Differential sensitivity of insulin- and adaptive-regulation-induced system A activation to microtubular function in skeletal muscle

Anna GUMÀ, Anna CASTELLÓ, Xavier TESTAR, Manuel PALACÍN and Antonio ZORZANO*

Departament de Bioquímica i Fisiologia, Facultat de Biologia, Universitat de Barcelona, Avda. Diagonal 647, 08028 Barcelona, Spain

1. Insulin and adaptive regulation are known to stimulate system A amino acid transport activity in skeletal muscle. The present study was designed to investigate whether activation of system A in muscle is a consequence of processes which rely on microtubule or microfilament function. To that end, extensor digitorum longus (EDL) muscles were incubated in the presence of colchicine and cytochalasin D, well-known inhibitors of microtubule and microfilament activity respectively. 2. Basal α -(methyl)aminoisobutyric acid (MeAIB) uptake decreased after incubation with 5 μ M-colchicine in a time-dependent manner. In keeping with this, adaptive regulation of MeAIB uptake caused by prolonged incubation in the absence of amino acids was substantially decreased in the presence of colchicine. 3. Under these conditions, stimulation of MeAIB uptake by insulin was unaltered in muscle in the presence of colchicine. This contrasted with the insulin-induced stimulation of MeAIB uptake by isolated rat hepatocytes, which was markedly decreased by colchicine. 4. Cytochalasin D, an agent that disrupts microfilaments, did not inhibit basal or insulin-stimulated MeAIB uptake by the incubated muscle. 5. Neither colchicine nor cytochalasin D modified the stimulatory effect of insulin on 3-*O*-methylglucose uptake by EDL muscle. 6. We conclude that up-regulation of system A by synthesis of new carriers depends on the integrity of microtubular function both in skeletal muscle and in hepatocytes. Microtubules might play a role in the movement of system A-containing vesicles from the Golgi network to the plasma membrane.

INTRODUCTION

Insulin is known to produce profound effects on membrane transport processes in a variety of tissues. In this regard, the most classical effect of insulin is the rapid activation of glucose transport that occurs in adipocytes and muscle. This activation is the result of a very complex process which, at least in isolated rat adipocytes, is partially due to the translocation of two different types of glucose transporters, GLUT-4 and GLUT-1 (Wheeler *et al.*, 1982; Shanahan *et al.*, 1982; James *et al.*, 1988; Zorzano *et al.*, 1989). Inhibition of microtubular function induced by colchicine in isolated rat adipocytes has been reported to delay somewhat the effect of insulin on glucose transport, but it does not decrease the maximal stimulation induced by the hormone (Haring *et al.*, 1979). In skeletal muscle, insulin has also been reported to promote the translocation of glucose carriers to surface membranes (Wardzala & Jeanrenaud, 1981, 1983; Klip *et al.*, 1987; Hirshman *et al.*, 1990).

Amino acid uptake is also modulated in response to insulin in hepatocytes and in skeletal muscle, owing to stimulation of system A transport activity (Kipnis & Noall, 1958; Kletzien *et al.*, 1976). In liver, the stimulatory effect of insulin on system A amino acid transport activity is dependent on protein and RNA synthesis and microtubular function, and this is characterized by an increased $V_{\max}$ (Fehlmann *et al.*, 1979; Prentki *et al.*, 1981). On the other hand, insulin-induced stimulation of system A transport activity in skeletal muscle is rapid, characterized by an increased $V_{\max}$, and independent of protein synthesis and the Na^+ electrochemical gradient (Zorzano *et al.*, 1986; Gumà *et al.*, 1988). Data obtained in muscle are compatible with insulin acting directly on the system A transporter; however, it is not possible to prove whether insulin induces the translocation of system A carriers or activates intrinsic activity of transporters in the plasma membrane, since no tools for quantification of these

carriers are available. This requires an advance in our knowledge on the structure/function relationship of the A carrier. In this regard, recently published data, which might be useful to develop methods for the quantification of system A, indicate that hepatic system A possesses essential histidine and cysteine residues (Pola *et al.*, 1990; Bertran *et al.*, 1991) and that it is encoded by mRNA of length 1.9–2.5 kb, as judged by its expression in *Xenopus laevis* oocytes (Palacín *et al.*, 1990; Tarnuzzer *et al.*, 1990).

Previous studies have described that glucagon- and insulin-induced stimulation of system A in hepatocytes is blocked by addition of colchicine to the incubation medium (Prentki *et al.*, 1981). In addition, insulin-stimulated α -aminoisobutyric acid uptake in KB cells, derived from human epidermoid carcinoma, was inhibited by pretreatment with agents that disrupt microfilaments (Goshima *et al.*, 1984). This allows us to postulate that microtubules or microfilaments might be essential for the arrival of newly synthesized system A carriers in the cell membrane. Thus we have examined the sensitivity of system A to colchicine and cytochalasin D in response to insulin and to adaptive regulation in skeletal muscle. In addition, we have also examined whether insulin-induced stimulation of glucose transport is sensitive to these blockers.

EXPERIMENTAL

Materials

Pig monocomponent insulin was a gift from Mr. T. L. Jeatran, Eli Lilly & Co. BSA (fraction V, fatty-acid-free), colchicine, cytochalasin D and most commonly used chemicals were from Sigma.

Muscle incubations

Muscle studies were performed in extensor digitorum longus (EDL) muscles from male Wistar rats (50–70 g), obtained from

Abbreviations used: EDL, extensor digitorum longus; MeAIB, α -(methyl)aminoisobutyric acid.

* To whom correspondence should be addressed.

our own colony. The rats were fed on Purina Laboratory chow *ad libitum*. Animals were housed in animal quarters maintained at 22 °C with a 12 h-light/12 h-dark cycle. The dissection and isolation of the EDL muscle was carried out under anaesthesia with pentobarbital (5–7 mg/100 g body wt., intraperitoneally) as described previously (Maizels *et al.*, 1977). The EDL muscle was fixed to a stainless-steel clip in order to maintain the muscle under slight tension (approximating to resting length) during the incubation. Such muscles (20–30 mg in weight) are able to maintain normal ATP and phosphocreatine concentrations during a 3 h incubation.

EDL muscles were incubated in a shaking incubator (45 cycles/min) at 37 °C for 3 h in 3 ml of Krebs–Henseleit buffer, pH 7.4, containing 5 mM-glucose, 0.2 % BSA and 20 mM-Hepes. Vials with intact muscles were gassed with O₂/CO₂ (19:1) during the whole incubation period. The incubation medium was kept for no longer than 90 min, and during prolonged incubations it was renewed thereafter. At different times, insulin (100 nM) was added to the incubation medium, as were several drugs such as colchicine or cytochalasin D (see details in Table legends). Experimental series were performed by comparing biological activity of one muscle with that of the contralateral one from the same rat (paired muscles).

Measurements of amino acid and glucose uptake by muscle

Amino acid uptake by system A was measured in EDL muscles by using the non-metabolizable amino acid analogue α -(methyl)aminoisobutyric acid (MeAIB). After the incubations with insulin and the above-mentioned agents, muscles were transferred to vials with 1.5 ml of Krebs–Henseleit buffer, pH 7.4, containing 5 mM-glucose, 0.2 % BSA, 20 mM-Hepes and 0.1 mM-[1-¹⁴C]MeAIB (800 μ Ci/mmol), 1 mM-[³H]mannitol (330 μ Ci/mmol) and insulin and modulators at the same concentrations as for the preceding incubation period. The vials were stoppered and incubated at 37 °C in a shaking incubator. The uptake of MeAIB was linear with time for at least 30 min (results not shown). Therefore, in all subsequent studies, uptake was measured over a 30 min period. In experiments designed to measure 3-*O*-methylglucose uptake, muscles were incubated in Krebs–Henseleit buffer containing 2 mM-pyruvate instead of glucose, and for the last 30 min of incubation the medium contained 0.1 mM-[¹⁴C]3-*O*-methylglucose (800 μ Ci/mmol) and 1 mM-[³H]mannitol (330 μ Ci/mmol). After incubation, muscles were digested in 0.25 ml of Protosol tissue solubilizer (New England Nuclear) and radioactivity of muscle digests and samples of the incubation media was measured. The amount of each radioisotope present in the samples was determined, and this information was used to calculate the extracellular space. The extracellular space of EDL muscles, estimated 30 min after addition of [³H]mannitol, was 0.222 ± 0.003 ml/mg and 0.219 ± 0.006 ml/mg in the absence and presence of insulin respectively. Incubation in the presence of cytochalasin D or colchicine did not alter the mannitol distribution space, under any of the conditions investigated (results not shown). The intracellular concentration of the ¹⁴C-labelled amino acid analogue or [¹⁴C]3-*O*-methylglucose was calculated by subtracting its amount in the extracellular space from the total label found in tissue, as previously reported (Zorzano *et al.*, 1985; Gumà *et al.*, 1988). Muscle ATP and phosphocreatine concentrations were determined spectrophotometrically (Lamprecht & Stein, 1963; Lamprecht & Trautschold, 1963). Student's *t* test was used for statistical analysis.

Isolation of hepatocytes and incubation procedure

Hepatocytes were isolated from fed male Wistar rats (200–250 g) by collagenase digestion of the liver as previously

described (Katz *et al.*, 1975). Hepatocytes were incubated at 37 °C in Krebs–Henseleit bicarbonate buffer, pH 7.4, containing 1 % BSA (dialysed and fatty-acid-free) and bacitracin (0.8 mg/ml) in the presence of O₂/CO₂ (19:1). Colchicine was added to the cell suspension 30 min after the initiation of the incubation, which was continued for 2.5 h. Insulin, when used, was added during the last 2 h of incubation.

Measurement of amino acid transport in hepatocytes

After incubation for 3 h, hepatocytes were collected by centrifugation (600 *g* for 10 s) and resuspended in Krebs–Henseleit bicarbonate buffer. Viability of hepatocytes was 88 ± 1 %, as assessed by Trypan Blue exclusion, after incubation for 3 h in the absence or presence of different hormones or blockers. MeAIB transport assays were initiated by the addition of 1.35 ml of the cell suspension [about $(0.5\text{--}1) \times 10^6$ cells/ml] to 90 μ l of Krebs–Henseleit bicarbonate buffer containing [¹⁴C]MeAIB (0.125 μ Ci) to give a final concentration of 0.1 mM-MeAIB. The tubes were incubated at 37 °C and the reaction was terminated at different times by a 30 s centrifugation of 750 μ l samples through 400 μ l of an oil mixture (dinonyl phthalate/dibutyl phthalate, 1:3, v/v) at 8000 *g* in a micro-centrifuge. After centrifugation, supernatants were obtained without removing the oil and the radioactivity of one sample was counted. Tubes were then refilled with cold saline poured on top of the oil, and saline plus oil were then discarded. The insides of the tubes above the cell pellets were then wiped with cotton swabs. Cell pellets were then solubilized in 100 μ l of 10 % SDS for 2 h at room temperature, and after addition of scintillation fluid their radioactivity was counted. Blank tubes were identical with the experimental ones, except that no cells were present. Blanks never exceeded 6 % of total radioactivity taken up at 0.1 mM-[¹⁴C]MeAIB. In keeping with this, the mannitol distribution space was only $0.8 \pm 0.1 \mu\text{l}/10^6$ cells. Thus the contamination of extracellular water in the cell pellet accounts for only 6 % of basal MeAIB uptake, and therefore it has a negligible effect on the measurement of MeAIB uptake. The uptake of MeAIB was linear with time for at least 20 min (results not shown). Therefore, in all subsequent studies transport was measured in triplicate over a 10 min period.

RESULTS

Effect of colchicine on system A transport activity in muscle and hepatocytes

Incubation of EDL muscles in the presence of 5 μ M-colchicine for the last 120 or 180 min caused a progressive decrease in basal MeAIB uptake (Table 1). This occurred in the absence of changes in the intracellular muscle concentration of ATP (3.4 and 3.0 μ mol/g in control and colchicine-treated groups respectively) or phosphocreatine (19.3 and 18.4 μ mol/g in control and colchicine-treated groups respectively). In spite of such an inhibition of basal system A transport activity, the stimulatory effect of insulin on MeAIB uptake was not altered to any extent in the presence of 5 μ M-colchicine (Table 1). Basal MeAIB uptake was not inhibited at 0.5 μ M-colchicine (results not shown).

In skeletal muscle, system A transport activity can be activated by adaptive regulation, i.e. by amino acid starvation (Guidotti *et al.*, 1975; Le Marchand-Brustel *et al.*, 1982; Logan *et al.*, 1982; Gumà *et al.*, 1988). Therefore we investigated whether the effect of colchicine on basal MeAIB uptake was a consequence of a blockade of adaptive regulation. To that end, EDL muscles were incubated for increasing periods of time (90 or 180 min) in the absence of amino acids in the medium, and MeAIB uptake was assayed thereafter. Basal MeAIB uptake was significantly

Table 1. Effect of colchicine on MeAIB uptake by EDL muscle

Results are means $\pm$ S.E.M. for 21–23 observations per group. EDL muscles were incubated for 180 min, in the absence or presence of 100 nM-insulin for the last 60 min of incubation. Muscles were incubated in the absence or presence of 5 μ M-colchicine, which was added either throughout the whole incubation period or during the last 120 min of incubation. MeAIB uptake was determined during the last 30 min of incubation. * indicates a significant difference between control and colchicine groups at $P < 0.05$. † indicates a significant difference between basal and insulin groups, at $P < 0.05$.

Treatment	Uptake (nmol of MeAIB/ 30 min per g)		Effect of insulin (% increase over basal)
	Basal	+ Insulin	
Control	27.5 $\pm$ 2.2	41.9 $\pm$ 3.5†	52
Colchicine (5 μ M, 120 min)	20.8 $\pm$ 2.6	37.7 $\pm$ 4.3†	81
Colchicine (5 μ M, 180 min)	17.4 $\pm$ 1.7*	34.5 $\pm$ 2.9†	98

stimulated by increasing the total incubation time from 90 to 180 min (Table 2). Under these conditions colchicine partially prevented such an increase, and in fact differences in MeAIB uptake between muscles incubated for 90 or 180 min in the presence of the blocker were insignificant (Table 2). Similar data were obtained when muscles were incubated in the presence of insulin. Thus, under these conditions a 56% increase in MeAIB uptake by EDL muscle was observed on increasing the total incubation time from 90 to 180 min (Table 2). However, in the presence of colchicine, the increase due to adaptive regulation was only 17% (Table 2). Again, under none of these conditions was the insulin effect blocked by the presence of colchicine (Table 2).

Hepatocytes were incubated in the presence of insulin for 120 min, and transport of the specific analogue MeAIB was estimated. Under these conditions, insulin caused a 2-fold increase in MeAIB transport (Table 3). Na⁺-independent MeAIB uptake was 0.22 $\pm$ 0.02 nmol/10 min per 10⁶ cells [it only accounted for 18% of total MeAIB uptake, in agreement with previous observations (Kilberg *et al.*, 1981)] and it was not influenced by insulin. When colchicine was present 30 min before insulin addition, the stimulation caused by insulin was largely prevented (Table 3). This is in keeping with a previous report indicating that colchicine blocks the activation by insulin and glucagon of α -aminoisobutyric acid transport by isolated rat hepatocytes (Prentki *et al.*, 1981).

Effect of cytochalasin D on MeAIB uptake by muscle

It has been previously reported that in KB cells (a cell line derived from human epidermoid carcinoma), AIB uptake stimulated by insulin is inhibited by pretreatment with cytochalasin D, a blocker of microfilament function (Goshima *et al.*, 1984). Based on that report, and in order to further investigate the mechanisms by which insulin stimulates system A in skeletal muscle, we tested the effect of 50 μ M-cytochalasin D on MeAIB uptake by EDL muscle. This concentration is high enough to exert maximal inhibitory effects in cultured cells or tissues (Goshima *et al.*, 1984; Huleux *et al.*, 1989). Data are shown in Table 4. Under basal conditions, insulin caused a 2-fold increase

Table 2. Effect of colchicine on adaptive regulation of system A by EDL muscle

Results are means $\pm$ S.E.M. for 9–11 observations per group. EDL muscles were incubated for 90 or 180 min, in the absence or presence of 100 nM-insulin for the last 60 min of incubation. Muscles were incubated in the absence or presence of 5 μ M-colchicine, which when present was added throughout the whole incubation period. MeAIB uptake was determined during the last 30 min of incubation. * indicates a significant difference between control and colchicine groups, at $P < 0.05$. † indicates a significant difference between basal and insulin groups, at $P < 0.05$.

Insulin (nM)	Duration of experiment (min)	Uptake (nmol of MeAIB/ 30 min per g)	
		Control	+ Colchicine
0	90	25.9 $\pm$ 1.6	21.0 $\pm$ 1.4
0	180	34.2 $\pm$ 1.8	25.5 $\pm$ 1.9*
100	90	39.1 $\pm$ 3.9†	38.5 $\pm$ 4.2†
100	180	60.9 $\pm$ 3.1†	45.0 $\pm$ 2.5*†

Table 3. Effect of colchicine on MeAIB uptake by isolated rat hepatocytes

Isolated rat hepatocytes were incubated for 180 min in the absence or presence of 100 nM-insulin and 0.5 μ M-colchicine. Cells were then incubated in the presence of [¹⁴C]MeAIB (0.1 mM, 0.125 μ Ci) for 10 min. * indicates a significant difference between control and colchicine groups, at $P < 0.05$. † indicates a significant difference between basal and insulin groups, at $P < 0.05$. Each value is the mean $\pm$ S.E.M. of three different experiments performed in triplicate.

Treatment	Uptake (nmol of MeAIB/ 10 min per 10 ⁶ cells)		Effect of insulin (% increase over basal)
	Basal	Insulin	
Control	1.22 $\pm$ 0.18	2.54 $\pm$ 0.13†	108
Colchicine (0.5 μ M, 120 min)	1.39 $\pm$ 0.22	1.78 $\pm$ 0.07*	28

Table 4. Effect of cytochalasin D on MeAIB uptake by EDL muscle

Results are means $\pm$ S.E.M. for 6–8 observations per group. EDL muscles were incubated for 180 min, in the absence or presence of 100 nM-insulin during the last 60 min of incubation. Muscles were incubated in the absence or presence of 50 μ M-cytochalasin D, which when present was added during the whole incubation period. MeAIB uptake was determined during the last 30 min of incubation. * indicates a significant difference between basal and insulin groups, at $P < 0.05$. Differences between control and cytochalasin D groups were insignificant.

	Uptake (nmol of MeAIB/30 min per g)	
	Control	Cytochalasin D
Basal	19.3 $\pm$ 2.2	23.6 $\pm$ 2.5
+ Insulin	39.8 $\pm$ 2.8*	36.2 $\pm$ 1.7*

in the rate of MeAIB uptake. However, neither basal nor insulin-stimulated MeAIB uptake by muscle was altered to any extent by the presence of cytochalasin D. Furthermore, 50 μ M-cytochalasin

Table 5. Effects of colchicine and cytochalasin D on 3-O-methylglucose uptake by EDL muscle

Results are means $\pm$ S.E.M. for 5–10 observations per group. EDL muscles were incubated for 180 min, in the absence or presence of 100 nM-insulin during the last 60 min of incubation. Muscles were incubated in the absence or presence of 5 μ M-colchicine or 50 μ M-cytochalasin D, which were added during the whole incubation period. 3-O-Methylglucose uptake was determined during the last 30 min of incubation. * indicates a significant difference between basal and insulin groups, at $P < 0.05$. Differences between control and experimental groups were insignificant.

Treatment	Uptake (nmol of 3-O- methylglucose/ 30 min per g)	
	Basal	+ Insulin
Control	7.8 $\pm$ 0.5	17.6 $\pm$ 1.0*
Colchicine	8.7 $\pm$ 1.1	17.7 $\pm$ 1.0*
Cytochalasin D	8.6 $\pm$ 0.9	16.3 $\pm$ 1.9*

D did not alter intracellular muscle concentration of either ATP (3.2 and 3.1 μ mol/g in control and cytochalasin D-treated groups respectively) or phosphocreatine (17.1 and 18.9 μ mol/g in control and cytochalasin D-treated groups respectively).

Effect of colchicine and cytochalasin D on insulin-stimulated 3-O-methylglucose uptake by EDL muscle

Insulin is known to stimulate glucose uptake and glucose-transporter translocation in skeletal muscle (Wardzala & Jeanrenaud, 1981, 1983; Klip *et al.*, 1987; Hirshman *et al.*, 1990). In a further set of experiments, we investigated whether insulin-induced stimulation of glucose transport was dependent on microtubule or microfilament function. To that end, the effect of insulin on 3-O-methylglucose uptake by EDL muscles previously incubated in the presence of 5 μ M-colchicine or 50 μ M-cytochalasin D was assessed (Table 5). Under control conditions, insulin caused a 125 % increase in 3-O-methylglucose uptake by muscle. Both basal and insulin-stimulated 3-O-methylglucose uptakes were unaltered by the presence of colchicine or cytochalasin D (Table 5).

DISCUSSION

In this study we have investigated the effects of colchicine and cytochalasin D in order to provide information on the dependence of insulin- and adaptive-regulation-induced stimulation of system A on activity of microtubules and microfilaments. We used concentrations of colchicine as high as 5 μ M, which cause depolymerization of microtubules and which do not induce non-specific effects in cultured skeletal myotubes or kidney cells (Vandenburgh & Kaufman, 1982; Díez *et al.*, 1987). For cytochalasin D, we used concentrations of 50 μ M, which are high enough to block microfilament function (Goshima *et al.*, 1984), but which do not substantially alter glucose transport activity (Griffin *et al.*, 1982).

Our results demonstrate that adaptive regulation of system A transport activity is sensitive to colchicine, in contrast with the stimulatory effect of insulin, which is clearly insensitive to colchicine. This is parallel to the differences previously reported in the sensitivity of insulin or adaptive regulation to blockers of protein synthesis. Thus, whereas amino acid deprivation causes

the activation of system A transport activity by a process which is blocked by inhibition of protein synthesis (Kelley & Potter, 1978; Handlogten *et al.*, 1982), the insulin effect in muscle is independent of protein synthesis (Elsas *et al.*, 1968; Le Marchand-Brustel *et al.*, 1982; Gumà *et al.*, 1988). These results further demonstrate that insulin causes the activation of system A transport activity in muscle and liver by completely different mechanisms. Thus insulin activates system A in rat hepatocytes by a process dependent on protein and RNA synthesis and on microtubular function (Fehlmann *et al.*, 1979; Prentki *et al.*, 1981). In contrast, insulin stimulates system A in muscle by a process independent of protein synthesis (Elsas *et al.*, 1968; Le Marchand-Brustel *et al.*, 1982; Gumà *et al.*, 1988) and microtubular function.

Up-regulation of system A provoked in muscle by adaptive regulation and in hepatocytes by insulin or glucagon share a common property, i.e. dependence on transcription, protein synthesis and microtubular function. Regarding the stimulatory effect of glucagon on system A transport activity in hepatocytes, it has been reported (a) that this effect is a consequence of a greater availability of the corresponding mRNA (Palacin *et al.*, 1990; Tarnuzzer *et al.*, 1990), and (b) that the enhanced transport activity can be recovered in proteoliposomes generated from hepatocyte plasma membranes (Bracy *et al.*, 1987). In all, these data support the proposal that glucagon regulates the hepatic system A gene, resulting in the synthesis *de novo* of carriers, followed by their transport to the plasma membrane. In this regard, it might be proposed that the process by which carriers move from Golgi apparatus to plasma membrane relies on an intact microtubular function. Colchicine also prevents the increased capacity for amino acid transport of concanavalin A-activated lymphocytes during the replicative phase (Greene *et al.*, 1976), of proliferating hepatocytes after partial hepatectomy (Walker & Whitfield, 1978) or of Morris hepatoma cells (Tauber & Reutter, 1980). Therefore one may postulate that, when activation of amino acid transport is due to the arrival of newly synthesized carriers at the plasma membrane, the vectorial movement of vesicles containing system A or other amino acid carriers towards the cell surface is dependent on microtubular function. It is unlikely that microtubules play a role in the transfer of intracellular information triggered by such different signals as insulin, hepatectomy, transformation or amino acid starvation.

In this study, we present evidence that microfilaments are not involved in the up-regulation of system A by insulin or adaptive regulation in skeletal muscle. In this regard, insulin-stimulated system A transport activity is blocked in KB cells by pretreatment with cytochalasin D (Goshima *et al.*, 1984), perhaps owing to the inhibition of insulin-induced formation of ruffling membranes. However, this may not be relevant to muscle, since muscle cells do not show ruffling movements. From all the above, we favour the view that the mechanisms involved in the modulation of system A differ widely according to cell type.

On the basis of data obtained in isolated rat adipocytes (Haring *et al.*, 1979) and our own from incubated skeletal muscle, we conclude that insulin-activated glucose transport is not dependent on microtubule or microfilament function. That suggests that blockers of microtubules or microfilaments do not inhibit insulin-mediated glucose-transporter translocation. The independence of insulin-stimulated glucose-transporter translocation and microtubule or microfilament function might be due to the fact that transporter-containing intracellular vesicles are located near the cell surface.

This work was supported by grants from the D.G.I.C.Y.T. (PB86-573 and PB89-0331) and from FIS (89/0174), Spain. A.G. and A.C. are

recipients of predoctoral fellowships from the 'Ministerio de Educación y Ciencia' and from C.I.R.I.T.-Generalitat de Catalunya.

REFERENCES

- Bertran, J., Roca, A., Pola, E., Testar, X., Zorzano, A. & Palacín, M. (1991) *J. Biol. Chem.* **266**, 798–802
- Bracy, D. S., Schenerman, M. A. & Kilberg, M. S. (1987) *Biochim. Biophys. Acta* **899**, 51–58
- Diez, J. C., Avila, J., Nieto, J. M. & Andreu, J. M. (1987) *Cell Motil. Cytoskel.* **7**, 178–186
- Elsas, L. J., Albrecht, I. & Rosenberg, L. E. (1968) *J. Biol. Chem.* **243**, 1846–1853
- Fehlmann, M., Le Cam, A. & Freychet, P. (1979) *J. Biol. Chem.* **254**, 10431–10437
- Goshima, K., Masuda, A. & Owaribe, K. (1984) *J. Cell Biol.* **98**, 801–809
- Greene, W. C., Parker, C. M. & Parker, C. W. (1976) *J. Immunol.* **117**, 1015–1022
- Griffin, J. F., Rampal, A. L. & Jung, C. Y. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3759–3763
- Guidotti, G. G., Gazzola, G. C., Borghetti, A. F. & Franchi-Gazzola, R. (1975) *Biochim. Biophys. Acta* **406**, 264–279
- Gumà, A., Testar, X., Palacín, M. & Zorzano, A. (1988) *Biochem. J.* **253**, 625–629
- Handlogten, M. E., Weissbach, L. & Kilberg, M. S. (1982) *Biochem. Biophys. Res. Commun.* **104**, 307–313
- Haring, H. U., Kemmler, W. & Hepp, K. D. (1979) *FEBS Lett.* **105**, 329–332
- Hirshman, M. F., Goodyear, L. J., Wardzala, L. J., Horton, E. D. & Horton, E. S. (1990) *J. Biol. Chem.* **265**, 987–991
- Huleux, C., Dreux, C., Imhoff, V., Chambaut-Guerin, A. M. & Rossignol, B. (1989) *Biol. Cell* **67**, 61–65
- James, D. E., Brown, R., Navarro, J. & Pilch, P. F. (1988) *Nature (London)* **333**, 183–185
- Katz, J., Wals, P. A., Golden, S. & Rongstad, R. (1975) *Eur. J. Biochem.* **60**, 91–101
- Kelley, D. S. & Potter, V. R. (1978) *J. Biol. Chem.* **253**, 9009–9017
- Kilberg, M. S., Handlogten, M. E. & Christensen, H. N. (1981) *J. Biol. Chem.* **256**, 3304–3312
- Kipnis, D. M. & Noall, M. W. (1958) *Biochim. Biophys. Acta* **28**, 226–227
- Kletzien, R. F., Pariza, M. W., Becker, J. E., Potter, V. R. & Butcher, F. R. (1976) *J. Biol. Chem.* **251**, 3014–3020
- Klip, A., Ramlal, T., Young, D. A. & Holloszy, J. O. (1987) *FEBS Lett.* **224**, 224–230
- Lamprecht, W. & Stein, P. (1963) in *Methods in Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 610–611, Academic Press, New York and London
- Lamprecht, W. & Trautschold, I. (1963) in *Methods in Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 543–551, Academic Press, New York and London
- Le Marchand-Brustel, Y., Moutard, N. & Freychet, P. (1982) *Am. J. Physiol.* **243**, E74–E79
- Logan, W. J., Klip, A. & Gagalang, E. (1982) *J. Cell. Physiol.* **112**, 229–236
- Maizels, E. Z., Ruderman, N. B., Goodman, M. N. & Lau, D. (1977) *Biochem. J.* **162**, 557–568
- Palacín, M., Werner, A., Dittmer, J., Murer, H. & Biber, J. (1990) *Biochem. J.* **270**, 189–195
- Pola, E., Bertran, J., Roca, A., Palacín, M., Zorzano, A. & Testar, X. (1990) *Biochem. J.* **271**, 297–303
- Prentki, M., Crettaz, M. & Jeanrenaud, B. (1981) *J. Biol. Chem.* **256**, 4336–4340
- Shanahan, M. F., Olson, S. A., Weber, M. J., Lienhard, G. E. & Gorga, J. C. (1982) *Biochem. Biophys. Res. Commun.* **107**, 38–43
- Tarnuzzer, R. W., Campa, M. J., Quian, N.-X., Englesberg, E. & Kilberg, M. S. (1990) *J. Biol. Chem.* **265**, 13914–13917
- Tauber, R. & Reutter, W. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5282–5286
- Vandenburgh, H. H. & Kaufman, S. (1982) *J. Biol. Chem.* **257**, 13448–13454
- Walker, P. R. & Whitfield, J. F. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1394–1398
- Wardzala, L. J. & Jeanrenaud, B. (1981) *J. Biol. Chem.* **256**, 7090–7093
- Wardzala, L. J. & Jeanrenaud, B. (1983) *Biochim. Biophys. Acta* **730**, 49–56
- Wheeler, T. J., Simpson, I. A., Sogin, D. C., Hinkle, P. C. & Cushman, S. W. (1982) *Biochem. Biophys. Res. Commun.* **105**, 89–95
- Zorzano, A., Balon, T. W., Garetto, L. P., Goodman, M. N. & Ruderman, N. B. (1985) *Am. J. Physiol.* **248**, E546–E552
- Zorzano, A., Balon, T. W., Goodman, M. N. & Ruderman, N. B. (1986) *Biochem. Biophys. Res. Commun.* **134**, 1342–1349
- Zorzano, A., Wilkinson, W., Kotliar, N., Thoidis, G., Wadzinsky, B. E., Ruoho, A. E. & Pilch, P. F. (1989) *J. Biol. Chem.* **264**, 12358–12363

Received 3 June 1991/6 August 1991; accepted 15 August 1991