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BLOOD FLOW AND UPTAKE OF GLUCOSE AND AMINO ACIDS IN ISCHEMIC MUSCLE

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SUMMARY

In order to examine muscle ischemia, muscle blood flow in the rat hindlimb was decreased by vessel ligation. Amino acid uptake, studied with [^{14}C] α -aminoisobutyric acid, was decreased in ischemic Type I (soleus) muscle. Glucose uptake, studied with [^{14}C]deoxyglucose, was increased in Type I muscle. These changes were temporally associated with histologic changes of ischemia in soleus muscle. Denervation, atrophy, and hypertrophy also produced uptake changes with these techniques, and although more prominent in soleus, the changes were also seen in Type II muscle.

INTRODUCTION

Ischemic muscle, produced by a number of methods, has been devised as an experimental model of muscle pathology (Hathaway, Engel and Zellweger 1970; Mendell, Engel and Derrer 1971; Karpati, Carpenter, Melmed and Eisen 1974; Yu, Wright, Dettbarn and Olson 1974; Meltzer 1976). Rat soleus muscle appears most subject to such manipulations (Karpati et al. 1974; Yu et al. 1974). The impetus for these models was the similarity between ischemic muscle and the changes of Duchenne muscular dystrophy (Engel 1965), but later studies of muscle blood flow in patients with this disorder gave normal results (Paulson, Engel and Gomez 1974). Patients with polymyositis, however, were shown to have reduced muscle blood flow using the same technique (Paulson et al. 1974), and others have demonstrated pathologic evidence for muscle ischemia in dermatomyositis (Banker and Victor 1966; Carpenter, Karpati, Rothman and Watters 1976).

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Thus, several clinical and experimental causes are known for muscle ischemia. We have examined some associated events. In looking for selective vulnerability of the soleus muscle in other situations, denervation, atrophy, and hypertrophy experiments were also performed. Blood flow was compared using [^{14}C]antipyrine. [^{14}C]Deoxyglucose (DG) and [^{14}C] α -aminoisobutyric acid (AIB) were then used to measure the uptake of glucose (Kennedy, DesRosier, Jehle, Reivich, Scharpe and Sokoloff 1975) and neutral amino acids (Yunis, Arimur and Kipnis 1962), respectively.

MATERIALS AND METHODS

Muscle ischemia

150–250 g male Sprague–Dawley rats were used in all experiments, and surgery was done under anesthesia with sodium pentobarbital (40 mg/kg i.p.). A decrease in blood flow to hindlimb muscles was produced by ligation of the descending aorta and vena cava just below the renal axis. Both the artery and vein were ligated in order to produce a maximum reduction in blood flow, since preliminary experiments showed that blood flow could not be consistently reduced by arterial ligation alone. After a 2-day interval, soleus, medial gastrocnemius, plantaris, and the long head of the triceps brachii (forelimb) were examined bilaterally for the uptake of [^{14}C]antipyrine, AIB and DG. A separate control group of animals was similarly examined without ligation.

Blood flow was compared in control and operated muscles by determining diffusion of [N-methyl ^{14}C]antipyrine (15.1 mCi/mmol) into tissue 20 sec after the intracardiac injection of 10 μCi of this isotope (Sapirstein 1958). Animals were anesthetized and inactive during the 20 sec period for measurement of blood flow. In all isotope studies, rats were killed, the muscle was rapidly dissected out, weighed, and dissolved at 40 °C in NCS tissue solubilizer (Amersham-Searle, Inc.) before 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.

The ability of muscle tissue to accumulate amino acids was estimated using the non-metabolizable amino acid analog, AIB (Noall, Riggs, Walker and Christensen 1957). Ten μCi of 2-amino-[1- ^{14}C]isobutyric acid (60 mCi/mmol) were injected intraperitoneally, and after 24 hr the relative radioactivity of control and experimental muscle was compared.

The uptake of [1- ^{14}C]2-deoxy-D-glucose (DG) by the various muscles was measured 2 hr after the intracardiac injection of 5–10 μCi (50 mCi/mmol). This injection was carried out during sodium pentobarbital anesthesia, and in the 2 hr period the animals remained inactive. DG is taken up by the glucose transport system of tissue and is then irreversibly phosphorylated. Since the phosphorylated 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 (Kennedy et al. 1975).

Radioactive isotope uptake in all muscles was calculated in counts per mg. From the control group, normal uptake ratios were established between the triceps brachii and each ipsilateral hindlimb muscle. This ratio for each muscle from the control

animals was then compared to like ratios from the experimental animals. In order to avoid skewing the data, the natural logarithm of each ratio was determined. Then, by taking the antilogarithm of the final mean log ratio, geometric means were calculated and constitute the values presented. In all experiments, results were taken to be significant at $P < 0.05$ with the two-tailed t -test.

Histology of the ischemic muscles was examined using the modified Gomori trichrome stain. Fiber typing was done using the adenosine triphosphatase (ATPase) series of reactions (pH 9.4 and pH 4.2 pre-incubation) (Brooke and Kaiser 1970).

Denervation, atrophy and hypertrophy

For denervation, a 1 cm segment of the tibial and common peroneal branches of the right sciatic nerve was removed. The soleus and medial portion of the gastrocnemius muscles from the denervated limbs were examined 7 days after denervation along with the corresponding muscles from the normal limb. Atrophy of the gastrocnemius, soleus, and plantaris muscles was produced in another group of animals by complete section of the right Achilles tendon. Nerves were left intact in this procedure. Examination of muscles from these animals was performed after 5 days. Hypertrophy of the soleus and plantaris muscles was produced in the third group by section of the synergistic gastrocnemius tendons on the right (Goldberg 1967b). This produces an increase in isometric work by the former two muscles, which were also examined 5 days after surgery. These time intervals were chosen to produce a maximal biochemical effect of each operation.

In all these comparisons, results of the experimental side (right) were divided by those of the normal side (left), constituting the data presented for the operated muscles. Statistics were derived from these ratios as deviation from unity and calculated with the two tailed t -test.

RESULTS

Normal

Relative uptake of the three radioactive tracers by normal soleus, medial gastrocnemius, and plantaris muscle (Table 1) is expressed as an average of the counts per minute (CPM) per mg in at least 6 animals. In all instances the soleus showed greater uptake of each tracer than either the plantaris or gastrocnemius muscle.

TABLE 1
RELATIVE UPTAKE IN NORMAL MUSCLES (cpm/mg)

	Soleus	Gastrocnemius	Plantaris	No. Animals
[^{14}C]Antipyrine	113.6 $\pm$ 26.8 (SEM)	38.0 $\pm$ 17.0	62.5 $\pm$ 16.7	9
[^{14}C] α -Aminoisobutyric acid	168.2 $\pm$ 7.8	92.7 $\pm$ 6.6	91.5 $\pm$ 2.0	6
[^{14}C]Deoxyglucose	79.9 $\pm$ 11.1	25.4 $\pm$ 4.6	49.5 $\pm$ 11.4	7

SEM = standard error of the mean.

TABLE 2

	Control	Number of Muscles	Experimental	Number of Muscles
<i>Blood flow with vessel ligation (expressed as ratio to triceps brachii)</i>				
Soleus	2.50 ± 0.24 (SEM)	8	0.44 ± 0.06 ^a	14
Plantaris	1.50 ± 0.43	8	0.43 ± 0.18 ^a	14
Gastrocnemius	1.00 ± 0.12	8	0.21 ± 0.14 ^a	14
<i>α-Aminoisobutyric acid uptake with vessel ligation (expressed as ratio to triceps brachii)</i>				
Soleus	2.22 ± 0.20	8	1.16 ± 0.15 ^a	12
Plantaris	1.12 ± 0.04	8	1.13 ± 0.06	12
Gastrocnemius	1.08 ± 0.04	8	0.95 ± 0.05	12
<i>Deoxyglucose uptake with vessel ligation (expressed as ratio to triceps brachii)</i>				
Soleus	3.97 ± 0.47	8	7.32 ± 0.79 ^a	12
Plantaris	2.59 ± 0.23	8	3.39 ± 0.62	12
Gastrocnemius	1.12 ± 0.12	8	1.39 ± 0.29	12

^a $P < 0.05$ (experimental significantly different from control).

SEM = standard error of the mean.

Fig. 1. Soleus muscle after vessel ligation demonstrates focal changes with increased internal nuclei. $\times 55$.

Histochemical ATPase reactions showed that the normal soleus muscle was predominantly Type I. The medial gastrocnemius was completely Type II. Plantaris muscles were predominantly Type II.

Muscle ischemia

Vessel ligation (Table 2) produced a significant decrease in blood flow in each hindlimb muscle. All values are ratios of the stated muscle to the ipsilateral triceps

brachii of each animal. Changes in AIB and DG uptake, however, were significant only in soleus muscles (Type I). AIB uptake was decreased, while DG uptake was increased.

Histology of the hindlimb muscles from the vessel-ligated animals showed focal coarsening of the intermyofibrillar network with increased internal nuclei. This was most prominent in the soleus muscle (Fig. 1).

Denervation, atrophy and hypertrophy

Denervation produced significant changes over controls in blood flow (soleus 40.85 ± 13.13 (SEM) and gastrocnemius 40.12 ± 12.86), AIB uptake (soleus 0.46 ± 0.06 and gastrocnemius 0.51 ± 0.03), and DG uptake (soleus 0.20 ± 0.04).

Atrophy produced significant decreases in soleus (0.56 ± 0.04) and gastrocnemius (0.875 ± 0.04) AIB uptake.

Hypertrophy significantly increased blood flow in soleus (2.18 ± 0.27) and AIB uptake in plantaris (1.53 ± 0.10).

DISCUSSION

Blood flow data derived from normal muscles with [^{14}C]antipyrine agree with results obtained via other methods (Smith and Giovacchini 1956; Wooten and Reis 1972), demonstrating the usefulness of this simple technique. The higher blood flow in the soleus supports the idea of a higher metabolic rate in Type I muscles, correlating with the known high oxidative metabolism in this muscle (Romanul 1964). We also found that AIB and DG accumulation follow blood flow in normal muscle. The high rate of AIB transport in soleus or Type I muscle confirms the earlier work of Goldberg (1967a).

When blood flow was reduced by vessel ligation, the muscle was able to compensate, perhaps by increasing transport, and take up normal or increased amounts of DG. This coincides with previous *in vitro* experiments showing that anoxia increases glucose uptake in muscle (Randle and Smith 1958). AIB accumulation, on the other hand, was significantly impaired in Type I (soleus) muscle with vessel ligation, while little changed in Type II muscle. The transport system for neutral amino acids requires energy and may be impaired by hypoxia (Bombara and Bergamini 1968). Thus, the more oxidative muscle would be the first affected.

Although other time points might have produced different results, the changes in AIB and DG uptake were temporally associated with histologic changes. Furthermore, all these observations were consistent with increased vulnerability of the soleus during experimental ischemia.

Denervation, atrophy and hypertrophy produced predictable changes in isotope uptake. Again, these tended to be more prominent in the soleus, though less so than with ischemia. No differential effect on AIB and DG uptake was observed.

We cannot comment on the relationship of these results to human diseases; but we suggest that these techniques might be helpful in a more complete examination of other models of human diseases causing reduced muscle blood flow.

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