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System A transport activity is stimulated in skeletal muscle in response to diabetes

Anna Gumà, Conchi Mora, Tomàs Santalucía, Francesc Viñals, Xavier Testar, Manuel Palacín and Antonio Zorzano

Departament de Bioquímica i Fisiologia, Facultat de Biologia, Universitat de Barcelona, Avda. Diagonal 645, 08028 Barcelona, Spain

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We have studied the activity of system A transport in skeletal muscle during experimental diabetes. Five days after streptozotocin injection, rats showed a marked hyperglycemia and a substantial decrease in the content of GLUT-4 protein in skeletal muscle and adipose tissue. Under these conditions, basal uptake of 2-(methyl)aminoisobutyric acid (MeAIB), an index of system A transport activity, was enhanced in extensor digitorum longus (EDL) muscles from diabetic rats compared to controls. Furthermore, insulin-stimulated MeAIB uptake by the incubated EDL and soleus muscles was markedly greater in diabetic than in control rats. The derepressive phase of adaptive regulation was partially blocked in the diabetic muscle, and incubation of muscles for 3 h in the absence of amino acids led to a lower stimulation of system A transport activity in muscles from diabetic groups compared to controls. We propose that the activated system A might participate in the enhanced alanine release from muscle cells that occurs in diabetes.

GLUT-4; System A transport; Amino acid transport; Diabetes; Skeletal muscle

1. INTRODUCTION

System A transport activity for neutral amino acids is subject to hormonal regulation, *trans*-inhibition, and adaptive regulation in a variety of cell types [1–3]. In skeletal muscle, system A transport activity is stimulated in response to amino acid starvation by a mechanism that requires protein synthesis and microtubular function [4–6]. System A transport activity is rapidly activated in skeletal muscle by insulin [7], acute exercise [8], vanadate and alkalinization of intracellular pH [9]. In skeletal muscle, the effect of insulin on system A transport activity is independent of protein synthesis, adaptive regulation, microtubular function and the sodium electrochemical gradient [6,10–12].

Here, in an attempt to obtain further insight into the regulatory characteristics of system A transport activity in skeletal muscle, we have evaluated the effect of acute streptozotocin-induced diabetes, a situation in which protein synthesis is markedly decreased [13,14], protein degradation has been reported to increase [15,16] and amino acid release is enhanced [17].

Correspondence address: A. Zorzano, Departament de Bioquímica i Fisiologia, Facultat de Biologia, Universitat de Barcelona, Avda. Diagonal 645, 08028 Barcelona, Spain.

Abbreviations: EDL, extensor digitorum longus; MeAIB, 2-(methyl)-aminoisobutyric acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HEPES, *N*-[2-hydroxyethyl]piperazine-*N*'-[ethanesulfonic acid].

2. MATERIALS AND METHODS

2.1. *Animals and dissection procedures*

Male Wistar rats (50–60 g) from our own colony were fed on Purina Laboratory chow ad libitum and housed in animal quarters at 22°C with a 12 h light/12 h dark cycle. Diabetes was produced by an i.p. injection of streptozotocin (150 mg/kg body weight) 5 days before the study. The dissection and isolation of the extensor digitorum longus (EDL) and soleus muscles were carried out under anaesthesia with pentobarbital (5–7 mg/100 g body wt, i.p.) as described [18].

2.2. *Muscle incubations*

EDL and soleus muscles were incubated in a shaking incubator at 37°C for 1.5–3 h in 3 ml of Kreb's–Henseleit buffer, pH 7.4, containing 5 mM glucose, 0.2% bovine serum albumin and 20 mM HEPES, as described [10]. Insulin (100 nM) was added to the incubation medium during the last 60 min of the incubation period. Amino acid uptake by system A was measured in muscles using the non-metabolizable amino acid analog, 2-(methylamino)isobutyric acid (MeAIB). Following incubation with insulin, muscles were transferred to vials with 1.5 ml of Kreb's–Henseleit buffer, pH 7.4, containing 5 mM glucose, 0.2% bovine serum albumin, 20 mM HEPES and 0.1 mM [1-^{14}C]MeAIB (800 μCi/mmol), 1 mM [^{3}H]mannitol (330 μCi/mmol) and insulin (100 nM). The vials were stoppered and incubated at 37°C in a shaking incubator for 30 min. The uptake of MeAIB was linear with time for at least 30 min. The gas phase in the vials was 95% O_2 and 5% CO_2. Following incubation, the muscles were processed as reported [10]. Intracellular concentration of [^{14}C]MeAIB in the extracellular space was calculated from the total label found in tissue, as in [10].

2.3. *Preparation of membrane fractions from tissues*

Tissues were homogenized in 10 vols. ice-cold buffer containing 25 mM HEPES, 250 mM sucrose, 4 mM EDTA, 1 trypsin inhibitory U/ml aprotinin, 25 mM benzamidine, 0.2 mM PMSF, 1 μM leupeptin and 1 μM pepstatin, pH 7.4. Homogenates from white and brown adipose tissue were centrifuged at 5,000 × *g* for 5 min at 4°C. The supernatant was then centrifuged at 150,000 × *g* for 2 h at 4°C to obtain the membrane fractions. The homogenates from tibialis anteri-

oris muscle were centrifuged at 15,000 × g for 20 min at 4°C. The supernatants were adjusted to 0.8 M KCl, incubated at 4°C for 30 min, and then centrifuged for 90 min at 200,000 × g at 4°C to obtain the membranes. The membrane pellets were resuspended in homogenization buffer and repeatedly passed through a 25 gauge needle before storage at −20°C. Proteins were measured by the method of Bradford [19] using gamma-globulin as a standard.

2.4. *Electrophoresis and immunoblotting of membranes*

SDS-PAGE was performed on membrane proteins in accordance with the method of Laemmli [20]. Proteins were transferred to Immobilon as previously reported [16] in buffer consisting of 20% methanol, 200 mM glycine, 25 mM Tris, pH 8.3. Following transfer, the filters were blocked with 5% non-fat dry milk and 0.02% sodium azide in PBS for 1 h at 37°C and incubated with polyclonal antibody, OSCRX, raised against the 15C-terminal peptide from GLUT-4 for the same time at the same temperature. Transfer was confirmed by Coomassie blue staining of the gel after the electroblot. OSCRX, purified by protein A chromatography, was used at 5–10 μg/ml in 1% non-fat dry milk and 0.02% sodium azide in PBS for immunoblotting. Antibody–antigen complexes were detected with [^{125}I]protein A and autoradiography. The autoradiograms were quantified using scanning densitometry (Ultrascan × L enhancer laser densitometer, LKB). Immunoblots were performed under conditions in which autoradiographic detection was in the linear response range, and data were expressed as a percentage of control values.

3. RESULTS AND DISCUSSION

We studied the effect of diabetes on the activity of system A transport in skeletal muscle. To this end, we injected 150 mg of streptozotocin per kg of body weight to young male Wistar rats (Table I). After 5 days of streptozotocin injection, rats showed marked hyperglycemia and a decreased growth rate, as assessed by body weight gain and EDL and soleus muscle weight (Table I). Under these conditions, diabetes led to a marked decrease in the content of GLUT-4 in white adipose tissue, brown adipose tissue and skeletal muscle, as determined by immunoblot (Fig. 1). The extent of the decrease was greater in white adipose tissue than in brown adipose tissue or skeletal muscle (Fig. 1). These results are in keeping with previous observations performed in adult rats after 7 days of streptozotocin administration [21–23].

System A was assessed by the uptake of the non-metabolizable analog, 2-(methyl)aminoisobutyric acid

Table I

Characteristics of control and diabetic animals

	Control	Diabetic
Initial body weight (g)	48 ±2 (8)	52 ±2 (19)
Final body weight (g)	73 ±5 (8)	59 ±3 (18) *
Blood glucose (mM)	9.0±0.4 (10)	32.2±0.7 (21)*
Weight of EDL muscles (mg)	33.2±1.1 (24)	25.3±1.3 (32)*
Weight of soleus muscles (mg)	30.3±1.1 (10)	24.1±1.5 (9)*

Values are mean ± S.E.M. for 8–21 rats (number of observations are in brackets). *Significant difference between control and diabetic groups, at $P < 0.05$.

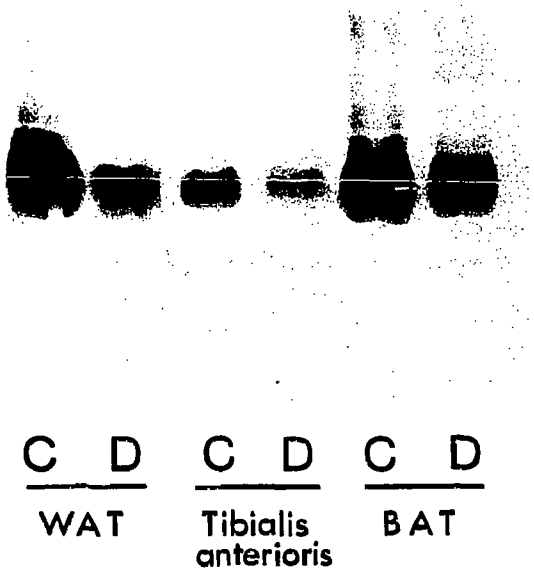

Fig. 1. Effect of diabetes on the expression of GLUT-4 protein in adipose tissue and muscle. Membrane proteins were purified from epididymal white adipose tissue (WAT), brown adipose tissue (BAT) and tibialis anterioris muscle obtained from control (C) and 5-day streptozotocin-induced diabetic rats (D); 100 μg of membrane proteins from control or diabetic rats was applied on gels. After blotting, GLUT-4 protein was detected by incubation with polyclonal antibody, OSCRX, raised against the C-terminus of GLUT-4. A representative autoradiogram is shown. GLUT-4 detected by OSCRX exhibited an apparent molecular weight of 45 kDa.

(MeAIB), in the incubated soleus muscle (mainly composed of slow-twitch red fibers) and EDL muscle (mainly composed of fast-twitch white fibres) and EDL muscle (mainly composed of fast-twitch white fibres) of control and 5-day diabetic rats. Initially, incubation of muscles was performed for 90 min, which was not long enough to show the triggering of adaptive regulation of system A transport activity [10]. Under these conditions, in EDL muscle, the diabetic group showed an enhanced MeAIB uptake compared to controls both in the absence (53% increase) and presence of insulin (53% increase) (Fig. 2). In soleus muscle, a significant rise in MeAIB uptake was noted in the diabetic group in the presence (54% increase) but not in the absence of insulin (Fig. 2). The increase in system A transport activity detected in diabetes as not a consequence of differences in muscle size (Table I). Thus, when data from control rats were considered, in which the EDL muscle weight varied over a wide range, no correlation was found between MeAIB uptake and EDL muscle weight (data not shown). It should be noted that insulin-induced MeAIB uptake was markedly greater in the diabetic EDL and soleus muscles than in control groups (Fig. 2); thus, the activatory effect of insulin was 102% and 174% in soleus muscles from control and diabetic groups, respectively, and 133% and 172% in EDL muscles from

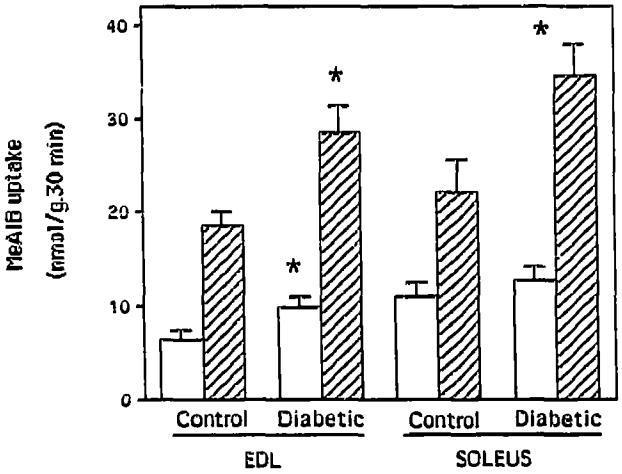

Fig. 2. Effect of diabetes on basal and insulin-stimulated MeAIB uptake by soleus and EDL muscles. Results are means ± S.E.M. for 6–10 observations per group. EDL and soleus muscles from control and 5-day streptozotocin-induced diabetic rats were incubated for 90 min in the absence (open bars) or presence of 100 nM insulin (striped bars) during the last 60 min of incubation. In the last 30 min of the incubation period, the MeAIB uptake was assessed. *Significant difference between control and diabetic groups, at $P < 0.05$.

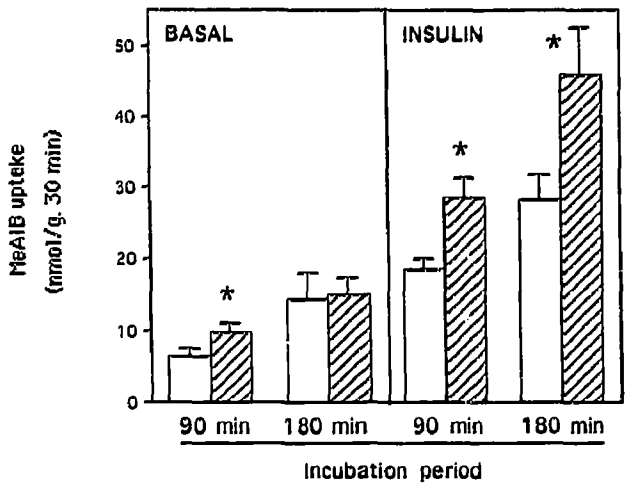

Fig. 3. Effect of adaptive regulation on basal and insulin-stimulated MeAIB uptake by EDL muscles from control and diabetic rats. Results are means ± S.E.M. for 6–12 observations per group. EDL muscles from control (open bars) and 5-day streptozotocin-induced diabetic (striped bars) rats were incubated for 90 and 180 min in the absence or presence of 100 nM insulin during the last 60 min of incubation. In the last 30 min of the incubation period, MeAIB uptake was assessed. *Significant difference between control and diabetic groups, at $P < 0.05$. Differences between groups incubated for 90 and 180 min were statistically significant under all conditions subjected to study, at $P < 0.05$.

control and diabetic groups, respectively. The increase in MeAIB uptake by muscle of diabetic rats was not due to an alteration in the interstitial space as assessed by data of mannitol distribution volume (data not shown).

We next determined whether the adaptive regulation, i.e. the activation of system A in response to amino acid starvation [4,5] was altered in the diabetic muscle. To that end, EDL muscles were incubated for 90 or 180 min in the absence of amino acids. In keeping with previous observations [4,5,10], the basal uptake of MeAIB was markedly enhanced in control EDL muscles by increasing the total incubation time (Fig. 3). The diabetic muscle also responsed to amino acid starvation by increasing the uptake of MeAIB (Fig. 3). However, the extent of the increase was markedly lower in the diabetic muscle (54% increase) than in the control group (122% increase) (Fig. 3). Under these conditions (derepressive phase of adaptive regulation), insulin again caused a greater stimulatory effect on MeAIB uptake in EDL muscles from diabetic rats (203% increase) than in controls (97%) (Fig. 3).

Our results indicate the existence of substantial modifications in the regulatory pattern of system A in skeletal muscle from acutely diabetic rats. This is based on the following observations: (i) diabetes causes an enhancement in the muscle transport activity of system A maximally stimulated by insulin, (ii) basal system A transport activity is either enhanced in EDL muscle or unaltered in soleus muscle from diabetic rats, and (iii) adaptive regulation of system A is impaired in the diabetic muscle, perhaps due to the enhanced muscle concentrations of amino acids found in this condition [24].

Our results agree with an earlier study in which an increased accumulation of α-aminoisobutyric acid was detected in diaphragm muscle from alloxan-induced diabetic rats [25].

Regarding the mechanisms by which diabetes leads to activation of muscle system A, it is unlikely that anti-insulinic hormones, such as catecholamines or glucocorticoids, are involved. Thus, incubation of EDL muscles for 3 h in the presence of either 1 μM isoproterenol or 100 nM dexamethasone did not modify system A transport activity (data not shown). System A transport activity might be activated during diabetes by modification of *trans*-inhibition or by an increase in the Na^+ electrochemical gradient, however, it is also unlikely that these factors contribute to diabetes-induced activation of system A, based on the following evidence: (i) the intracellular concentration of amino acids is markedly increased in muscle from diabetic rats [24], which in theory should enhance the *trans*-inhibition of system A, and therefore lead to its inhibition, and (ii) it has been reported that streptozotocin-induced diabetes causes an increase in the intracellular concentration of Na^+ in rat soleus muscle [26], i.e. a decrease in the Na^+ electrochemical gradient. Based on all these considerations, we favor the view that short-term diabetes either increases the number of A carriers present in skeletal muscle at the cell surface or activates the intrinsic activity of carriers already localized at the plasma membrane.

Also of interest, we have found that insulin-induced

system A is enhanced in muscle from diabetic rats compared to controls. This has allowed us to detect a dissociation between basal and insulin-stimulated system A transport activities in control and diabetic muscles. Bearing in mind that insulin stimulates system A by a rapid mechanism which increases the V_{max} of transport and which is independent of protein synthesis, microtubule and microfilament function and Na^+ electrochemical gradient [6,10], the existence of a dissociation between basal and insulin-stimulated system A transport might be explained either by an insulin-stimulated translocation of system A from an intracellular site to the cell surface or via activation of the intrinsic activity of carriers.

The release of alanine from muscle is increased in experimental diabetes [17], which has been explained by a decreased protein synthesis and an increased proteolysis [13–16], however, based on measurements of muscle cell/plasma ratios and arteriovenous differences across the hindquarter performed in diabetic rats for alanine, Ruderman and co-workers suggested that alterations in the transport of alanine across the muscle cell membrane might have a major role in determining the magnitude and the direction of alanine flux in the diabetic condition [27]. Our results suggest that the activated system A might participate in the enhanced alanine transport out of the muscle cell that occurs in diabetes.

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