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Insulin Effect on Creatine Transport in Skeletal Muscle[1] (38464)

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(Introduced by H. J. Ralston)

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Radiation-induced creatinuria in rats was found to be a direct consequence of the decreased ability of irradiated skeletal muscle to take up newly synthesized creatine (1). Koszalka and Andrew (2, 3) reported that insulin decreases hypercreatinemia and creatinuria in rats which have been either creatine-loaded by a high dose ip injection or exposed to X-rays.

Insulin has long been known to decrease plasma levels of amino acids and sugars. More recently insulin has been shown to bind to the cell membrane (4) and in skeletal muscle to directly enhance the translocation of some sugars and amino acids by increasing both the rate of entry and the total uptake (5, 6).

Insulin action in preventing creatinuria in X-ray irradiated or creatine-loaded rats could be due to stimulation of creatine transport in skeletal muscle. This was, in fact, the conclusion reached by Koszalka and Andrew (3). However, other hormones such as testosterone and some of its analogues (7), growth hormone and corticosteroids (8) have all been shown to influence creatine metabolism. The *in vivo* effect of insulin could therefore be secondary to a modification of the hormonal balance or to enhanced utilization of other hormonal substances. Only *in vitro* experiments with isolated muscle can demonstrate a direct effect of insulin on creatine transport.

The present investigation of creatine transport in isolated extensor digitorum longus (EDL) of the rat confirms that insulin does indeed enhance creatine uptake by skeletal muscle.

Materials and Methods. Muscles and source. EDL's from normal male Sprague–Dawley rats, weighing 100–150 g and maintained on commercial rat chow and water *ad libitum* were used.

The rats were anesthetized with pentobarbital or urethane. No differences in creatine transport were found between EDL's from pentobarbital or urethane anesthetized rats or from decapitated rats, indicating that the anesthetic did not affect creatine transport.

Incubation procedure. The incubation procedure was similar to that of Fitch and Shields (9). The EDL was quickly removed, weighed and incubated in 7 ml of Krebs–Ringer bicarbonate buffer containing 0.13% glucose, 0.1 m*M* creatine and about 0.2 μCi/ml 1-^{14}C-creatine (Amersham–Searle). Insulin (Sigma or Squibb "regular" Insulin) was added to a final concentration of 0.2 units/ml. The muscles were incubated at 37° under 95% O_2–5% CO_2 atmosphere, quickly rinsed, blotted and weighed. Creatine was extracted by freezing the muscle in liquid nitrogen, powdering and homogenizing it in distilled water. Trichloroacetic acid (TCA) solution was added to a final TCA concentration of 10%. The mixture was centrifuged and a portion of the supernatant counted in Bray's scintillation fluid. Values of creatine transport, corrected for the extracellular space, were expressed as distribution ratio of the isotope:

$$\frac{\text{cpm}/\mu\text{l intracellular water}}{\text{cpm}/\mu\text{l medium}}$$

The extracellular space was determined by adding ^{14}C-inuline to the incubation medium as described by Fitch and Shields (9). Values practically identical to those that they reported were obtained.

Creatine and creatine phosphate measurement. After incubation, the EDL's were frozen in liquid nitrogen, powdered and ex-

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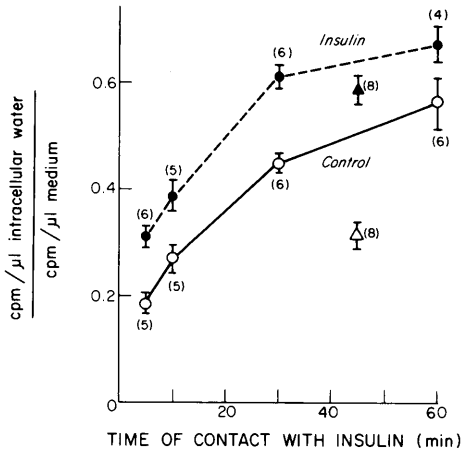

FIG. 1. Time course of insulin effect on creatine transport in rat EDL. The muscles were preincubated for 15 min in 0.1 m*M* creatine containing 1-^{14}C-creatine prior to the addition of insulin (0.1 unit/ml). Glucose (0.15%) was present in the medium except for a group indicated with open triangle (control) and filled triangle (insulin). Number in brackets indicates number of muscles for each point. The incubation medium was Krebs–Ringer bicarbonate under 95% oxygen and 5% carbon dioxide atmosphere, at the temperature of 37°. (Incubation procedure was identical in all the experiments presented in this paper.)

tracted with 50 m*M* bicarbonate buffer pH 9 and the extract deproteinized by ultra filtration. These conditions have been found in our laboratory to maximally prevent hydrolysis of creatine phosphate. Creatine concentration in the muscle extract was measured by the enzymatic assay described by Tanzer and Gilvarg (11) and by Bernt *et al.* (12). Total creatine was measured after 20 minutes acid hydrolysis of the extract in 0.33 *N* HCl, and creatine–phosphate determined as the difference between the free and total creatine (Table I). The hydrolysate was neutralized to pH 8–9 prior to the assay.

TABLE I. DISTRIBUTION RATIO OF ISOTOPIC CREATINE IN EDL FROM CONTROL AND ALLOXAN-DIABETIC RATS[a]

Group	Control	Alloxan-Diabetic
Distribution ratio ± S.E.M.	0.65 ± 0.04 (10)	0.68 ± 0.02 (14)

[a] The muscles were incubated for 1 hr in the presence of 0.1 m*M* creatine, containing 1-^{14}C-creatine. Value in brackets represents number of muscles for each group.

Alloxan treatment. Diabetic rats were obtained by the method described by Krahl (13). Alloxan (50 mg/kg) was injected into the saphena vein. Before sacrifice blood sugar was determined by the "Glucostat" method (Worthington Biochemicals). Only rats with a value of blood sugar four times higher than that of the controls were considered diabetic.

Results and Discussion. Figure 1 shows the distribution ratio of isotopic creatine in the EDL's incubated for different lengths of time in the presence or absence of insulin. Incubation in medium containing the physiological concentration, 0.1 m*M* creatine, was found by Fitch and Shields (9) and also in our experiments to give the maximum distribution ratio. Insulin increased the distribution ratio about 40% in 30 min, and stimulation of creatine transport was evident within 5 min after addition of the hormone to the incubation medium.

To exclude the possibility that the insulin effect on creatine transport was due to increased utilization of glucose promoted by the hormone, a group of muscles was incubated without glucose. In this case, insulin action on creatine uptake appeared to be even greater, giving a 75% increase in the isotope distribution ratio in the insulin-treated EDL.

Creatine transport in rat skeletal muscle is a saturable process (9). Under our experimental conditions the "half saturating" concentration of creatine was about 0.6 m*M* in both control and insulin-treated muscles (Fig. 2). The effect of insulin appeared to be independent of the creatine concentration in the incubation medium and, as reported for amino acids and sugars it appeared to involve both the rate of transport and the "space" in which creatine is distributed.

As indicated in Table I we were unable to find any defect in the ability to transport creatine in EDL from alloxan-diabetic rats. Similar results have been reported for the transport of amino acids (4). It is possible, however, that alloxan-treated rats do not represent an adequate experimental model for diabetes and that a defect in creatine

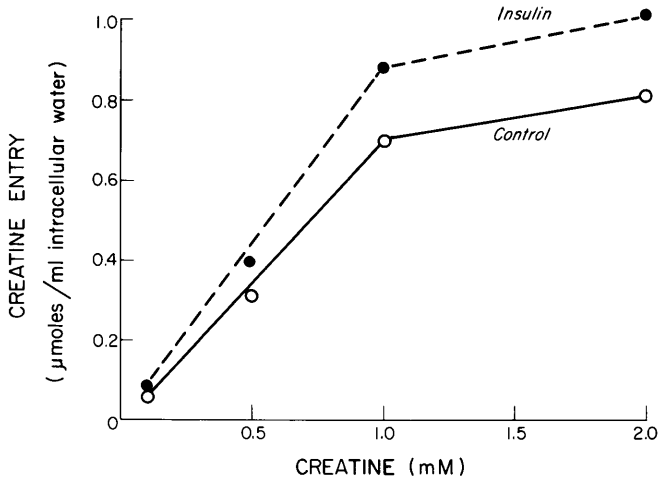

FIG. 2. Effect of creatine concentration and of insulin (0.2 unit/ml) on creatine uptake by isolate EDL.

TABLE II. FREE CREATINE, TOTAL CREATINE, AND CREATINE-PHOSPHATE CONTENT OF EDL.[a]

μmoles/g wet wt	Control	Insulin	*P*
Free creatine	14.45 ±0.46	17.12 ±0.34	<0.002
Total creatine	16.95 ±1.00	19.20 ±0.24	<0.05
Creatine-phosphate	3.50 ±0.16	3.35 ±0.18	—

[a] The muscles were incubated for 30 min in 1 m*M* creatine and in presence or absence of insulin (0.1 unit/ml).

transport may contribute to the phenomenon of fatigue in diabetic patients.

Insulin is known to increase transport of some sugars and amino acids, independently of the type of mechanism by which they are transported (13). In our laboratory we have some evidence (14) that creatine accumulation is not due to an ATPase-sustained membrane pump but rather to the phosphorylating activity of creatine kinase. If this proves correct, a greater amount of creatine transported should result in a higher concentration, not only of creatine, but also of creatine phosphate within the muscle. To investigate this possibility we have measured the concentrations of creatine and creatine-phosphate in EDL after 30 min incubation with 1 m*M* creatine in the presence or absence of insulin. The results reported in Table II show that creatine concentration in the treated muscle is significantly increased, and that creatine appears to enter the muscle fibers against even a higher concentration gradient. An increase in total creatine was observed, as expected, but no change was found in the creatine-phosphate concentration. The latter could be explained by the inability of the incubated EDL to maintain a level of respiration sufficient to keep up with the increased metabolic rate caused by the hormone. Consequently the observed increase in creatine transport could be due not only to an effect of insulin at the membrane level but also to an increased creatine-phosphate turnover. Insulin could also have an indirect effect on creatine kinase by increasing the Mg^{2+} concentration in the muscle fibers (15) and making it more available for creatine kinase.

The results reported recently by Morgan and collaborators (16) obtained with perfused rat hemicorpus indicate that our interpretation (that creatine accumulates as a result of creatine kinase activity) may be correct.

Summary. Insulin is shown to directly enhance both the rate of transport and the uptake of creatine *in vitro* in isolated rat skeletal muscle. This action of the hormone on creatine transport is similar to that reported for the transport of some amino acids and sugars.

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