Hormonal regulation of glucose transport in a brown adipose cell preparation isolated from rats that shows a large response to insulin

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Isolated brown adipose cells from rats are prepared whose viability is indicated by the expected stimulation of oxygen consumption by noradrenaline and counter-regulation of this oxygen consumption response by insulin. Insulin stimulates 3-*O*-methyl-D-glucose transport by approx. 15-fold in the absence of adenosine, and adenosine augments this response at least 2-fold. The insulin-stimulated translocation of the glucose transporter GLUT4 from an intracellular compartment to the plasma membrane is readily detected by subcellular fractionation and Western blotting, and the appearance of GLUT4 on the cell surface in response to insulin is demonstrated by bis-mannose photolabelling. Isoprenaline also stimulates glucose transport

activity but only by approx. 3-fold; this effect is not altered by adenosine. Isoprenaline increases insulin-stimulated glucose transport activity in the absence of adenosine but decreases it in the presence of adenosine. These results demonstrate that although the regulation of glucose transport by insulin in brown adipose cells is qualitatively similar to that in white adipose cells, counter-regulation by adenosine and isoprenaline is at least quantitatively and may be qualitatively different. Isolated brown adipose cells from rats thus represent an excellent model for further examination of the mechanism by which multiple hormone signalling pathways interact to control glucose transport and GLUT4 subcellular trafficking.

INTRODUCTION

Brown adipose tissue (BAT) is the major site of thermogenesis in mammals; by techniques *in vivo* such as the euglycaemic clamp in combination with 2-deoxyglucose uptake it is known to be one of the most responsive of the insulin target tissues with respect to glucose transport activity [1]. However, the quantitative contribution of BAT to the removal of glucose from the blood as a fuel supply for thermogenesis is equivocal [2–5]. Nevertheless, ever since Slot et al. [6] showed by immunohistochemistry that insulin stimulates translocation of the glucose transporter GLUT4 in this tissue in the rat, BAT has been thought to represent a potentially useful model for study of the regulation of glucose transport. Recently a number of studies have been reported on the regulation of the expression of GLUT4 in rat and goat BAT [7–12] and the effects of overexpression of GLUT4 in mouse BAT [13,14]. In addition, noradrenaline and other β -adrenergic agonists as well as insulin have been reported to stimulate glucose uptake in BAT in the rat both *in vivo* [2,15,16] and *in vitro* [16–18] and also *in vitro* in primary cultured brown adipocytes from rats [19]. Contrary to expectations, however, the magnitude of the stimulation of glucose transport activity by insulin previously reported in isolated brown adipose cells from rats is extremely small, 4–8-fold, compared with that reported in isolated white adipose cells from rats [20], presumably because of the difficulty in isolating these cells in a fully viable state. Here we report a preparation of isolated brown adipose cells from rats that is highly responsive to insulin and characterize the regulation of glucose transport in these cells by insulin, isoprenaline and adenosine.

EXPERIMENTAL

Materials

Male 6-week-old Sprague–Dawley rats were purchased from

Charles River Breeding Laboratories Inc. (Boston, MA, U.S.A.) and fed with standard NIH chow *ad libitum* for at least 5 d before use. Fraction V BSA was purchased from Interger (Purchase, NY, U.S.A.), class II crude collagenase from Worthington Biochemical (Freehold, NJ, U.S.A.), and silicone fluid from Dow Corning (New Redford, MA, U.S.A.). Insulin was a gift from Dr. R. B. Chance (Eli Lilly, Indianapolis, IN, U.S.A.). 3-*O*-Methyl-D-glucose, L-glucose, (–)-isoprenaline bitartrate salt, D,L-propranolol hydrochloride, adenosine and *N*⁶-phenylisopropyladenosine (PIA) were obtained from Sigma (St. Louis, MO, U.S.A.). 5'-(*N*-Ethyl)carboxyaminoadenosine (NECA) was a generous gift from Dr. C. Londos (NIDDK/NIH). D,L-Noradrenaline hydrochloride was purchased from Fluka (Ronkonkoma, NY, U.S.A.), and adenosine deaminase (ADA), C₁₂E₉ (Thesit) and DNase I were obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN, U.S.A.). Protein A-Sepharose was purchased from Pharmacia (Piscataway, NJ, U.S.A.). Rabbit polyclonal antisera prepared against peptides of 19 amino acids and 20 amino acids corresponding to the C-terminal sequences of GLUT4 and GLUT1 respectively were kindly supplied by Hoffmann-La Roche (Nutley, NJ, U.S.A.). 2-*N*-4-(1-azi-2,2,2-trifluoroethyl)benzoyl-[2-³H]1,3-bis(D-mannos-4-yloxy)-2-propylamine (ATB-[2-³H]BMPA; specific activity approx. 10 Ci/mmol) was a gift from Dr. G. D. Holman (University of Bath, U.K.) and was prepared as described [21]. All other radiolabelled compounds were from Du Pont-New England Nuclear (Boston, MA, U.S.A.).

Isolation of rat brown adipose cells

The method used for isolating brown adipose cells was a modification of that previously described [17,18]. Rats were anaesthetized with a mixture of CO₂/O₂ (70:30) and killed by decapitation. BAT was carefully dissected from the interscapular

Abbreviations used: ADA, adenosine deaminase; ATB-[2-³H]BMPA, 2-*N*-4-(1-azi-2,2,2-trifluoroethyl)benzoyl-[2-³H]1,3-bis(D-mannos-4-yloxy)-2-propylamine; BAT, brown adipose tissue; KRBH, Krebs–Ringer bicarbonate Hepes buffer; NECA, 5'-(*N*-ethyl)carboxyaminoadenosine; PIA, *N*⁶-phenylisopropyladenosine.

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Table 1 Protein yields and 5'-nucleotidase specific activities in subcellular membrane fractions in isolated brown adipose cells from rats

Membrane fractions were obtained as described in the Experimental section. Results are the means $\pm$ S.D. obtained from triplicate samples in a typical experiment. Abbreviations used: BAS, basal state; INS, insulin-stimulated state; NM, not measured.

Fraction	Hormone treatment	Protein (mg per g of tissue)	5'-Nucleotidase activity (μ mol/min per mg of protein)
Total homogenate	BAS	16.1 $\pm$ 0.4	NM
	INS	17.5 $\pm$ 2.0	NM
Plasma membranes	BAS	0.08 $\pm$ 0.03	7.0 $\pm$ 0.14
	INS	0.09 $\pm$ 0.03	6.0 $\pm$ 0.11
High-density microsomes	BAS	0.04 $\pm$ 0.01	1.87 $\pm$ 0.01
	INS	0.05 $\pm$ 0.01	1.52 $\pm$ 0.02
Low-density microsomes	BAS	0.18 $\pm$ 0.02	0.57 $\pm$ 0.08
	INS	0.19 $\pm$ 0.01	0.51 $\pm$ 0.01

regions and placed in Krebs–Ringer bicarbonate Hepes buffer (KRBH), pH 7.4, containing 1% (w/v) BSA and 200 nM adenosine. Muscle, white adipose tissue and other tissues were carefully trimmed away from the BAT mass with round-tipped forceps under a dissecting microscope to prevent any damage of the surface of the BAT. This dissection procedure is critical in obtaining a good cell preparation. Dissected BAT was minced into pieces and digested with a combination of collagenase/DNase I (7.5 and 0.5 mg/ml respectively) at 37 °C for 35 min with rapid shaking. After digesting, the reaction mixture was filtered through 400 μ m mesh nylon screen. The filtrate was centrifuged at 100 *g* for 60 s and the buffer was removed with a syringe fitted with a long metal needle. Cells were resuspended in KRBH, BSA (1%), adenosine buffer and filtered through 200 μ m mesh nylon screen. The filtrate was centrifuged (100 *g* for 60 s) at room temperature and the buffer discarded. Cells were then suspended in KRBH buffer containing 5% BSA with 200 nM adenosine and washed twice more with centrifugation in this buffer. After final washing, floating brown adipose cells were resuspended in the same buffer and diluted to the concentration designed for each experiment. All procedures were performed in plastic containers.

Cell size and number were determined by measuring cell lipid content by the method of lipid extraction, osmic acid fixation and Coulter electronic counting [22]. The size of the isolated brown adipose cells used here was approx. 0.035 μ g of lipid per cell, whereas that of the white adipose cells isolated from the epididymal fat tissue of rats of the same age was approx. 0.09 μ g of lipid per cell.

Assays

Cell oxygen consumption was measured by using a Biological Oxygen Monitor, MODEL 5300 (YSI Inc., Yellow Springs, OH, U.S.A.) with an oxygen-sensitive electrode. Isolated brown adipose cells [(1–3) $\times$ 10⁵ cells per ml] were incubated in KRBH buffer containing 5% BSA and 200 nM adenosine in a chamber attached to a water jacket to maintain 37 °C with continuous mixing by a magnetic stirrer. The inside of the chamber was coated with silicone to prevent cell breakage.

Glucose transport activity in isolated brown adipose cells was measured by using the 3-*O*-methyl-D-glucose double isotope (L-[³H]glucose, 3-*O*-methyl-D-[¹⁴C]glucose, each at a final concentration of 0.1 mM) technique with oil flotation [23]. Cells were separated from the medium by using an oil made by mixing two silicone fluids of different density, DC200 and DC550 (1:3, final specific gravity 0.9985) to accommodate the smaller lipid content of the brown adipose cells compared with white adipose cells.

Cells [(1–3) $\times$ 10⁶ cells per ml] were incubated in KRBH containing 5% BSA and 200 nM adenosine with or without insulin or other agents at 37 °C for the designated times before assay. In the experiments requiring the absence of adenosine, 1 unit/ml ADA was added to the buffer to deplete the adenosine. The glucose transport activity assay was performed in the same buffer as used for the incubation.

For photoaffinity labelling experiments, isolated brown adipose cells [(2–4) $\times$ 10⁶ cells per ml] were incubated with or without 700 nM insulin in KRBH buffer containing 2% BSA in the presence or absence of 200 nM adenosine at 37 °C for 30 min. For experiments in the absence of adenosine, ADA was added to the incubation medium at 1 unit/ml. After incubation, 2 ml samples of cells were transferred to 35 mm Petri dishes and 250 μ Ci of ATB-[2-³H]BMPA in KRBH buffer without BSA was added. The dishes were irradiated, without lids, twice for one min each by using a Rayonet photochemical reactor with 300 nm lamps. The photolabelled cell suspension was centrifuged through the silicone mixture at 100 *g* for 60 s at room temperature and the buffer removed. Cells were solubilized in 1.2 ml of a 2% (w/v) C₁₂E₉ (Thesit) detergent buffer in PBS containing protease inhibitors, and GLUT4 was immunoprecipitated with an anti-GLUT4 antiserum as described previously [24]. SDS/PAGE analysis of the immunoprecipitates was followed by gel slicing and ³H counting.

Subcellular fractionation

The method for fractionating isolated brown adipose cells was based on the method previously described for isolated white adipose cells [23]. After incubating 5 ml of cells [(2–5) $\times$ 10⁶ cells per ml] with or without 700 nM insulin, the cell suspension was centrifuged at 100 *g* for 60 s at room temperature and the buffer removed. Cells were suspended in 10 ml of Tris/EDTA/sucrose buffer (20 mM Tris/HCl, 1 mM EDTA and 255 mM sucrose, pH 7.4) at 18–20 °C and homogenized with a glass–Teflon homogenizer at 4 °C. The homogenate was centrifuged in a Sorvall SS34 rotor at 12000 rev./min (18000 *g*) for 15 min at –2 °C. The infranatant was saved for preparation of the microsomal membranes. The fat cake was then removed with cotton swabs. The first pellet was resuspended in 500 μ l of Tris/EDTA/sucrose buffer by using a glass–Teflon homogenizer, layered on a 600 μ l cushion of 1.12 M sucrose in 20 mM Tris/1 mM EDTA buffer (pH 7.4), and centrifuged in a Beckman TLS55 rotor at 35000 rev./min (8150 *g*) at 4 °C for 30 min. Plasma membranes collected at the cushion interface were suspended in 2.5 ml of Tris/EDTA/sucrose buffer and centrifuged in a Beckman TLA100.3 rotor at 100000 rev./min

(410000 g) at 4 °C for 20 min. The final plasma membrane pellet was resuspended in approx. 60 μ l of buffer. The initial supernatant was centrifuged in a Sorvall SS34 rotor at 20000 rev./min (48000 g) at 4 °C for 15 min and the high-density microsome pellet was resuspended in approx. 40 μ l of buffer. The supernatant was further centrifuged in a Beckman 70.1Ti rotor at 65000 rev./min (300000 g) at 4 °C for 75 min and the low-density microsome pellet was resuspended in approx. 60 μ l of buffer.

The membrane fractions were characterized by the distribution of 5'-nucleotidase activity, a marker enzyme of the plasma membrane, compared with the data obtained with white adipose cells [23]. 5'-Nucleotidase activity was measured as previously described [25]. Protein was measured with the BCA Protein assay kit (Pierce, Rockford, IN, U.S.A.). Protein recoveries and the specific activity of 5'-nucleotidase in subcellular membrane fractions from brown adipose cells are shown in Table 1. Membranes were stored at -70 °C until use.

For Western blotting, 10–20 μ g of each membrane fraction was solubilized in sample buffer [final concentrations, 2.3 M urea, 1.5% (w/v) SDS, 15 mM Tris/HCl and 100 mM dithiothreitol, pH 6.8] at room temperature for 30 min, subjected to SDS/PAGE [10% (w/v) gel], and transferred overnight to nitrocellulose membranes. The nitrocellulose membranes were immunoblotted with either anti-GLUT1 or anti-GLUT4 anti-

serum and the immunoreactive proteins were detected with [125 I]-labelled protein A. Measurement of the glucose transporter signals was performed with a phosphorimager.

RESULTS

Oxygen consumption

Figure 1 shows the typical oxygen consumption response of isolated brown adipose cells from rats to noradrenaline and insulin. Noradrenaline (10 nM) stimulated oxygen consumption by approx. 10-fold (Figure 1A). Addition of insulin to noradrenaline-stimulated cells caused a partial decrease in the oxygen consumption rate. Further addition of propranolol to the cell suspension rapidly and completely blocked the effect of noradrenaline. Insulin did not change the oxygen consumption rate in the resting state (Figure 1B). In addition, when insulin was added first, 10 nM noradrenaline showed almost the same stimulatory effect on oxygen consumption rate as observed in the absence of insulin.

Effects of insulin and adenosine on glucose transport activity

Figure 2A shows the concentration-dependent stimulation of glucose transport activity by insulin as assessed by 3-O-methyl-D-glucose transport in isolated brown adipose cells from rat. In the presence of 200 nM adenosine, the maximum stimulation by insulin is approx. 30-fold compared with the basal level. In the

Figure 1 Oxygen consumption of brown adipose cells

Isolated cells were incubated in the Biological Oxygen Monitor chamber at 37 °C with continuous stirring during the measurement of oxygen consumption with an O_2 -specific electrode. (A) response when noradrenaline added first; (B) response when insulin added first.

Figure 2 Effect of adenosine on the insulin concentration dependent stimulation of glucose transport activity in brown adipose cells

Isolated cells were incubated with various concentrations of insulin in the presence (●) and absence (○) of 200 nM adenosine at 37 °C for 30 min before measurement of 3-O-methyl-D-glucose transport. Results are the means $\pm$ S.E.M. of the mean values obtained from triplicate samples in three individual experiments. (A) transport rate; (B) % of maximum response.

Figure 3 Gel analysis of cell-surface ATB-[2-³H]BMPA-labelled GLUT4 in rat brown adipose cells

Isolated cells were incubated at 37 °C for 30 min with (●,▲) or without (○,△) 700 nM of insulin in the presence (●,○) and absence (▲,△) of 200 nM adenosine respectively, and then photolabelled with ATB-[2-³H]BMPA. Photolabelled cells were solubilized, GLUT4 was immunoprecipitated, and the immunoprecipitates were analysed by SDS/PAGE followed by gel slicing and counting as described in the Experimental section. Results are the averages of duplicate samples obtained in a typical experiment.

absence of adenosine, glucose transport activity is slightly increased in basal cells, but the maximum stimulation by insulin is about half that in the presence of adenosine. However, the sensitivity to insulin is not changed by adenosine. The concentration of insulin producing a half-maximal response is 0.14 nM both in the presence and absence of adenosine (Figure 2B). Similar effects of insulin and adenosine were observed when

assessing glucose transport activity using tracer D-[U-¹⁴C]glucose (results not shown).

Quantification of cell-surface GLUT4

Cell-surface GLUT4 was quantified in isolated brown adipose cells from rats by using ATB-[³H]BMPA as illustrated in Figure 3. In insulin-treated cells, a large ³H peak is observed at approx. 45 kDa both in the presence and in the absence of adenosine. In basal cells the areas under the GLUT4 peaks are 39 and 46 d.p.m. per 2 mg of total cell protein in the presence and absence of adenosine respectively. In insulin-treated cells the GLUT4 peak is 279 d.p.m. per 2 mg of protein in the presence of adenosine, which is 7.4 times higher than that in the basal state. In the absence of adenosine, the cell-surface GLUT4 in insulin-treated cells is 172 d.p.m. per 2 mg of protein, which is approx. 60 % of that observed in that in the presence of adenosine.

Subcellular distributions of GLUT4 and GLUT1

Figure 4 shows the subcellular distributions, detected by Western blotting, of GLUT4 and GLUT1 in isolated brown and white adipose cells from rats. In the presence of adenosine, insulin increases the amount of GLUT4 in the plasma membranes by 3-fold and decreases that in the low-density microsomes by 36 %. In white adipose cells, insulin increases the amount of GLUT4 in the plasma membranes by 6-fold and decreases that in the low-density microsomes by 54 %. GLUT1 is also detectable by Western blotting in both cell types and is redistributed from the intracellular membranes to the plasma membranes in response to insulin.

Effects of PIA and NECA on glucose transport activity

To examine the receptor type to which adenosine binds in rat brown adipose cells, glucose transport activity was measured in response to PIA, an agonist of the A1 receptor for adenosine,

Figure 4 Subcellular distributions of GLUT4 and GLUT1 in brown and white adipose cells

Isolated brown (C,D) and white (A,B) cells were incubated with or without 700 nM insulin at 37 °C for 30 min in the presence of 200 nM adenosine. Subcellular fractions were then prepared and analysed by SDS/PAGE and Western blotting as described in the Experimental section. Plasma membrane (PM) [10 µg for GLUT4 (B,D) or 20 µg for GLUT1 (A,C)], high-density microsome (HDM), and low-density microsome (LDM) proteins were analysed.

Figure 5 Concentration-dependent effects of PIA and NECA on insulin-stimulated glucose transport activity in brown adipose cells

Isolated cells were incubated with 700 nM insulin and various concentrations of PIA (▲) or NECA (●) in the absence of adenosine at 37 °C for 30 min before measurement of 3-*O*-methyl-D-glucose transport. Results are the means $\pm$ S.E.M. of the mean values obtained from triplicate samples in three individual experiments.

Figure 6 Effect of adenosine on the isoprenaline concentration-dependent modulation of glucose transport activity in the absence and presence of insulin in brown adipose cells

Isolated cells were incubated in the absence (A) and presence (B) of 700 nM insulin at 37 °C for 30 min in the presence (●, ■) and absence (○, □) of 200 nM adenosine. Cells were then further incubated with various concentrations of isoprenaline for 10 min before measurement of 3-*O*-methyl-D-glucose transport. Results are the means $\pm$ S.E.M. of the mean values obtained from triplicate samples in three individual experiments.

and NECA, an agonist of the A2 receptor for adenosine. Figure 5 shows that PIA reverses the effect of adenosine removal by adenosine deaminase in the presence of insulin while NECA has a small stimulatory effect on glucose transport activity in the presence of insulin and absence of adenosine, but not enough to substitute for the effect of adenosine (Figure 5).

Effects of isoprenaline and adenosine on glucose transport activity

Figure 6 shows the concentration-dependent modulation of 3-*O*-methyl-D-glucose transport by isoprenaline in isolated brown adipose cells from rats. In the absence of insulin and in both the presence and absence of adenosine (Figure 6A), isoprenaline stimulates glucose transport activity up to 3-fold and the magnitude of this increase is approx. 10% of that induced by insulin (Figure 2). The concentration of isoprenaline producing a maximum stimulation is 10 nM; higher concentrations produce diminishing responses. No significant difference is observed in the isoprenaline response between the presence and absence of adenosine. However, in the presence of a maximally stimulating concentration of insulin (Figure 6B), isoprenaline inhibits glucose transport activity in the presence of adenosine and only slightly stimulates glucose transport activity in the absence of adenosine. The maximal effects of isoprenaline are also shifted from 10 to 100 nM in the presence of insulin.

Figure 7 shows the effect of 10 nM isoprenaline on the stimulation of 3-*O*-methyl-D-glucose transport by insulin in the presence and absence of adenosine. In the presence of adenosine, isoprenaline partly inhibits the stimulation of glucose transport activity by insulin, whereas in the absence of adenosine, isoprenaline increases the stimulation of glucose transport activity by insulin. However, the sensitivity to insulin is not changed by isoprenaline.

DISCUSSION

Despite efforts by many investigators, preparations *in vitro* of isolated brown adipose cells from rats have typically exhibited small glucose transport responses to insulin in comparison with rat white adipose cells *in vitro* and brown adipose cells *in vivo* as assessed by the euglycaemic clamp in combination with 2-deoxyglucose [2–5,15–19]. These results have been attributed to difficulties in cell isolation. In the present study we describe the isolation of rat brown adipose cells that show a 30-fold increase in glucose transport activity in response to insulin in the presence of adenosine (Figure 2). It is well known that β -adrenergic agents promote heat production in BAT, and that this effect is specifically blocked by β -blockers such as propranolol and partly suppressed by insulin [17]. The preparation of isolated brown adipose cells from rats presented here shows typical responses to these agents (Figure 1). The magnitude of the stimulation of glucose transport activity by insulin in rat brown adipose cells is almost the same as that reported in rat white adipose cells [20] but the accessibility of cell-surface GLUT4 to photolabelling (Figure 3) and the concentration of GLUT1 and GLUT4 in the plasma membranes from insulin-stimulated cells (Figure 4) are somewhat smaller than those in white adipose cells [26].

The biggest difference in insulin-enhanced glucose transport between brown and white adipose cells is the stimulatory effect of extracellular adenosine (Figure 2). In white adipose cells, we have previously reported that removing adenosine from the incubation medium causes only an approx. 25% decrease in the

Figure 7 Effects of isoprenaline on the insulin concentration-dependent stimulation of glucose transport activity in the presence and absence of adenosine in brown adipose cells

Isolated cells were incubated with various concentrations of insulin at 37 °C for 30 min in the presence (A,C) and absence (B,D) of 200 nM adenosine. Cells were then further incubated with (▲,△) or without (●,○) 10 nM isoprenaline for 10 min before measurement of 3-*O*-methyl-D-glucose transport. Results are the means $\pm$ S.E.M. of the mean values obtained from triplicate samples in three individual experiments, and are expressed as glucose transport activity (A,B) and percentage of maximal response (C,D).

maximum stimulation of 3-*O*-methyl-D-glucose transport by insulin through a reduction in the transport $V_{\max}$ [27]. Here we show that the effect of adenosine on insulin-stimulated glucose transport in brown adipose cells is more than twice that in white adipose cells. In contrast, Marette and Bukowiecki [17] reported that stimulation of glucose transport in brown adipose cells is independent of extracellular adenosine. The discrepancy is probably related to the incubation conditions used. We added 200 nM adenosine in the incubation medium even during the collagenase incubation while preparing the cells to prevent potential damage to the cells by the lipolytic release of fatty acids [28] and then added ADA to this medium to remove the adenosine. Marette and Bukowiecki did not control for the likely presence of adenosine in their 'adenosine-free' medium because of cell breakage, frequently enough to produce a maximum adenosine effect [28]. This interpretation is supported by the PIA effect seen here. PIA, an agonist of the A1 receptor for adenosine, reverses the inhibitory effects of ADA on glucose transport activity (Figure 5), an observation compatible with that in white adipose cells [27]. The response to adenosine in brown adipose cells is thought to be the same as that in white adipose cells, that is, a reduction in intracellular cyclic AMP levels via an A1 receptor/ G_i -protein mechanism. However, adenosine does not seem to influence the insulin sensitivity in brown adipose cells (Figure 2) as it does in white adipose cells [29].

The amount of cell-surface GLUT4 detected in insulin-stimulated brown adipose cells by photolabelling is also increased by extracellular adenosine (Figure 3) in the absence of differences in GLUT4 concentrations between subcellular fractions determined by Western blotting (results not shown). These observations are compatible with those reported in white adipose

cells [30] where they were interpreted to suggest that adenosine might be affecting the translocation process at a step specifically localized to the plasma membrane such as vesicle fusion. Thus the membrane-impermeant bis-mannose photolabel might specifically detect GLUT4 in the plasma membrane only after fusion of the translocated vesicles, whereas subcellular fractionation cannot distinguish between pre-fusion and post-fusion GLUT4 in the plasma membrane fraction.

We also performed Western blotting analyses of other glucose transporter isoforms (results not shown). GLUT2 is not detected at all in any subcellular membrane fractions and only very faint signals for GLUT3 are detected in the plasma membrane fraction. In contrast, clear and specific signals for GLUT5 are detected in the plasma membranes and high-density microsomes, but not in the low-density microsomes, and no effects of insulin are observed. These results are consistent with the known capacity of white adipose cells to take up and metabolize fructose in a non-insulin-dependent fashion.

It is well established that noradrenaline and other β -adrenergic agonists stimulate glucose transport in brown adipose cells [2,15–19]. The approx. 3-fold increase in glucose transport stimulated by isoprenaline in the present study (Figure 6A) is compatible with previous reports [15–19]. In the present study, isoprenaline is also shown to counteract the effect of insulin in the presence of adenosine, but to augment the effect of insulin in the absence of adenosine (Figure 6B and 7). No effects are observed on the insulin dose-response curves. However, a previous report suggests that isoprenaline has an additive stimulatory effect with insulin in brown adipose cells, although this work was done in incubation medium without controlling the extracellular adenosine concentration [17,18]. Thus this

discrepancy is also thought to be due to the incubation conditions and differences in the cell viability as described above. We have previously reported that catecholamines counter-regulate insulin-stimulated glucose transport in white adipose cells in the absence of adenosine [27,31] but the present study highlights the qualitative differences in the interaction between the catecholamines and insulin between brown and white adipose cells.

Rat brown adipose cells isolated by the present method exhibit marked and highly sensitive glucose transport responses to a variety of regulatory stimuli. These cells thus represent an excellent model for further examination of the mechanisms through which multiple signalling pathways interact to control glucose transport and GLUT4 subcellular trafficking.

We thank Dena R. Yver and Steven R. Richards for their expert technical assistance. We also thank Dr. G.D. Holman for generously providing the ATB-[2-³H]BMPA, Dr. P. Boon Chock (NHLBI/NIH) for providing the oxygen monitoring system and Dr. Sergei Khangulov and Dr. Luis Netto (NHLBI/NIH) for assisting in its use. This work was done while M.O.K. was on leave of absence from the Department of Physiology, Shiga University of Medical Science, Ohtsu 520-21 Japan.

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