Rapid Activation and Nuclear Translocation of Mitogen-activated Protein Kinases in Response to Physiological Concentration of Glucose in the MIN6 Pancreatic β Cell Line*

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MIN6 is one of the few pancreatic β cell lines that respond to physiological concentrations of glucose by secreting insulin, and little is known about the triggered molecular mechanisms. We report below that the response to glucose in the MIN6 cells includes an activation of the p42 and p44 mitogen-activated protein (MAP) kinases (ERK2 and ERK1). This activation also occurred with the antidiabetic sulfonylureas glibenclamide and kainate, a specific agonist of a subtype of the ionotropic glutamate receptors, which depolarize the cytoplasmic membrane. The requirement for a calcium entry through the L-type voltage-gated channels and other characteristics of the regulation of the MAP kinase activity, such as the effect of the elevation of the cAMP concentration by forskolin, were similar to those of the secretion of insulin. However, the activation of the MAP kinases is not required for the secretion of insulin, inasmuch as this effect of glucose was not abolished when the MAP kinases were prevented from activation by PD98059, an inhibitor of the MAP kinase kinase. However, as the MAP kinases were translocated into the nucleus, they might be implicated in the calcium-dependent transcriptional response of the cells to glucose and thus regulate the expression of the insulin gene.

The secretion of insulin by the pancreatic β cells in response to the elevation of the blood glucose level is controlled at the level of insulin synthesis and exocytosis. Insulin synthesis is itself regulated both at the level of transcription and translation. Glucose also exerts a mitogenic effect on the pancreatic β cells through insulin-independent mechanisms. The pancreatic β cells are also regulated by numerous hormones and neurotransmitters such as glucagon, glucagon-like peptide-1 (GLP-1),1 vasoactive intestinal polypeptide, acetylcholine, or epinephrine, which enable them to respond to different physiological conditions (1). The signaling pathways mediating the short term action of the secretagogues on the exocytosis and the long term regulation of insulin production are not well understood. However, several enzymatic activities mediating cell signaling have been shown to be regulated by glucose in the pancreatic β cells. Protein kinase C (PKC), phospholipase C, and phospholipase A2 activities regulate insulin secretion in response to glucose (2–7), whereas cAMP-dependent protein kinase activity mediates the effect of GLP-1 on glucose-induced insulin secretion (1).

The pivotal role of calcium in the triggering and the regulation of exocytosis in the endocrine cells is well documented (8–10). The mechanisms leading to insulin exocytosis have been shown to depend on the entry of calcium through the voltage-gated calcium channels (1). In the pancreatic β cells the depolarization that induces the opening of voltage-gated channels is the result of the closure of the KATP potassium channels. The KATP channels, which are sensitive to the intracellular ATP/ADP ratio (with ATP inducing the closure of the channel) are the target of the widely used antidiabetic drug sulfonylureas (such as glibenclamide).

Recently, the activation of the mitogen-activated protein (MAP) kinases by calcium was demonstrated in several cellular systems (11–15). The MAP kinases p42 and p44, also known as ERK2 and ERK1, are serine/threonine kinases involved in the proliferation, differentiation, and adaptation of many cells to their environment (16). The calcium-activated MAP kinase pathway seems at least partially identical to the pathway activated in response to growth factors, a pathway known to involve a MAP kinase kinase (MEK), a MEK kinase, and a small GTPase such as Ras (17–20). Several isoforms of PKC, known as conventional PKCs (α, β1, βII, and γ isoforms), are directly regulated by calcium (21), and some of them may regulate the MAP kinase pathway (22). Among the calcium-regulated isoforms, the MIN6 cells express the α and βII isoforms (23). The elevation of the cyclic AMP concentration, which activates cAMP-dependent protein kinase, can either inhibit the MAP kinase activation through Ras/Raf in some systems (24–26), or enhance it in others (27, 28).

The MIN6 pancreatic β cell line was derived from transgenic mice expressing the SV40 T antigen under the control of the insulin promoter. These cells secrete normal levels of insulin, and the kinetics and the dose response of the glucose-induced insulin secretion are similar to the response of the primary cultures of the β islet cells (29). Interestingly, like the Langerhans β islet cells, this cell line expresses several functional glutamate receptors previously characterized in the central nervous system of mammals (30, 31). Stimulation of these receptors allows the entry of calcium into the cells and induces insulin secretion (32). Remarkably, the MIN6 cells express the...
kainate-receptor subunits that allow the formation of the kainate-sensitive sodium/potassium channels.

The present work focuses on the regulation of the ERKs/MAP kinases by calcium in response to several secretagogues and the effect of cAMP in a cell line that is very similar to the normal pancreatic islet β cells.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Stimulation**—The MIN6 cells were cultured as described previously (29), in Dulbecco’s modified Eagle’s medium containing 25 mM glucose and supplemented with 15% heat-inactivated fetal calf serum. Before treatment with the different reagents, the cells were washed twice in incubation buffer containing: 124 mM NaCl, 5.6 mM KCl, 2.5 mM CaCl2, 2.5 mM MgCl2, 20 mM HEPES, pH 7.0, 1 mM glucose. Before stimulation, the cells were incubated 1 h in this buffer at 37 °C. The stimulation with the different reagents (16 mM glucose, 500 μM kainate, 100 μM glibenclamide, or 100 μM forskolin) was performed at 37 °C and stopped at different times by the removal of the buffer and immediate cell lysis. The cells were lysed in cold lysis buffer containing: 124 mM NaCl, 5.6 mM KCl, 2.5 mM CaCl2, 2.5 mM MgCl2, 20 mM HEPES, pH 7.0, 1 mM glucose. Before stimulation, the cells were incubated 1 h in this buffer at 37 °C. The stimulation with the different reagents (16 mM glucose, 500 μM kainate, 100 μM glibenclamide, or 100 μM forskolin) was performed at 37 °C and stopped at different times by the removal of the buffer and immediate cell lysis. The cells were lysed in cold lysis buffer containing: 1% Nonidet P-40, 0.1% SDS, 158 mM NaCl, 10 mM Tris, pH 7.8, 1 mM phenylmethylsulfonyl fluoride, and 1 mM Na3VO4. Where indicated, nifedipine was used at 10 μM, the MEK inhibitor (PD98059) at 50 μM or 100 μM, and PKC inhibitors GF109203X and RO 31–8220 at 1 μM or 10 μM, during preincubation (1 h, 37 °C) and during stimulation. In the calcium-free experiments, the cells were incubated for 15 min in a calcium-free medium containing 1 mM EGTA and stimulated in the same buffer. The stimulation with elevated extracellular potassium was done by increasing the potassium concentration to 50 mM and reducing the sodium to 79.6 mM to maintain isotonicity. K50 (79.6 mM NaCl, 50 mM KCl, 2.5 mM CaCl2, 2.5 mM MgCl2, 20 mM HEPES, pH 7.0, 1 mM glucose).

**Preparation of Protein Extracts**—The protein extracts were prepared at 4 °C. After lysis with lysis buffer, the lysates were collected and centrifuged for 15 min at 12,000 × g. The pellets were discarded. Using γ-globulin as a standard, the protein concentration in the supernatants was determined by the Bradford microassay method (Bio-Rad) (33). The protein extracts were either used immediately for Western blotting, or stored at −20 °C before the determination of the MAP kinase activity.

**Immunoblotting**—Equal amounts of protein (50–100 μg) were separated by SDS-polyacrylamide gel electrophoresis in a 5% or 10% gel and transferred electrophoretically onto a nitrocellulose membrane. The nitrocellulose membrane was incubated for 1 h with 1% gelatin in Tris-buffered saline (10 mM Tris-HCl, pH 7.5, 150 mM NaCl) containing 0.05% Tween 20 (TBST) to block nonspecific binding. The antibodies used were: the monoclonal 4G10 anti-phosphoryosine antibody (a generous gift from Dr Brian Drucker, Oregon University) (1:1000, 1 h, room temperature) or the anti-active ERK antibody (1:20,000; Promega) (this antibody recognizes the dually phosphorylated MAP kinases ERK1 and ERK2 on the activating sites corresponding to residues 183 and 185 in ERK2); the anti-ERK2 C-14 antibody (1:1000; sc154, Santa Cruz) (this antibody recognizes ERK2 and also weakly ERK1), or (iii) the anti-active MAP kinase antibody 283 (a generous gift from Dr. Marsha Rich-Rosner, Ben May Institute, Chicago, IL) (1:500) (this antibody recognizes ERK2 and also weakly ERK1), or (iii) the anti-active MAP kinase antibody.

**MAP Kinase Assay**—The MAP kinase activity was determined at 30 °C in 25 μl of a buffer containing: 75 mM β-glycerophosphate, pH 7.2, 3.75 mM EGTA, 30 mM MgCl2, 1.5 mM dithiothreitol, 6 μM Ki peptide (cAMP-dependent protein kinase inhibitor), 0.15 mM Na2VO4, and 1 mM synthetic peptide (APRTPGGRR) (Upstate Biotechnology, Inc.), corresponding to the major site of phosphorylation of the myelin basic protein (MBP) by MAP kinase, and to which 12.5 μl of cytosolic fractions containing 6 μg of protein was added. The reaction was started with the addition of [γ-32P]PATP (0.25 μCi/assay) (Amersham Pharmacia Biotech). After 10 min, the reaction was stopped by the addition of 17 μl of 24% trichloroacetic acid and 100 μg of bovine serum albumin. After centrifugation for 5 min at 12,000 × g, 30 μl of supernatant were spotted onto P-81 phosphocellulose papers, the papers were washed four times with 175 μl orthophosphoric acid and transferred to vials, and the radioactivity was counted (34, 15). Under the conditions used, the MAP kinase activity was linear with time and protein concentration.

**Insulin Secretion**—The MIN6 cells grown in 2-cm diameter wells at 50% confluence were incubated for 1 h in the incubation buffer containing 5 mM glucose and 0.2% bovine serum albumin. The cells were then placed in 1 ml of the same buffer containing the different effectors or in 1 ml of 50 mM potassium buffer. After 1 h, the supernatants were collected and centrifuged (12,000 × g, 5 min), and the insulin content was determined by radioimmunoassay using the Sanofi Diagnostics Pasteur or Sorin (INSIK-5) kits.

**Immunofluorescence**—The cells were grown on glass coverslips, and the immunofluorescence was performed as described above; the stimulation was stopped by fixation of cells with 4% paraformaldehyde in PBS for 10 min, at room temperature. The permeabilization was done with 0.1% Triton X-100 in PBS. Incubation with a nonimmune goat serum (15 min, room temperature) was followed by 1 h incubation with anti-ERK2 polyclonal antibody (SC154) at a 1:100 dilution. After washing in PBS supplemented with 5% fetal calf serum, the secondary fluorescent antibody (fluorescein isothiocyanate-coupled goat antibody, Sigma) was added for 30 min. The final washes were followed by mounting in Vectashield containing 4,6-diamidino-2-phenylindole (Vector). Confocal microscopy was performed with a Bio-Rad MRC 1000.

**RESULTS**

Glucose Increases the MAP Kinase Activity of the MIN6 Cells—Addition of glucose to the cells induces the phosphorylation of a 42-kDa protein on tyrosine, which can be shown by Western blotting (Fig. 1A). The p42 protein can be identified as ERK2 when the blot is reprobed with a polyclonal anti-ERK antibody (anti-p42 and -p44 MAP kinase, respectively, ERK2 and ERK1). The phosphorylation of p42 peaked at 5 min after the addition of glucose, and decreased rapidly to the basal level afterward. The kinetics of the phosphorylation correlated well with the MAP kinase activity, which was measured with a synthetic peptide corresponding to a fragment of the MBP (Fig. 1C). A more sensitive assay, using an antibody specific for the active biphosphorylated form of ERK1 and ERK2, showed a low level of activation in response to glucose, which lasted at least 5 h (see Fig. 5). The activation was maximal with 16 mM glucose. This concentration is known to induce a maximal secretory response to a kainate receptor stimulation in the pancreatic β cells (29). The level of activation was not higher with higher concentrations of glucose (data not shown).

**The Activation of the MAP Kinases Is Mediated by the Glutamate Receptors**—The stimulation of the glutamate receptors expressed in the β cells also increases insulin secretion (32); therefore, this stimulation may also affect the MAP kinase activity. Indeed, when the cells were stimulated with kainate, a specific agonist of the kainate receptor (a subtype of the ionotropic glutamate receptors), it increased the phosphorylation of ERK2 (Fig. 1B). As with glucose, the MAP kinase activity was also increased when measured with the synthetic MBP peptide (Fig. 1C). The response to kainate was faster and sharper than to glucose, with a peak at 2 min (Fig. 1C). This transient activation was also detected by immunoblotting with the anti-active ERK antibody (data not shown). To our knowledge, this is the first report of an activation of the ERKs in response to a kainate receptor stimulation in the pancreatic β cells.

Kainate is likely to affect the membrane depolarization generated by glucose, so the cells were stimulated by the simultaneous addition of glucose and kainate. In several instances, the resulting activation was over 3 times the basal level, with a peak at 5–8 min (Fig. 2, A and B), whereas the activation by either glucose or kainate alone was just over 1.6 times the basal level (Fig. 1C and Table I).

**The Activation of the MAP Kinases Requires the Presence of Calcium**—Glucose depolarizes the plasma membrane, which leads to an entry of calcium. When the MIN6 cells were stimulated with glucose and/or kainate in the absence of calcium...
and in the presence of EGTA (1 mM), the ERKs were no longer activated (Fig. 3 and Table I). The glucose-stimulated calcium entry into the cells is known to occur mostly through the L-type voltage-gated calcium channels, and it was shown that this entry induces the secretion of insulin (36). Incubation of the MIN6 cells with 10 mM nifedipine, a specific inhibitor of the L-type voltage-gated calcium channels, blocked the activation of the ERKs by glucose and/or kainate (Fig. 3 and Table I). Therefore, the calcium entry through the L-type voltage-gated calcium channels appears to be necessary for the stimulation of the ERKs by glucose and/or kainate.

The MAP Kinases Are Also Activated by a Depolarization Induced by the Sulfonylurea Glibenclamide and by a High Extracellular Concentration of Potassium—It is known that glucose can by itself initiate a calcium-independent signaling. In order to estimate the involvement of the calcium independent signal in the activation of the ERKs by glucose, the antidiabetic sulfonylurea glibenclamide was used. Glibenclamide blocks the K_ATP channels (3, 37), thus mimicking the depolarizing effect of glucose, which triggers the entry of calcium. When 100 μM glibenclamide was added to the MIN6 cells, the ERKs were phosphorylated (Fig. 4). The activation of the ERKs by glucose was not significantly modified by glibenclamide (Fig. 4 and data not shown). The activation by glibenclamide was also blocked in the presence of EGTA (data not shown).

A cell membrane depolarization by an elevated extracellular potassium concentration is known to induce a calcium entry and a MAP kinase activation in several cell lines (12, 13, 35). When the MIN6 cells were stimulated with 50 mM extracellular potassium, the ERKs were rapidly activated (Fig. 4). This activation was larger (8.2 ± 0.54-fold, four independent experiments) than the one induced by glucose or kainate, but it was
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not sustained, and ended approximately after 1 h (Fig. 5, A and C). The activation of the ERKs by potassium was also blocked by EGTA and nifedipine (data not shown). These results imply that a calcium entry is by itself sufficient to activate the ERKs.

Forskolin Activates the MAP Kinases and Enhances the Glucose- but Not the Potassium-induced Activation of the MAP Kinases—The activity of the pancreatic β cells is regulated through several hormone receptors some of which are linked to the activation of adenylate cyclase. For example, GLP-I potentiates insulin secretion in response to glucose (38). In the presence of a nonstimulating concentration of glucose (3 mM), forskolin (which activates adenylate cyclase) led to a very low activation of the ERKs (Fig. 5). Additionally, when the cells were incubated in the presence of both a stimulating glucose concentration and forskolin, the activation of the ERKs was more than additive. The effect of forskolin was most clearly seen with a 10 mM concentration of glucose (Fig. 5A, a). However, forskolin also increased significantly the glucose-induced ERK activation, even at the 16 mM optimal glucose concentration (Fig. 5, A and B). Forskolin also had a potentiating effect on the low and sustained ERKs activation that occurred between 1 and 3.5 h (Fig. 5C). It appears that the elevation of the cytoplasmic concentration of cAMP by hormones such as GLP-I could modulate the activation of the ERKs by glucose. Interestingly, forskolin did not potentiate the stimulation by the potassium-induced depolarization (see Fig. 5A, b, and “Discussion”).

The Activation of the MAP Kinases by Glucose May Require PKC Activity—PKC activity is known to play an important role in the secretion of insulin in response to glucose and several isoforms of PKC are regulated by calcium. Two bisindolylmaleimide derivatives, GF109203X and RO31–8220, that are specific inhibitors of PKC were used to investigate its involvement in the activation of the ERKs by calcium in the MIN6 cells. Indeed, the two inhibitors blocked the activation of the ERKs by glucose in a concentration-dependent manner (Fig. 6). PMA (phorbol 12-myristate 13-acetate), which is a direct activator of some isoforms of PKC, was able to activate the ERKs in the MIN6 cells. The activation of the ERKs by PMA was blocked by RO 31–8220 with a concentration dependence similar to that seen with glucose (Fig. 6A).

The activation of the ERKs by a potassium depolarization was also blocked by the two PKC inhibitors within the same range of concentrations (Fig. 6B, b).

These results suggest that the activation of the ERKs by the entry of calcium into the MIN6 cells depends on one or several PKC isoforms. However, due to the high concentrations of the inhibitors used, the possibility that kinases other than PKC were inhibited cannot be excluded.

The MAP Kinase Activity Is Not Involved in the Immediate Regulation of Insulin Secretion—Previous studies on insulin secretion and the results described above show that: (i) secretagogues activate the ERKs, (ii) the maximal activation of the ERKs precedes the maximal initial secretion by 5–8 min, (iii) both the secretion of insulin and the activation of the ERKs depend on the entry of calcium through the L-type voltage-gated channels, and (iv) both responses depend on the activity of PKC. Therefore, the ERKs may be involved in the immediate regulation of insulin secretion. The recently described MEK inhibitor PD098059 (39) was used to test this possibility. Its addition to the MIN6 cells blocked the activation of ERKs by glucose (Fig. 7A) or potassium (data not shown). However, the secretion of insulin after 1 h in response to glucose or potassium was not affected by it (Fig. 7B). Thus, it appears that the activation of the ERKs and the secretion of insulin, both of which are triggered by an entry of calcium in response to glucose, become independent at some point in the signaling pathways.

ERK2 Is Translocated into the Nucleus in Response to Secretagogues—Because the activation of the ERKs is not involved in the immediate regulation of insulin secretion and because in other cells the ERKs have been shown to translocate into the nucleus in response to various stimuli, glucose and other secretagogues could also promote a nuclear translocation of the ERKs. The MIN6 cells were stimulated as above, and the

| Glucose | K50 |
|---------|-----|
| 1.65 ± 0.06 | 0.95 ± 0.06 |
| 1.60 ± 0.09 | 1.02 ± 0.05 |

**Table 1**

**Effect of EGTA and nifedipine on the MAP kinase activation by glucose and kainate**

The MIN6 cells were stimulated for 5 min with 16 mM glucose, for 2 min with 500 µM kainate, or without 10 µM nifedipine (Nif) or 1 mM EGTA. The MAP kinase activity was determined as described in Fig. 1 and compared to the activity in unstimulated cells. The data are expressed as the average ± standard deviation. n, number of independent experiments. ND, not done.

**Fig. 3.** EGTA and nifedipine abolish the stimulation of the ERKs by glucose and kainate. The MIN6 cells were stimulated for 2 min with 500 µM kainate (Ka), for 5 min with 16 mM glucose (G), or for 5 min with 16 mM glucose and 500 µM kainate, or not stimulated (C). 15 min before the stimulation 1 mM EGTA in calcium-free buffer and 10 µM nifedipine were added. Activation was determined by Western blotting with the anti-active ERK antibody (upper panel), an antibody that recognizes specifically the phosphorylated (on the two activating sites) ERK1 and ERK2 molecules. Lower panel, reprobing with the anti-ERK2 antibody.

**Fig. 4.** The ERKs are activated by a depolarization induced by glibenclamide or potassium. The MIN6 cells were stimulated for 5 min with 16 mM glucose (G), for 5 min with 16 mM glucose and 100 µM glibenclamide (G+Glib), for the indicated times (1–4 min) with 100 µM glibenclamide, or for 3 min with 50 mM KCl (K50). Western blots were carried out with the 4G10 anti-phosphothesine antibody (A), the anti-ERK2 antibody (B), or the anti-ERK2 plus the anti-active ERK antibody (C).
The subcellular location of ERK2 was determined by indirect immunofluorescence (Fig. 8). In the basal state (3 mM glucose), ERK2 was almost exclusively located in the cytoplasm, and only a very faint nuclear labeling could be seen in a few cells. In response to K50, a clear and rapid translocation of ERK2 into the nucleus was seen by confocal microscopy. Glucose alone or with forskolin also induced the translocation of ERK2 into the nucleus but with a lower efficiency, correlating with the level of the activation of the ERKs induced by the different treatments. Moreover, the translocation induced by glucose was already maximal at 5 min. These results show that the entry of calcium is sufficient to promote a nuclear translocation of the ERKs that may regulate the transcription of the insulin as well as other genes.

**DISCUSSION**

In this work, three different stimuli, glucose, kainate, and elevated extracellular potassium, are found to stimulate the activity of the ERKs in the MIN6 cells. The fact that such an activation of the ERKs was not previously detected in freshly isolated β cells after 15 min of stimulation by glucose (40) may be due to a difference in the sensitivity of the methods; the persistence of a low activity for at least 5 h was detected only with the anti-active ERK antibody. When the entry of calcium into the cells was blocked, either by incubation of the cells in the absence of calcium or by blocking the L-type calcium channels with nifedipine, the stimulation of the ERKs was abolished, indicating that calcium entry is a necessary component of this effect. The ERKs are also activated by glibenclamide, which, similarly to glucose, induces the entry of calcium into the cells by blocking the $K_{ATP}$ channels, thus depolarizing the membrane. The calcium component of the response of the MIN6 cells to glucose appears therefore sufficient to activate the ERKs. Furthermore, the magnitude of the activation of the ERKs was correlated with...
the extent of the calcium entry. For instance, potassium, which induces a higher rise in cytoplasmic calcium than either glucose or kainate (41), induced a higher activation of the ERKs. In addition, the concentration of kainate (0.5–1 mM) required to obtain a maximal activation of the ERKs was identical to the concentration that induced the maximal calcium entry into the cells (32). Although the magnitude of the activation of the ERKs was found to be clearly correlated with the extent of calcium entry, it decreased 15 min after the addition of glucose, despite the fact that glucose induces a sustained entry of calcium in the MIN6 cells (41). It appears that an inhibitory mechanism, which is independent of the decrease of calcium entry, regulates the activity of the ERKs. The level of the activation of the ERKs by stimulating the cells with both glucose and kainate showed some variability. This may be due to the fact that the calcium entry that takes place in response to this costimulation is itself variable. Analysis of the cytoplasmic concentration of calcium may help to elucidate this point.

Kainate was shown to be neurotoxic in the central nervous system by injection into the cerebral ventricles (42, 43). It specifies a subtype of the glutamate receptors, but its physiological counterpart is unknown. In the MIN6 cells, it stimulates the secretion of insulin (32). In the present study, the finding that kainate by itself induced a transient activation of the ERKs, and enhanced the activation in response to glucose, suggests that the kainate receptor may regulate the activity of the β cell in coordination with glucose. In fact, such a role was recently suggested for the kainate receptor in the pancreas; in an isolated rat pancreas, kainate stimulates the secretion of glucagon when the pancreas is perfused with 2.8 mM glucose (44), but it switches to the secretion of insulin when the concentration of glucose is raised to 8.3 mM. This concentration is slightly stimulatory by itself (45). Thus, the function of the kainate receptor may be to enhance the response of the pancreas to glucose, its main stimulus.

Both glucose and potassium trigger the entry of calcium into the cell, activate the ERKs, and induce the secretion of insulin. Forskolin by itself has little effect on the activity of the ERKs, but in the presence of glucose, it can bring about a quasi-maximal activation of the ERKs for up to 3.5 h. Such an effect does not occur with potassium. In contrast to glucose, the entry of calcium triggered by potassium is not mediated by the binding of ATP to the KATP channels. If the entry of calcium triggered either by glucose or potassium activates the ERKs by the same pathway, then the components mediating the enhancement of the activation of the ERKs by forskolin may be the

![Fig. 7. Inhibition of the phosphorylation of the ERKs in response to glucose by the MEK inhibitor PD098059: effect on insulin secretion. A, the MIN6 cells were stimulated 5 min with 16 mM glucose alone (G); glucose and Me2SO (same final concentration than for the MEK inhibitor, G + D); glucose and MEK inhibitor, (50 μM PD098059 added 1 h before the stimulation) (G + In). Western blots were carried out with the 4G10 anti-phosphotyrosine antibody (a), the anti-ERK2 antibody (b), or the anti-ERK2 plus the anti-active ERK antibody (c). B, the insulin secretion was determined by radioimmunoassay. The MIN6 cells were stimulated in buffer containing 0.2% bovine serum albumin and 100 μM PD098059 where indicated (In), with 3 mM glucose (Ctl), 16 mM glucose (Glc), or 50 mM potassium (K50). The results shown are the average of duplicate measurements of three to six samples in one experiment. Error bars represent the standard deviation between the samples. Three independent experiments were carried out with similar results.](http://www.jbc.org/)
K\textsubscript{ATP} channels themselves, possibly by phosphorylation. Finally, it is interesting to relate the absence of activation of the ERKs by forskolin alone and its ability to enhance the effect of glucose to the fact that the triggering of the insulin secretion by cAMP-dependent hormones such as GLP-1 also depends on the presence of a stimulatory concentration of glucose (1). This regulation by forskolin may be physiologically significant because cAMP may have a part in maintaining the transactivation potential of target transcription factors.

The kinetics of the activation of the ERKs suggested their direct involvement in the regulation of the insulin secretory process. However, the present work demonstrates that the MEK inhibitor (PD 098059) was unable to inhibit the insulin secretion after addition of glucose, either after 15 min (data not shown) or 1 h (Fig. 7), whereas it could efficiently block the activation of the ERKs. Similar results were described recently in the INS-1 cells (46). These observations are in agreement with the fact that nerve growth factor, which stimulated the ERKs in INS-1 cells (35), does not induce insulin secretion and with the fact that inhibitors of tyrosine phosphatases that stimulate the ERKs are also able to induce insulin secretion (40). Therefore, the regulation of insulin secretion appears to be independent of the activation of the ERKs. The long term regulation of insulin production at the transcriptional level is a more likely target of the activated ERKs. In this work, we demonstrate an occurrence of a nuclear translocation of the ERKs in the MIN6 cells, which is maximal after 5 min of stimulation with glucose. This is consistent with the rapid activation of the ERKs. It is important to note that, as opposed to what was reported in other systems (47), a sustained activation of the ERKs is not required for a nuclear translocation. Furthermore, inasmuch as a potassium-induced depolarization is able to trigger this translocation, it is clear that the entry of calcium into the cells is by itself sufficient to induce a fast nuclear translocation of the ERKs.

Altogether, the results presented above suggest that the ERKs are important intermediates in the regulation of the pancreatic \(\beta\) cell activity by calcium-dependent mechanisms. The secretory process itself, however, appears to be independent of the activation of the ERKs. The rapid translocation of the ERKs into the nucleus suggests their involvement in a transcriptional regulation. Such involvement was recently shown in the MIN6 cells for another MAP kinase, p38, which mediates stress responses, and was found to regulate the insulin gene expression in response to glucose (48). Recently, it was shown that, in contrast to the primary islet cells, in the INS-1 cells (49) the transcriptional activity of the insulin gene was diminished in response to high concentrations of glucose. Because the regulation of insulin secretion was almost identical in the primary islet cells and in the MIN6 cells, they may provide a valuable tool to study the transduction pathways involved in the transcriptional response of the insulin gene to glucose.

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