<tr>
<th></th>
<th>Soleus Muscle</th>
<th>Plantaris Muscle</th>
<th>Gastrocnemius Muscle</th>
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<tbody>
<tr>
<td>Basal 2-DG uptake</td>
<td>100</td>
<td>68</td>
<td>40</td>
</tr>
<tr>
<td>Insulin-stimulated 2-DG uptake</td>
<td>100</td>
<td>54</td>
<td>34</td>
</tr>
<tr>
<td>Blood flow</td>
<td>100</td>
<td>21</td>
<td>19</td>
</tr>
</tbody>
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Data are expressed as a percentage of those in the soleus muscle. 2-DG, 2-deoxy-D-glucose.

Table 2. Changes in denervated soleus, plantaris, and gastrocnemius muscles compared with corresponding sham counterparts

<table>
<thead>
<tr>
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<th>1 day Postdenervation</th>
<th>3 day Postdenervation</th>
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<tbody>
<tr>
<td></td>
<td>Soleus muscle</td>
<td>Plantaris muscle</td>
</tr>
<tr>
<td>Basal 2-DG uptake</td>
<td>↓</td>
<td>NC</td>
</tr>
<tr>
<td>Insulin-stimulated 2-DG uptake</td>
<td>↓</td>
<td>↓</td>
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<tr>
<td>Blood flow</td>
<td>↑↑↑</td>
<td>↑↑↑↑↑</td>
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NC, no change. Number of arrows is proportional to the magnitude of change induced by denervation.
~300% increase in blood flow through denervated plantaris and gastrocnemius muscles did not increase basal 2-deoxy-D-glucose uptake above the level seen in contralateral sham muscles. In the denervated soleus muscle, a 63% increase in blood flow was, in fact, associated with a 58% reduction in basal 2-deoxy-D-glucose uptake. Our data are consistent with observations that the bradykinin-induced increase in leg skeletal muscle blood flow in human volunteers does not result in augmentation of muscle glucose uptake in vivo (20).

The mechanism of changes in blood flow through denervated hindlimb muscles remains to be fully elucidated. The initial increase in blood flow at 1 day after denervation is most likely due to interruption of sympathetic nerve supply within the sectioned sciatic nerve. The restoration of blood flow through denervated plantaris and gastrocnemius muscles to control levels and a pronounced reduction of soleus muscle blood flow below control level, at 3 days after denervation, are unexplained. It might be due to the development of hypersensitivity to circulating catecholamines and/or other vasconstrictors.

In summary, the present in vivo studies show that 1) basal and insulin-stimulated 2-deoxy-D-glucose uptake and blood flow are lower in fast-twitch muscles than in slow-twitch muscles; 2) changes in blood flow do not contribute to the insulin resistance in denervated muscles; and 3) an increase in muscle blood flow per se does not cause an increase in muscle glucose uptake.

Perspectives

The single hindlimb denervation model has several important advantages for studying the mechanism of insulin resistance. The development of insulin resistance is rapid and highly reproducible. The presence, in the same animal, of muscles that exhibit insulin resistance (denervated hindlimb) and muscles that respond to insulin in a normal fashion (contralateral sham, control hindlimb) provides an internal control and decreases variability of results. Perfusion of both hindlimbs with the same blood in vivo eliminates differences in plasma concentrations of metabolic substrates, hormones, and cytokines as potential causal or contributing factors in the development of insulin resistance. This creates “cleaner” conditions for studying cellular signaling events and increases the probability of uncovering new important mechanisms underlying insulin resistance.

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REFERENCES


