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Activation of the Ras/Mitogen-Activated Protein Kinase Signaling Pathway Alone Is Not Sufficient To Induce Glucose Uptake in 3T3-L1 Adipocytes

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The signal transduction pathway by which insulin stimulates glucose transport is largely unknown, but a role for tyrosine and serine/threonine kinases has been proposed. Since mitogen-activated protein (MAP) kinase is activated by insulin through phosphorylation on both tyrosine and threonine residues, we investigated whether MAP kinase and its upstream regulator, p21^{ras}, are involved in insulin-mediated glucose transport. We did this by examining the time- and dose-dependent stimulation of glucose uptake in relation to the activation of Ras-GTP formation and MAP kinase by thrombin, epidermal growth factor (EGF), and insulin in 3T3-L1 adipocytes. Ras-GTP formation was stimulated transiently by all three agonists, with a peak at 5 to 10 min. Thrombin induced a second peak at ≈30 min. The activation of p21^{ras} was paralleled by both the phosphorylation and the activation of MAP kinase: transient for insulin and EGF and biphasic for thrombin. However, despite the strong activation of Ras-GTP formation and MAP kinase by EGF and thrombin, glucose uptake was not stimulated by these agonists, in contrast to the eightfold stimulation of 2-deoxy-D-[^{14}C]glucose uptake by insulin. In addition, insulin-mediated glucose transport was not potentiated by thrombin or EGF. Although these results cannot exclude the possibility that p21^{ras} and/or MAP kinase is needed in conjunction with other signaling molecules that are activated by insulin and not by thrombin or EGF, they show that the Ras/MAP kinase signaling pathway alone is not sufficient to induce insulin-mediated glucose transport.

Type 2 or non-insulin-dependent diabetes mellitus (NIDDM) is a disease in which insulin-mediated glucose uptake by adipocytes and cardiac and skeletal muscle cells is impaired (33). In these insulin-sensitive tissues, glucose uptake is stimulated by insulin-induced translocation and activation of a specific glucose transporter, GLUT 4 (2, 12, 24). GLUT 4 belongs to a family of glucose transporters composed of at least five isoforms (GLUT 1 to 5), which were identified by cDNA cloning (2, 12, 16). In the insulin-sensitive tissues, two types of glucose transporters are expressed: GLUT 4, which is restricted to these tissues, and GLUT 1, the more uniformly distributed isoform (15, 16).

Although the existence of genetic components in the etiology of NIDDM is well established, for the majority of NIDDM patients the gene defects have not been identified yet. A subgroup of NIDDM may be the result of mutations in genes encoding proteins involved in the intracellular signaling of insulin to glucose transport. Studies of their role in the pathogenesis of NIDDM are hampered by the poor characterization of these signaling intermediates. Some of the early effects of insulin are the phosphorylation of insulin receptor substrate 1 (IRS-1) (40) and the activation of the Ras/mitogen-activated protein (MAP) kinase signaling pathway (8). Treatment of adipocytes with the phosphatase inhibitor okadaic acid partly mimics the effect of insulin on 2-deoxy-D-glucose (2DOG) uptake (21). One of the effects of this toxin is the activation of MAP kinase (11). Furthermore, tyrosine as well as serine/threonine phosphorylation has been shown to be essential for this process (13, 17, 20, 21, 36), while MAP kinase is activated by phosphorylation on threonine and tyrosine (7, 32). In addition, GTP-binding proteins are involved in the translocation of GLUT 4-containing vesicles to the plasma membrane (1). Together, these data could suggest that the Ras/MAP kinase pathway acts in the signaling pathway of insulin toward glucose uptake.

The role of p21^{ras} and its downstream component, MAP kinase, in the activation of glucose transport is controversial. In an earlier report, we presented data showing that Ras-GTP formation is not needed to provoke GLUT 1-mediated glucose transport (28). Kozma et al. showed that transfection of activated p21^{ras} in 3T3-L1 adipocytes stimulated glucose uptake, although it is unclear whether this approach reflects the natural situation (18). As 3T3-L1 adipocytes express GLUT 1 and GLUT 4, these results could also suggest that GLUT 4-mediated glucose transport is activated by Ras-GTP, whereas GLUT 1-mediated glucose uptake proceeds via a p21^{ras}-independent pathway.

In the present study, we attempted to clarify the roles of p21^{ras} and MAP kinase in insulin-stimulated glucose transport in 3T3-L1 adipocytes expressing GLUT 4. To do this, we compared the time- and dose-dependent activation of p21^{ras}, MAP kinase, and glucose uptake by insulin, thrombin, and epidermal growth factor (EGF). In addition, we inhibited the downstream coupling of Ras-GTP to MAP kinase by using 8-bromo-cyclic AMP (8-bromo-cAMP). The data show that the activation of both Ras-GTP formation and MAP kinase is not sufficient to activate GLUT 4-mediated glucose uptake. This finding corroborates our previous suggestion (28) that the

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activation of the Ras/MAP kinase signaling pathway alone is not sufficient to stimulate glucose transport.

MATERIALS AND METHODS

Materials. 2DOG phosphate-free Dulbecco modified Eagle medium (DMEM), 8-bromo-cAMP, bovine insulin, mouse EGF, and bovine thrombin were purchased from Sigma; 2-deoxy-D-[^{14}C]glucose (45 to 55 mCi/mmol) was purchased from NEN-Dupont. Tissue culture media and fetal calf serum were purchased from GIBCO. Polyethyleneimine-cellulose F plates were obtained from Merck, and protein G-Sepharose 4 fast flow beads were obtained from Pharmacia. Anti-MAP kinase antiserum used for Western blotting (immunoblotting) was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, Calif.; anti-ERK1/2). Anti-ERK2 antiserum used for immunoprecipitation was a gift from A. M. M. de Vries-Smits and G. J. Pronk (Utrecht University, Utrecht, The Netherlands) (31). Monoclonal anti-p21^{ras} (Y13-259) and rabbit polyclonal anti-rat antisera were provided by A. Zantema (University of Leiden, Leiden, The Netherlands). Rainbow protein molecular weight markers and $^{32}P_i$ (acid free and carrier free) were obtained from Amersham International (Amersham, Buckinghamshire, United Kingdom).

Cell culture. 3T3-L1 fibroblasts were grown to confluence in DMEM containing a high glucose concentration, 10% fetal calf serum, 50 U of penicillin per ml, and 50 μg of streptomycin per ml (standard medium) and maintained in a 7.5% CO_2-humidified atmosphere at 37°C. This medium was changed every 3 to 4 days. Fibroblasts were routinely subcultured at a surface ratio of 1:100 after trypsin treatment, when they had just reached confluency. Two days after the fibroblasts achieved confluence, differentiation to adipocytes was induced by incubating the cells for 2 days in standard medium supplemented with 10 μg of insulin per ml, 0.25 μM dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX). The cells were then incubated for an additional 6 days in the same medium without dexamethasone and IBMX. This medium was changed every 2 days. The cells were then maintained on standard medium for at least another 6 days. At this time, >95% of the cells expressed the adipocyte phenotype. All experiments were performed with cells grown on six-well plates, which were serum starved overnight with DMEM supplemented with 0.5% fetal calf serum.

Assay of 2DOG uptake in 3T3-L1 adipocytes. One hour before the 2DOG uptake experiment started, the serum-starved medium was replaced with 750 μl of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (50 mM HEPES, 0.14 M NaCl, 1.85 mM $CaCl_2$, 1.3 mM $MgSO_4$, 4.8 mM KCl [pH 7.4]) at 37°C. The cells were stimulated with insulin, thrombin, or EGF in this HEPES buffer at 37°C. The dose and time of incubation of these agonists varied and are indicated in the legends to the figures. 2DOG uptake was initiated by the addition of 2-deoxy-D-[^{14}C]glucose (0.075 μCi per well) in 3 mM 2DOG. After 10 min, the assay was terminated by two quick washes with ice-cold 0.9% NaCl and the addition of 1 ml of 0.1 M NaOH. Incorporated 2-deoxy-D-[^{14}C]glucose was counted in an LKB 1217 Rackbeta liquid scintillation counter with Ultima Gold scintillation fluid (Packard).

Assay of MAP kinase phosphorylation. Quiescent 3T3-L1 adipocytes were treated with various concentrations of insulin and/or EGF or thrombin for different time periods as indicated in the legends to the figures. The incubation was stopped by two washes with ice-cold 0.9% NaCl and the addition of sodium dodecyl sulfate (SDS) sample buffer (0.1 M Tris, 1.7% SDS, 6.7% glycerol, 0.7 M β-mercaptoethanol, 0.1% bromophenol blue [pH 7]). DNA was sheared by repeated resuspension through a 25-gauge 5/8 needle and then was centrifuged for 10 min at 4°C (10,000 × *g*). The supernatant (cell homogenate) was boiled for 3 min and separated on 10% polyacrylamide slab gels (30 μg of protein per lane). SDS-polyacrylamide gel electrophoresis (PAGE) was performed as described by Laemmli (19). Immunoblotting was performed as described previously (38). MAP kinase was visualized with goat anti-rabbit antiserum coupled to alkaline phosphatase. Phosphorylation of MAP kinase induces a shift in its migration.

Assay of MAP kinase activity. Quiescent 3T3-L1 adipocytes were treated with insulin, EGF, or thrombin for various time periods as indicated in the legends to the figures. The incubation was stopped by two washes with ice-cold 0.9% NaCl. The cells were then lysed in 20 mM Tris (pH 8)–40 mM PP_i–50 mM NaF–5 mM $MgCl_2$–0.1 mM sodium orthovanadate–10 mM EDTA–0.8% Triton X-100–0.4% deoxycholate–0.08% SDS–17 μg of leupeptin per ml–17 μg of aprotinin per ml–2.5 mM phenylmethylsulfonyl fluoride. Three hundred micrograms of protein from this total cell lysate was used to immunoprecipitate MAP kinase. The washed immunoprecipitate was incubated with 0.6 μCi of [^{32}P]ATP in 30 mM Tris (pH 8.0)–20 mM $MgCl_2$–2 mM $MnCl_2$–10 μM ATP for 30 min and with 7.5 μg of myelin basic protein as the substrate. The mixture was electrophoresed on a 15% polyacrylamide gel and then autoradiographed.

Cell labeling and analysis of guanine nucleotides bound to p21^{ras}. 3T3-L1 adipocytes were serum starved overnight, labeled for 3 h with $^{32}P_i$ (0.5 mCi per well), and then stimulated with insulin, EGF, or thrombin. The conditions are given in the legends to the figures. Ras-GTP formation was measured as described previously (4, 28). In short, the cells were lysed in a buffer containing 1% Triton X-114. The Triton X-114-soluble fraction was collected by means of a short temperature shift from 0 to 37°C and diluted 10-fold with lysis buffer containing 0.5% deoxycholate and 0.005% SDS. NaCl was added to 500 mM. The diluted Triton X-114 detergent phase was precleared for 15 min with protein G-Sepharose beads coupled to rabbit anti-rat immunoglobulin G. The supernatant was used for the immunoprecipitation of p21^{ras} with monoclonal antiserum Y13-259 coupled to protein G-Sepharose beads. The immunoprecipitates were extensively washed, and GDP and GTP were eluted and separated on polyethyleneimine-cellulose F plates. GDP and GTP spots were visualized by autoradiography, and the radioactivity in GTP and GDP was quantitated by liquid scintillation counting.

Other procedures. The protein concentration was determined by use of a protein assay kit from Pierce, Rockford, Ill., with bovine serum albumin as a standard.

RESULTS

The differentiation of 3T3-L1 fibroblasts to 3T3-L1 adipocytes was paralleled by the induction of insulin-sensitive glucose transport, the expression of the GLUT 4 protein, and the translocation of this protein from the low-density microsomes to the plasma membrane following insulin stimulation, in concordance with results obtained by others (16, 39) (data not shown). These observations indicate that insulin stimulates glucose uptake in these 3T3-L1 adipocytes, at least in part, through GLUT 4.

Insulin, thrombin, and EGF are known to activate the Ras/MAP kinase signaling pathway in fibroblast-derived cell lines. In Fig. 1, it is shown that these agonists also stimulated Ras-GTP formation in 3T3-L1 adipocytes, with the following

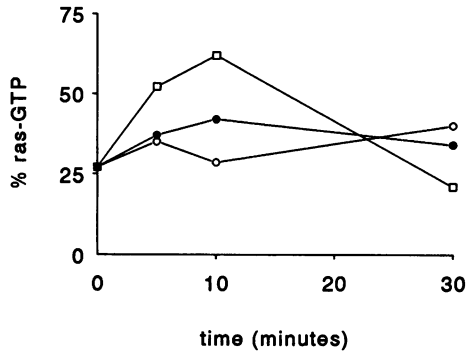
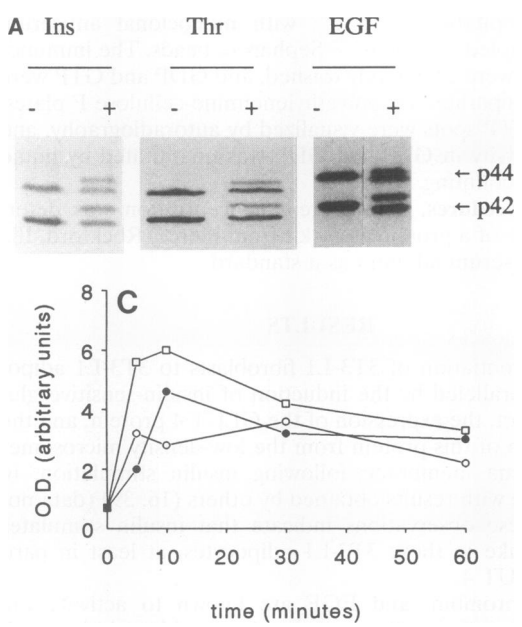


FIG. 1. Time course of insulin-, thrombin-, or EGF-induced effects on Ras-GTP formation. 3T3-L1 adipocytes were $^{32}P_i$ labeled and incubated for 5, 10, or 30 min with insulin (1 μM; ●), thrombin (2 U/ml; ○), or EGF (50 ng/ml; □). The radioactivity in $p21^{ras}$-bound GDP and GTP was determined, and the percentage of Ras-GTP was calculated. Because of differences in the basal levels of Ras-GTP (21.5 to 30.3%), together with the fact that not all three agonists were added together in each experiment, the data are not presented as the mean ± standard error of the mean but are shown as an experiment representative of at least two other independently performed experiments with one well per condition (insulin, $n = 5$; thrombin and EGF, $n = 3$).

order of potency: EGF > insulin > thrombin. The activation of $p21^{ras}$ was transient, with a maximum after 5 to 10 min, in response to 1 μM insulin and 50 ng of EGF per ml and biphasic in response to 2 U of thrombin per ml, with a peak at 5 min and another peak at ≈30 min. $p21^{ras}$ is known to be the upstream regulator of MAP kinase in fibroblasts. We measured to what extent the activation of $p21^{ras}$ was paralleled by the activation of MAP kinase in 3T3-L1 adipocytes following stimulation by insulin, thrombin, and EGF. The activation of MAP kinase was determined by the appearance of a more slowly migrating form in gel electrophoresis because of the phosphorylation of specific threonine and tyrosine residues (10, 14, 22, 29, 30). As shown in Fig. 2A, all agonists induced a shift in the migration of two isoforms of MAP kinase: extracellularly regulated kinases 1 (p44) and 2 (p42). The activation of MAP kinase by these hormones was confirmed by an in vitro kinase assay with immunoprecipitated MAP kinase from stimulated 3T3-L1 adipocytes and with myelin basic protein as the substrate (Fig. 2B). The time dependence of Ras-GTP formation correlated with the profile of MAP kinase activation for all three agonists: transient for EGF and insulin and biphasic for thrombin (Fig. 2B and C), suggesting that MAP kinase is predominantly activated through Ras-GTP formation. The decrease observed after 10 min with thrombin was found to be consistent.

The effects of insulin, thrombin, and EGF on 2DOG uptake were subsequently determined. 3T3-L1 adipocytes were treated with insulin (1 μM), thrombin (2 U/ml), or EGF (50 ng/ml) for up to 30 min. No effect on 2DOG uptake was observed, even after prolonged incubation with thrombin or EGF (Fig. 3A). In contrast, insulin was found to stimulate glucose uptake maximally after 5 to 15 min, and stimulation remained at that level for at least 30 min (Fig. 3A). We also measured the effect of high doses of thrombin on 2DOG uptake. However, even concentrations of up to 50 U of thrombin per ml were unable to stimulate glucose uptake (Fig. 3B).

It is possible that the receptors for thrombin or EGF and insulin share some common signaling intermediates involved in the stimulation of glucose transport. In such a situation, an effect of insulin and thrombin or EGF on glucose transport would be expected. To test this possibility, we measured the dose response of insulin in the presence and absence of thrombin or EGF. The dose-dependent activation of 2DOG uptake by insulin is shown in Fig. 4. This dose dependency was similar to that for MAP kinase activation by insulin (Fig. 5A). The additional presence of either 2 U of thrombin per ml or 50 ng of EGF per ml did not significantly influence the dose-



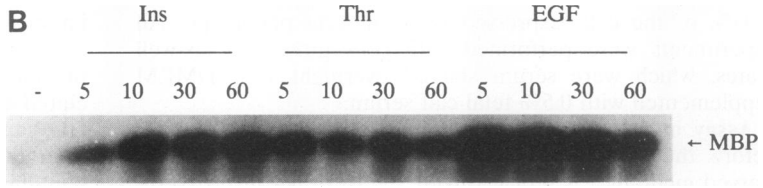


FIG. 2. Insulin-, thrombin-, and EGF-induced activation of MAP kinase in 3T3-L1 adipocytes. (A) Activation of MAP kinase measured as a phosphorylation-induced shift in its migration in SDS-PAGE. The cells either were not treated (−) or were stimulated (+) for 5 min with 1 μM insulin (Ins), 2 U of thrombin (Thr) per ml, or 50 ng of EGF per ml. The cells were then solubilized in SDS sample buffer, and cell homogenates were separated by SDS-PAGE (30 μg of protein per lane) as described in Materials and Methods. Two forms of MAP kinase, ERK1 (p44) and ERK2 (p42), were recognized by the MAP kinase-specific antisera and were visualized with alkaline phosphatase. The same results were obtained in six other experiments. (B) Activation of MAP kinase measured by an in vitro kinase assay with myelin basic protein (MBP) as a substrate. The cells either were not treated (−) or were incubated for 5, 10, 30, or 60 min with 1 μM insulin, 2 U of thrombin per ml, or 50 ng of EGF per ml. The data are representative of two experiments. The same pattern of activation as that in the MAP kinase shift assay was found. (C) Degree of phosphorylation of MBP quantified by densitometry. The two experiments differed by less than 10%. Symbols: ●, insulin; ○, thrombin; □, EGF. O.D., optical density.

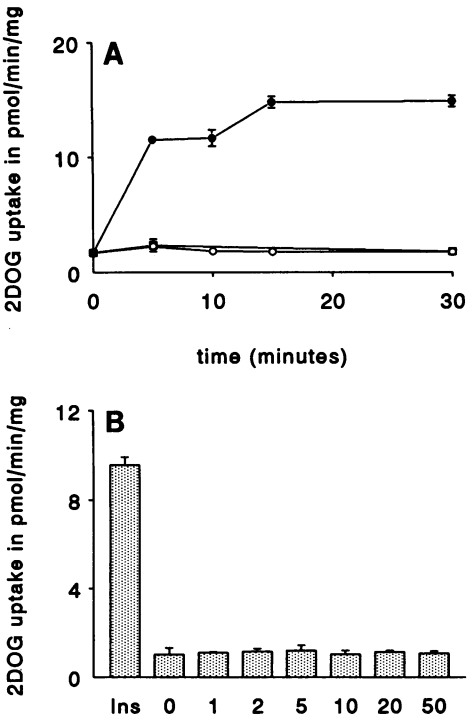


FIG. 3. Time course of insulin-, thrombin-, or EGF-induced effects on 2DOG uptake. (A) 3T3-L1 adipocytes were incubated for various times with insulin (1 μM; ●), thrombin (2 U/ml; ○), or EGF (50 ng/ml; □), and then 2DOG uptake was measured. Basal 2DOG uptake was 1.5 ± 0.4 pmol/min/mg (mean ± standard deviation, n = 17) and was stimulated 8.2- ± 1.7-fold (n = 13) by insulin, 1.1- ± 0.2-fold (n = 9) by thrombin (5 min), 1.3- ± 0.2-fold (n = 8) by EGF (5 min), and 1.0- ± 0.1-fold (n = 7) by thrombin or EGF (30 min). The data are presented as the mean ± standard deviation for a representative experiment done in triplicate. (B) The cells either were not treated (−) or were incubated for 5 min with 1 μM insulin (Ins) or increasing thrombin concentrations (1 to 50 U/ml). After these treatments, 2DOG uptake was measured for 10 min as described in Materials and Methods. Data are expressed as the mean ± standard deviation and are representative of at least three other experiments done in triplicate.

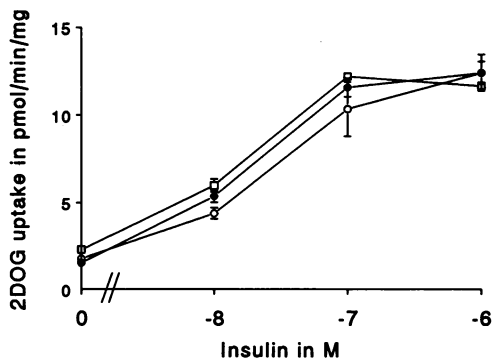


FIG. 4. Effects of thrombin and EGF on the dose-dependent stimulation of 2DOG uptake by insulin in 3T3-L1 adipocytes. 3T3-L1 adipocytes either were incubated for 15 min with increasing insulin concentrations (●) or were pretreated for 5 min with either 2 U of thrombin per ml (○) or 50 ng of EGF per ml (□) and then were incubated for 15 min with increasing concentrations of insulin. After these treatments, 2DOG uptake was measured for 10 min as described in Materials and Methods. Data are expressed as the mean ± standard deviation and are representative of at least two other experiments done in triplicate.

response curve of insulin (Fig. 4). The same results were obtained for cells pretreated for 5 min with thrombin and then incubated with insulin for 5, 15, or 30 min or when thrombin (or EGF) and insulin were added simultaneously for 5 min (data not shown).

The recent observation that cyclic AMP inhibits insulin-stimulated MAP kinase activation in some cell types at a step downstream of $p21^{ras}$ activation (5) prompted us to examine the effect of 8-bromo-cAMP on insulin-induced MAP kinase activation and glucose transport in 3T3-L1 adipocytes as well. The cyclic AMP analog by itself had no effect on MAP kinase phosphorylation (Fig. 5A). However, the insulin-induced phosphorylation of MAP kinase was inhibited when the cells were pretreated for 10 min with 0.5 mM 8-bromo-cAMP. This inhibition was only found at submaximal doses of 10^{-8} and 10^{-9} M insulin (Fig. 5A). In contrast to this inhibition of MAP kinase phosphorylation, 2DOG uptake was slightly stimulated by 8-bromo-cAMP. The small increase in glucose uptake in response to 8-bromo-cAMP was additive to that induced by insulin, even at a submaximal insulin concentration (Fig. 5B). This finding strengthens our idea that MAP kinase is not needed to provoke glucose uptake.

DISCUSSION

The steps that link the insulin receptor to the activation of glucose transport are unknown. It was recently shown that the activated insulin receptor, like many other tyrosine kinases, induces the formation of Ras-GTP and the activation of MAP kinase (4, 8). Evidence is emerging that the stimulation of an exchange factor (SOS, son of sevenless) leads to the activation of $p21^{ras}$ by enhancing the exchange of GDP for GTP on $p21^{ras}$ (3, 26). SOS binds via Grb2 to Tyr-phosphorylated IRS-1 (35, 37) and Tyr-phosphorylated Shc (9, 23, 25, 34). IRS-1 and Shc may be the direct substrates of the insulin receptor. Somehow, the signal generated by the activated insulin receptor results in the translocation of GLUT 4-containing vesicles from intracellular stores to the plasma membrane. GTP-binding proteins seem to be involved in this process (1, 6).

In this report, we present data showing that the activation of the Ras/MAP kinase signaling pathway is not sufficient to induce glucose transport in 3T3-L1 adipocytes, insulin-responsive cells in which glucose uptake is mediated predominantly via GLUT 4 (16, 39). Insulin, thrombin, and EGF all induced Ras-GTP formation and the activation of MAP kinase (Fig. 1 and 2), but only insulin provoked glucose uptake in these cells (Fig. 3). Also, the insulin-mediated glucose uptake was not potentiated by thrombin or EGF (Fig. 4). In addition, the inhibition of insulin-induced MAP kinase activation by 8-bromo-cAMP did not lead to an attenuation of insulin-mediated glucose uptake (Fig. 5).

In the case of EGF, it could be argued that the additional time needed to measure glucose uptake compared with that required for the MAP kinase activity assay and the Ras-GTP determination could lead to a misinterpretation of our results, as MAP kinase and $p21^{ras}$ were transiently activated, with a maximum at 5 min of EGF incubation. This situation seems unlikely, as thrombin induced a second peak of MAP kinase activity and Ras-GTP formation after ≈30 min but had no effect on glucose uptake for up to 30 min of incubation (Fig. 3). Furthermore, the time course of EGF-induced MAP kinase activation and Ras-GTP formation was rather similar to that observed for insulin (Fig. 1 and 2). Nevertheless, EGF did not stimulate glucose uptake, in sharp contrast to insulin (Fig. 3).

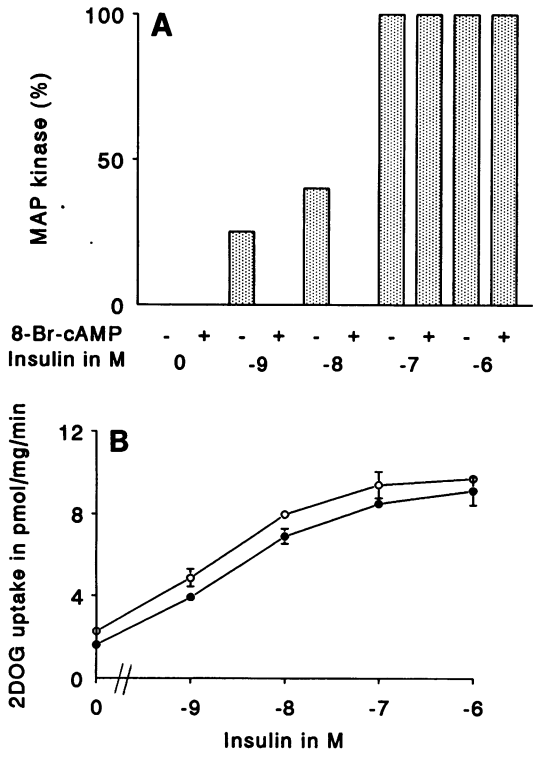


FIG. 5. Effects of 8-bromo-cAMP on insulin-mediated MAP kinase activation and glucose transport in 3T3-L1 adipocytes. 3T3-L1 adipocytes either were incubated for 10 min with increasing insulin concentrations (●) or were pretreated for 10 min with 0.5 mM 8-bromo-cAMP (8-Br-cAMP) (○) and then were incubated for 10 min with increasing concentrations of insulin. After these treatments, MAP kinase activation (A) or 2DOG uptake (B) was measured. MAP kinase activation was determined by the mobility shift assay with SDS-PAGE and then by Western blotting. The proportion of activated MAP kinase is given in percentages relative to the maximal response. The same results were obtained in another independently performed experiment. 2DOG uptake was measured for 10 min as described in Materials and Methods. Data are expressed as the mean ± standard deviation and are representative of two other experiments done in triplicate.

In addition, insulin was still capable of inducing glucose uptake after 30 min, a time point at which MAP kinase was inactivated again. This result suggests that MAP kinase is not involved in insulin-mediated glucose transport or that the transient activation of MAP kinase is enough to trigger more sustained processes needed to provoke glucose uptake.

The lack of effect of thrombin or EGF on glucose transport was not due to low-level activation of $p21^{ras}$ or MAP kinase by these hormones, as the order of potency for $p21^{ras}$ and MAP kinase activation was EGF > insulin > thrombin. Additional evidence that activated MAP kinase is not involved in the stimulation of glucose uptake is provided by experiments in which the effect of 8-bromo-cAMP on insulin-mediated glucose uptake and MAP kinase activation was examined. A rise in intracellular cyclic AMP levels in fibroblasts was recently shown to inhibit MAP kinase activation by insulin (5). The inhibition occurred at a step between Raf and $p21^{ras}$. We also found an inhibitory effect of 8-bromo-cAMP on the insulin-mediated activation of MAP kinase in 3T3-L1 adipocytes (Fig. 5A). However, no effect of 8-bromo-cAMP on the dose-response relationship between insulin and 2DOG uptake was observed (Fig. 5B).

Since thrombin, EGF, and insulin activate different upstream signaling pathways, it is possible that another signaling molecule that is activated by insulin but not by thrombin and EGF is needed in conjunction with $p21^{ras}$ and/or MAP kinase to provoke glucose uptake. Although we cannot exclude this possibility, our data provide no support for it, as we observed no effect of thrombin or EGF on the dose-response curve for insulin-stimulated glucose uptake (Fig. 4). Furthermore, the lack of inhibition of insulin-mediated glucose uptake by thrombin and EGF also indicated that the signaling pathway that is activated by these hormones did not block the insulin response, e.g., by competing for important substrates involved in glucose transport. We previously showed that some insulin receptor mutants were unable to increase Ras-GTP levels but still activated glucose uptake in CHO-derived cell lines (28). A dissociation of Ras/MAP kinase signaling and several metabolic, but not mitogenic, effects of insulin was also recently reported for PC12 cells (27). Very recently, Yang and Farese (41) also concluded from their studies with rat adipocytes that MAP kinase is not involved in insulin-mediated glucose uptake.

Our results are in contrast to the data presented by Kozma et al. (18). These authors obtained evidence that the introduction of activated $p21^{ras}$ mimics insulin action on glucose transporter translocation in 3T3-L1 adipocytes. The setups of our experiments and those of Kozma et al. are quite different. Our approach involved the transient activation of physiological levels of $p21^{ras}$ by extracellular stimuli, whereas Kozma et al. used 3T3-L1 adipocytes in which $p21^{ras}$ was semipermanently overexpressed. It is possible that under those conditions, secondary effects, e.g., an altered expression of genes, which may result in glucose transporter translocation are introduced. For example, 95% of the GLUT 4 protein was lost when activated $p21^{ras}$ was introduced into 3T3-L1 adipocytes. It is clear that more experiments are required to distinguish between direct and indirect effects of activated $p21^{ras}$ on glucose transport.

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