G_{q}-coupled Receptors Transmit the Signal for GLUT4 Translocation via an Insulin-independent Pathway*

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Guanosine 5′-O-(3-thiotriphosphate) (GTPγS) induces the translocation of glucose transporter type 4 (GLUT4) from an intracellular pool to the cell surface and increases glucose uptake in adipocytes. The GTP-binding protein(s) involved in the GLUT4 translocation has remained to be identified. Using a sensitive and quantitative method to assess the translocation of c-MYC epitope-tagged GLUT4, we obtained evidence that the activation of receptor-coupled G_{q} (neither G_{i} nor G_{s}) triggered GLUT4 translocation in cells, independently of insulin signaling pathway(s). Platelet-activating factor (PAF) induced GLUT4 translocation in the cells expressing the G_{q} and G_{i} coupled PAF receptor, but the translocation was induced even after pretreatment with wortmannin, an islet-activating protein and phorbol 12,13-dibutyrate. Norepinephrine triggered GLUT4 translocation in cells expressing the G_{q} and G_{i} coupled adrenergic receptor; but prostaglandin E2 did not cause GLUT4 translocation in cells expressing the G_{q} coupled EP1 receptor or the G_{i} coupled EP3 receptor. The norepinephrine-stimulated GLUT4 translocation and glucose uptake via G_{i} may possibly contribute to the fuel supply required for thermogenesis in brown adipocytes and for the enhanced contractility in cardiomyocytes, both of which have an abundant endogenous GLUT4.

Glucose transporter type 4 (GLUT4)1 is expressed exclu-

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† The abbreviations used are: GLUT4, glucose transporter type 4; CHO, Chinese hamster ovary; PIP2, phosphatidylinositol; GTPγS, guanosine 5′-O-(3-thiotriphosphate); PIPγS, phosphatidylinositol 4,5-bisphosphate; PAF, 1-O-hexadecyl-2-acyl-sn-glycero-3-phosphocholine; PAFR, 1-O-hexadecyl-2-acyl-sn-glycero-3-phosphocholine receptor; IAP, islet-activating protein; BAPTA-AM, 1,2-bis(O-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid; A2AM, Fura2-acetoxymethyl ester; A2AM, Fura2-acetoxyethyl ester; AR; α_{1A} or α_{1B} or α_{1D} adrenergic receptors; PM, phosphoribol 12-myristate 13-acetate; PDBu, phorbol 12,13-dibutyrate; PLC, phosphoinositide-specific phospholipase C; GDPβS, guanosine 5′-O-(2-thiodiphosphate).
meric GTP-binding proteins. However, the molecular mechanism of insulin-independent GLUT4 translocation was not elucidated.

We report here that Gα-coupled receptors, but not Gγ- or Gβγ-coupled receptors, trigger GLUT4 translocation in an insulin-independent manner in 3T3-L1 adipocytes and CHO cells.

MATERIALS AND METHODS

Cells and Materials—The parent cell lines used in this study were CHO-GLUT4myc, a CHO cell line expressing GLUT4myc, constructed by inserting a human c-MYC epitope (14 amino acids) into the first ectodomain of GLUT4, and 3T3-L1-GLUT4myc, a 3T3-L1 fibroblast line expressing GLUT4myc. The 3T3-L1-GLUT4myc fibroblasts were induced to differentiate into adipocytes as described previously (6). All other reagents were of analytical grade.

Establishment of Stable Cell Lines Expressing Specifically Gα-, Gβγ-, or Gγ-coupled Receptors—The human platelet-activating factor (PAF) receptor (PAFR) cDNA (28), mouse prostaglandin E2 (PGE2) receptors EP3α (29) and EP4 (30) cDNAs, and human adrenergic receptors α1a-, α1b- and α2a-AR cDNAs (31, 32) were subcloned into a mammalian expression vector, pSV2-bsd (35). These plasmids were co-transfected into CHO cells, CHO-GLUT4myc cells, and 3T3-L1-GLUT4myc cells with pSV2-bsd, a blasticidin S deaminase expression plasmid, and selected with blasticidin S hydrochloride (Funakoshi). Several independent clones expressing each receptor were established and designated as follows, CHO-GLUT4myc-PAFR, CHO-GLUT4myc-EP3a, CHO-GLUT4myc-EP4, and CHO-GLUT4myc-α1aAR cells were CHO-GLUT4myc cells stably expressing the PAFR, PGE2 receptors EP3a, EP4, and α1a-AR, respectively. CHO-PAFR and CHO-α1aAR cells were CHO cells stably expressing the PAFR and α1a-AR, respectively. 3T3-L1-GLUT4myc-PAFR and 3T3-L1-GLUT4myc-α1aAR cells were 3T3-L1-GLUT4myc cells stably expressing the PAFR and α1a-AR, respectively.

Cell Surface Anti-c-MYC Antibody Binding Assay (GLUT4myc Translocation Assay)—The CHO-GLUT4myc cells expressing various receptors in 24-well plates were incubated in 500 μl of KRH buffer (6) for 30 min at 37 °C and then with indicated concentrations of ligands for indicated periods at 37 °C. GLUT4myc translocation was measured as described previously (6).

The 3T3-L1-GLUT4myc-PAFR and 3T3-L1-GLUT4myc-α1aAR adipocytes in 24-well plates were incubated in 500 μl of KRH buffer for 20 min at 37 °C and then treated with indicated concentrations of ligands for indicated periods at 37 °C. GLUT4myc translocation was measured after fixation with 2% paraformaldehyde, as described previously (6).

2-Deoxyglucose Uptake Measurement—Cells in 24-well plates were treated with indicated concentrations of ligands for indicated periods at 37 °C. 2-Deoxyglucose uptake was measured as described previously (6).

Down-regulation of Protein Kinase C with Phorbol 12,13-Dibutyrate (PDBu) and Pretreatment with Inlet-activating Protein (IAP) or Wortmannin—Cells were treated with or without PDBu (100 ng/ml) for 24 h at 37 °C in medium containing 5% FBS and with or without IAP (100 ng/ml) for 24 h at 37 °C in medium that abolishes Gα-coupled pathway(s). To inactivate PI 3-kinases, the cells were pretreated with or without indicated concentrations of wortmannin for 20 min at 37 °C.

cAMP Assay—The cAMP levels were measured using Yamasawa radioimmunoassay kits as described (36). [Ca2+]i Measurements—[Ca2+]i, was determined by loading 4 μM Fura2-acetoxymethylester (AM) and using a CAS-110 fluorescence spectrophotometer (Nihon Bunkoh, Tokyo) as described previously (37). [Ca2+]i was calculated based on the formula by Grynkiewicz et al. (38).

RESULTS

PAP-stimulated GLUT4 Translocation—GTPγS or NaF plus AlCl3 treatments triggered the GLUT4myc translocation in both 3T3-L1-GLUT4myc adipocytes and CHO-GLUT4myc cells, as did the endogenous GLUT4 translocation in 3T3-L1 adipocytes (6, 13, 14, 23).

To examine which heterotrimeric GTP-binding protein(s) is responsible for the GTPγS-stimulated GLUT4 translocation, we co-transfected 3T3-L1-GLUT4myc adipocytes and CHO-GLUT4myc cells with platelet-activating factor (PAFR) receptor (PAFR) is thought to transmit the signal via IAP-sensitive (Gγ) and -insensitive (Gγ) heterotrimeric GTP-binding proteins (28, 37, 39). As shown in Fig. 1, 3T3-L1-GLUT4myc adipocytes stably expressing PAF receptors showed that PAF-stimulated GLUT4myc translocation in a dose- and time-dependent manner, whereas the parent 3T3-L1-GLUT4myc adipocytes did not respond to any concentrations of PAF (Fig. 1, A and B). PAF treatment also increased the rate of glucose uptake in 3T3-L1-GLUT4myc-PAFR adipocytes, in proportion to GLUT4myc translocation (Fig. 1, A and C). Almost the same dose- and time-dependent GLUT4myc translocation and glucose uptake in response to PAF were observed in CHO-GLUT4myc-PAFR cells but not in the parent CHO-GLUT4myc cells (Fig. 1, D–F).

The CHO-GLUT4myc cells have relatively large amounts of endogenous GLUT1 compared with GLUT4myc, and the GLUT1 might affect the rate of glucose uptake. The translocated GLUT4myc in response to PAF increased the rate of glucose uptake in CHO-PAFR-GLUT4myc cells, compared with CHO-PAFR cells (Table I). The enhanced glucose uptake in CHO-PAFR cells was attributed to GLUT1 translocation, because PAF-stimulated GLUT1myc translocation in CHO-
The CHO-PAFR cells and CHO-PAFR-GLUT4myc cells were estimated to have almost the same amount of PAF receptors and were treated with $2 \times 10^{-8}$ M PAF or buffer alone (−) for 10 min. 2-Deoxyglucose uptake was measured, as described under "Materials and Methods." Values are means ± S.E. of three determinations. Asterisk shows significant difference ($p < 0.005$, Student's t test) from the PAF-stimulated increment of CHO-PAFR cells.

### Table I

**2-Deoxyglucose uptake (pmol/min/2 × 10⁶ cells) in CHO-PAFR cells and CHO-PAFR-GLUT4myc cells**

|       | CHO-PAFR     | CHO-PAFR-GLUT4myc |
|-------|--------------|-------------------|
| −     | 32.9 ± 1.4   | 129.7 ± 5.2       |
| PAF   | 49.0 ± 2.7   | 164.6 ± 8.3       |

Increment of CHO-PAFR-GLUT4myc translocations were calculated by subtracting the (−) values from PAF values.

Next, we examined the effect of wortmannin on the PAF-stimulated GLUT4 translocation in 3T3-L1-GLUT4myc-PAFR adipocytes and CHO-GLUT4myc-PAFR cells; wortmannin completely inhibited the insulin-stimulated GLUT4 translocation and glucose uptake in 3T3-L1 adipocytes and CHO-GLUT4myc cells, by abolishing phosphatidylinositol (PI) 3-kinase activity (7, 12). As shown in Figs. 2A and 3A, wortmannin inhibited the insulin-stimulated GLUT4myc translocation dose dependently and abolished the translocation at $10^{-7}$ M in both lines of cells. This inhibitory effect on the insulin-stimulated translocation was closely related to that on PI 3-kinase (p85/p110 heterodimer type) activity. However, the PAF-induced GLUT4myc translocations were 3.8-fold in 3T3-L1-GLUT4myc-PAFR adipocytes and 9.4-fold in CHO-GLUT4myc-PAFR cells, even in the presence of $10^{-6}$ M wortmannin. Therefore, the PAF-stimulated GLUT4myc translocation pathway was independent of PI 3-kinases including p110γ (40).

Some PAF-stimulated physiological responses are mediated through Gq, that is sensitive to IAP (39). The PAF-stimulated GLUT4myc translocation of 3T3-L1-GLUT4myc-PAFR adipocytes was partially inhibited but remained to a certain extent (from 4.4-fold increase to 2.4-fold increase) in CHO-PAFR cells. This inhibitory effect on the insulin-stimulated GLUT4myc translocation was closely related to that on PI 3-kinase (p85/p110 heterodimer type) activity. However, the PAF-induced GLUT4myc translocations were 3.8-fold in 3T3-L1-GLUT4myc-PAFR adipocytes and 9.4-fold in CHO-GLUT4myc-PAFR cells, even in the presence of $10^{-6}$ M wortmannin. Therefore, the PAF-stimulated GLUT4myc translocation was partially inhibited but remained to a certain extent (from 4.4-fold increase to 2.4-fold increase) in CHO-PAFR cells. This inhibitory effect on the insulin-stimulated GLUT4myc translocation was closely related to that on PI 3-kinase (p85/p110 heterodimer type) activity. However, the PAF-induced GLUT4myc translocations were 3.8-fold in 3T3-L1-GLUT4myc-PAFR adipocytes and 9.4-fold in CHO-GLUT4myc-PAFR cells, even in the presence of $10^{-6}$ M wortmannin. Therefore, the PAF-stimulated GLUT4myc translocation pathway was independent of PI 3-kinases including p110γ (40).

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Some PAF-stimulated physiological responses are mediated through Gq, that is sensitive to IAP (39). The PAF-stimulated GLUT4myc translocation of 3T3-L1-GLUT4myc-PAFR adipocytes was partially inhibited but remained to a certain extent (from 4.4-fold increase to 2.4-fold increase) (Fig. 2B) (see “Discussion”). On the other hand, the PAF-stimulated GLUT4myc translocation of CHO-GLUT4myc-PAFR cells was not affected significantly by treatment of 100 ng/ml IAP (Fig. 3B), a treatment that abolished the Gq coupling (39). The IAP-insensitive pathway observed in the both cell lines is thought to be mediated by Gq, which activates phosphoinositide-specific phospholipase C β (PLCβ) to hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP2) (41, 42). The breakdown products, inositol 1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol, activate a Ca²⁺ channel and protein kinase C, respectively (43, 44). Therefore, we asked whether the IAP-insensitive GLUT4myc translocation by PAF is due to protein kinase C activation or to [Ca²⁺]i increase.

PM demonstrates GLUT4 translocation by activating protein kinase C in 3T3-L1 adipocytes and CHO-GLUT4myc cells (6, 22, 45, 46). The PMA-stimulated GLUT4myc translocations in 3T3-L1-GLUT4myc-PAFR adipocytes (1.3-fold increase) and CHO-GLUT4myc-PAFR cells (4.2-fold increase) were abolished by PDBu pretreatment that down-regulates protein kinase C, albeit the extent of translocation differing between the two lines (Figs. 2C and 3C). However, the same PDBu pretreatment had no apparent effects on the PAF-stimulated GLUT4myc translocation, in the both lines. In addition, PAF triggered GLUT4myc translocation (1.9–3.3-fold) and the resultant glucose uptake (2.3-fold) in both lines, even after simultaneous IAP, PDBu, and wortmannin treatment (Figs. 2, D and E, and 3D).

To examine the effects of [Ca²⁺]i, on the PAF-stimulated GLUT4myc translocation, we used two different Ca²⁺ ionophores. Ionomycin (1 μM) and PAF (2 × 10⁻⁸ M) treatments elevated [Ca²⁺] in CHO-GLUT4myc-PAFR cells, from basal levels (lower than 100 nM) up to about 900 and 600 nM, respectively, as in Fig. 4E. However, neither ionomycin (1 μM) nor A23187 (1 μM) led to GLUT4myc translocation in 3T3-L1-GLUT4myc-PAFR adipocytes and CHO-GLUT4myc-PAFR cells, whereas PAF evoked GLUT4myc translocation (Fig. 4, A and C). Depletion of [Ca²⁺]i, by 1,2-bis(O-aminophenyl)
Abundant receptors specifically coupled to Gq, Gi, and Gs have been identified (47). To confirm that GLUT4 translocation is triggered by Gq activation, we used the well-characterized α1b-adrenergic receptor, prostaglandin E2 receptors EP3α, and EP4 as Gq-, Gi-, and Gs-coupled receptors, respectively (29, 30, 33, 34). Prostaglandin E2 did not translocate GLUT4myc in CHO-GLUT4myc cells expressing the prostaglandin E2 receptor EP4 (CHO-GLUT4myc-EP4) (Fig. 5A), although it did stimulate cAMP formation in the same cells by activating Gs in a dose-dependent manner (Fig. 5B). Prostaglandin E2 inhibited the forskolin-stimulated cAMP accumulation dose-dependently by coupling Gs in CHO-GLUT4myc cells expressing the prostaglandin E2 receptor EP3α (CHO-GLUT4myc-EP3α) (Fig. 5D), but prostaglandin E2 did not cause GLUT4myc translocation in the same cells (Fig. 5C). In contrast, norepinephrine stimulated GLUT4myc translocation by 4.3-4.4-fold in CHO-GLUT4myc cells stably expressing α1b-adrenergic receptors (CHO-GLUT4myc-α1bAR) (Fig. 5E) and raised [Ca2+]i (Fig. 5F) presumably by coupling Gq.

As shown in Fig. 6, A and B, norepinephrine stimulated GLUT4myc translocation in a dose- and time-dependent manner in CHO-GLUT4myc-α1bAR cells but not in the parent CHO-GLUT4myc cells. Norepinephrine stimulated glucose uptake in CHO-GLUT4myc-α1bAR in almost the same dose-dependent manner as GLUT4myc translocation (Fig. 6C). The fold increase of glucose uptake, however, is not in proportion to that of GLUT4myc translocation, because the norepinephrine-stimulated glucose uptake resulted from the translocations of both the endogenous GLUT1 and exogenous GLUT4myc in CHO cells. The GLUT4myc translocated to the cell surface in response to norepinephrine took up more glucose in CHO-α1bAR-GLUT4myc cells, compared with CHO-α1bAR cells (Table II). The enhanced glucose uptake in the CHO-α1bAR cells was probably due to GLUT1 translocation, because norepinephrine stimulated GLUT1myc translocation in CHO-

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**Fig. 3. Effects of wortmannin, IAP, and PDBu on the GLUT4myc translocation in CHO-GLUT4myc-PAFR cells.** A. CHO-GLUT4myc-PAFR cells were stimulated with 2 × 10⁻³ M PAF (●, 10⁻³ M insulin (▲), or buffer alone (○)) for 10 min at 37°C after pretreatment with the indicated concentrations of wortmannin for 20 min at 37°C. GLUT4myc translocations are shown. B. CHO-GLUT4myc-PAFR cells were treated with 2 × 10⁻³ M PAF (solid bar), 10⁻³ M insulin (shaded bar), or buffer alone (open bar) for 10 min at 37°C after pretreatment with 100 ng/ml IAP or medium alone (—) for 24 h at 37°C. The GLUT4myc translocations are shown. C. CHO-GLUT4myc-PAFR cells were treated with 2 × 10⁻³ M PAF (solid bar), 10⁻³ M insulin (shaded bar), 10⁻⁶ M PMA (hatched bar), or buffer alone (open bar) for 10 min at 37°C after pretreatment with 100 ng/ml PDBu or medium alone (—) for 24 h at 37°C. The GLUT4myc translocations are shown. Values represent means ± S.E. for three separate experiments done in triplicate.

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**Fig. 4. Effects of Ca²⁺ ionophors (ionomycin and A23187) and Ca³⁺ chelator (BAPTA-AM) on the GLUT4myc translocations in 3T3-L1-GLUT4myc-PAFR adipocytes and CHO-GLUT4myc-PAFR cells.** A and C, 3T3-L1-GLUT4myc-PAFR adipocytes (A) and CHO-GLUT4myc-PAFR cells (C) were incubated with 1 μM ionomycin (stippled bar), 1 μM A23187 (hatched bar), or 2 × 10⁻³ M PAF (solid bar), or buffer alone (open bar) for 10 min at 37°C. The GLUT4myc translocations are shown. B, the parent cells (3T3-L1-GLUT4myc adipocytes) (○) and those expressing PAFR (3T3-L1-GLUT4myc-PAFR adipocytes) (●) were pretreated with various concentrations of BAPTA-AM for 20 min at 37°C and stimulated with 2 × 10⁻³ M PAF for 10 min at 37°C. The GLUT4myc translocations are shown. D, CHO-GLUT4myc cells (○) and CHO-GLUT4myc-PAFR cells (●) were incubated with 2 × 10⁻³ M PAF for 10 min at 37°C after pretreatment with various concentrations of BAPTA-AM for 20 min at 37°C. The GLUT4myc translocations are shown. E, CHO-GLUT4myc PAF cells were loaded with Fura2-AM in the absence or presence of 20 μM BAPTA-AM and then challenged with 1 μM ionomycin or 2 × 10⁻³ M PAF at time 0 (indicated with arrow). [Ca²⁺]i was monitored using CAF-110, as described under “Materials and Methods.” The panel shows typical traces from three different experiments (for each stimulation).
translocation was reduced by guanosine 5’-O-(2-thiodiphosphate) (GDPβS) pretreatment, while norepinephrine-activated Gα accelerates PIP2 breakdown via PLCβ. Therefore, the norepinephrine-stimulated GLUT4 translocation was thought to be mediated by Gα directly or through unknown pathways(s) after Gα activation but probably not to be a secondary phenomenon by PIP2 breakdown.

Finally, we examined whether norepinephrine would stimulate glucose uptake in 3T3-L1 adipocytes, because 3T3-L1 adipocytes carry endogenous GLUT4 and adrenergic receptors. Norepinephrine stimulated glucose uptake in a dose- and time-dependent manner in 3T3-L1 adipocytes (Fig. 7, A and B). The glucose uptake stimulated by norepinephrine was not substan-
Gq-mediated GLUT4 Translocation

2-Deoxyglucose uptake (pmol/min/10^6 cells) in CHO-α1AR cells and CHO-α1AR-GLUT4myc cells

| CHO-α1AR | CHO-α1AR-GLUT4myc |
|----------|-----------------|
| NE       | 30.1 ± 2.0      | 75.9 ± 5.4 |
| Increment | 19.3 ± 2.3      | 34.1 ± 5.0 |

a CHO-α1AR cells; CHO cells expressing α1a-adrenergic receptors.  
b CHO-α1AR-GLUT4myc cells; CHO cells expressing α1a-adrenergic receptors and a large amount of GLUT4myc.

c Increments were calculated by subtracting the (−) values from norepinephrine values.

Discussed Heterotrimeric GTP-binding Proteins and (Ca^{2+})—The heterotrimeric GTP-binding proteins are associated with signal transduction from cell surface receptors (48). α-Subunits of the GTP-binding proteins have been classified into four, Gα, Gβ, Gγ, and G12, based on amino acid sequence homology (47). We find that Gα class can mediate GLUT4 translocation and the resultant glucose uptake, because three independent Gα-coupled receptors triggered GLUT4 translocation in 3T3-L1 adipocytes and CHO cells. The use of CHO cells to study the molecular mechanisms of GLUT4 translocation has been controversial (49). In CHO cells, a relatively smaller amount of intracellular GLUT4myc translocation was observed in CHO cells, even after insulin treatment (50). The high sensitivity of our method made feasible the detection of GLUT4 on the cell surface, following insulin-induced translocation in CHO cells. The machinery for insulin-stimulated GLUT4 translocation in CHO cells is not completely identical with that in 3T3-L1 adipocytes, but CHO cells seem to possess a basic machinery for insulin-stimulated translocation of exogenously expressed GLUT4, which mimics that of adipocytes (6, 13, 14, 23, 45, 46, 50, 51).

Molecular mechanisms involved in GLUT4 translocation, in addition to 3T3-L1 adipocytes.
pathway(s), but not by an insulin-dependent pathway(s). Among $G_i$, $G_{12}$, $G_{14}$, and $G_{15}$ (or $G_{16}$) isotypes have different activities to stimulate PLC $\beta$ subtypes (47, 52). We have not identified which isotype of $G_i$ class is important for the GLUT4 translocation. $G_{16}$-deficient transgenic mice have been generated, and insulin-stimulated glucose uptake was impaired in these mice (53). An IAP-sensitive pathway involved in PAF-stimulated GLUT4 translocation in 3T3-L1-GLUT4myc adipocytes (Fig. 2B) may be related to $G_{16}$. Of course, the involvement of $G_{12}$ class or $\beta \gamma$ subunits in the GLUT4 translocation cannot be excluded.

The GLUT4 translocations triggered by $G_{12}$-coupled receptors were not inhibited by down-regulating protein kinase C (Figs. 2, 3, and 6), and $Ca^{2+}$ mobilization with ionophores did not cause GLUT4 translocation (Fig. 4, A, C, and E). Therefore, GLUT4 translocation does not seem to be a secondary phenomenon following PIP$_2$ breakdown, as evoked by $G_i$ activation. However, the GLUT4 translocation required a certain amount of $[Ca^{2+}]_i$, because 20–40 $\mu M$ BAPTA-AM inhibited the GLUT4 translocation and $Ca^{2+}$ mobilization (Fig. 4, B, D, and E). It is considered that $[Ca^{2+}]_i$, and some $Ca^{2+}$-binding proteins play important roles in membrane traffic (54). Considering that the GLUT4 translocation is one of the regulated membrane traffic systems, it seems reasonable that GLUT4 translocation requires a certain amount of $[Ca^{2+}]_i$. However, the possibility that BAPTA-AM affects the GLUT4 translocation by effects other than chelating $Ca^{2+}$ would need to be ruled out.

**Physiological Aspects of PAF-stimulated GLUT4 Translocation**—PAF receptors are expressed mainly in hematopoietic cells, and GLUT1 (but not GLUT4) is expressed in hematopoietic cells. PAF induces GLUT1 translocation and glucose uptake by about 1.5–2-fold in CHO-GLUT1myc-PAFR cells. PAF exerts reactions such as chemotaxis, phagocytosis, and smooth muscle contraction (37) that require fuel for ATP. To supply the fuel, GLUT1 may translocate to the cell surface and take up glucose via $G_i$ coupling in response to PAF. Numerous receptors couple with $G_i$, and most ligand-stimulated reactions require an energy supply. Therefore, our evidence provides new insights into $G_i$ functions.

**Physiological Aspects of Norepinephrine-stimulated GLUT4 Translocation**—The finding that norepinephrine stimulates glucose uptake via $G_i$ might seem inconsistent with data that catecholamines usually antagonize many insulin actions, especially in glucose and lipid metabolism in adipocytes and hepatocytes. However, norepinephrine did not antagonize the insulin-stimulated GLUT4 translocation in CHO-GLUT4myc-$\alpha_{1b}$AR cells, and the simultaneous stimulation of norepinephrine and insulin worked additively as for GLUT4 translocation (4). CHO cells are not physiologically adrenergic target cells. However, norepinephrine treatment or physical exercise stimulates glucose uptake by translocating GLUT4 in cardiomyocytes that have a large amount of endogenous GLUT4, independently of insulin (24, 25); they reported that norepinephrine-stimulated glucose uptake was mediated by $\alpha_1$-adrenergic receptor but not by $\alpha_2$- or $\beta$-adrenergic receptors. The $\alpha_1$-adrenergic receptors are classified into at least three types, $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$ (31–34), and all the three types of receptors couple to $G_i$ class (52). This is consistent with our results (Figs. 5–7). In cardiomyocytes not only fatty acid but also endogenous glycogen and glucose supplied from extracellular fluid are consumed to generate ATP during physical exercises (55). Interestingly, dysfunction and hypertrophy of cardiomyocytes were observed in GLUT4-knockout mice (56). They considered that GLUT4 played an important role in cardiomyocytes and that a low energy supply to cardiomyocytes owing to deficient GLUT4 might lead to dysfunction and hypertrophy. Therefore, it seems reasonable that norepinephrine translocates GLUT4 via $G_i$ coupling and takes up glucose to supply the fuel for ATP during physical exercises, independently of insulin signaling pathway(s).

Glucose uptake into brown adipocytes is enhanced directly by norepinephrine released from sympathetic nerves (26, 27). As the enhanced glucose uptake occurs without increase in plasma insulin levels and is not inhibited by wortmannin, a PI 3-kinase inhibitor, the phenomenon is probably independent of insulin actions. This is consistent with our findings with 3T3-L1 adipocytes (Fig. 7). However, there is controversy regarding GLUT4 translocation by subcellular fractionation, despite the enhanced glucose uptake (26, 27). The discrepancy may relate to different sensitivities of tests to assess GLUT4 translocation or by different cell origins. We examined norepinephrine-stimulated GLUT4myc translocation (Fig. 7, E and F) and glucose uptake (Fig. 7G) in 3T3-L1-GLUT4myc adipocytes expressing $\alpha_{1a}$-AR, because $\alpha_{1a}$-AR also activated $G_i$ and translocated GLUT4myc in CHO-GLUT4myc-$\alpha_{1a}$AR cells, like $\alpha_{1b}$-AR. As shown in Fig. 7E, the norepinephrine treatment increased the anti-c-MYC antibody binding on the cell surface in 3T3-L1 adipocytes that do not express exogenous GLUT4myc. The GLUT4myc-unrelated increase of the antibody binding in 3T3-L1 adipocytes was only observed by the norepinephrine treatment but not by the treatments with insulin, platelet-derived growth factor, and epidermal growth factor (6, 8, 9). In CHO cells, the GLUT4myc-unrelated increase of the antibody binding was not observed by the treatment of norepinephrine. We have found an approximate 120-kDa protein in 3T3-L1 and 3T3-L1-GLUT4myc-$\alpha_{1a}$AR adipocytes, which is different from GLUT4myc protein and cross-reacts with the anti-c-MYC antibody (4). The adipocyte-specific 120-kDa protein may translocate to the cell surface by the norepinephrine treatment and increase the surface antibody binding, in addition to GLUT4myc. Therefore, we assessed the norepinephrine-stimulated GLUT4myc translocation in 3T3-L1-GLUT4myc-$\alpha_{1a}$-AR adipocytes by subtracting each surface antibody binding of 3T3-L1 adipocytes in the absence and presence of norepinephrine (Fig. 7F). Our results were consistent with those in brown adipocytes reported by Omatsu-Kanbe and Kitasato (26). Brown adipocytes play a key role in thermogenesis especially in newborn animals. Norepinephrine-induced glucose uptake may thus contribute to the thermogenesis in brown adipocytes. Another possible target of norepinephrine is capillary endothelial cells. Endothelial cells have diverse characteristics, depending on organs or tissues. Endothelial cells of muscle and fat have abundant GLUT4, and the GLUT4 translocated to the luminal plasma membrane was thought to help take more glucose into the muscle and fat, in response to insulin (57). Considering that endothelial cells are dominated by the autonomic nervous system, norepinephrine may stimulate the GLUT4 translocation.

Contractile stimuli were seen to enhance the glucose uptake of skeletal myocytes, independently of insulin (58–61), but the exact mechanism has not been identified. The released bradykinin by muscle contraction (62) may via $G_i$ activate bradykinin receptors (63, 64) and the translocated GLUT4 may help to take up glucose to generate ATP for repeated muscle contractions.

As we find that three independent $G_i$-coupled receptors trigger GLUT4 translocation to take up glucose, new insights into $G_i$ function have been forthcoming.

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1. Stremmel, W., and S. Ohara for reading the manuscript.
G,,-coupled Receptors Transmit the Signal for GLUT4 Translocation via an Insulin-independent Pathway

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