Vol. 189, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1992 Pages 572-580

STIMULATION OF GLUCOSE TRANSPORT BY GUANINE NUCLEOTIDES IN PERMEABILIZED RAT ADIPOCYTES

Yoich Suzuki, Hiroshi Shibata, Shuji Inoue* and Itaru Kojima

Cell Biology Research Unit Institute of Endocrinology Gunma University, Japan

***Third Department of Internal Medicine Yokohama City University, School of Medicine, Japan**

Received October 19, 1992

Effects of guanine nucleotides on glucose transport were studied in permeabilized rat epididymal fat cells. GTPγS and $G_{pp}(NH)_p$, but not $A_{pp}(NH)_p$, stimulated 3-0-methylglucose transport. Effect of GTPγS was dose-dependent, being detectable at 0.1 mM, and 1.0 mM GTPγS stimulated glucose transport to the same extent as insulin. GTPγS (0.3 mM) enhanced insulin-stimulated glucose transport while 1 mM GTPγS did not affect insulin-mediated transport. GDPβS had no effect on glucose transport by itself but rather enhanced insulin action. NaF, which is known to activate trimeric G proteins, increased glucose transport to the same extent as insulin. Likewise, mastoparan augmented glucose transport. These results indicate that a certain type of trimeric G protein(s) is involved in the regulation of glucose transport.

Insulin elicits diverse metabolic effects in variety of target cells. One of the most important actions of insulin is stimulation of glucose transport in target cells such as muscle and adipose tissue cells. In those cells, insulin induces a marked increase in the rate of glucose transport. Evidence obtained so far indicates that insulin induces translocation of the insulin-sensitive glucose transporter, GLUT 4, and increases the number of GLUT 4 in plasma membrane (1,2). Insulin also enhances the activity of individual glucose transporter. Morphological studies have confirmed that insulin induces translocation of GLUT 4 in adipocytes (3,4). Thus, upon stimulation by insulin, GLUT 4 is recruited from endosome to plasma membrane. Although kinetics of GLUT 4 in insulin-stimulated cells has been well characterized (1-6), the mechanism by which insulin induces translocation of GLUT 4 is poorly understood. In this regard, Valdini et al. (7) have recently demonstrated that GTPγS, a non-hydrolyzable

0006-291X/92 $4.00

analogue of GTP, induces translocation of GLUT 4 in permeabilized adipocytes. Their report raises a possibility that a certain class of G protein may be involved in translocation of glucose transporter. Yet, their study does not address clearly whether or not the G protein is involved in insulin-mediated translocation of glucose transporter.

The present study was conducted to further characterize the effect of guanine nucleotides on glucose transport in rat adipocytes. To this end, we measured changes in glucose transport in electrically permeabilized adipocytes (8,9). This method enables us quantitative analysis of changes in glucose transport in adipocytes. Our results suggest that a certain type of trimeric G protein may be involved in GTPγS-mediated increase in glucose transport in adipocytes.

Materials and Methods

Materials

3-0-[^{3}H]Methy-l-D-glucose was purchased from Dupont New England Nuclear (Boston, MA, U.S.A.). 3-0-Methyl-D-glucose, bovine serum albumin (fraction V), phloretin, sodium pyruvate were obtained from Sigma (St.Louis, MO, U.S.A.). Mastoparan was from Penninsula Laboratories.

Cell Preparation

Isolated adipocytes were prepared by the collagenase method (10) from epididymal adipose tissue of Sprague-Dowley rats. Unless otherwise stated, isolated cells were incubated at 37°C in buffer A [Krebs-Henseleit Hepes (25 mM) buffer (pH7.4), supplemented with 20 mg/ml bovine serum albumin (fraction V) and 3 mM sodium pyruvate] (8). The cells to be electroporated were washed and suspended in buffer designated as buffer X (4.74 mM NaCl, 118.0 mM KCl, 0.38 mM $CaCl_2$, 1.00 mM EGTA, 1.19 mM $MaSO_4$, 1.19 mM KH_2PO_4, 20 mg/ml bovine serum albumin, 3 mM sodium pyruvate, and 25.0 mM Hepes/KOH, pH7.4). The electroporation was carried out using a Gene Pulser (Bio-Rad) which was set at 25 μF and 0.8 kv/cuvette (or 2 KV/cm) as described previously (8). The cells to be electroporated were suspended in buffer X (see above) at a concentration 20 % (v/v) and electroporated four times at room temperature unless otherwise specified. After the second treatment, the cell suspension was shaked gently and then electroporated two times more (8).

Measurement of Glucose Transport Activity

The glucose transport activity was assessed by measuring cellular uptake of 3-0-methylglucose by modification (8) of the oil-floatation method.(10). The transport reaction was terminated by the addition of 300 μl of 1 mM phloretin in 154 mM NaCl that contained 0.0125 % bovine serum albumin and 1 % ethanol (initial solvent for phloretin).

Results

Effects of GTPγS on Glucose Transport in Intact Cells and Electrically Permeabilized Cells

Permeabilized adipocytes (8) were prepared as described in Methods. Both intact and permeabilized cells were divided into a number of aliquots and incubated at 37°C for 10 min in the presence and absence of 0.3 mM GTPγS. Insulin (10 nM in intact cells and 100 nM in permeabilized cells, respectively) was then added and the cells were further incubated for 10 min. After the incubation, glucose transport activity was assessed by measuring the rate of 3-0-methyl-D-glucose uptake. Our previous study indicates that 100 nM insulin induces the maximal effect in permeabilized cells while 10 nM insulin elicits the maximal effect in intact cells (9). Figure 1 illustrates the effects of GTPγS on glucose transport activity in intact cells and in permeabilized cells. In intact cells, insulin induced approximately 7-fold increase in glucose transport. GTPγS (0.3 mM) had no effect on either basal or insulin-stimulated glucose transport. In permeabilized cells, the effect of insulin was also observed as in intact cells even though the magnitude is smaller. GTPγS stimulated the basal glucose transport activity by approximately 3-fold in permeabilized cells. GTPγS also enhanced insulin-stimulated glucose transport. The effect of GTPγS plus insulin was

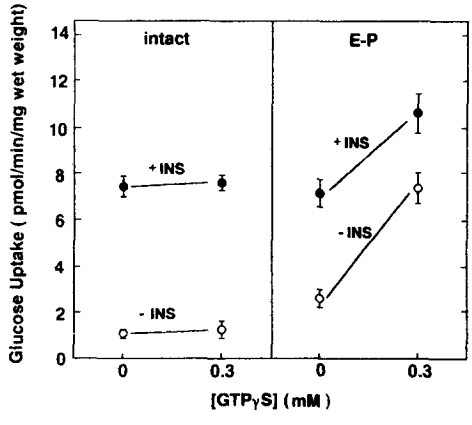

Figure 1. Effect of GTPγS on Glucose Transport in Intact and Permeabilized Adipocytes.
Intact or electrically-permeabilized (E-P) cells were incubated with or without 0.3 mM GTP-γS in the presence and absence of insulin. Glucose transport was measured as described in Methods. Values are the mean±S.E. for four determinations and the representative of three experiments.

Table 1. Effect of Nucleotides on Glucose Transport in Permeabilized Adipocytes

Addition	Glucose Uptake (pmol/min/mg wet weight)
none	2.8±0.19 (10)
GTPγS(1 mM)	8.4±0.60 (8)
$G_{pp}(NH)_p$ (1 mM)	5.7±0.56 (8)
$A_{pp}(NH)_p$ (1 mM)	3.0±0.45 (5)

Glucose uptake was measured in permeabilized adipocytes in the presence and absence of various nucleotides. Values are the mean ±S.E. of number of determinations shown in the parentheses from three independent experiments.

greater than the maximal effect of insulin. As shown in Table 1, the stimulatory effect was specific to guanine nucleotides since $G_{pp}(HH)_p$ had similar stimulatory effect as GTPγS whereas $A_{pp}(NH)_p$ did not affect glucose transport.

Dose Response Relationship for the Effect of GTPγS on Glucose Transport in Electrically Permeabilized Cells

Figure 2 depicts dose-response relationship for GTPγS-induced glucose uptake in permeabilized cells. In the absence of insulin, GTPγS evoked a dose

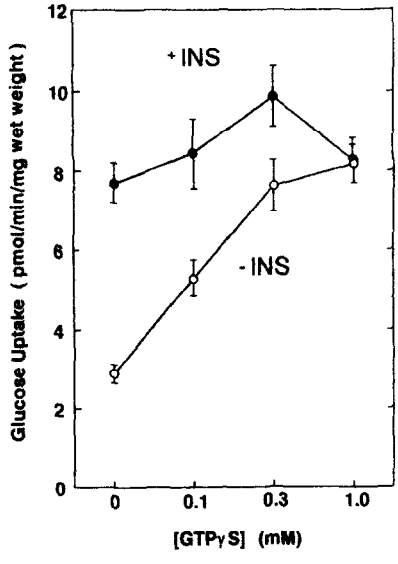

Figure 2. Dose Response Relationship for the Effect of GTPγS on Glucose Transport in Permeabilized Adipocytes.
Electrically permeabilized adipocytes were incubated with various concentrations of GTP-γS in the presence and absence of 100 nM insulin. Glucose transport was measured as described in Methods. Values are the mean±S.E. for three determinations and the representative of four experiments.

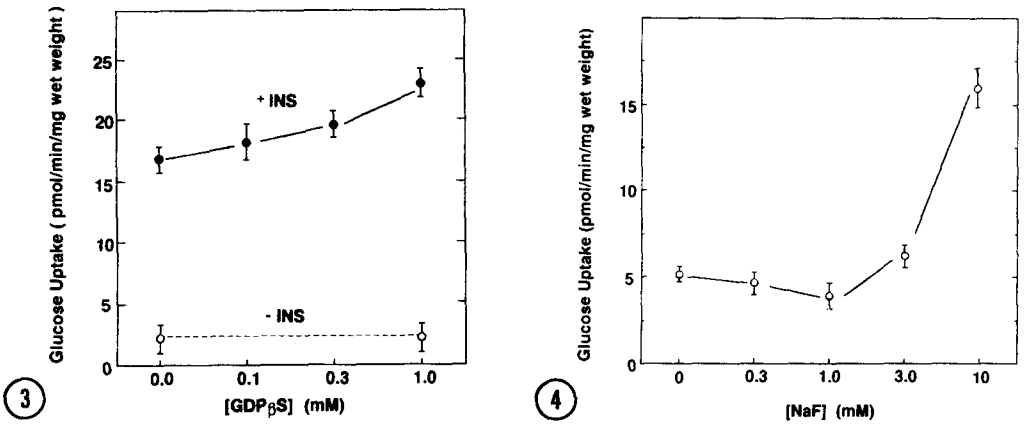

Figure 3. Effect of GDPβS on Insulin-stimulated Glucose Transport in Permeabilized Adipocytes.
Electrically permeabilized cells were incubated with various concentrations of GDPβS in the presence and absence of 100 nM insulin. Values are the mean ±S.E. for four determinations and the representative of six experiments.

Figure 4. Effect of NaF on Glucose Transport in Permeabilized Adipocytes.
Electrically permeabilized cells were incubated with various concentrations of NaF and glucose transport was measured. Values are the mean ±S.E. for four determinations and the representative of four experiments.

dependent increase in glucose transport. The effect was almost saturated at a concentration of 1.0 mM. At this concentration, GTPγS augmented glucose transport to the same extent as insulin. In the presence of insulin, GTPγS elicited a small but significant stimulatory effect at a concentration of 0.3 mM. The effect of combination of insulin and 0.3 mM GTPγS was greater than the maximal effect of GTPγS. However, further stimulatory effect was not observed at a GTPγS concentration of 1.0 mM.

Effect of GDPβS on Glucose Transport in Electrically Permeabilized Cells

Figure 3 depicts dose-response relationship for the effect of GDPβS on glucose transport in permeabilized cells. In the absence of insulin, GDPβS had essentially no effect on glucose transport. In the presence of insulin, rather than inhibition of insulin-mediated glucose transport, GDPβS had a small stimulatory effect: 1.0 mM GDPβS enhanced insulin-mediated glucose transport by 140 %

Effect of NaF and Mastoparan on Glucose Transport in Permeabilized Cells

To further examine an involvement of G protein in glucose transport, we determined the effect of NaF on glucose transport in permeabilized cells. As shown in Figure 4, NaF augmented glucose transport at a concentration of 3.0

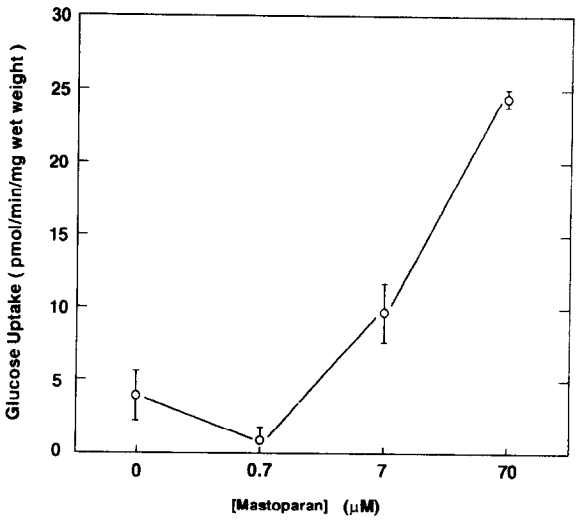

Figure 5. Effect of Mastoparan on Glucose Transport in Permeabilized Adipocytes. Electrically permeabilized cells were incubated with various concentrations of mastoparan and glucose transport was measured. Values are the mean ±S.E. for four determinations and the representative of four experiments.

mM. At 10 mM, NaF induced a three fold stimulation of glucose transport. The stimulatory effect of NaF was probably due to AlF_3 since addition of desferroxamine, a chelator of Al^{3+}, completely blocked NaF-induced stimulation of glucose transport (data not shown). It is to be noted that NaF also stimulated glucose transport in intact cells. However, magnitude of the response was smaller than that in permeabilized cells.

To further examine an involvement of G protein, we employed mastoparan, a bee venom which activates certain types of G proteins (12). As demonstrated in Figure 5, 0.7 μM mastoparan exerted an inhibition while higher concentration of mastoparan increased glucose transport, the increase of which being dose-dependent. At 70 μM, mastoparan induced six fold stimulation of glucose transport.

Discussion

In the present study, we examined the effect of guanine nucleotides on glucose transport in rat adipocytes by measuring glucose uptake in electrically permeabilized cells. This method enables us quantitative analysis of the effect of guanine nucleotides on glucose transport. Our results confirm and extend the report by Valdini et al. (7) by showing that GTPγS actually increases glucose

transport in permeabilized adipocytes. Our results further indicate that the stimulatory effect is specific to guanosine triphosphate and its non-hydrolyzable analogues, GTPγS and $G_{pp}(NH)_p$. It is therefore plausible that a certain type(s) of G protein is involved in the stimulation of glucose transport. Supports for this concept come from observations that both NaF and mastoparan, agents known to activate G proteins (9,10), are capable of stimulating glucose transport in adipocytes. Of particular importance is that NaF augments glucose transport in adipocytes. NaF activates trimeric G proteins without affecting low molecular weight G proteins including p21 ras, ADP ribosylation factor and rab protein (13). Existence of G various proteins in rat adipocytes was reported (14-16). In particular, low molecular weight G protein is shown to be localized in vesicles containing GLUT 4 (16). Our present results suggest that a certain type of trimeric G protein is involved in NaF-induced and, presumably, GTP-induced stimulation of glucose transport.

Recent studies indicate that G proteins are involved in some actions of insulin (17-19). Also, certain actions of insulin are blocked by pertussis toxin (20,21). With regard to glucose transport, however, Honnor et al. have shown that pertussis toxin does not affect glucose transport induced by insulin in adipocytes (22). As shown in Figure 3, GDPβS does not block insulin-mediated glucose transport. A simple interpretation is that G protein is involved in GTPγS-stimulated but not insulin-stimulated glucose tranport. Nevertheless, a recent morphological study by Robinson et al. (23) indicates that both insulin and GTPγS induce translocation of GLUT 4 in a similar manner. It is therefore still possible that both GTPγS and insulin induce translocation of GLUT 4 through the same mechanism. Results shown in Figure 3 indicate that GDPβS rather enhances insulin-stimulated glucose transport. In addition, insulin-mediated glucose transport in the presence of 0.3 mM GTPγS is higher than in the presence of 1 mM GTPγS. Therefore, another possibility exists that a second type of G protein, which is inhibitory on glucose transport is involved in the action of insulin. This concept is in agreement with a report by Schurmann et al. (24) that GTP and GTPγS reduced insulin-stimulated glucose transport activity in reconstituted adipocyte membrane fraction. Taken together, it is possible that

multiple types of G proteins are involved in insulin-mediated stimulation of glucose transport.

Acknowledgments

The present study was supported in part by Grants-in-Aid for Scientific Research from The Ministry of Education, Science and Culture of Japan and a grant from The Naito Foundation. The authors are grateful to Ms. Romi Nobusawa for secretarial assistance during the preparation of the manuscript.

References

1) Suzuki,K. and Kono,T. (1980) Proc.Natl.Acad.Sci.USA. 77,2542-2545.
2) Cushman,S.V. and Wardzala,L.J. (1980) J.Biol.Chem. 255,4758-4762.
3) Slot,J.W.,Geuze,H.J.,Gigengack,S.,Leinhard,G.E. and James,D.E. (1991) J.Cell.Biol. 113,123-135.
4) Smith,R.M.,Charron,M.J.,Shah,N.,Lodish,H.F. and Jarrett,L. (1991) Proc.Natl.Acad.Sci.USA. 88,6893-6897.
5) Holman,G.D.,Kozka,I.J.,Clark,A.E.,Flower,C.J.,Saltis,J.,Habberfield,A.D., Simpson,I.A. and Cushman,S.W. (1990) J.Biol.Chem. 265,18172-18179.
6) Harrison,S.A.,Clancy,B.M.,Pessino,A. and Czech,M.P.(1992) J.Biol.Chem. 267, 3783-3788.
7) Baldini,G.,Hohman,R.,Charron,M.J. and Lodish,H.F.(1991) J.Biol.Chem. 266, 4037-4040.
8) Shibata,H.,Robinson,F.,Benzing,C.F. and Kono,T.(1991) Arch.Biochem.Biophys 285, 97-104.
9) Shibata,H.,Robinson,F.W.,Soderling,T.R. and Kono,T. (1991) J.Biol.Chem. 266,17948-17953.
10) Rodbell,M. (1964) J.Biol.Chem. 239,375-380.
11) Whitesell,R.R. and Gliemann,J. (1979) J.Biol.Chem. 254,5276-5283.
12) Higashijima,T.,Sonoko,U.,Nakajima,T. and Ross,E.M. (1988) J.Biol.Chem. 263 6491-6494.
13) Kahn,R.A. (1991) J.Biol.Chem. 266,15595-15597.
14) Schurmann,A.,Rosenthal,W.,Shultz,G. and Joost,H.G. (1992) Biochem.J. 283,795-801.
15) Baldini,G.,Hohl,T.,Lin,H.Y. and Lodish,H.F. (1992) Proc.N atl.Acad.Sci.USA. 283,795-801.
16) Cormont,M.,Tanti,J.,Gremeaux,T.,Van Obberghen,E. and Marchand-Brustel,Y.L. (1991) Endocrinology 129,3343-3350.
17) Korn,L.J.,Siebel,C.W.,McCormick,F. and Roth,R.A. (1987) Science 236, 840-843.
18) Davis,H.W. and McDonald,J.M. (1990) Biochem.J. 270,401-407.
19) Keleller,M.,Obermaier-Kusser,B.,Profrock,A.,Schleicher,E.,Seffer,E.,Mushack,J Ermel,B. and Haring,H.U. (1991) Biochem.J. 276,103-108.
20) Luttrell,L.M.,Hewlett,E.L..Romeo,G. and Rogol,A.D. (1988) J.Biol.Chem. 263, 6134-6141.

21) Luttrell,L.M.,Kilgour,E.,Larner,J. and Romeo,G. (1990) J.Biol.Chem. 265, 16873-16879.
22) Honnor,R.C.,Naghshineh,S.,Cushman,S.W.,Wolff,J.,Simpson,I.A and Londos,C. (1992) Cell.Signall. 4,87-98.
23) Robinson,L.J.,Pang,S.,Harris,D.S.,Heuser,J. and James,D.E. (1992) J.Cell.Biol. 117,1181-1196.
24) Schurmann,A.,Rosenthal,W.,Hinsch,K.D. and Joost,H.G. (1989) FEBS Lett. 255,259-264.