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The Rate of Nutrient Supply to Normal and Denervated Slow and Fast Muscle, and Its Relation to Muscle Blood Flow

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Muscle blood flow, glucose uptake, and the ability of muscle to concentrate an inert amino acid have been studied *in vivo* using a variety of radiosotopes. The rate of blood flow is four times greater in the slow soleus muscle than in the fast gastrocnemius muscle of the rat, but the rate of deoxyglucose uptake is 14 times higher in the slow muscle relative to the fast muscle. Slow muscle is also able to accumulate the inert amino acid, α-aminoisobutyric acid, more effectively than does fast muscle. A week after denervation, the rate of blood flow through denervated muscles was increased 30- to 40-fold over control values. However, deoxyglucose uptake was not significantly elevated in the gastrocnemius and was reduced to 20% of control levels in the soleus. The ability of the denervated muscles to concentrate α-aminoisobutyric acid was also impaired. These changes could not be accounted for in terms of an altered inulin space. There is no correlation between the rate of muscle blood flow and the ability of muscle to extract blood-borne nutrients. Impaired transport mechanisms precede and may initiate the major morphological changes that follow muscle denervation.

INTRODUCTION

There are several reports of an increase of blood flow to denervated muscle tissue (14). In spite of this increase in blood supply, striated muscle undergoes rapid atrophy after denervation with concomitant loss of glycogen and phosphocreatine reserves (15). We have investigated this apparent discrepancy by determining blood flow, deoxyglucose uptake,

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and the ability to concentrate an inert amino acid in both type I ("red") and type II ("white") muscle.

We are presenting data indicating that impaired transport mechanisms in denervated muscle prevent the uptake of nutrients, although there is an accompanying increase in the velocity of blood flow. This anomaly underlines the failure of anabolic processes in denervated muscle. Large differences in the relative ability of normal fast and slow muscle to take up glucose and amino acid analogues from the bloodstream are also reported.

METHODS

A 1-cm segment of the common peroneal and tibial branches of the right sciatic nerve was removed from 200- to 250-g, male Sprague–Dawley rats. The operation denervated the soleus and medial gastrocnemius muscles but left enough musculature innervated so that animals were able to walk using both hind legs. This procedure minimized the possibility of hypertrophy of control muscles contralateral to the transected nerve. Such a compensatory increase has not been found in control muscles for periods up to 14 days after unilateral sciatic nerve transsection in 200-g rats (12). One week later, various determinations were carried out on control and denervated muscles. The soleus and medial head of the gastrocnemius were taken as representative of predominantly "red" and "white" muscle, respectively (2). One week after denervation, the soleus had undergone considerable reduction in weight. However, using the modified Gomori trichrome and haematoxylin stains, no major denervation changes such as group atrophy or pyknotic nuclear clumps were seen at this time (7). This was probably due to the short period that the muscles were denervated.

Blood flow was measured by determining the amount of *N*[methyl-^{14}C] antipyrine (15.1 mCi/mole) that had diffused into tissues, 20 sec after intracardiac injection of 10 μCi of this isotope (18). Antipyrine was used rather than tritiated water (^3H_2O), since preliminary studies showed that reproducibility of data with ^3H_2O was lower. The use of ^3H_2O has previously been found inadequate to measure muscle blood flow (9). While at high flow rates both isotopes may give reduced values due to diffusion limitation (8), the nonvolatility of antipyrine made this compound more convenient to use. To ensure that data were not affected by large changes in extracellular water, the inulin space of tissues was determined 10 min after intracardiac injection of 100 μCi of [methoxy-^{3}H]inulin (875 mCi/mole). Plasma inulin was determined in blood from the jugular vein rather than from the site of isotope injection.

The uptake of 1-^{14}C-2-deoxy-D-glucose into muscle was measured 2 hr after intracardiac injection of 5–10 μCi (50 m Ci/mmole). At that time, serum [^{14}C]deoxyglucose values are very low since this compound is taken

up by the glucose transport system of tissue and is then irreversibly phosphorylated. Because the phosporylated derivative cannot be further metabolized and cannot readily cross the cell membrane, it is essentially trapped within the cell. Thus its concentration in tissue is proportional to the rate of glucose uptake by that tissue (16).

The ability of tissue to accumulate amino acids was estimated using the nonmetabolizable α-aminoisobutyric acid (17). Ten microcuries of 2-amino [1-^{14}C]isobutyric acid (60 mCi/mmole) were injected intraperitoneally and after 24 hr the relative radioactivity of muscle and serum was compared. In all studies, muscle tissue was rapidly dissected out, weighed, and dissolved at 40°C in NCS tissue solublizer (Amersham-Searle Inc.) before the determination of radioactivity. The use of a small amount of muscle tissue (less than 100 mg) and a large volume (20 ml) of scintillation fluid, minimized color quenching.

RESULTS AND DISCUSSION

Radioactivity within normal muscle after the various experimental procedures is presented in Table 1. At least five animals were used for each determination. The rate of blood flow and the ability to accumulate α-aminoisobutyric acid was approximately four times higher in the soleus than the gastrocnemius. Similar results for blood flow have been described previously (14, 19).

The rate of deoxyglucose uptake by soleus muscle was about 14 times that of the gastrocnemius. The magnitude of this effect makes it unlikely that it is solely due to the higher blood flow through the soleus. When differences in blood flow are taken into account, the probability that the soleus takes up deoxyglucose to a greater extent than the gastrocnemius muscle is highly significant—more than 98% using Student's 2-tailed t test. Red muscle thus appears to have a more active glucose transport mechanism than white muscle. As 24 hr was allowed for the complete equilibration of α-aminoisobutyric acid, the higher uptake by red muscle could not have been caused by varying rates of blood flow. The concentrations of α-aminoisobutyric acid in muscle relative to those of blood were 2.0 ± 0.2 in the gastrocnemius and 9.0 ± 0.6 in the soleus, implying a more active amino acid accumulation mechanism in red muscle.

The inulin space was larger in the soleus than in the gastrocnemius. The proportion of muscle inulin relative to that in plasma was $23.9 \pm 4.8\%$ for the soleus and $12.5 \pm 1.8\%$ for the gastrocnemius.

The ratios of the specific radioactivity of denervated muscle relative to those of the corresponding control muscles, using the isotopes described, are shown in Table 2. To avoid skewing data, the natural logarithm of this ratio was determined for each animal. Then, by taking the antilogarithm

TABLE 1

Concentration of Various Labeled Compounds in Normal Rat Muscle after Their Intracardiac or Intraperitoneal Injection

Isotope	Counts/min/100 mg wet muscle	
	Gastrocnemius	Soleus
[^{14}C]antipyrine	152 ± 22[a]	614 ± 249
[^{14}C]deoxyglucose	1381 ± 128	19032 ± 3202
[^{14}C]α-aminoisobutyric acid	5800 ± 209	16015 ± 1739
[^{3}H]inulin	4210 ± 799	8960 ± 1144

[a] Standard errors of the mean are given. For details, see text.

of the final mean log ratio, the geometric means were calculated and constitute the values presented.

A week after denervation the rate of blood flow through denervated muscles had increased enormously. This increase was probably largely due to loss of sympathetic vasoconstrictor tone. While there are earlier reports of altered blood flow after nerve transsection (13), no such major changes have been described. The use of diffusible indicators make it likely that the increased flow is through capillaries rather than anastomoses. Essentially similar results were obtained when tritiated water was used as the diffusible isotope. The relatively minor increase in the inulin space following denervation suggests that extracellular space changes cannot account for most of the increased blood flow. Increased inulin space has been reported for the denervated tibialis anterior muscle (6).

In spite of increased blood flow, the ability to take up glucose was not significantly increased in the denervated gastrocnemius and was dramati-

TABLE 2

Proportion of Label within Denervated Muscle Relative to that within Control Muscle from the Same Animal

Isotope	Radioactivity/g denervated[a]	
	Radioactivity/g control	
	Gastrocnemius	Soleus
[^{14}C]antipyrine	31.5 ± 2.8[b]	46.5 ± 3.8[b]
[^{14}C]deoxyglucose	1.50 ± 0.56	0.20 ± 0.02[b]
[^{14}C]α-aminoisobutyric acid	0.51 ± 0.05[b]	0.44 ± 0.07[b]
[^{3}H]inulin	1.23 ± 0.04[b]	1.12 ± 0.09

[a] See text for method of calculation. Means ± SEM are given.
[b] $P < 0.05$.

cally reduced in the soleus. The capacity to take up the experimental amino acid was also depressed, especially in the soleus. The rapid weight loss of the soleus after denervation may be due in part to the loss of its ability to take up amino acids or glucose from the bloodstream. This deficiency could account for the reduced rate of protein synthesis of denervated muscle (10). As early as 1 hr after denervation, blood flow was elevated 8.0 ± 1.7 times in gastrocnemius and 4.5 ± 1.1 times in soleus, relative to control muscles. At that interval there was no significant difference in deoxyglucose uptake by control and experimental muscles.

The decrease in the uptake of α-aminoisobutyric acid by denervated muscle has previously been reported (11), but an increase has also been described (5) as well as biphasic changes (11). These discrepancies may be explained by the failure of some workers to allow sufficient time for full equilibration of α-aminoisobutyric acid (4). Under such circumstances, increased blood flow to denervated muscle could lead to an apparent increase in amino acid uptake.

α-Aminoisobutyric acid uptake into muscle is increased by insulin (1). We are examining at present whether or not the reduced uptake of deoxyglucose and α-aminoisobutyric acid after denervation is due to loss of muscle insulin receptors. The rate of glucose uptake by resting muscle is low but this can dramatically increase with exercise (3). This may be due to changes in plasma insulin as well as increased blood flow.

The alterations in the supply of compounds to muscle reported here may be directly due to loss of neural influence or to changes in muscle resulting from an altered level of activity. Denervated muscle tissue has a clearly reduced ability to take up blood-borne nutrients in the face of vastly increased blood flow. Thus it is unlikely that the resulting muscle atrophy could be reversed by further stimulation of blood flow. The impariment of muscle transport mechanisms precede, and may initiate, the major morphologic changes resulting from denervation.

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