Noradrenaline increases glucose transport into brown adipocytes in culture by a mechanism different from that of insulin

Yasutake SHIMIZU*, Danuta KIELAR, Yasuhiko MINOKOSHI and Takashi SHIMAZU

Department of Medical Biochemistry, Ehime University, School of Medicine, Shigenobu, Ehime 791-02, Japan

Glucose uptake into brown adipose tissue has been shown to be enhanced directly by noradrenaline (norepinephrine) released from sympathetic nerves. In this study we characterized the glucose transport system in cultured brown adipocytes, which responds to noradrenaline as well as insulin, and analysed the mechanism underlying the noradrenaline-induced increase in glucose transport. Insulin increased 2-deoxyglucose (dGlc) uptake progressively at concentrations from 10^{-11} to 10^{-6} M, with maximal stimulation at 10^{-7} M. Noradrenaline concentrations ranging from 10^{-8} to 10^{-6} M also enhanced dGlc uptake, even in the absence of insulin. The effects of noradrenaline and insulin on dGlc uptake were additive. The stimulatory effect of noradrenaline was mimicked by the β_3-adrenergic agonist, BRL37344, at concentrations two orders lower than noradrenaline. Dibutyryl cyclic AMP also mimicked the stimulatory effect of noradrenaline, and the antagonist of cyclic AMP, cyclic AMP-S Rp-isomer, blocked the enhancement of glucose uptake due to noradrenaline. Furthermore Western blot analysis with an anti-phosphotyrosine antibody revealed that, in contrast with insulin, noradrenaline apparently does not stimulate intracellular phosphorylation of tyrosine, suggesting that the noradrenaline-induced increase in dGlc uptake depends on elevation of the intracellular cyclic AMP level and not on the signal chain common to insulin. When cells were incubated with insulin, the content of the muscle/adipocyte type of glucose transporter (GLUT4) in the plasma membrane increased, with a corresponding decrease in the amount in the microsomal membrane. In contrast, noradrenaline did not affect the subcellular distribution of GLUT4 or that of the HepG2/erythrocyte type of glucose transporter. Although insulin increased $V_{max.}$ and decreased the K_m value for glucose uptake, the effect of noradrenaline was restricted to a pronounced decrease in K_m. These results suggest that the mechanism by which noradrenaline stimulates glucose transport into brown adipocytes is not due to translocation of GLUT but is probably due to an increase in the intrinsic activity of GLUT, which is mediated by a cyclic AMP-dependent pathway.

INTRODUCTION

The entry of glucose into mammalian cells occurs through facilitated diffusion, which is mediated by a family of specific glucose transporter (GLUT) molecules. The GLUT family is composed of at least six species with similar primary structures, and each member of the family is expressed in a tissue-specific manner [1,2]. It is now evident that the mechanism underlying the insulin-stimulated glucose transport in adipocytes and muscle cells involves translocation of the insulin-regulatable glucose transporter (GLUT4), which is exclusively expressed in these cell types, from an intracellular pool to the plasma membrane [3–6]. Certain conditions such as hypoxia, inhibition of oxidative phosphorylation, and exposure to protein synthesis inhibitors also markedly stimulate glucose transport in the absence of insulin [7–10]. However, several lines of evidence suggest that insulin-independent acceleration of glucose transport is not associated with the translocation of GLUT.

Recently we demonstrated that exposure to cold or stimulation of the ventromedial hypothalamic nucleus in rats, either of which promotes activation of sympathetic nerves, markedly enhances glucose uptake by brown adipose tissue (BAT) without an increase in the plasma insulin level [11–15]. Although the chronic effect of sympathetic nerve activation was accompanied by *de novo* synthesis of GLUT4 in BAT [15], another mechanism without an increase in the cellular content of GLUT could account for the enhanced glucose uptake in response to acute exposure to cold or intravenous infusion of the sympathetic neurotransmitter noradrenaline (norepinephrine) [11,15].

To explore this latter mechanism we established conditions for primary cultures of brown adipocytes and showed that noradrenaline increases glucose uptake even in the absence of insulin, confirming that these cultured cells are suitable for studying the mechanism underlying glucose transport stimulated by noradrenaline [16]. In the present study we characterized the noradrenaline-induced increase in glucose uptake by cultured brown adipocytes, showing that the mechanism by which noradrenaline stimulates glucose transport in brown adipocytes does not involve translocation of GLUT but presumably activates the intrinsic catalytic activity of GLUT.

EXPERIMENTAL

Materials

2-Deoxy-D-[^{3}H]glucose (dGlc) and [^{14}C]sucrose were obtained from American Radiolabeled Chemicals. Insulin, noradrenaline,

Abbreviations used: ATB-BMPA, 2-*N*-[4-(1-azitrifluoroethyl)benzoyl]-1,3-bis-(D-mannos-4-yloxy)-2-propylamine; BAT, brown adipose tissue; db-cAMP, dibutyryl cyclic AMP; dGlc, 2-deoxyglucose; DMEM, Dulbecco's modified Eagle's medium; ECL, enhanced chemiluminescence; FCS, fetal calf serum; GLUT, glucose transporter; GLUT1, HepG2/erythrocyte type of glucose transporter; GLUT4, muscle/adipocyte type of glucose transporter; RpcAMP, cyclic AMP-S Rp-isomer.

* To whom correspondence should be addressed.

dexamethasone, tri-iodothyronine, collagenase (type I) and BSA were from Sigma. Dibutyryl cyclic AMP (db-cAMP) was from Wako Chemicals. The antagonist of cyclic AMP, cAMP-S Rp-isomer (RpcAMP), was from Boehringer Mannheim. The β_3-adrenergic agonist BRL37344, was a gift from Dr. M. A. Cawthorne of SmithKline Beecham, Epsom, Surrey, U.K. Dulbecco's modified Eagle's medium (DMEM) was from Nissui Pharmaceutical Co. Ltd. Fetal calf serum (FCS) was from Whittaker Bioproducts Inc. Antisera against the C-terminal peptides of GLUT1 and GLUT4 were prepared as previously described [15]. The anti-phosphotyrosine antibody (4G10; Upstate Biotechnology, U.S.A.) was kindly provided by Dr. N. Okumura of the Protein Institute, Osaka University, Osaka, Japan. The ECL (enhanced chemiluminescence) system was from Amersham Corp.

Cell isolation and culture

Brown-fat precursor cells were isolated as the stromal-vascular fraction from the interscapular brown fat of newborn rats by the procedure described previously [16]. Cells isolated by collagenase digestion were suspended in DMEM supplemented with 10% (v/v) FCS, 17 μM D-pantothenic acid, 33 μM D-biotin, 100 μM ascorbic acid, 1 μM octanoic acid, 100 i.u./ml penicillin, 0.1 mg/ml streptomycin, 50 nM insulin and 50 nM tri-iodothyronine, and were seeded on collagen-coated dishes. The cells were incubated at 37 °C under an atmosphere of 5% CO_2 in air. When the cultured cells reached confluence, they were treated with 1 μM dexamethasone for 48 h [16]. The cells were used 3–4 d after reaching confluence.

Glucose uptake measurement

dGlc uptake was measured with differentiated brown adipocytes as described previously [16]. Briefly, cells seeded in 35 mm dishes were washed three times with a Hepes-buffered saline solution (140 mM NaCl, 1 mM $CaCl_2$, 5 mM KCl, 2.5 mM $MgSO_4$ and 20 mM Hepes, pH 7.4) and then preincubated with plain DMEM for 3 h at 37 °C, to reduce the effects of serum and supplements. Where indicated, hormones were added during the last 1 h of the preincubation. Then the cells were rinsed twice with the Hepes-buffered saline to remove the glucose in the medium. After incubation for 20 min in Hepes-buffered saline containing 2% (w/v) fatty acid-free BSA in the presence or absence of the same hormones, the cells were further incubated with 50 μM dGlc (0.01 μCi/nmol) in the same solution. dGlc uptake was allowed to proceed for 2 min and then terminated by rapid suction and subsequent washing with ice-cold 3 mM $HgCl_2$ in PBS. Contamination of the extracellular space by the isotope was quantified using [^{14}C]sucrose. The net uptake of dGlc was expressed in pmol/min per mg protein ± S.E.M.

In experiments where the relationship between the external glucose concentration and the isotope uptake rate was examined, glucose was added to the medium at the desired concentrations. The glucose uptake was calculated as nmol/min per mg protein, with the assumption that GLUT does not distinguish between glucose and dGlc. Although this assumption was made for simplicity, the results would not be affected if the relative affinities of the transporter for glucose and dGlc were unaffected by the treatment with insulin or noradrenaline. Kinetic parameters were determined by linear regression of the relationships between the ratio of the uptake rate to the substrate concentration (V/[S]) and the uptake rate (V) (Eadie–Hofstee plot). In this analysis the slope of the line gives the apparent K_m, whereas the Y-intercept gives the maximal uptake velocity ($V_{max.}$) [17].

Detection of protein tyrosine phosphorylation

After incubation with insulin or noradrenaline for 1–60 min, cells were taken up in SDS sample buffer containing 100 μM sodium orthovanadate and 1 mM PMSF. Equal volumes of the samples were subjected to SDS/PAGE on 6% (w/v) linear gels and then transferred to nitrocellulose membranes. The blots were incubated with the anti-phosphotyrosine antibody, followed by the peroxidase-labelled second antibody. Immunostained proteins were revealed with the ECL system.

Subcellular fractionation and Western blot analysis of GLUT

To study the translocation of GLUT, cultured brown adipocytes were collected after incubation with noradrenaline or insulin for 1 h. The cells from eight dishes of 60 mm diameter were pooled into one preparation of the plasma and microsomal membranes according to the method described previously [3]. The GLUT1 and GLUT4 contents of each membrane fraction were determined by Western blot analysis, by using rabbit antisera against the respective C-terminal peptide and ^{125}I-labelled protein A. Immunoreactivity was analysed with BAS-1000 (Fuji Film, Japan), and expressed relative to control.

Data analysis

All values are presented as means ± S.E.M. Statistical significance was examined by analysis of variance, with *post hoc* testing by means of Duncan's multiple range test.

RESULTS

Effects of insulin and noradrenaline on dGlc uptake by cultured brown adipocytes

Cultured brown adipocytes were differentiated and maintained in DMEM containing supplements such as FCS and insulin. In preliminary experiments these cells did not respond significantly to further addition of insulin ranging from 10^{-11} to 10^{-6} M (results not shown). The absence of a response could be explained if the transport systems were operating at maximum rates under these conditions and thus were not stimulated further by the hormone. To reduce the rate of uptake before the addition of insulin or noradrenaline, the cells were deprived of the supplements for 3 h. The uptake rate measured after deprivation is hereafter referred to as the basal uptake. When these cells were exposed to insulin or noradrenaline, dGlc uptake was enhanced about 5-fold and 2.5-fold respectively within 30 min (Figure 1), and essentially remained elevated for at least 1 h. In all experiments described below, incubation with hormones for 1 h was performed accordingly.

Figure 2 shows dose–response curves for the actions of insulin and noradrenaline on dGlc uptake by cultured brown adipocytes. Insulin increased dGlc uptake progressively at concentrations from 10^{-11} to 10^{-6} M, with maximal stimulation at 10^{-7} M. In contrast, noradrenaline concentrations ranging from 10^{-8} to 10^{-6} M enhanced dGlc uptake, with a maximal effect at 10^{-6} M. These results indicate that brown adipocytes differentiated *in vitro* are highly sensitive to both insulin and noradrenaline, as are their counterparts *in vivo*.

We also examined the effect of the β_3-adrenergic agonist BRL37344, which increased dGlc uptake in the same way as noradrenaline (Figure 2). The maximum rate of dGlc uptake with the β_3-agonist was comparable to that with noradrenaline, but the dose–response curve was shifted to the left, towards concentrations two orders lower than those of noradrenaline.

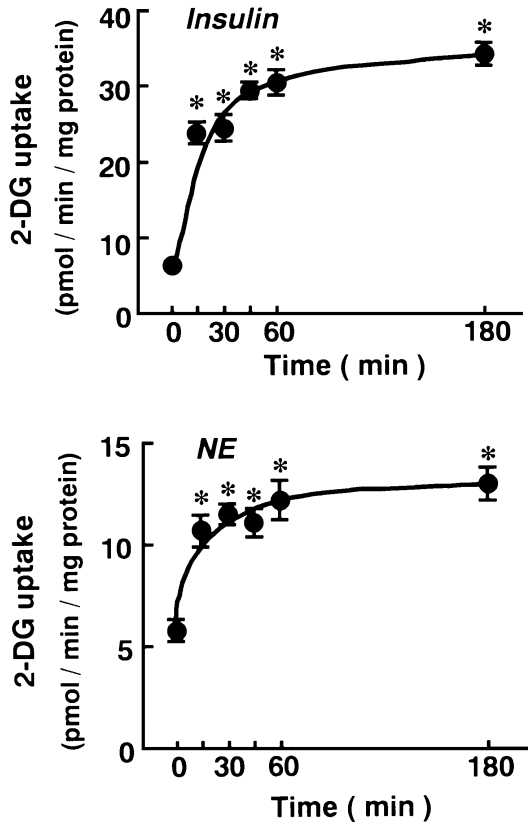

Figure 1 Time courses of increased dGlc (2-DG) uptake after treatment with insulin and noradrenaline by cultured brown adipocytes

Cultured brown adipocytes were treated with 0.1 μM insulin or 1 μM noradrenaline (NE) for the times indicated on the abscissa and dGlc uptake was measured as described in the Experimental section. Values are means ± S.E.M. ($n = 5$ or 6). *$P < 0.05$ against basal.

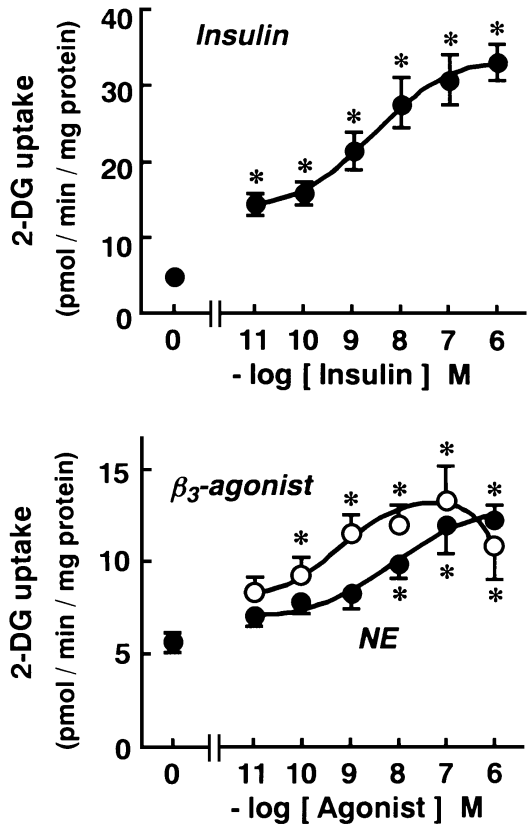

Figure 2 Dose–response curves for insulin, noradrenaline and a β_3-adrenergic agonist to dGlc (2-DG) uptake by cultured brown adipocytes

Cultured brown adipocytes were incubated for 1 h with increasing doses of insulin, noradrenaline (NE) or the β_3-adrenergic agonist BRL37344, and dGlc uptake was measured as described in the Experimental section. Values are means ± S.E.M. ($n = 6$–8). *$P < 0.05$ against basal.

To determine whether there is synergism or additivity between the actions of insulin and noradrenaline, brown adipocytes were incubated for 1 h together with the two agents at the maximally effective concentrations, and then dGlc uptake was measured. As shown in Table 1, the effects of insulin and noradrenaline were completely additive, suggesting that these two agents stimulate glucose uptake by brown adipocytes through different mechanisms.

Table 1 Additivity of the effects of noradrenaline and insulin on dGlc uptake by cultured brown adipocytes

dGlc uptake was measured in cultured brown adipocytes in the basal state, after treatment with 1 μM noradrenaline or 0.1 μM insulin, or both insulin and noradrenaline, as described in the Experimental section. Values are means ± S.E.M. ($n = 4$–6). *$P < 0.05$ against basal; †$P < 0.05$ against insulin alone.

Noradrenaline	Insulin	dGlc uptake (pmol/min per mg of protein)
−	−	5.89 ± 0.69
−	+	24.16 ± 1.96*
+	−	12.57 ± 0.62*
+	+	38.42 ± 1.90*†

Effects of insulin and noradrenaline on protein tyrosine phosphorylation in cultured brown adipocytes

To clarify the difference between the effects of insulin and noradrenaline, the possibility of protein tyrosine phosphorylation by insulin and noradrenaline was examined by using phosphotyrosine immunoblotting. When cultured cells were treated with 0.1 μM insulin, three major phosphoproteins were detected (Figure 3), the first a 185 kDa protein corresponding to the major insulin receptor substrate, IRS-1; the second a 95 kDa protein corresponding to the insulin receptor β-subunit; and the third a 60 kDa protein corresponding to pp60. These proteins were phosphorylated within 1 min and remained essentially unchanged at least for 60 min after treatment with insulin. In contrast, noradrenaline did not stimulate tyrosine phosphorylation, at least with respect to these proteins (Figure 3).

Subcellular distribution of GLUT1 and GLUT4

Brown adipocytes in primary culture express at least two types of GLUT: GLUT1 and GLUT4 [16]. To determine whether noradrenaline promotes the translocation of GLUT from an intracellular pool to the plasma membrane, the subcellular distributions of these transporters after treatment of cells with noradrenaline or insulin for 1 h were analysed by Western

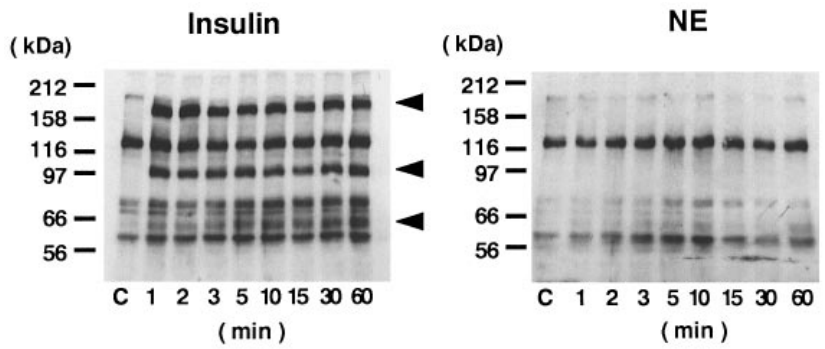

Figure 3 Effects of insulin and noradrenaline on tyrosine phosphorylation in cultured brown adipocytes

Cultured brown adipocytes were treated with 0.1 μM insulin or 1 μM noradrenaline (NE) for 1–60 min as indicated on the abscissa. C denotes the untreated control. Protein tyrosine phosphorylation was detected after analysis of cell lysates by SDS/PAGE and immunoblotting with an anti-phosphotyrosine antibody. Molecular size markers are indicated. The arrowheads indicate three major phosphoproteins observed after insulin treatment.

Table 2 Effects of insulin and noradrenaline on the subcellular distributions of GLUT4 and GLUT1 in cultured brown adipocytes

Cultured brown adipocytes were incubated for 1 h with either 0.1 μM insulin or 1 μM noradrenaline, then subcellular fractionation and Western blotting were performed as described in the Experimental section. The amounts of GLUT4 and GLUT1 were determined with a BAS1000 image analyser, and the values were expressed as percentages ± S.E.M. ($n = 6$–8) relative to the means of respective controls. $^*P < 0.05$ against control.

Condition	Plasma membrane fraction (% of control)	Microsomal fraction (% of control)
GLUT4		
Control	100 ± 10	100 ± 10
Insulin	164 ± 13*	70 ± 8*
Noradrenaline	96 ± 14	89 ± 12
GLUT1		
Control	100 ± 16	100 ± 20
Insulin	109 ± 20	109 ± 24
Noradrenaline	103 ± 22	121 ± 26

blotting. Table 2 shows the relative amounts of GLUT in the plasma membrane and the microsomal fraction, comprising the intracellular pool of GLUT. Insulin increased the GLUT4 content in the plasma membrane with a corresponding decrease in the microsomal fraction, which is indicative of promotion of the translocation of GLUT4. In contrast, noradrenaline did not change the subcellular distribution of GLUT4, confirming our recent observation [18]. However, the GLUT1 distribution was not significantly influenced by either insulin or noradrenaline.

Effects of insulin and noradrenaline on the kinetic parameters of glucose uptake

It could be expected that the enhancement of dGlc uptake induced by noradrenaline reflects an increase in the affinity of GLUT to the substrate, because noradrenaline stimulates dGlc uptake without the translocation of GLUT (Table 2). The rates of dGlc uptake were then measured with different concentrations of glucose, and the results are shown in Figure 4 as Eadie–Hofstee plots. For cells in the basal state, the K_m value was 5.34 ± 0.50 mM and $V_{max.}$ was 1.74 ± 0.01 ng/min per mg protein. Insulin increased $V_{max.}$ and decreased K_m. In contrast, the effect of noradrenaline was restricted to K_m, which decreased significantly to 2.19 ± 0.17 mM.

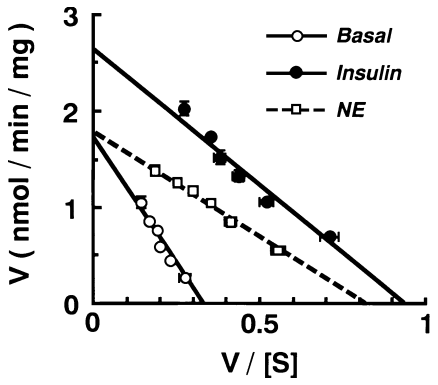

Figure 4 Eadie–Hofstee plots of glucose uptake by cultured brown adipocytes in the basal, insulin- and noradrenaline-stimulated states

Cultured brown adipocytes were treated for 1 h with 0.1 μM insulin or 1 μM noradrenaline (NE), and dGlc uptake was measured in the presence of different concentrations of glucose. The rates of uptake (ng/min per mg protein) were plotted against V/[glucose] ([ng/min per mg protein]/[mM]). With this method of analysis, a negative slope represents K_m and the Y-intercept corresponds to $V_{max.}$. The lines drawn through the points were calculated by linear-least regression ($r > 0.90$ in each instance). Values are means ± S.E.M. ($n = 5$ or 6). When standard error bars are not visible, they are within the symbol.

Table 3 Effect of db-cAMP on dGlc uptake by cultured brown adipocytes

Cultured brown adipocytes were incubated for 1 h with or without 1 mM db-cAMP in the absence or presence of either 0.1 μM insulin or 1 μM noradrenaline. dGlc uptake was then measured as described in the Experimental section. Values are means ± S.E.M. ($n = 4$–6). *$P < 0.05$ against basal; †$P < 0.05$ against absence of db-cAMP.

Condition	db-cAMP	dGlc uptake (pmol/min per mg of protein)
Basal	−	5.27 ± 0.59
	+	13.89 ± 0.50*†
Insulin	−	23.83 ± 1.61*
	+	33.10 ± 2.25*†
Noradrenaline	−	13.39 ± 1.50*
	+	13.87 ± 1.56*

Effect of cyclic AMP on dGlc uptake by cultured brown adipocytes

To determine whether or not cyclic AMP mediates the effect of noradrenaline, the effect of db-cAMP on dGlc uptake was investigated. When db-cAMP was added at 1 mM, it increased dGlc uptake from the basal level of 5.27 to 13.89 pmol/min per mg protein (Table 3), this being comparable to the maximal effect of noradrenaline. In addition, the effect of db-cAMP on dGlc uptake was additive to that of insulin (Table 3). The simultaneous addition of noradrenaline and db-cAMP did not produce higher dGlc uptake than with noradrenaline alone (Table 3). Furthermore when the antagonist of cyclic AMP, RpcAMP (10^{-4} M) was added 15 min before incubation with 10^{-7} M noradrenaline, the enhancement of dGlc uptake was completely suppressed (Table 4). These results suggest that the stimulatory effect of noradrenaline on hexose uptake by brown adipocytes is mediated by cyclic AMP.

DISCUSSION

In this study we characterized the glucose transport system in cultured brown adipocytes, which is regulated by both insulin and noradrenaline, and analysed the mechanism underlying the noradrenaline-induced increase in glucose transport. Our principal findings are: (1) noradrenaline, in the absence of insulin, stimulates glucose uptake in a dose-dependent manner; (2) the stimulatory effects of noradrenaline and insulin on glucose uptake are completely additive; (3) the effect of noradrenaline is mimicked by a β_3-adrenergic agonist at much lower concentrations; (4) the noradrenaline-induced increase in glucose uptake is dependent on cyclic AMP; (5) noradrenaline does not promote the translocation of either GLUT1 or GLUT4; and (6) noradrenaline decreases K_m for glucose uptake without altering $V_{max.}$.

Glucose uptake by BAT is stimulated not only by insulin but also by the sympathetic nerves distributed abundantly to this tissue [11–13]. It is now evident that sympathetic activation of glucose uptake by BAT is mediated by the neurotransmitter noradrenaline [11,19,20]. In the present study we have shown that noradrenaline, without the co-existence of insulin, enhances dGlc uptake in dose- and time-dependent manners, demonstrating that noradrenaline directly stimulates the insulin-independent pathway of glucose transport in brown adipocytes. The β_3-adrenergic agonist BRL37344 mimicked the effect of noradrenaline, but the dose–response curve was shifted to the left, the agonist being effective at concentrations two orders lower than noradrenaline (Figure 2). Although we cannot rule out the contributions of β_1- and β_2-receptors without a more comprehensive pharmacological study, the above results indicate that β_3-receptors may take part in the noradrenaline-induced increase in glucose uptake. In support of this notion we observed that the expression of β_3-receptors was induced during the differentiation of cultured brown adipocytes in parallel with the appearance of a response of glucose uptake to noradrenaline [21].

It was previously reported that noradrenaline augments the insulin sensitivity of brown adipocytes [20]. Thus an alternative interpretation for the noradrenaline-induced increase in glucose uptake could be that a small amount of insulin, which remained in the reaction medium even after washing, exerted its action more efficiently in the presence of noradrenaline. However, this is unlikely because treatment of the cells with noradrenaline did not stimulate autophosphorylation of the insulin receptor β-subunit or tyrosine phosphorylation of IRS-1 (Figure 3), both of which are signalling events essential for the insulin-induced increase in glucose transport [22–24]. Further support for this is the observation that inhibition of phosphatidylinositol 3-kinase, which plays a predominant role in the insulin-induced translocation of GLUT4 [25], does not significantly affect the noradrenaline action [18]. It is thus concluded that noradrenaline directly augments glucose uptake via an insulin-independent pathway in brown adipocytes.

It has been well established that insulin promotes the translocation of GLUT4 from an intracellular pool to the plasma membrane [3–6]. This is the major mechanism responsible for the insulin-induced increase in glucose transport in adipocytes and muscle cells. On the other hand, it is not clear whether the translocation mechanism is able to account for the increased glucose transport caused by noradrenaline in brown adipocytes. Although we found in this study, by using a subcellular fractionation procedure, that noradrenaline did not increase the content of either GLUT4 or GLUT1 in the plasma membrane (Table 2), fractionation methods commonly underestimate the degree of translocation, and this is therefore not enough to exclude the possibility that the translocation of GLUT takes place in response to noradrenaline. However, several lines of evidence allow us to conclude that the mechanism by which noradrenaline stimulates glucose transport is unrelated to the translocation of GLUT. First, the maximal effects of insulin and noradrenaline were completely additive (Table 1). Secondly, as mentioned above, neither tyrosine kinase nor phosphatidylinositol 3-kinase is involved in the noradrenaline-induced increase in glucose uptake (Figure 3) [18]. Thirdly, noradrenaline decreased the K_m for glucose uptake without affecting $V_{max.}$, whereas insulin increased the $V_{max.}$ greatly (Figure 4). Fourthly, preliminary experiments indicated that exofacial photo-affinity labelling of GLUT4 with the membrane-impermeant bismannose derivative ATB-BMPA was not increased by treatment of cultured brown adipocytes with noradrenaline (Y. Shimizu, S. Satoh and T. Shimazu, unpublished work). Finally, it has been shown *in vivo* that acute cold exposure or stimulation of the ventromedial hypothalamic nucleus in rats, either of which treatments increases dGlc uptake through the activation of the sympathetic nerves, does not induce apparent changes in the subcellular distribution of GLUT [6,13]. Taken together, it seems probable that the noradrenaline-induced increase in glucose transport is due not to the translocation of GLUT but possibly to an increase in the intrinsic activity of GLUT already present in the plasma membrane.

It is interesting to speculate which isoform of GLUT is affected by noradrenaline. Two types of GLUT, GLUT1 and GLUT4, are known to be expressed in brown adipocytes. GLUT1 is primarily located in the plasma membrane, being responsible

Table 4 Effect of RpcAMP on noradrenaline-induced dGlc uptake by cultured brown adipocytes

Cultured brown adipocytes were treated with or without 0.1 μM noradrenaline in the absence or presence of 100 μM RpcAMP, with preincubation for 15 min. dGlc uptake was then measured as described in the Experimental section. Values are means ± S.E.M. ($n = 3$–5). *$P < 0.05$ against basal; †$P < 0.05$ against noradrenaline alone.

Condition	dGlc uptake (pmol/min per mg of protein)
Basal	5.89 ± 0.32
Noradrenaline	12.53 ± 0.10*
Noradrenaline + RpcAMP	6.38 ± 0.52†

mainly for basal glucose uptake. GLUT4, which is expressed exclusively in insulin-sensitive tissues, is located predominantly in intracellular vesicles and is translocated to the plasma membrane in response to insulin [1,2]. Thus the stimulatory effect of insulin on glucose transport seems to depend largely on the presence of the GLUT4 isoform. At present it remains unknown which isoform(s) of GLUT is responsible for the noradrenaline-induced increase in glucose transport. However, on the basis of our present results showing that noradrenaline does not promote the translocation of GLUT4, it is possible that noradrenaline increases the intrinsic catalytic activity of GLUT1 residing in the plasma membrane. This assumption convincingly explains the completely additive effects of noradrenaline and insulin on glucose uptake by brown adipocytes. To corroborate the significant role of GLUT1 in this effect of noradrenaline will require further study, such as cell-surface labelling with ATB-BMPA. Indeed, by using this membrane-impermeable, photoactive glucose analogue, Harrison et al. [26] recently demonstrated that in 3T3-L1 adipocytes inhibition of protein synthesis by anisomycin stimulates hexose transport primarily by enhancing the intrinsic catalytic activity of cell surface GLUT1.

The present study indicates that the enhancement of glucose transport by noradrenaline is probably mediated by cyclic AMP (Tables 3 and 4). The sequence of events after the production of cyclic AMP leading to enhanced glucose uptake is not known at present. In this context it should be noted that GLUT4 is phosphorylated at its C-terminal region by cyclic AMP-dependent protein kinase [27]. However, this phosphorylation of GLUT4 seems unlikely to be a predominant mechanism responsible for the increase in glucose transport caused by noradrenaline, because phosphorylation of GLUT4 leads to a decrease, rather than an increase, in its transport activity [28]. Alternatively, Czech and co-workers [8,10,26] suggested that the catalytic activity of GLUT1 in the plasma membrane is suppressed by the interaction with an inhibitory regulator protein, on the basis of the observation that the protein synthesis inhibitor anisomycin or cycloheximide leads to a marked increase in glucose transport in 3T3-L1 adipocytes without detectable changes in the levels of cell-surface GLUT1 and GLUT4. In preliminary experiments we confirmed the enhancement of glucose uptake by cycloheximide in cultured brown adipocytes. Interestingly, the noradrenaline-induced increase in glucose uptake was no longer observed after treatment with the protein synthesis inhibitor, whereas the insulin effect was still observed (Y. Shimizu and T. Shimazu, unpublished work). Thus it is possible that cyclic AMP dissociates a hypothetical inhibitory regulator protein from GLUT1, in a direct manner (like the release of the catalytic subunit of cyclic AMP-dependent protein kinase from its regulatory subunit) or an indirect manner (as might occur on phosphorylation through cyclic AMP-dependent protein kinase), thereby removing presumed tonic inhibition of transport function. The precise mechanism of such dissociation from the regulator protein remains to be determined.

In summary, the results of this study demonstrate that noradrenaline and a β_3-adrenoceptor agonist (at much lower concentrations) stimulate an insulin-independent pathway for glucose transport in brown adipocytes. In addition, the cellular mechanism by which noradrenaline enhances the glucose uptake is not associated with the translocation of GLUT but possibly with an increase in the intrinsic transport activity of GLUT through cyclic AMP. However, the molecular basis of the cyclic AMP-induced increase in glucose transport has not yet been established. Further studies are required to clarify the nature of this mechanism.

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