The Effect of Low ATP on Glucose Uptake in Soleus Muscle During Hemorrhagic Shock (37582)

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Considerable controversy exists concerning the peripheral utilization of glucose (1–4) during hemorrhagic shock. Drucker and Dekiewiet (5) found increased glucose uptake by hemidiaphragms of rats subjected to severe hemorrhagic shock and suggested that the increased uptake was due to severe depletion of intracellular energy.

One of the problems in evaluating the results of the experiments of Drucker and Dekiewiet (5) and indeed those of most of the papers cited above, had been the "leaky" nature of the hemidiaphragm preparation (6). Thus it is difficult to distinguish between hemorrhagic shock effects at the site of glucose transport and those concerned with its subsequent metabolism, and effects due to the leakage of intracellular constituents into the medium. This problem could be minimized by the use of an intact muscle preparation (6). Rat soleus muscle can be removed from the animal without cutting or damaging the fibers, and has proved very suitable for *in vitro* experimentation (7–10).

We have recently shown that there is a significant decrease in ATP levels in rat soleus muscle during late hemorrhagic shock ("shock muscle") (11) which could be due to hypoxic conditions as a result of decreased blood flow to this tissue (12). Since ATP is considered to act as a feedback inhibitor of glucose uptake (13), one would expect that a decrease in this nucleotide should enhance glucose uptake. In the work reported here, we have demonstrated that the so-called cause–effect relationship between glucose uptake and ATP levels does not exist in skeletal muscle during late hemorrhagic shock.

Methods. The hemorrhagic shock procedure and isolation of soleus muscle. Albino Holtzman rats, weighing 180–210 g were allowed water *ad libitum* but not food for 16 hr prior to study. The rats were anesthetized lightly with ether and both subclavian arteries were cannulated with polyethylene tubing (PE-50). Heparin, 400 units, was injected into each subclavian artery. One of the subclavian artery cannulae was connected to a 1.75 m length of PE-50 tubing which was calibrated in millimeters Hg for monitoring arterial blood pressure (BP). The other cannula was connected to a 10 ml heparinized glass syringe which served as a blood reservoir. The animals were restrained in the supine position and were allowed to awaken. The duration of anesthesia was approximately 40 min. They were then bled rapidly within 10 min to a mean arterial pressure of 40 mm Hg. About 45 min after the beginning of bleeding the BP usually began to fall below 40 mm Hg and approximately 60% of the shed blood had to be returned gradually to maintain the above pressure for 2 hr. At the end of 2 hr, the animals were sacrificed and 2 soleus muscles from each animal were quickly removed (7) and treated as described below. Control muscles were obtained from rats which were treated in the same manner but were not bled.

Determination of glucose uptake. As soon as the soleus muscles were removed, they were incubated in 1.0 ml Krebs–Henseleit bicarbonate buffer (pH 7.4), containing 10 m*M* glucose or 20 m*M* raffinose. In order to increase the accuracy of the determination, from 2 to 4 muscles were placed in each beaker. Incubations were carried out in a Gallenkamp metabolic shaker for 1 hr at 37° under an atmosphere of either O_2–CO_2 (95:5,

v/v) or N_2–CO_2 (95:5, v/v); shaking rate 110 cycles/min. Glucose uptake, glucose space and raffinose space were determined as described previously (7).

Lactate and ATP determination. For the determination of ATP and lactate at the end of the incubation, the muscles were quickly rinsed in ice-cold water, blotted on filter paper and frozen between aluminum blocks chilled in dry ice, then homogenized in a trichoroacetic acid (10%)–HCl (0.1 *M*) mixture and centrifuged. A portion of the supernatant solution was withdrawn at this point for the assay of lactate, using the method of Barker and Summerson (14), the remainder was extracted 4 times with ether, then neutralized with 1 *M* Tris base. ATP was assayed spectrophotometrically using glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and hexokinase (EC 2.7.1.1).

Results. Influence of muscle size. The present studies were conducted on isolated rat soleus muscles weighing approximately 80 mg. Previous work with soleus muscle has shown that glucose uptake was proportional to the muscle weight only up to a weight of 60 mg (7). From this it may be concluded that diffusion of glucose into the muscle weighing 80 mg would be a rate-limiting factor and this point should be kept in mind. It is, however, extremely difficult to produce hemorrhagic shock in smaller rats as the animals die before the termination of the experiment. Since the corresponding control muscles were obtained from rats of the same weight, this would appear to justify the direct comparison of the glucose uptake values of control and "shock muscles."

Glucose uptake during hemorrhagic shock. Randle and Smith (13) found that anoxia and certain metabolic poisons would increase sugar transport, and from this proposed that the stimulation of sugar entry was due to the lowering of inhibitory levels of ATP. Since ATP levels were significantly lowered in soleus muscle during late hemorrhagic shock (11), it was therefore desirable to see if increased glucose uptake would also be observed in these muscles. As shown in Table I, glucose uptake under aerobic conditions in control and shock muscles was the same despite the significant lowering of ATP levels in muscles from "shock" rats. In order to determine whether the lack of increased glucose uptake in shock muscles was due to an accumulation of free glucose in the muscle mass, we have measured both the glucose space and the raffinose space. The raffinose space is taken to indicate the extracellular space of the muscle. The glucose space, determined at the end of 1 hr incubation is also shown in Table I, along with raffinose space. In control muscles as well as in shock muscles, the glucose space did not exceed the extracellular space thereby indicating that there was no accumulation of free glucose in the muscle mass. Thus the lack of increased glucose uptake in shock muscles was not due to an impaired phosphorylation mechanism in these muscles.

TABLE I. Glucose Uptake, ATP Levels and Sugar Spaces of Muscles.[a]

	Control muscles	"Shock muscles"
Glucose uptake (μmoles/g/hr)	8.8 ± 0.68	8.1 ± 0.74
ATP (μmoles/g)	2.07 ± 0.07	1.05 ± 0.09
Glucose space (ml/g)	0.10 ± 0.02	0.09 ± 0.01
Raffinose space (ml/g)	0.21 ± 0.02	0.21 ± 0.01

[a] Soleus muscles from control and "shock" rats were incubated for 1 hr at 37° in 1.0 ml Krebs–Henseleit buffer (pH 7.4) containing glucose (10 m*M*) or raffinose (20 m*M*); atmosphere 95% O_2–5% CO_2. Glucose uptake and ATP were determined as described in Methods. Values are mean of 8 determinations ± SE.

Effect of anoxia. As shown above (Table I), muscles from shock rats showed no increase in glucose uptake although ATP levels were reduced. This led us to determine whether these muscles would respond to anoxia *in vitro*. Table II shows that glucose uptake under anaerobic conditions increased both in control muscles and in shock muscles. This increase in glucose uptake was associated with the normal anoxic effect, *i.e.*, increased lactate production and decrease in ATP levels.

Discussion. The present experiments have shown that glucose uptake under aerobic con-

TABLE II. Effect of Anoxia on Glucose Uptake, Lactate Production and ATP Concentration in Shock Muscles.[a]

Conditions	Glucose uptake (μmoles/g/hr)		Lactate produced (μmoles/g/hr)		ATP (μmoles/g)	
	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
Control muscles	8.76 ± 0.22	15.94 ± 1.03	26.10 ± 2.33	67.78 ± 1.32	1.99 ± 0.12	1.09 ± 0.06
Shock muscles	8.62 ± 0.24	15.41 ± 0.60	40.89 ± 2.68	77.83 ± 1.74	1.12 ± 0.03	0.64 ± 0.03

[a] Soleus muscles were incubated for 1 hr at 37° in 1.0 ml Krebs–Henseleit buffer (pH 7.4) containing glucose (10 m*M*); atmosphere 95% O_2–5% CO_2 or 95% N_2–5% CO_2. The lactate values represent the total lactate present in both muscle and medium. For details, see Methods. Values are mean of 8 determinations ± SE.

ditions in control muscle was the same as that observed in shock muscle (Table I). This would suggest that hemorrhagic shock *per se* did not produce any damage or alterations at the glucose carrier site. Nor was there any effect of shock on the subsequent utilization of glucose as demonstrated by the fact that glucose spaces of these muscles did not exceed the extracellular space (Table I).

Little information is available concerning glucose uptake by tissues isolated from shock animals. Drucker and Dekiewiet (5) observed an increase in glucose uptake by hemidiaphragms from rats subjected to hemorrhagic shock. However, since tissue Na^+ concentration is increased in hemorrhagic shock (15, 16) and since the preparation used by the above investigators contained severely damaged muscle fibers (6), it is possible that Na^+ from the cells leaked out into the medium. The stimulatory effect of *in vitro* Na^+ on glucose uptake has been well documented (17–19). Thus it appears that the increased glucose uptake by hemidiaphragms as observed by Drucker and Dekiewiet (5) may not be due to hemorrhagic shock *per se* but to leakage of Na^+ from the muscle into the medium.

Although glucose uptake in soleus muscle was unaffected by hemorrhagic shock, ATP levels of these muscles were significantly lowered. The present experiments may thus be considered in terms of the mechanism proposed by Drucker and Dekiewiet (5) by which sugar transport is affected during hemorrhagic shock. It has been suggested by these workers that increased uptake by hemidiaphragms of rats subjected to hemorrhagic shock was due to depletion of intracellular energy as a result of anoxic conditions prevailing during shock. This is in accordance with the hypothesis of Randle and Smith (13) that ATP might control sugar transport by functioning as a feedback inhibitor. According to this hypothesis, anoxia "depressed" the feedback system by lowering the intracellular ATP concentration. However, as shown in Table I, when animals were subjected to severe hemorrhagic shock, although this resulted in a lowering of the muscle ATP levels, there was no effect on glucose uptake. Severe hemorrhagic shock thus uncoupled any relationship which might have existed between the lowering of ATP concentrations and stimulation of sugar transport. If the effect of such a relationship were to restrain the process of sugar transport, it seems reasonable to assume that when the links were broken, this would have removed the restraint, resulting in increased sugar transport. That glucose uptake remained unaffected in muscle in which the ATP concentrations were lowered, supports our earlier conclusions (9) that there is no direct cause–effect relationship between oxidative phosphorylation and glucose uptake.

Although glucose uptake was not affected in muscles in which ATP concentrations were lowered, these muscles still responded to the *in vitro* effect of anoxia by showing increased glucose uptake, further reduction in ATP levels and increased lactic acid production (Table II). Muscles from late hemorrhagic shock animals showed increased lactate production when incubated under aerobic conditions and decreased ATP concentrations, suggesting

that these muscles were anoxic. Since these muscles responded to the *in vitro* effect of anoxia, it may be concluded that whatever the mechanism of action of anoxia *in vitro* might be, it is quite different from that of *in vivo*. Previous work by Chaudry and Gould (9) led them to conclude that the mechanism of anoxia in promoting glucose uptake by normal soleus muscle was not through lowering of muscle ATP levels. The present experiments indicate that severe hemorrhagic shock also uncoupled any relationship which might have existed between ATP depletion and increase in glucose uptake.

Although peripheral to the main theme of these studies, the observation that shock muscles responded to the *in vitro* effect of anoxia deserves further comment. Gould and Chaudry (20) using rat soleus muscles found that endogenous insulin was not required for basal transport, but its presence was essential for anoxia to produce its stimulatory effect. Since divalent cations but not insulin were present in the incubation medium and since shock muscles responded to the *in vitro* effect of anoxia, this suggests that hemorrhagic shock *per se* did not produce any alteration or modification in the function of endogenous insulin.

The present experiments may also be considered in terms of the mechanism by which anaerobic muscles replenish ATP when subsequently transferred to an aerobic environment. Normal soleus muscles when incubated for 15 min under anaerobic conditions show a decrease in their ATP levels. However, when these muscles were transferred to aerobic environment following anaerobic incubation, their ATP levels increase and show approximately the same values as those muscles which were incubated under aerobic conditions only (Table III). This suggests that a brief period of anoxia did not cause a permanent damage to the ATP synthesizing system. Since shock muscles showed a further decrease in ATP levels when incubated aerobically, this indicates that some essential component of ATP synthesizing system was lost during severe hemorrhagic shock. This observation might support the hypothesis of Crowell, Jones and Smith (21) that purine base is lost during shock. Thus under the conditions when ATP resynthesis or production became limited causing ATP levels to decrease, glucose uptake was not increased.

TABLE III. ATP Levels under Aerobic and Anaerobic Conditions.[a]

Conditions	Muscle ATP (μmoles/g)
Incubation under O_2–CO_2	2.15 ± 0.10
Incubation under N_2–CO_2 for 15 min	1.51 ± 0.08
Incubation under N_2–CO_2 for 15 min followed by incubation under O_2–CO_2	1.89 ± 0.11
Shock muscles without incubation	1.57 ± 0.12
Shock muscles incubated under O_2–CO_2	1.07 ± 0.12

[a] Soleus muscles were incubated in 1.0 ml of Krebs–Henseleit bicarbonate buffer containing 10 m*M* glucose for 1 hr under 95% O_2–5% CO_2 or under 95% N_2–5% CO_2 for 15 min followed by incubation under 95% O_2–5% CO_2 for additional 45 min. The ATP values reported above are those at the end of the incubation period. Values are mean of 8 determinations ± SE.

Summary. Subclavian arteries of rats were cannulated and the animals were bled rapidly to a pressure of 40 mm Hg and maintained at that level for 2 hr. Glucose uptake by isolated soleus muscles from normal rats and rats which were subjected to severe hemorrhagic shock ("shock muscles") was the same although ATP levels were significantly lowered in muscles of the latter rats. Whereas shock muscles did not show increased glucose uptake, these muscles still responded to the *in vitro* effect of anoxia by showing increased glucose uptake, further depletion of ATP and increased lactic acid production. The present experiments indicate that: (a) during severe hemorrhagic shock, there is no cause–effect relationship between glucose uptake and ATP depletion and (b) whatever the mechanism of action of anoxia in promoting glucose uptake *in vitro* might be, it is quite different from that of *in vivo*.

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