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Nigericin Inhibits Insulin-Stimulated Glucose Transport in 3T3-L1 Adipocytes

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Abstract We used nigericin, a K^+/H^+ exchanger, to test whether glucose transport in 3T3-L1 adipocytes was modulated by changes in intracellular pH. Our results showed that nigericin increased basal but decreased insulin-stimulated glucose uptake in a time- and dose-dependent manner. Whereas the basal translocation of GLUT1 was enhanced, insulin-stimulated GLUT4 translocation was inhibited by nigericin. On the other hand, the total amount of neither transporter protein was altered. The finding that insulin-stimulated phosphoinositide 3-kinase (PI 3-kinase) activity was not affected by nigericin implies that nigericin exerted its inhibition at a step downstream of PI 3-kinase activation. At maximal dose, nigericin rapidly lowered cytosolic pH to 6.7; however, this effect was transient and cytosolic pH was back to normal in 20 min. Removal of nigericin from the incubation medium after 20 min abolished its enhancing effect on basal but had little influence on its inhibition of insulin-stimulated glucose transport. Moreover, lowering cytosolic pH to 6.7 with an exogenously added HCl solution had no effect on glucose transport. Taken together, it appears that nigericin may inhibit insulin-stimulated glucose transport mainly by interfering with GLUT4 translocation, probably by a mechanism not related to changes in cytosolic pH. J. Cell. Biochem. 85: 83–91, 2002.

Key words: intracellular pH; GLUT1; GLUT4; vesicular trafficking; translocation; PI 3-kinase

A major metabolic effect of insulin is to promote glucose uptake into muscle cells and adipocytes [Czech, 1995]. Two isoforms of glucose transporter, namely, GLUT1 and GLUT4, are present in adipocytes [Gould et al., 1989; James et al., 1989]. In adipocytes, although both GLUT1 and GLUT4 transporters are recruited by insulin from intracellular pools to plasma membranes, it is believed that the increased glucose uptake evoked by insulin is mainly contributed by redistribution of GLUT4 [Cushman and Wardzala, 1980; Suzuki and Kono, 1980; Birnbaum, 1992]. Based on morphological and biochemical studies, GLUT4 is associated with endosomal-tubulovesicular structures in the trans-Golgi apparatus in adipocytes, and its unique interaction with endosomal fractions may play an essential role in its redistribution in basal or insulin-stimulated states [Smith et al., 1991; Malide et al., 1997; Millar et al., 1997]. Most endomembranes compartments are endowed with H^+-pumping ATPase to keep its luminal compartment acidic [Merzendorfer et al., 1997]. Endosomal acidification is thought to be involved in vesicular trafficking and modulation of this acidification may play a role in GLUT4 redistribution [van Weert et al., 1995; Merzendorfer et al., 1997]. A recent finding by Chinni and Shisheva [1999] demonstrated that arrest of the acidification of endosomal compartments by bafilomycin A_1, a selective inhibitor of the vacuolar proton pump [Bowman et al., 1988], induced GLUT4 translocation in 3T3-L1 adipocytes and implied that alkalinization of internal stores might be involved in insulin's effect on GLUT4 translocation. Conversely, another study suggested that transmembrane pH gradient was not involved in the insertion of GLUT4 vesicles into plasma membranes [Romanek et al., 1993]. Thus, whether intracellular alkalization is involved in insulin-induced glucose transport remains unclear.

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In the present study, we have used nigericin, a K^+/H^+ exchanger well known for its acidifying effect on cytosolic pH (pHi)[Pressman and Fahim, 1982], to examine if changes in intracellular pH may have any influence on glucose transport in 3T3-L1 adipocytes. Our results showed that nigericin increased basal but inhibited insulin-stimulated glucose transport by interference with GLUT4 translocation. Although nigericin lowered cytosolic pH, it inhibited GLUT4 translocation probably by a mechanism not related to changes in pHi.

MATERIALS AND METHODS

Materials

Dexamethasone, 3-isobutyl-1-methylxanthine (IBMX), 2-deoxy-D-glucose (2-DG), phenylmethylsulfonyl fluoride, nigericin, insulin, okadaic acid, H_2O_2, BCECF/AM, L-α-phosphatidylinositol, thapsigargin, bovine serum albumin (BSA), and 4β-phorbol 12β-myristate 13α-acetate (PMA) were obtained from Sigma Chemicals (St. Louis, MO). [^{3}H]2-deoxyglucose (8 mCi/mmol) and [γ-^{32}P]ATP (100 μCi/μmol) were obtained from New England Nuclear (Boston, MA).

Cell Culture

3T3-L1 cells, obtained from American Type Culture Collection, were grown and differentiated in 10-cm dishes (Corning) or 12-well plates (Costar) exactly as described by Fong [1990].

Nigericin Treatment of Differentiated Adipocytes

For each experiment, differentiated adipocytes (day 8–12) were pre-incubated for 2 h in Dulbecco's modified Eagle's medium (DMEM), and then incubated further in the control medium (DMEM containing 0.5% BSA) or control medium also containing nigericin or other reagents for indicated times. The cells were then washed twice with a KRP buffer containing 128 mM NaCl, 4.7 mM KCl, 1.25 mM $CaCl_2$, 1.25 mM $MgSO_4$, and 10 mM sodium phosphate (pH 7.4), and assayed for glucose transport activity.

Measurement of Glucose Transport Activity

Glucose transport activity was analyzed by measuring uptake of 2-DG into the cells as described previously [Fong et al., 1996].

Preparation of Membrane Fractions

Plasma and total cellular membranes were prepared exactly as described earlier [Fong et al., 1996]. All final membrane suspensions included 1 mM phenylmethylsulfonyl fluoride.

Immunoblot Analysis

For each experiment, membrane samples were subjected to SDS–polyacrylamide (10%) gel electrophoresis (SDS–PAGE) under reducing conditions as described by Laemmli [1970], and transferred to a nitrocellulose membrane. The GLUT1 and GLUT4 transporter proteins were immunodetected by using polyclonal rabbit anti-GLUT1 (1:1,000; East Acres Biologicals, Southbridge, MA) and monoclonal mouse anti-GLUT4 (IF8, 1.3 μg/ml; Genzyme, Cambridge, MA) antibodies, respectively, and the blots were developed by the enhanced chemiluminescence method (ECL, Amersham, Buckinghamshire, England) employing horseradish peroxidase-conjugated donkey anti-rabbit IgG (1:5,000) for GLUT1 and sheep anti-mouse IgG (1:9,000) for GLUT4, respectively. Quantification of relative band intensity was performed by laser scanning densitometry.

Measurement of pHi

Briefly, after cells were incubated in the presence of BCECF/AM for 30 min at 37°C, cells were washed and transferred to a cuvette equipped with a magnetic stirrer at 37°C. Fluorescence was monitored with a spectrofluorometer (SPEX CM) at an excitation wavelength of 510 nm and an emission wavelength of 540 nm. Calibration of the fluorescence vs. pH was performed by comparing to a standard curve obtained as described earlier [Arsenis, 1995].

Determination of Phosphoinositide 3-Kinase (PI 3-Kinase) Activity

After various treatments, cells were lyzed in the lysis buffer (5 mM $NaHPO_4$, pH 7.2, 0.4 mM Na_3VO_4, 0.5% NP-40, 1 μg/ml each of pepstatin A, leupeptin and aprotinin) on ice for 40 min. The lysates were centrifuged at 20,000*g* for 30 min at 4°C to remove the fat cake and insoluble materials and then incubated with rabbit anti-insulin receptor substrate-1 (IRS-1) antibodies (3 μg/ml, Upstate Biotechnology, Inc., Lake Placid, NY) for 8–12 h at 4°C. Immunoprecipitates were collected by protein G PLUS/protein A-Agarose (Oncogene, Cambridge, MA)

and washed three times with lysis buffer, three times with 10 mM Tris, pH 7.4, 145 mM NaCl, and twice with assay buffer (40 mM Tris, pH 7.4, 5 mM $MgCl_2$, and 0.5 mM EGTA). The immunocomplexes obtained were then assayed for PI 3-kinase activity as described by Ridderstrale et al. [1995], using phosphatidylinositol and [γ-^{32}P]ATP as the substrates.

Measurement of Intracellular Calcium Concentrations ([Ca^{2+}]i) by Fura-2 Analysis

After washing monolayer cells in 10-cm dish three times with 10 ml each of modified KRB buffer (120 mM NaCl, 4.75 mM KCl, 1 mM KH_2PO_4, 1.44 mM $MgSO_4$, 25 mM HEPES, 5 mM $NaHCO_3$, 1.1 mM $CaCl_2$, 0.1 mM EGTA, and 2.8 mM glucose, pH 7.4), cells were removed from the dish by pipetting for several times. Cells were harvested by centrifugation at 500*g* for 5 min and incubated in 2 ml KRB buffer also containing 2.5 μM Fura-2/AM for 30 min at 37°C. At the end of incubation, cells were diluted to 25 ml with KRB buffer, centrifuged and the pellet was resuspended in KRB buffer at a density of $2-3 \times 10^6$ cells/ml in a thermostated cuvette with constant stirring at 25°C. The fluorescence of the Fura-2 loaded cells was measured with a spectrofluorometer (SPEX CM) and [Ca^{2+}]i was determined as described previously [Grynkiewicz et al., 1986]. Statistical differences were determined by Student's *t*-test.

RESULTS

Effect of Nigericin on 2-DG Uptake

As shown in Figures 1 and 2, nigericin pretreatment of 3T3-L1 adipocytes enhanced basal but inhibited insulin-stimulated glucose uptake. The effect of nigericin on glucose uptake was both time- and dose-dependent. At a concentration of 7 μg/ml, the maximal enhancing effect on basal (800 ± 120% of control, means ± SE) or inhibitory effect on insulin-stimulated glucose uptake (↓68 ± 8%) was reached at 5 h incubation. The increase in 2-DG uptake in nigericin-pretreated cells was nearly eliminated (↓92 ± 4%) when 10 μM cytochalasin B was also present in the assay buffer, indicating that the observed increase in glucose uptake was mediated by specific glucose transporter. Compared to other reported studies, we used higher concentrations of nigericin, due to the presence of 0.5% BSA in the incubation medium, which reduced the effective concentrations of nigericin.

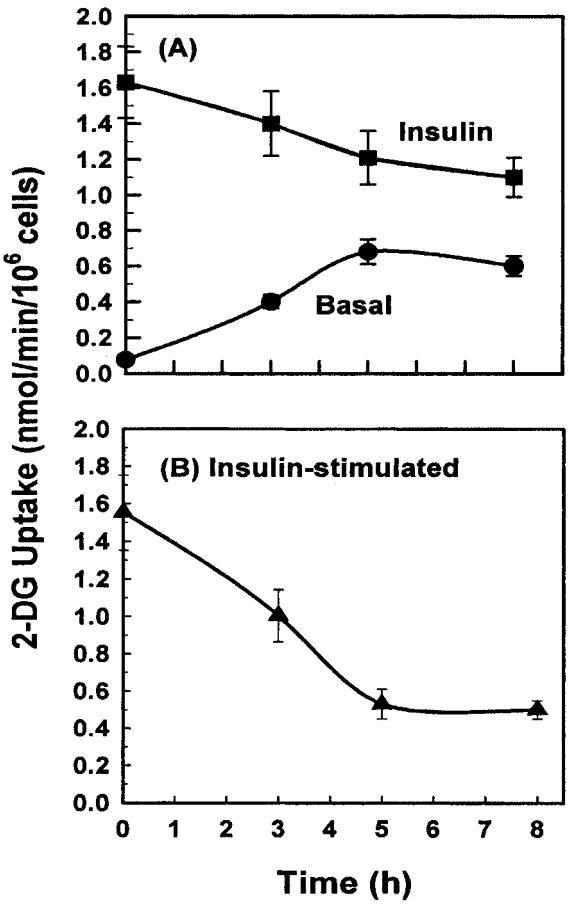

Fig. 1. Time-dependent effect of nigericin on basal and insulin-stimulated 2-DG uptake. **A**: 3T3-L1 adipocytes were pretreated with nigericin (7 μg/ml) for various times and measured for 2-DG uptake in the absence (basal) or presence of insulin as described in Materials and Methods. **B**: Insulin-stimulated activities after being corrected for basal. Values are means ± SE (n = 3).

Immunoblot Analysis of the Amount of Glucose Transporters in Total and Plasma Membranes

To determine the possible mechanism responsible for the observed effect of nigericin on glucose uptake, we performed immunoblot analysis of both GLUT1 and GLUT4 in total and plasma membranes from control and nigericin-pretreated cells, after they were activated by insulin. As shown in Figure 3, whereas nigericin pretreatment did not alter the total amount of neither transporter, it enhanced the amount of GLUT1 to 175 ± 25% of control in plasma membranes. As expected, insulin induced the

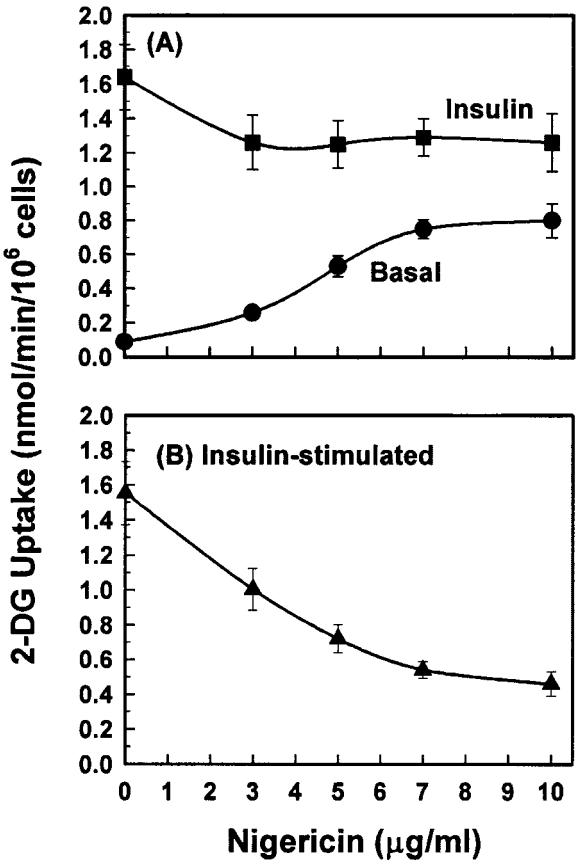

Fig. 2. Dose-dependent effect of nigericin on basal and insulin-stimulated 2-DG uptake. **A**: 3T3-L1 adipocytes were pretreated with various concentrations of nigericin for 5 h and measured for 2-DG uptake in the absence (basal) or presence of insulin as described in Materials and Methods. **B**: Insulin-stimulated activities after being corrected for basal. Values are means ± SE (n = 3).

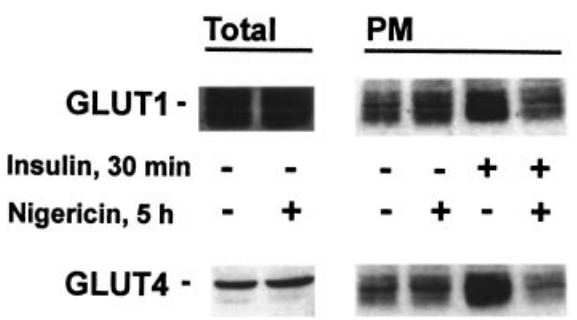

Fig. 3. Effect of nigericin pretreatment on the membrane contents of GLUT1 and GLUT4 transporter proteins in control or insulin-stimulated cells. After pretreatment of 3T3-L1 adipocytes without or with nigericin (7 μg/ml) for 5 h, cells were further incubated in the absence or presence of 10^{-7} M insulin for 30 min. Total cellular membranes and plasma membranes (PM) were then isolated, and were subjected to SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting using antiserum against GLUT1 or GLUT4 as described in Materials and Methods. Two other independent experiments gave similar results.

translocation of both GLUT1 and GLUT4 from internal pools to plasma membranes in control cells; however, the insulin-induced increases of both transporters in plasma membranes were diminished in nigericin-pretreated cells (↓85 ± 10% and ↓95 ± 15% for GLUT1 and GLUT4, respectively). Thus, it appears that nigericin exerts its effect on glucose transport by interference with the translocation processes of both transporters. Nevertheless, if we compare the extent of stimulation by nigericin on 2-DG uptake (~8-fold) to that on GLUT1 translocation (~2-fold), it appears that in addition to inducing GLUT1 redistribution from intracellular pools to plasma membranes, nigericin may also increase the intrinsic activity of GLUT1 in plasma membranes.

Effect of Nigericin on the Activity of PI 3-Kinase

Since it is well established that the initial event of insulin action on GLUT4 translocation is mediated by sequential activation of insulin receptor kinase, tyrosine phosphorylation of IRS-1 and subsequent association and activation of PI 3-kinase [Myers and White, 1993; Rice and Garner, 1994; White and Kahn, 1994], we examined whether IRS-1 associated PI 3-kinase activity upon insulin activation was altered by nigericin-pretreatment. As shown in Figure 4, the increased PI 3-kinase activity in response to insulin was not changed by nigericin, thus indicating that nigericin exerted its inhibitory effect on insulin-stimulated GLUT4 translocation at a step downstream of insulin activation of PI 3-kinase.

Effect of Nigericin on 2-DG Uptake Stimulated by Various Agents in 3T3-L1 Adipocytes

Besides insulin, H_2O_2, okadaic acid and PMA, a phorbol ester, were also reported to have stimulated glucose transport by altering the redistribution of GLUT4 [Hayes and Lockwood, 1987; Saltis et al., 1991; Rampal et al., 1995]. Whereas H_2O_2 is a protein tyrosine phosphatase inhibitor and may activate insulin receptor kinase [Heffetz et al., 1990], okadaic acid seems to activate aPKC-ζ/λ [Standaert et al., 1999] via inhibiting protein phosphatase 1 and 2A. While both H_2O_2 and okadaic acid seem to stimulate glucose transport via some steps also involved in insulin's signaling cascade, PMA may induce the redistribution of both GLUT1 and GLUT4 by an insulin-independent mechanism [Saltis

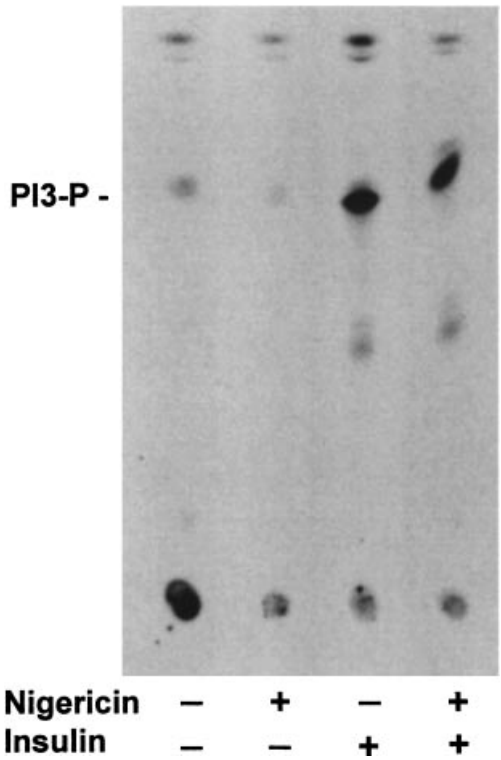

Fig. 4. Effect of nigericin on PI 3-kinase activity in control and insulin-stimulated cells. 3T3-L1 adipocytes were pretreated with or without nigericin (7 μg/ml) for 5 h and were further incubated in the absence or presence of 10^{-7} M insulin for 30 min. IRS-1 and associated proteins were collected by immunoprecipitation. PI 3-kinase activity in the immunocomplex was then measured as described in Materials and Methods, and the product, phosphatidylinositol-3-^{32}P (PI3-P) was developed by thin-layer chromatography on a Silica gel plate. Two other independent experiments gave similar results.

et al., 1991]. Thus, it is of interest to examine if glucose uptakes evoked by these different agents are also influenced by nigericin pretreatment. As shown in Table I, whereas all three agents enhanced 2-DG uptake, although at a lower stimulation as compared to insulin (19.7-, 5.4-, 9.9-, and 10.2-fold for insulin-, PMA-, H_2O_2-, and okadaic acid-stimulated, respectively), they were unable to further increase glucose transport significantly in nigericin-pretreated cells. Since it is unlikely that these agents share a common pathway with nigericin in stimulating glucose transport, the experimental results suggest that nigericin exerts its inhibitory effect at a step or steps common to all these GLUT4-translocating agents.

Effect of Nigericin on pHi

As shown in Figure 5, addition of nigericin to the incubation buffer rapidly induced lowering

TABLE I. Effect of Nigericin on 2-DG Uptake Stimulated by Various Agents in 3T3-L1 Adipocytes

	2-DG uptake (nmol/min/10^6 cells)	
	Control	Nigericin
Basal	0.067 ± 0.006	0.416 ± 0.060^b
Insulin	1.323 ± 0.130^a	0.943 ± 0.100^a
PMA	0.363 ± 0.027^a	0.454 ± 0.086
H_2O_2	0.663 ± 0.076^a	0.492 ± 0.095
Okadaic acid	0.685 ± 0.028^a	0.662 ± 0.130

After 3T3-L1 adipocytes were pretreated without (control) or with nigericin (7 μg/ml) for 5 h, cells were incubated in the absence (basal) or presence of insulin (10^{-7} M), PMA (1 μM), H_2O_2 (10 mM), and okadaic acid (1 μM) for 30 min. 2-DG uptake was then measured as described in Materials and Methods. Values are means ± SE (n = 3).
[a,b]Values are significantly different from the corresponding basal and control, respectively.

of pHi in 3T3-L1 adipocytes in a dose-dependent manner. At 3 and 5 μg/ml of nigericin, the decrease in pHi was rather slow; it took approximately 6 and 3 min, respectively, to reduce pHi from 7.2 to about 6.8. Thereafter, the pHi remained little changed till the end of the 10 min-incubation period. At higher concentrations (7 and 10 μg/ml) of nigericin, the pHi was rapidly decreased from 7.2 to ~ 6.7 within 2 min, and then reverted towards the initial state slowly. If the incubation was prolonged to 20 min, the pHi would eventually return to the original value (Fig. 6). The biphasic phenomena of pHi changes probably reflect the activation of a H^+-exporting process that was triggered by a pHi below 6.8. Indeed, activation of Na^+/H^+ exchanger by acidic pHi has been observed in rat adipocytes [Arsenis, 1995].

Effect of Washing

The recovery of pHi within 20 min of incubation in the presence of nigericin prompted us to test if a short exposure to nigericin to reduce pHi for a short period of time was sufficient to exert its effect on glucose transport. After exposure of 3T3-L1 adipocytes to nigericin (7 μg/ml) for 20 min, 1, 3, or 5 h, nigericin was removed from the incubation medium by washing and incubation was continued till the end of 5 h-incubation period. Cells were then measured for basal and insulin-stimulated 2-DG uptake. As shown in Figure 7, whereas the effect of nigericin on basal glucose transport required the sustained presence of this drug, the inhibition of insulin-stimulated glucose transport remained little

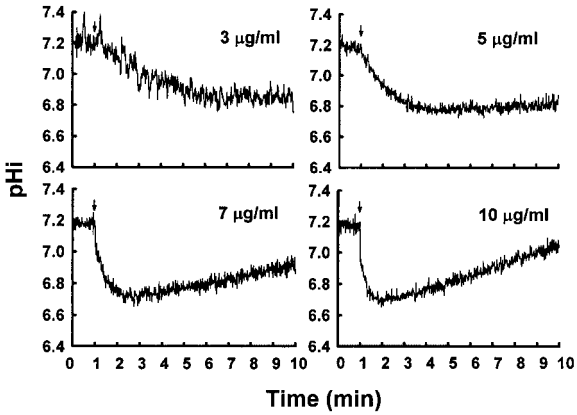

Fig. 5. Dose-dependent effect of nigericin on pHi in 3T3-L1 adipocytes. Cells were loaded with BCECF as described in Materials and Methods, and were incubated in the buffer for 1 min before the addition of various concentrations of nigericin as indicated by the arrow.

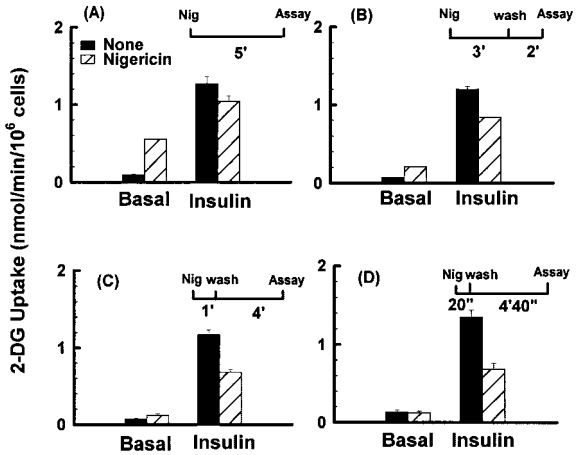

Fig. 7. Effects of nigericin on basal and insulin-stimulated 2-DG uptake were differently affected by washing. After being treated without (none) or with nigericin (7 μg/ml) for (**A**) 5 h, (**B**) 3 h, (**C**) 1 h, or (**D**) 20 min, cells were washed and incubation was continued for another (A) 0 h, (B) 2 h, (C) 4 h, or (D) 4 h and 40 min. 2-DG uptake in the absence (basal) or presence of insulin was then measured.

changed after initial exposure to nigericin for 20 min. The extents of inhibition of insulin-stimulated 2-DG uptake as shown in Figure 7A,B,C,D are 59 ± 7, 47 ± 3, 51 ± 3, and $57 \pm 10\%$, respectively. Since removal of nigericin from the incubation medium probably does not abstract inserted nigericin from the plasma membranes, the washing out effect may reflect the endocytosis of nigericin from plasma membranes into internal compartments. Once inside the cell, nigericin probably lost its ability to effect greatly, if at all, cytosolic pH. If this is the case, it raises two possibilities that may explain nigericin's effect on GLUT4 translocation. Firstly, a short exposure to acidic pHi may induce some irreversible processes such as phosphorylation/dephosphorylation or degradation of certain proteins. In other words, activities of protein kinases, phosphatases, or proteases may be altered by nigericin. Previous reports that intracellular acidification may activate c-Src [Yamaji et al., 1997], protein kinase C [Yanagawa and Jo, 1997], or ICE-like protease activity [Furlong et al., 1997] are consistent with this view. Secondly, GLUT4 translocation may be influenced by nigericin in internal compartments or vesicles that are involved in the translocation process. The presence of nigericin may alter the luminal pH in these internal compartments or vesicles.

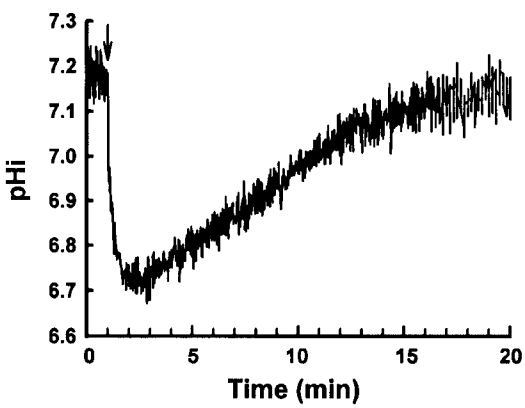

Fig. 6. Reversal of pHi in cells treated with nigericin (7 μg/ml) for 20 min.

Effect of Exogenously Added HCl Solution on Glucose Uptake

In an attempt to differentiate the aforementioned possibilities, we further examined if lowering pHi for short period time by exogenously added HCl solution could have the same result. Previous studies have demonstrated that exposure of cultured cells to acidic media resulted in decreases in pHi [L'Allemain et al., 1984]. As shown in Figure 8A, by adding exogenously an appropriate aliquot of HCl solution, we could deliberately lower pHi to ~ 6.7 (pH in the incubation buffer $= 6.2$), which was similar to that obtained by nigericin (7 μg/ml). In contrast to nigericin, lowering pHi to 6.7 by exogenously added acid had no significant effect on either basal or insulin-stimulated 2-DG uptake (Fig. 8B). These results seem to

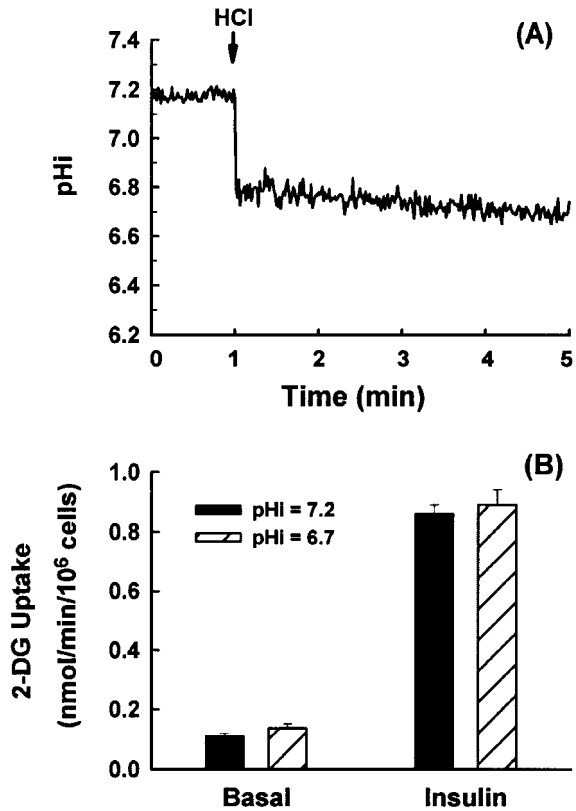

Fig. 8. **A**: Effect of an exogenously added HCl solution on pHi. **B**: Effect of low pHi (pHi = 6.7) on basal and insulin-stimulated 2-DG uptake. An adequate HCl solution was added to the incubation buffer to lower pHi to 6.7 as shown in (A). Cells were then incubated for 5 h, washed, and measured for basal and insulin-stimulated 2-DG uptake. Values are means ± SE (n = 2).

indicate that lowering cytosolic pH by nigericin is not responsible for the observed effect of nigericin on glucose transport.

Effect of Nigericin on Intracellular Ca^{2+} Concentrations

Since nigericin has been shown to increase $[Ca^{2+}]i$ [Tornquist and Tashjian, 1991], we examined if nigericin may induce an elevation of $[Ca^{2+}]i$ in 3T3-L1 adipocytes and compared the response to that induced by thapsigargin, an inhibitor of the Ca^{2+}-ATPase activity of endoplasmic reticulum [Kwan et al., 1990]. As shown in Figure 9, nigericin slowly induced an increase in $[Ca^{2+}]i$ in a similar pattern with a smaller magnitude to that induced by thapsigargin. Based on our observation that thapsigargin had no significant effect on either basal or insulin-stimulated 2-DG uptake (Table II), we conclude that the effect of nigericin on glucose transport is independent of its effect on $[Ca^{2+}]i$.

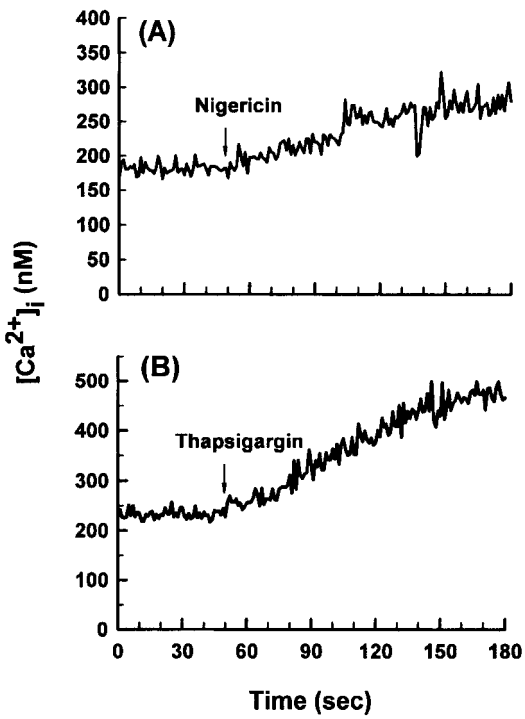

Fig. 9. Effect of (**A**) nigericin and (**B**) thapsigargin on $[Ca^{2+}]i$. Cells were loaded with Fura-2 as described in Materials and Methods and were incubated in the buffer for 50 s before the addition of nigericin (7 μg/ml) or thapsigargin (100 nM) as indicated by the arrows.

DISCUSSION

The present study has demonstrated that nigericin enhances basal but inhibits insulin-stimulated glucose transport in 3T3-L1 adipocytes. Due to its nature as an electroneutral K^+/H^+ exchanger, it induced acidic pHi in adipocytes. Since a change in pHi has been suggested to be involved in a variety of cellular responses, such as regulation of fertilization, proliferation, and metabolism [Madshus, 1988] and some of insulin's metabolic effect might be mediated by intracellular alkalinization [Civelek et al., 1996], it is tempting to suggest that the effect

TABLE II. Effect of Thapsigargin on Basal and Insulin-Stimulated Glucose Uptake

	2-DG uptake (nmol/min/10^6 cells)	
	Control	Thapsigargin
Basal	0.095 ± 0.016	0.116 ± 0.010
Insulin	1.305 ± 0.125[a]	1.030 ± 0.130[a]

After 3T3-L1 adipocytes were pretreated without (control) or with thapsigargin (100 nM) for 5 h; cells were incubated in the absence (basal) or presence of insulin (10^{-7} M) for 30 min. 2-DG uptake was then measured as described in Materials and Methods. Values are means ± SE (n = 2).
[a]$P < 0.02$ compared with the corresponding basal.

of nigericin on glucose transport is related to its effect of pHi. However, as shown by our experimental results, it appears that nigericin's effect on insulin-stimulated glucose transport is not related to changes in cytosolic pH. This conclusion is reached by the finding that inhibition of insulin-stimulated glucose transport required only a short exposure to nigericin with a transient effect on lowering cytosolic pH and reducing cytosolic pH to the same level as nigericin did, with an exogenously added acidic solution, had no effect on insulin-stimulated glucose uptake. It is further supported by the observation that amiloride and 4,4'-diisothiocyanostilbene 2,2-disulfonic acid, inhibitors of Na^+/H^+ and $HCO3^-/Cl^-$ exchangers, respectively [Rotin et al., 1987], had no significant influence on the effect of nigericin on glucose uptake (data not shown).

The result of washing experiment was consistent with our observation that basal and insulin-stimulated glucose transports were differently modulated by nigericin. Whereas GLUT1 activity is likely to be influenced by nigericin remained in the plasma membranes, GLUT4 translocation seems to be affected by nigericin inside the cells, probably through modulation of luminal pH in internal compartments or vesicles that are involved in vesicular trafficking. In accordance with this notion, nigericin has been suggested to induce an internal alkalinization of nonmitochondrial calcium stores in RINm5F rat insulinoma cells [Bode et al., 1994]. The lack of effect of exogenously added HCl solution could be ascribed to, presumably, its inability to induce such changes in internal compartments as induced by nigericin.

Since nigericin may induce pHi changes by exchanging K^+ for H^+ in the medium or in the internal compartments such as endosomal-vesicular structures, it raises the question whether it is possible that the effect of nigericin could be mediated by changes of K^+ concentrations in cytosol or in internal compartments. However, decreasing pHi from 7.2 to 6.7 only denotes an increase of H^+ concentration from 6.3×10^{-8} to 20×10^{-8} M. In other words, an equivalent and very minor amount of K^+ ions are moved out of cells, into internal compartments or both. Considering the high K^+ concentrations in cells ($\gg$ mM), it becomes obvious that nigericin has negligible influence on the concentrations of K^+ ions in cells. In agreement with this, we have found that replacing 70 mM NaCl in the incubation medium with 70 mM KCl/70 mM choline, or inhibiting Na^+/K^+ ATPase with ouabain has no significant effect on basal or insulin-stimulated glucose transport in control or nigericin-pretreated cells (data not shown).

In summary, the present study has demonstrated that nigericin may enhance basal but inhibit insulin-stimulated glucose transport in 3T3-L1 adipocytes by distinct mechanisms. Translocation processes of both GLUT1 and GLUT4 transporters appear to be influenced. Although nigericin may lower cytosolic pH, its effect on insulin-stimulated glucose transport does not seem to be related. Rather, our results suggest that stimulated glucose transport requires an appropriate pH in GLUT4-containing or other vesicles/compartments involved in vesicular trafficking, which may be altered by the presence of nigericin in these vesicles or compartments.

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