We showed previously that ERK1/2 were activated by glucose and amino acids in pancreatic β cells. Here we examine and compare signaling events that are necessary for ERK1/2 activation by glucose and other stimuli in β cells. We find that agents that interrupt Ca$^{2+}$ signaling by a variety of mechanisms interfere with glucose- and glucagon-like peptide (GLP-1)-stimulated ERK1/2 activity. In particular, calmodulin antagonists, FK506, and cyclosporin, immunosuppressants that inhibit the calcium-dependent phosphatase calcineurin, suppress ERK1/2 activation by both glucose and GLP-1. Ca$^{2+}$ signaling from intracellular stores is also essential for ERK1/2 activation, because thapsigargin blocks ERK1/2 activation by glucose or GLP-1. The glucose-sensitive mechanism is distinct from that used by phorbol ester or insulin to stimulate ERK1/2 but shares common features with that used by GLP-1.

Insulin is produced by β cells in the pancreatic islets of Langerhans in mammals. It is the key hormone that promotes the utilization and storage of glucose. Glucose, on the other hand, is the most important regulator of the secretion and biosynthesis of insulin by β cells, creating a deceptively simple primary loop controlling sugar metabolism. Signals from Ca$^{2+}$, inositol phospholipids, and cAMP are believed to mediate glucose effects on β cells, but detailed knowledge of the pathways that control β cell function is limited (1–6). Van Obberghen and co-workers (7) were the first to show that glucose activates the mitogen-activated protein (MAP)1 kinases ERK1 and ERK2 in islet-derived cells. We and others have confirmed this finding (8–12). MAP kinases, also known as ERKs, are components of kinase cascades important for transmitting extracellular information to coordinate cellular responses. MAP kinases have been implicated in many physiological events ranging from cellular proliferation and differentiation to cell survival (13, 14).

Glucose over its normal physiological concentration range increases the activity of ERK1/2 in pancreatic β cell lines and intact islets (7, 8, 10). Glucose metabolism is required for ERK1/2 activation (8), as it is for insulin secretion. Glucose regulation of ERK1/2 has been reported in adipocytes, for example, which also have some capacity for glucose sensing (15), but in few other cell types. Potentiators of insulin secretion, including forskolin, glucagon-like peptide 1 (GLP-1), and glucose-dependent insulinotropic polypeptide, which promote cAMP synthesis, potentiate ERK1/2 activation, leading to the view that ERK1/2 perform functions that depend on the glucose-sensing machinery of β cells (8, 12, 16).

Exposure of INS-1 cells to KCl induces Ca$^{2+}$ uptake and ERK activation (8). Inclusion of EDTA or EGTA in the medium blocks activation of ERKs by glucose in INS-1 cells and in islets. Experiments with chelators and with artificially low glucose suggest that ERKs are activated to a small but significant extent even at subthreshold glucose concentrations, because the activity in the presence of chelators or at 0–1 mM glucose is lower than activity at 2.8–3 mM glucose in the absence of chelators (8). As confirmation that agents that stimulate insulin secretion via an effect on Ca$^{2+}$ influx also activate ERKs, effects of the oral hypoglycemic drugs glyburide and tolbutamide, which cause closure of the ATP-dependent potassium channel in the β cell plasma membrane, were also examined (8, 10). Treatment of INS-1 cells with 10 mM glyburide or 100 mM tolbutamide for 2 h increased immunoreactive insulin in the medium by 2-fold in the absence of glucose and also caused a discernible increase in ERK activity. Finally, blockers of L-type Ca$^{2+}$ channels interfere with glucose-induced ERK1/2 activation, suggesting the importance of Ca$^{2+}$ influx on this kinase pathway (10).

Efforts to elucidate the mechanism of ERK1/2 activation have suggested a role for several signaling molecules as intermediates in the pathway. Notable among these is protein kinase C (PKC), which is implicated in one report but discounted by others (11, 17). PKC causes activation of ERK1/2 downstream of G protein-coupled receptors that act through G$_{i}$/G$_{o}$ and is also involved in prolonged activation caused by some other ligands (18, 19). Likewise, glucose induces tyrosine phosphorylation, but its role in ERK1/2 activation by glucose is controversial (11, 17, 20).

In this study we have examined regulation of ERK1/2 by several extracellular cues, including glucose, GLP-1, and insulin. Our goals were to determine to what extent these agents shared common mechanisms for ERK1/2 activation and to be-
gin to define essential components of the pathways. Our studies reveal that glucose and GLP-1 converge on a common mechanism for ERK1/2 regulation that is distinct from that used by insulin. The mechanism we propose has significant differences from those previously suggested to control ERK1/2 in \(H9252\) cells; we find a dependence on the natural release of \(Ca^{2+}\) from intracellular stores and a sensitivity to inhibition by immunosuppressants that block the calmodulin-regulated phosphatase calcineurin.

**EXPERIMENTAL PROCEDURES**

**Materials**—Recombinant adenoviruses were prepared in this laboratory (K52R ERK2) as described (21) or were kind gifts of L. Klesse (MEK1 S217A, MEK1 S217E, S221E, Raf C4B, Raf BXB, G15A H-Ras) (22) and B. Rothermel (myocyte-enriched calcineurin interacting protein) (23, 24). Forskolin, cyclosporin A, and GLP-1 were purchased from Sigma. KN62, KN93, GF109203X, pituitary adenylyl cyclase-activating peptide (PACAP), the calmodulin antagonist W7, thapsigargin, dantrolene, 2-aminoethoxy-diphenyl borate, nifedipine, dazoxiben, rapamycin, the Src inhibitor PP2, and wortmannin were purchased from Calbiochem.

**Cells**—INS-1 cells, either from early passages or subclones selected for increased glucose-stimulated insulin secretion kindly provided by Chris Newgard (6), were grown in RPMI 1640 medium containing 10% fetal bovine serum, 0.5 mM Hepes, pH 7.4, 10.2 mM l-glutamine, 50 mM sodium pyruvate, and 2.5 mM \(\text{H}_2\text{S}_2\text{O}_3\) (25). Cells that were 60–80% confluent were preincubated for 1–2 h in Krebs-Ringer-bicarbonate-Hepes (KRBH) with 0 or 2 mM glucose prior to treatment. In the indicated experiments, the cells were infected with recombinant adenoviruses at multiplicities of infection of 10–100 for 1 h, 24 or 48 h prior to cell treatment. After treatment with the agents indicated in figure legends, the medium was removed, and the cells were washed with cold phosphate-buffered saline and harvested in 0.2 ml of cold lysis buffer (50 mM Hepes, pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.2 mg/ml phenylmethylsulfonyl fluoride, 0.1 M NaF, 2 mM Na\(_3\)VO\(_4\), 10 mg/ml aprotinin, 5 \(\mu\)g/ml pepstatin A, 5 mg/ml leupeptin (7)). After 20 min on ice, the supernatants were collected following centrifugation for 10–30 min at 14,000 rpm in an Eppendorf microcentrifuge and were stored at \(-80°\)

**Measurement of ERK1/2 Activity**—Equal amounts of lysate proteins (20–40 \(\mu\)g) were resolved in 10% polyacrylamide gels in sodium dodecyl sulfate and subjected to electrophoresis. The proteins were transferred...
Nitrocellulose at 700 mA for 1.5 h at 4 °C. The membranes were incubated in 5% nonfat milk, 0.05% Tween-Tris-buffered saline (TBS) for 1 h, then in 1:3000 anti-phospho-ERK1/2 antibody (BioSource or Sigma) in 1% nonfat milk, 1% bovine serum albumin, 0.05% Tween-TBS for 2 h, and finally in 1:5000 anti-rabbit IgG in 1% nonfat milk, 1% bovine serum albumin, 0.05% Tween-TBS for 1 h at room temperature. The membranes were washed twice in 0.05% Tween-TBS and twice in TBS. After detection of phosphorylated ERK1/2 bands (43 and 41 kDa, respectively) by enhanced chemiluminescence and autoradiography, the membranes were stripped in 62.5 mM Tris-HCl, pH 6.7, 100 mM β-mercaptoethanol, and 2% SDS for 30 min at 60 °C, rinsed with 0.05% Tween-TBS, and immunoblotted as above with 1:5000 Y691 anti-ERK1/2 rabbit polyclonal antibody (26).

RESULTS

Upstream Components of the Glucose-stimulated ERK1/2 Pathway in INS-1 Cells—To examine upstream components of the glucose-dependent MAP kinase cascade, we infected INS-1 cells with adenoviruses expressing kinase-defective or interfering mutants of proteins viewed as core components of the ERK1/2 pathway in other cell types (Ras, Raf, MEK1, and ERK2) (14, 22). Expression of mutants of the core components that have been shown to inhibit ERK1/2 activation in other cell types (13, 14), K52R ERK2, S217A MEK1, Raf C4B, and G15A H-Ras, blocked glucose-stimulated ERK1/2 activity (Fig. 1, A–C). In these experiments ERK1/2 activity was monitored in cell lysates with antibodies that selectively recognize the doubly phosphorylated, active forms of the kinases. Total ERK1/2 immunoreactivity demonstrated that equal amounts of the two proteins were present in each lane. Consistent with its inhibitory action, the kinase-defective mutant K52R ERK2 reduced phosphorylation of endogenous ERK1/2. We previously showed that MEK1 but not MEK2 was activated by glucose in INS-1 cells (9), consistent with the finding that the phosphorylation-defective mutant S217A MEK1 blocked ERK2 activation by glucose (Fig. 1B). The effects of the inhibitory Ras mutant and the Raf N-terminal fragment support the conclusion that a small GTP-binding protein, either a Ras isoform or another GTPase that uses a common guanine nucleotide exchange factor, is required for ERK1/2 activation in INS-1 cells.

Tyrosine kinases often participate in ERK1/2 activation, and glucose has been reported to induce tyrosine phosphorylation in β cells (15, 20). We examined the potential roles of two tyrosine kinases, Src and PYK2, in inducing ERK1/2 activation. We found no evidence of glucose-induced changes in tyrosine phosphorylation of PYK2 in INS-1 cells (data not shown), suggesting that it is not involved. Src inhibitors including PP2 (27) caused a partial but significant reduction in ERK1/2 phosphorylation in response to glucose (Fig. 1D), suggesting a role for a Src-like kinase.

Calmodulin Is the Calcium-dependent Effector Required for Stimulation of ERK1/2 by Glucose in INS-1 Cells—We previously showed that extracellular Ca²⁺ was required for glucose-dependent ERK1/2 activation (8). Thus, we evaluated the effects of inhibitors that block calcium-dependent signaling molecules. First, we tested PKC inhibitors (27) because PKC is required in several systems for ERK1/2 activation, and it has been suggested that this is also the case in β cells (11, 28). None of the inhibitors tested, including bis-indoymaleimide...
Because we had clear evidence that calmodulin/calcineurin were required for ERK1/2 activation by glucose, we wished to determine whether other agents that activate ERK1/2 also employ a mechanism dependent on these factors. We examined the potential involvement of calmodulin/calcineurin in ERK1/2 activation by phorbol ester, which has not generally been found to use these signal transducers (Fig. 4A). Consistent with an independent mechanism of action, phorbol ester-stimulated activity was not affected by calcineurin inhibitors.

Hormones that stimulate adenyl cyclase including GLP-1 (Fig. 4, B and C) and PACAP (not shown) activate ERK1/2 (Refs. 12, 16, and 40 and this study). Although the extent of activation was usually less than with either glucose or forskolin, increased ERK1/2 phosphorylation was detected within 2–5 min and persisted for at least 10 min with either GLP-1 or PACAP. The effect on kinase activity was greater if 2 mM glucose was present in the medium. Activation of ERK1/2 by GLP-1 was sensitive to inhibition by W7 but like glucose largely insensitive to wortmannin (Fig. 4B). Although activity stimulated by forskolin alone was apparently less sensitive to the phosphatase inhibitors (Fig. 2B and not shown), activity stimulated by glucose plus GLP-1 or glucose plus forskolin was blocked by FK506 (Fig. 4C). In each of these cases, rapamycin did not reduce ERK1/2 activity.

Because insulin stimulates ERK1/2 in many tissues and the agents above that trigger ERK1/2 also enhance insulin secretion, we wished to determine whether induction of ERK1/2 activity by insulin in β cells uses a calmodulin/calcineurin-dependent mechanism and is involved in their activation by glucose. Insulin and IGF-1 (not shown) stimulated ERK1/2 in INS-1 cells, but to a much reduced extent compared with glucose (Fig. 4D). Interestingly, activation of ERK1/2 by insulin was not blocked by wortmannin (lower panel), which inhibits phosphatidylinositol 3-kinase, a major mediator of insulin action (41), although activation of Akt was inhibited (not shown). Wortmannin also had little effect on glucose-stimulated ERK1/2 activity (Fig. 4B). Stimulation of ERK1/2 by insulin, in contrast to glucose, was not reduced by W7 or cyclosporin A (Fig. 4D). These findings suggest that glucose and insulin use distinct mechanisms to trigger ERK1/2.

Source of Ca\(^{2+}\) Required for Glucose-dependent ERK1/2 Activation—Ca\(^{2+}\) was shown to be required in the earliest studies of this response (7, 8). To explore this requirement further, we first depolarized cells by exposing them to 25 mM KCl (Fig. 5A). KCl caused a rapid but transient increase in ERK1/2 activity, which had returned to control values by 10 min; glucose activated ERK1/2 more following a 30-min incubation in the presence of KCl than in its absence (not shown). Like activation by glucose, activation of ERK1/2 by KCl was blocked by wortmannin (Fig. 5A).

We next examined the source of calcium required for ERK1/2 activation. Nifedipine, a blocker of L-type voltage-gated Ca\(^{2+}\) channels (42), partially blocked glucose stimulation of ERK1/2 (Fig. 5B), as was reported (11, 12). Similar results were observed with the related blockers nisoldipine and nitrendipine (not shown). Under no condition was a complete blockade produced with these agents. Dazoxiben is a thiazide, which maintains ATP-sensitive potassium channels in the open state, and is used therapeutically to inhibit insulin release from insulin-secreting tumors. This agent at millimolar concentrations blocked glucose activation of ERK1/2 (Fig. 5C); high micromolar dazoxiben caused a modest reduction in ERK1/2 activity (not shown). These results indicate that Ca\(^{2+}\) influx is important for ERK1/2 activation.

The Ca\(^{2+}\) that is necessary to trigger the kinase cascade could be that resulting from the influx of extracellular Ca\(^{2+}\) or
that released from intracellular stores. To determine the impact of the release of Ca\(^{2+}\) from intracellular stores on activation of ERK1/2 by glucose, cells were pretreated with 1 mM thapsigargin for 15 min, a time sufficient for this ATPase inhibitor to exhaust the intracellular Ca\(^{2+}\) pool (43). Thapsigargin itself did not increase ERK1/2 activity following 5, 10, or 15 min of exposure (not shown). However, activation of ERK1/2 by glucose was completely blocked by thapsigargin (Fig. 6A), suggesting that glucose causes release of intracellular Ca\(^{2+}\) to promote ERK1/2 activation. To compare the effects of glucose to membrane depolarization induced by KCl, we determined whether the effects of KCl were also blocked by thapsigargin. Thapsigargin significantly reduced but did not completely block ERK1/2 activation by KCl (Fig. 6B), consistent with the idea that the Ca\(^{2+}\) that activates ERK1/2 is the intracellular pool. For comparison, we also examined the effects of thapsi-
gargin on stimulation of ERK1/2 by insulin (Fig. 4D) and GLP-1 (Fig. 6C). No blockade of insulin-increased ERK1/2 activity was detected, but GLP-1-induced activity was inhibited.

Ca\textsuperscript{2+} influx can induce the release of this pool through ryanodine receptors, and glucose-induced production of inositol trisphosphate (IP\textsubscript{3}) may activate IP\textsubscript{3} receptors (44–48). Thus, we tested dantrolene and 2-aminoethoxy-diphenyl borate, inhibitors of Ca\textsuperscript{2+}/H\textsuperscript{11001} release mediated by ryanodine and IP\textsubscript{3} receptors, respectively (49, 50), to seek independent evidence that intracellular stores are required (Fig. 6D). Both blocked ERK1/2 activation by glucose.

**DISCUSSION**

Glucose causes the rapid and continuous activation of ERK1/2 in \( \beta \) cells. The effects of GLP-1 and PACAP, although also very rapid, are short-lived. In comparison, ERK1/2 activation by forskolin is slower but prolonged, perhaps because of actions independent of its ability to increase cAMP or to the much greater accumulation of cAMP it elicits than GLP-1. Because drugs that block GLP-1 are variably effective in blocking forskolin, forskolin may bypass some otherwise essential steps, thereby forcing a cAMP-dependent pathway that does not normally occur in pancreatic \( \beta \) cells in response to hormones that produce cAMP as a second messenger. Our results further suggest that glucose and GLP-1 converge on a common mechanism of action. In contrast, insulin is generally a weaker ERK1/2 stimulus and clearly regulates ERK1/2 through a different mechanism. Agents that block glucose- and GLP-1-induced ERK1/2 activity, including calmodulin antagonists and thapsigargin, are ineffective in blocking induction of kinase activity by insulin. Glucose-induced secretion of insulin does not require the release of intracellular Ca\textsuperscript{2+} stores (51, 52). In addition, FK506 appears to have little effect on insulin secretion within the first few hours of exposure (34, 53–55), although it inhibits glucose-stimulated ERK1/2 activation. These observations further support the idea that the control of ERK1/2 activity exerted by glucose has little to do with the autocrine action of insulin on these cells.

The terminal components of the signaling pathway implicated by the use of interfering mutants include several of the usual suspects. Most clear, MEK1 activity is required; pharmacological inhibitors as well as an inhibitory mutant of MEK1 block ERK1/2 activation, consistent with the finding that MEK1 but not MEK2 is responsive to glucose (9). A small G protein, most likely Ras, is also required, based on the inhibitory effects of a dominant-interfering Ras mutant and of an N-terminal fragment of Raf1, which is thought to act by se-

---

**Fig. 5. Role of calcium influx in ERK1/2 activation.** A, INS-1 cells preincubated for 4 h in KRBH plus 2 mM Glc were exposed to 30 mM KCl for the indicated times either without or with a 15-min preincubation in 100 nM FK520 or rapamycin. B, cells treated as for Fig. 1 were preincubated in 5 mM nifedipine (Nif) or 100 nM FK506 for 15 min and then stimulated with 15 mM Glc for 30 min. C, cells treated as for Fig. 1 were incubated with 1 mM diazoxide (Diaz) for 15 min and then stimulated with 15 mM Glc for 30 min. ERK1/2 activities were analyzed as above. In B, duplicate lanes show samples from independent replicates. The data shown are representative of three or more similar experiments.
questering activated Ras, preventing it from binding to endogenous Raf proteins. The work of Bos and co-workers (56) suggests that Rap cannot directly activate Raf, despite the fact that the Rap effector domain can bind to Raf. The inhibitory effect of the Src inhibitor PP2 suggests that a Src family or other tyrosine kinase leads to stimulation of Ras. Glucose-induced tyrosine phosphorylation has been documented in pancreatic β cells (20). In contrast to previous reports, we find no evidence for a role of conventional isoforms of PKC, which might act upstream of Ras (11, 17). Furthermore, phorbol ester stimulation of ERK1/2 is insensitive to inhibition by the immunosuppressants that completely block the action of glucose on the kinases.

Less clear is the MAP kinase kinase kinase involved in ERK1/2 activation. Kinase-dead MEK1 would probably interfere with any relevant MAP kinase kinase kinase, and the inhibitory N-terminal Raf fragment that contains the Ras-binding domain most likely blocks the pathway by targeting Ras, not a Raf family member. In numerous experiments we have found inconsistent activation of Raf-1 and even less evidence for B-Raf activation by glucose in INS-1 cells. However, our findings do not yet convince us that glucose or GLP-1 employs a Raf-independent mechanism, as was recently suggested (12). This is not the only setting in which Raf activity has not been well correlated with ERK1/2 activation (57). Aside from the difficulties of the assay itself, two possibilities seem worthy of consideration. First, cAMP may suppress phosphatase activity that normally inactivates MEK1, and second, cAMP may enhance the formation of Raf1-MEK1 complexes. In either case the efficiency of MEK1 activation would be increased, despite minimal Raf activation.

Two conclusions about mechanism are straightforward from our findings. First, the essential mediator of ERK1/2 activation by glucose and GLP-1 is Ca^{2+}. Inhibiting Ca^{2+} signaling prevents ERK1/2 activation by either type of agent. Epac2, a cAMP-dependent guanine nucleotide exchange factor for Ras family small GTPases (58, 59), is present in pancreatic β cells.
and may mediate the actions of cAMP, perhaps directly on Ras. However, our findings suggest that if this factor is involved, it likely acts upstream not downstream of Ca$^{2+}$; this conclusion is consistent with a report from Holz and co-workers (59), who suggest that Epac2 activates Ca$^{2+}$ release in β cells through effects on the related G protein Rap. Second, an important source of Ca$^{2+}$ for glucose activation of ERK1/2 is the intracellular storage compartment. By blocking a Ca$^{2+}$- ATPase, thapsigargin depletes Ca$^{2+}$ from this pool. Furthermore, dantrolene blocks calcium-induced release from this pool. The impaired release of Ca$^{2+}$ from the storage compartment completely prevents ERK1/2 activation by glucose or GLP-1, strongly suggesting that the intracellular release of Ca$^{2+}$ is an essential part of the mechanism of ERK1/2 activation by these agents.

Given that both glucose and cAMP cause release of intracellular Ca$^{2+}$ from this pool, perhaps via ryanodine receptors (2, 44, 59, 60), Ca$^{2+}$-stimulated Ca$^{2+}$ release may be the key process on which these agents converge. Inhibition of glucose stimulation of ERK1/2 by dantrolene supports this conclusion. However, 2-aminoethoxy-diphenyl borate also blocks glucose activation. This inhibitor also reportedly interferes with Ca$^{2+}$ entry (61); thus, we can only speculate that the IP$_3$ receptor is also involved in this process. These Ca$^{2+}$ release receptors are reportedly regulated by calcineurin, suggesting that the requirement for calcineurin in activating ERK1/2 may come from its effects on Ca$^{2+}$ release (37, 38). If this is correct, calcineurin may be viewed as a gatekeeper rather than acting directly as a participant, in ERK1/2 activation. Some evidence suggests that the Ca$^{2+}$ release pool may be linked directly to the plasma membrane (62). This juxtaposition may facilitate compartmentalized signaling from receptor and channel complexes; if so, that may account for the rapid effects of GLP-1 and PACAP relative to forskolin.

Inhibitor studies have implicated CaMKII, which is known to be activated by glucose in β cells (31). It may act downstream of the release of intracellular Ca$^{2+}$ stores, as suggested below. The CaMKII inhibitors have been reported to influence not only other CaMks but also Ca$^{2+}$-channels (63); thus, the inhibition we observed may have been caused by effects on Ca$^{2+}$ signaling that is not mediated by CaMks at all. This remains to be determined.

Because so many glucose-stimulated components have been implicated in multiple ways by inhibitor studies, it has been difficult to define the signaling pathway leading to stimulation of ERK1/2. We can envision two scenarios that might account for the apparent complexity of our findings. One possibility is that ERK1/2 act as a coincidence monitor in this system in that more than one type of signal must be triggered for activation of the cascade by glucose; for example, based on the comments above, calcineurin might provide the coincident signal. A second scenario is that the kinases are activated by a complex but linear series of events that have not been previously defined for this pathway.

For future investigation and with the many caveats discussed above, we propose the following pathway (Fig. 7), which incorporates all of the signaling molecules implicated by our inhibitor studies. Glucose metabolism is coupled to Ca$^{2+}$ influx, which causes Ca$^{2+}$ release from intracellular stores, through ryanodine and perhaps also IP$_3$ receptors (44). GLP-1 through cAMP also induces Ca$^{2+}$ release via Epac2 from intracellular stores (2, 59, 64). The actions of GLP-1 and glucose converge at this Ca$^{2+}$ release step, and potentiation of the response may occur as a consequence of the mechanisms by which each agent works on Ca$^{2+}$ release. Depending on calcineurin activity, Ca$^{2+}$ release will occur or be prevented. Release of intracellular Ca$^{2+}$ in a discrete location is coupled to activation of a CaM kinase family member. The CaM kinase then employs a tyrosine kinase (e.g., epidermal growth factor receptors (65, 66)) to activate the Ras/ERK1/2 cascade. We are currently devising experiments to test the key steps in this pathway.

ERK1/2 are most frequently implicated in cell proliferation programs. Nevertheless, they are highly expressed in terminally differentiated cells including neurons (67). They play a role in long term potentiation and in synaptic modulation, providing a means of storing signaling information on a longer time scale than individual action potentials (68–71). Their actions in β cells may be analogous in that they offer a means of integrating the complex and ever changing nutrient and hormonal signals that acutely control insulin secretion to ensure that β cells maintain their secretory capacity.

Acknowledgments—We thank Joe Albanesi, Shmuel Mualem, Mike White, James Chen, Kim Orth, and members of the Cobb laboratory (University of Texas Southwestern Medical Center) and Barbara Ehrlich (Yale) for comments about the manuscript, Cherry Ballard Croft for some early work on this project, and Dionne Ware for administrative assistance.

REFERENCES

1. Antinoozi, P. A., Ishihara, H., Newgard, C. B., and Wollheim, C. B. (2002) J. Biol. Chem. 277, 11164–11175
2. Holz, G. G., Luech, C. A., Heller, R. S., Castonguay, M., and Habener, J. F. (1996) J. Biol. Chem. 271, 14147–14156
3. Nielsen, D. A., Welsh, M., Casadaanan, M. J., and Steiner, D. F. (1985) J. Biol. Chem. 260, 13585–13589
4. Liu, Y.-J., Grupenengesser, E., Gilfr, E., and Hellman, B. (1996) Arch. Biochem. Biophys. 334, 295–302
5. Ma, Z., Ramanadatham, S., Wohltmann, M., Behr, W., Hsu, F. U., and Turk, J. (2001) J. Biol. Chem. 276, 13189–13208
6. Hohtsner, H. E., Mulder, H., Chen, G., Henkel-Rieger, R., Prentki, M., and Newgard, C. B. (2000) Diabetes 49, 424–430
7. Frodin, M., Sekein, N., Roche, E., Fillouco, C., Prentki, M., Wollheim, C. B., and van Ooerkbergen, E. (1996) J. Biol. Chem. 271, 7892–7898
8. Xue, S., Khoo, D., and Cobb, M. H. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5509–5604
9. Xue, S., Khoo, D., Wang, A., Witt, S., Do, V., Zhen, E., Schaefer, E., and Cobb, M. H. (1997) Mol. Endocrinol. 11, 1615–1625
10. Benes, C., Ruisins, M. P., Van Tan, H., Creuzet, C., Miyazaki, J., and Fargard, R. (1998) J. Biol. Chem. 273, 15507–15513
11. Benes, C., Poitout, V., Marie, J. C., Martin-Perez, J., Rouin, M. P., and Fargard, R. (1999) Biochem. J. 340, 219–222
12. Gomez, E., Pritchard, C., and Herbert, T. P. (2002) J. Biol. Chem. 277, 43572–43581
13. Lewis, T. S., Shapiro, P. S., and Ahn, N. G. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 13205–13209
14. Chen, Z., Gibson, T. B., Robinson, F., Silvestro, L., Pearson, G., Xu, W., Wright, A., Vanderlith, C., and Cobb, M. H. (2001) J. Biol. Chem. 276, 24459–24476
15. Rothermel, B., Vega, R. B., Yang, J., Wu, H., Bassel-Duby, R., and Williams, R. S. (2000) Mol. Endocrinol. 14, 15507–15513
16. Benes, C., Ruisins, M. P., Van Tan, H., Creuzet, C., Miyazaki, J., and Fargard, R. (1998) J. Biol. Chem. 273, 15507–15513
17. Benes, C., Poitout, V., Marie, J. C., Martin-Perez, J., Rouin, M. P., and Fargard, R. (1999) Biochem. J. 340, 219–222
18. Corbit, K. C., Foster, D. A., and Rosner, M. R. (1999) Mol. Cell. Biol. 19, 4209–4218
19. Rothermel, B., Vega, R. B., Yang, J., Wu, H., Bassel-Duby, R., and Williams, R. S. (2000) Mol. Endocrinol. 14, 15507–15513
20. Konrad, R. J., Dean, R. M., Young, R. A., Billings, C. F., and Wolf, B. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6928–6928
Regulation of ERK1 and ERK2 in Pancreatic β Cells

32525

142, 1218–1227
35. Redmon, J. B., Olsen, L. K., Armstrong, M. B., Greene, M. J., and Robertson, R. P. (1996) J. Clin. Invest. 98, 2786–2793
36. Renstrom, E., Ding, W. G., Bokvist, K., and Rorsman, P. (1996) Neuron 17, 515–522
37. Cameron, A. M., Steiner, J. P., Roskams, A. J., Ali, S. M., Ronnett, G. V., and Soderling, T. R. (1995) J. Biol. Chem. 270, 340–346
38. Summers, S. A., Whiteman, E. L., and Birnbaum, M. J. (2000) Biochem. J. 352, 61–70
39. Zawalich, W. S., Zawalich, K. C., and Kelley, G. G. (1996) Pflugers Arch. Eur. J. Physiol. 432, 589–596
40. Bandyopadhyay, A., Shin, D. W., Ahn, J. O., and Kim, D. H. (2000) Cell 103, 463–472
41. Mitterdorfer, J., Grabner, M., Kraus, R. L., Hering, S., Prinz, H., Glossmann, H., and Striessnig, J. (1998) J. Bioenerg. Biomembr. 30, 319–334
42. Shin, D. M., Zhao, X. S., Luo, X., Bera, A. K., and Muallem, S. (2000) J. Biol. Chem. 275, 15963–15968
43. Le, C. T., and Peppiatt, C. M. (2002) FASEB J. 16, 5425–5436
44. Lemmens, R., Larsson, O., Berggren, P. O., and Islam, M. S. (2001) EMBO J. 20, 3463–3468
45. Maechler, P., Kennedy, E. D., Sebo, E., Valeva, A., Pozzan, T., and Wollheim, C. B. (1999) J. Biol. Chem. 274, 13813–13818
46. Takasawa, S., Kiymar, T., Naka, K., Kuroki, M., Kohgo, Y., Noguchi, N., Kobayashi, S., Ito, K., Katada, T., and Okamoto, H. (1998) J. Biol. Chem. 273, 2497–2500
47. Hagar, R. E., and Ehrlich, B. E. (2000) Cell Mol. Life Sci. 57, 1938–1949
48. Boulton, T. G., Sebo, E., Katsuki, T., Naka, K., Kuroki, M., Kohgo, Y., Noguchi, N., Kobayashi, S., Ito, K., Katada, T., and Okamoto, H. (1998) J. Biol. Chem. 273, 2497–2500
49. Macchler, P., Kennedy, E. D., Sebo, E., Valeva, A., Seba, M., and Wallheim, C. B. (1999) J. Biol. Chem. 274, 12583–12592
50. Teubl, T., da Silva Xavier, G., Hols, G. G., Jouaville, L. S., Thomas, A. P., and Rutter, G. A. (2003) Biochem. J. 360, 29–39
51. Paul-Pletzer, K., Yamamoto, T., Shut, M. B., Ma, J., Ikemoto, N., Jimenez, L. S., Morimoto, H., Williams, P. G., and Parmess, J. (2002) J. Biol. Chem. 277, 34918–34923
52. Boutilier, D. J., Christensen, A. E., de Rooij, J., van Triest, M., Schwede, F., Genieser, H. G., Dockeck, S. O., Blank, J. L., and Bos, J. L. (2001) Nat. Cell Biol. 4, 901–906
53. Dhillon, A. S., and Kelch, W. (2002) Arch. Biochem. Biophys. 404, 3–9
54. de Rooij, J., Zwartkruis, F. J., Verheijen, M. H., Cool, R. H., Nijman, S. M., Wittinghofer, A., and Bos, J. L. (1998) Nature 396, 474–477
55. Kang, G., Chepurny, O. G., and Holz, G. G. (2001) J. Physiol. (Lond.) 536, 375–385
56. Enserink, J. M., Christensen, A. E., de Rooij, J., van Triest, M., Schwede, F., Genieser, H. G., Dockeck, S. O., Blank, J. L., and Bos, J. L. (2001) Nat. Cell Biol. 4, 901–906
57. Herold, K. C., Nagamatsu, S., Buse, J. B., Kalsakudin, P., and Steiner, D. F. (1993) Transplantation 55, 186–192
58. Fuhrer, D. K., Kobayashi, M., and Jiang, H. (2001) Diabetes Obes. Metab. 3, 393–492
59. Robertson, P. R. (1986) Diabetes 35, 1016–1019
60. Mithieux, P., Christensen, A. E., de Rooij, J., van Triest, M., Schwede, F., Genieser, H. G., Dockeck, S. O., Blank, J. L., and Bos, J. L. (2001) Nat. Cell Biol. 4, 901–906
61. Herold, K. C., Nagamatsu, S., Buse, J. B., Kalsakudin, P., and Steiner, D. F. (1993) Transplantation 55, 186–192
62. Fuhrer, D. K., Kobayashi, M., and Jiang, H. (2001) Diabetes Obes. Metab. 3, 393–492
63. Robertson, P. R. (1986) Diabetes 35, 1016–1019
64. Mithieux, P., Christensen, A. E., de Rooij, J., van Triest, M., Schwede, F., Genieser, H. G., Dockeck, S. O., Blank, J. L., and Bos, J. L. (2001) Nat. Cell Biol. 4, 901–906
65. Herold, K. C., Nagamatsu, S., Buse, J. B., Kalsakudin, P., and Steiner, D. F. (1993) Transplantation 55, 186–192
66. Fuhrer, D. K., Kobayashi, M., and Jiang, H. (2001) Diabetes Obes. Metab. 3, 393–492
67. Robertson, P. R. (1986) Diabetes 35, 1016–1019
68. Mithieux, P., Christensen, A. E., de Rooij, J., van Triest, M., Schwede, F., Genieser, H. G., Dockeck, S. O., Blank, J. L., and Bos, J. L. (2001) Nat. Cell Biol. 4, 901–906
69. Herold, K. C., Nagamatsu, S., Buse, J. B., Kalsakudin, P., and Steiner, D. F. (1993) Transplantation 55, 186–192
70. Fuhrer, D. K., Kobayashi, M., and Jiang, H. (2001) Diabetes Obes. Metab. 3, 393–492
61. Diver, J. M., Sage, S. O., and Rosado, J. A. (2000) Cell Calcium. 28, 323–329
71. Kiselevy, K. I., Shin, D. M., Wang, Y., Pessah, I. N., Allen, P. D., and Muallem, S. (2000) Mol. Cell. 6, 421–431
72. Diver, J. M., Sage, S. O., and Rosado, J. A. (2000) Cell Calcium. 28, 323–329
73. Kiselevy, K. I., Shin, D. M., Wang, Y., Pessah, I. N., Allen, P. D., and Muallem, S. (2000) Mol. Cell. 6, 421–431
74. Ihara, T. S., and Pearson, H. A. (1995) Neuropharmacology 34, 731–741
75. Ihara, T. S., and Pearson, H. A. (1995) Neuropharmacology 34, 731–741
76. Ihara, T. S., and Pearson, H. A. (1995) Neuropharmacology 34, 731–741
77. Ihara, T. S., and Pearson, H. A. (1995) Neuropharmacology 34, 731–741