Original Article

Inhibition of Glucose-Stimulated Activation of Extracellular Signal–Regulated Protein Kinases 1 and 2 by Epinephrine in Pancreatic β-Cells

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Glucose sensing is essential for the ability of pancreatic β-cells to produce insulin in sufficient quantities to maintain blood glucose within the normal range. Stress causes the release of adrenergic hormones that increase circulating glucose by promoting glucose production and inhibiting insulin release. We have shown that extracellular signal–regulated kinases 1 and 2 (ERK1/2) are responsive to glucose in pancreatic β-cells and that glucose activates ERK1/2 by mechanisms independent of insulin. Here we show that glucose-induced activation of ERK1/2 is inhibited by epinephrine through the α_2-adrenergic receptor. Epinephrine and the selective α_2-adrenergic agonist UK14304 reduced insulin secretion and glucose-stimulated ERK1/2 activation in a pertussis toxin–sensitive manner, implicating the α subunit of a Gi family member. α_2-adrenergic agonists also reduced stimulation of ERK1/2 by glucagon-like peptide 1 and KCl, but not by phorbol ester or nerve growth factor. Our findings suggest that α_2-adrenergic agonists act via a Gi family member on early steps in ERK1/2 activation, supporting the idea that ERK1/2 are regulated in a manner that reflects insulin demand. *Diabetes* 55:1066–1073, 2006

Insulin is produced by β-cells within pancreatic islets to regulate glucose uptake and promote glucose utilization. Glucose, the major regulator of insulin production and release, activates nutrient-sensing and signal transduction pathways, including the mitogen-activated protein (MAP) kinases and extracellular signal–regulated protein kinases 1 and 2 (ERK1/2), in islets and β-cell lines (1–4). ERK1/2 act in many signal transduction pathways (5,6).

In β-cells ERK1/2 are stimulated over the physiologic range of glucose concentrations (2–10 mmol/l); this range also stimulates insulin secretion and biosynthesis. Nutrients and hormones, such as glucagon-like peptide (GLP)-1, also activate ERK1/2 in proportion to their ability to induce insulin secretion. We have previously shown that glucose, GLP-1, and KCl activate ERK1/2 by a calcium-, calmodulin-, and calcineurin-dependent mechanism in β-cells (4). In contrast, insulin and phorbol ester activate ERK1/2 by a calcineurin-independent mechanism (4). A major action of ERK1/2 in β-cells is the stimulation of insulin gene transcription through phosphorylation of multiple transcriptional regulators that interact with E and A elements within the insulin gene promoter (7,8).

Release of epinephrine in response to stress increases circulating glucose by promoting glucose production by the liver and by inhibiting insulin release (9). Pertussis toxin, originally known as islet activating protein, blocks this adrenergic effect, implicating the action of the Gi family of heterotrimeric G proteins. To test the hypothesis that ERK1/2 are activated in proportion to insulin demand (2,4), we examined effects of epinephrine on ERK1/2 activation by glucose and other secretagogues. We find that epinephrine suppresses ERK1/2 activation by an α_2-adrenergic mechanism dependent on a Gi family member, suggesting that the site of blockade is early in the stimulatory pathway. Thus, a relationship between ERK1/2 activation and insulin demand is maintained not only under conditions in which insulin secretion is stimulated, but also under conditions in which insulin secretion is reduced.

RESEARCH DESIGN AND METHODS

The following reagents were purchased from the indicated sources: GLP-1(7–36), yohimbine, and UK14304 (Sigma-Aldrich, St. Louis, MO); active ERK1/2 antibody (BioSource, Camarillo, CA); ERK antibody Y691 (2); phospho-Rsk antibody (Thr359/Ser365; Cell Signaling Technology); pertussis toxin, N^6-benzoyl cAMP, and 2′,5′-dideoxyadenosine (Calbiochem); Rp-8-CPT-cAMPS, Rp-8-Br-cAMPS, and 8-pCPT-2′-*O*-Me-cAMP (Biolog).

Cell culture and harvest. INS-1 cells were maintained in RPMI 1640 medium (7). Cells at ~80% confluence were incubated for 2 h in Krebs-Ringer bicarbonate HEPES (KRBH) buffer before treatment. In some experiments, cells were treated with pertussis toxin as described (10). After treatment with the agents indicated in figure legends, medium was removed, and cells were washed with cold PBS and harvested in 0.2 ml cold lysis buffer (50 mmol/l HEPES, pH 7.5, 0.15 mol/l NaCl, 1% Triton X-100, 0.2 mg/ml phenylmethylsulfonyl fluoride, 0.1 mol/l NaF, 2 mmol/l Na_3VO_4, 10 μg/ml aprotinin, 5 μg/ml pepstatin A, and 5 μg/ml leupeptin). After 20 min on ice, lysates were

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ER, endoplasmic reticulum; ERK1/2, extracellular signal–regulated kinases 1 and 2; GLP, glucagon-like peptide; KRBH, Krebs-Ringer bicarbonate HEPES; MAP, mitogen-activated protein; PKA, protein kinase A; SERCA, sarcoplasmic reticulum/endoplasmic reticulum ATPase.

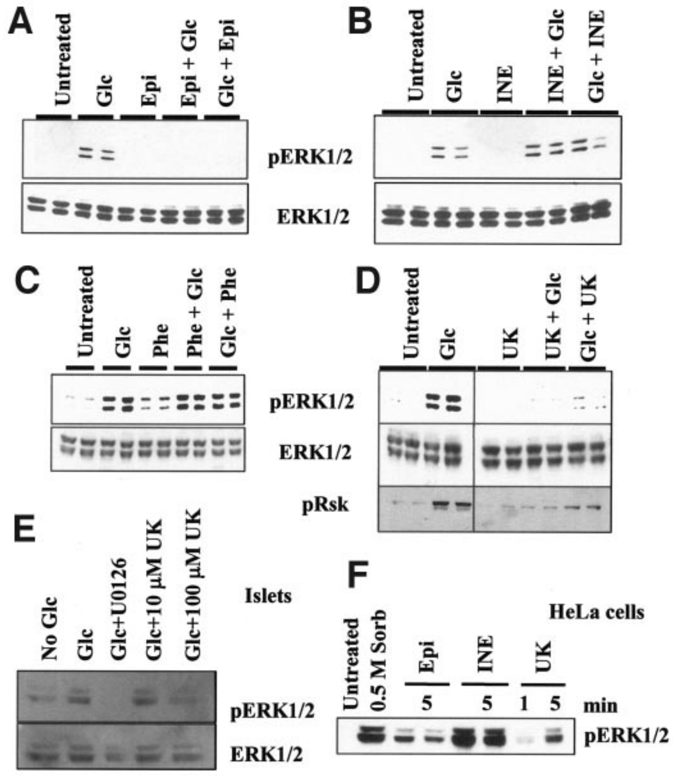

FIG. 1. Inhibition of glucose-stimulated ERK1/2 activity by adrenergic agonists. Replicate samples of INS-1 cell lysates immunoblotted with the indicated antibodies. *A*: Fifteen millimoles per liter glucose (Glc) or 10 μmol/l epinephrine (Epi) for 30 min alone, 15 min pretreatment with epinephrine followed by glucose for 30 min, or 15 min pretreatment with glucose followed by 15 min with epinephrine. *B*: As in *A* with 10 μmol/l isoproterenol (INE). *C*: As in *A* with 10 μmol/l phenylephrine (Phe). *D*: As in *A* with 10 μmol/l UK14304 (UK). *E*. Lysates from human pancreatic islets incubated without glucose for 2 h then stimulated with 11 mmol/l glucose in the presence or absence of 25 μmol/l U0126 or UK14304. *F*. Lysates of HeLa cells treated with the positive control 0.5 mol/l sorbitol for 30 min, 10 μmol/l epinephrine, 10 μmol/l isoproterenol, or 10 μmol/l UK14304 each for 1 or 5 min. Experiments were repeated three or more times.

sedimented for 15 min at 14,000 rpm in a microcentrifuge at 4°C. Supernatants were stored at −80°C until further analysis. Protein concentrations were measured using the BioRad Bradford reagent.

Isolation of human pancreatic islets. Handpicked islets were washed in KRBH-BSA, resuspended in RPMI (11 mmol/l glucose), and cultured overnight at 37°C, 10% CO_2. Islets were then washed twice, incubated without glucose in RPMI for 2 h, and divided into ~4,000 islet equivalents per condition for each experiment, all as described (11). The islets were treated as indicated in Fig. 1*E*, harvested as described above, and analyzed as described below.

Analysis of ERK1/2 phosphorylation. Equal amounts of lysate proteins (15 μg) were resolved by electrophoresis in 10% polyacrylamide gels in sodium dodecyl sulfate and transferred to nitrocellulose membranes. Immunoblotting was as described (7).

Insulin secretion assays. Radioimmunoassays were performed according to manufacturer's instructions (Linco Research, St. Charles, MO). Medium (2 ml) was collected after treatment with indicated reagents, before cell harvest, and 0.1 ml of each sample was assayed.

cAMP measurements. Total intracellular cAMP was measured using a cAMP Biotrak Enzymeimmunoassay kit (Amersham Biosciences). After the indicated treatments, cells were harvested in 0.5 ml 70% ethanol. Each dish was washed with an additional 0.5 ml. The combined lysate and wash were lyophilized and resuspended in 0.5 ml assay buffer.

Measurements of calcium signaling. Cells on 22-mm^2 glass coverslips at 60–80% confluence were incubated for 2 h in KRBH, 2 mmol/l glucose. For the final 30 min, cells were bathed in 6 μmol/l Fluo-4AM (Invitrogen, Carlsbad, CA). Indicated agents were added for the times noted and throughout recording. Control conditions are defined as incubated in 0.1% (vol/vol) dimethyl sulfoxide (DMSO). Coverslips were mounted in a RC-26 open-bath imaging chamber (Warner Instruments, Hamden, CT) on a Zeiss LSM 510 confocal imaging system (Zeiss), and bathed in KRBH containing the indicated agents. Images were collected using an excitation laser of 488 nm and bandpass emission filter of 505–550 nm, every 1 s. After recording for 1 min to obtain baseline fluorescence, solution was added to maintain glucose at 2 mmol/l or to bring the bath solution to a final concentration of 15 mmol/l, and then cells were recorded for an additional 9 min. Cells were included for analysis if they displayed oscillations (at least three peaks) in intracellular calcium after glucose was added; cells were excluded if they displayed a calcium response before glucose addition. Oscillation frequency was analyzed using a program described previously (12). Data are displayed as means ± SE. *P* values were obtained via Student's *t* test.

RESULTS

Inhibition of glucose-dependent ERK1/2 activity by epinephrine. Glucose and other secretagogues, such as GLP-1 and KCl, activate ERK1/2 in β-cell lines and intact islets (1,2,7,13). Secretagogues stimulate ERK1/2 by mechanisms that share some common features with those used by glucose (4). In this study, we examined the effects of agents that interfere with insulin secretion on ERK1/2 activity to determine whether decreased insulin demand resulted in decreased ERK1/2 activation and to gain further information about the mechanism of ERK1/2 activation by glucose.

Even though insulin increases ERK1/2 activity due to its high concentration around β-cells, we previously concluded that activation of ERK1/2 by glucose can occur independently of insulin because agents that block ERK1/2 activation by glucose, such as antagonists of calmodulin or calcineurin, have little effect on ERK1/2 activation by insulin (4). Thus, agents that interfere with insulin secretion will not significantly impact glucose-induced ERK1/2 activation directly through reduced insulin release.

To determine whether the stimulation of ERK1/2 is sensitive to agents that inhibit glucose-dependent insulin secretion, we treated INS-1 cells with epinephrine, which reduces secretion from β-cells within islets and INS-1 cells (14), and assessed the effects on ERK1/2 phosphorylation in response to glucose (Fig. 1*A*). Activation of ERK1/2 was monitored by immunoblotting with antiphosphoERK1/2 antibodies (1,2,4,13). Epinephrine did not stimulate ERK1/2 activity, but it strongly inhibited ERK1/2 phosphorylation induced by glucose (Fig. 1*A*). Because insulin does not mediate ERK1/2 activation by glucose, we attribute the inhibition caused by epinephrine to a direct effect on β-cells.

Inhibition of ERK1/2 is mediated through α_2-adrenergic receptors. Epinephrine binds to α- and β-adrenergic receptors. To determine which adrenergic receptor(s) mediated this inhibitory effect, we examined effects of receptor subtype-selective agonists. Neither isoproterenol, a β_2-adrenergic receptor agonist, nor phenylephrine, an α_1-adrenergic receptor agonist, blocked glucose stimulation of ERK1/2 (Fig. 1*B* and *C*). The α_2-adrenergic receptor agonist UK14304, like epinephrine, inhibited ERK1/2 phosphorylation induced by glucose in INS-1 cells (Fig. 1*D*) and in human islets (Fig. 1*E*), consistent with studies showing blockade of insulin secretion by α_2-adrenergic receptor agonists (9,14,15). Epinephrine and UK14304 also blocked glucose-stimulation of the protein kinase Rsk, a downstream target of ERK1/2 (data not shown) (Fig. 1*D*). In contrast to their effects in β-cells, epinephrine, isoproterenol, and UK14304 all activated ERK1/2 phosphorylation in HeLa cells (Fig. 1*F*).

As further confirmation of the involvement of α_2-adrenergic receptors in blocking ERK1/2 activation, we treated glucose-stimulated INS-1 cells with the α_2-adrenergic an-

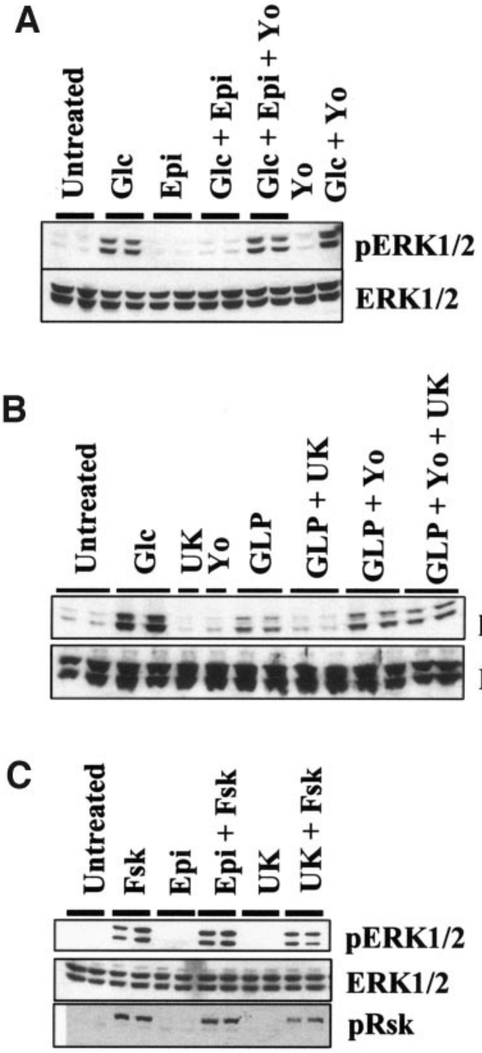

FIG. 2. Effects of α_2-adrenergic agonists and an antagonist. Lysates of INS-1 cells treated as indicated were immunoblotted with the indicated antibodies. *A*: Thirty-minute treatment with glucose (Glc), 10 μmol/l epinephrine (Epi), glucose + epinephrine; 15-min pretreatment with 10 μmol/l yohimbine (Yo) then glucose + epinephrine, yohimbine alone; and 5-min pretreatment with yohimbine then glucose. *B*: Five-minute treatment with 15 mmol/l glucose, 10 μmol/l UK14304 (UK), 10 μmol/l yohimbine, 30 nmol/l GLP, GLP + UK14304; 15-min pretreatment with yohimbine then GLP; and 15-min pretreatment with yohimbine then GLP + UK14304. *C*: Treatment with 10 μmol/l forskolin (fsk), 10 μmol/l epinephrine, epinephrine + forskolin, 10 μmol/l UK14304, and UK14304 + forskolin for 30 min. Experiments were performed three or more times.

tagonist yohimbine. Yohimbine alone had little effect on ERK1/2 activity. Incubation of cells with yohimbine before treatment with glucose and epinephrine or UK14304 resulted in full restoration of glucose stimulation of ERK1/2 phosphorylation (data not shown) (Fig. 2*A*).

Inhibition of GLP-1–dependent activation of ERK1/2 by UK14304. Hormones that stimulate adenylyl cyclase, such as glucagon and GLP-1, also stimulate ERK1/2 activation (4). Binding of GLP-1 to its receptor engages the heterotrimeric G protein Gs and activates adenylyl cyclase. GLP-1 caused a small but rapid increase in ERK1/2 phosphorylation. Addition of UK14304 to INS-1 cells partially blocked GLP-1–stimulated phosphorylation of ERK1/2 (Fig. 2*B*). Yohimbine countered the inhibitory effect of UK14304 on GLP-1–stimulated ERK1/2 activation. When cells were treated with yohimbine and GLP-1, there was a slight enhancement of ERK1/2 phosphorylation compared with that induced by GLP-1 alone.

We also determined the effects of epinephrine and UK14304 on the ability of forskolin to stimulate phosphorylation of ERK1/2 (Fig. 2*C*). These agonists had little or no effect on forskolin-induced ERK1/2 phosphorylation or phosphorylation of its downstream substrate Rsk, nor did yohimbine influence forskolin-stimulated ERK1/2 phosphorylation (data not shown). These results are consistent with earlier findings suggesting that forskolin activates ERK1/2 by a mechanism in part distinct from that employed by GLP-1 (4).

α_2-adrenergic agonists inhibit ERK1/2 phosphorylation through the Gai family. In most cell types, α_2-adrenergic receptors couple to the Gi family of heterotrimeric G proteins. To determine whether this is also the case in β-cells, we pretreated INS-1 cells with pertussis toxin overnight before treatment with glucose and either UK14304 or epinephrine (Fig. 3*A*). Pertussis toxin completely blocked the inhibitory effects of UK14304 and epinephrine on glucose-stimulated ERK1/2 activation, indicating that inhibition requires the α subunit of a Gi/o family member (16). Glucose-activated insulin secretion in INS-1 cells was significantly reduced by UK14304, but reduction by epinephrine was not statistically significant (Fig. 3*B*). Near-normal insulin secretion was restored by pretreatment with pertussis toxin. Like α_2-adrenergic agonists, somatostatin blocked stimulation of ERK1/2 by glucose in INS-1 cells; the inhibitory effect of somatostatin also proved to be pertussis toxin sensitive (Fig. 3*C*).

α_2-adrenergic agonists suppress cAMP accumulation. One of the best documented actions of the Gi family is the inhibition of adenylyl cyclase activity. It seems possible that α_2-adrenergic agonists could be preventing glucose- or GLP-1–stimulated ERK1/2 activation by damping the cAMP response. It has been suggested that cAMP is permissive for the actions of glucose (17). Therefore, we measured cAMP production under various conditions. Glucose caused a small accumulation of cAMP in INS-1 cells that became significantly greater than control values after 30 min of treatment (Fig. 4*A* and *B*). The addition of UK14304 inhibited cAMP accumulation caused by glucose plus GLP-1. Forskolin treatment of INS-1 cells increased cAMP levels twofold; however, the addition of UK14304 did not reduce cAMP accumulation induced by forskolin. There was no significant difference between GLP-1–induced cAMP production in the presence of 2 or 15 mmol/l glucose within the first 5 min of stimulation (Fig. 4*B*). The effect of GLP-1 on cAMP production was greatest within the first 5 min of treatment (data not shown). Addition of UK14304 inhibited cAMP production at all time points.

Role of PKA and Epac in the activation of ERK1/2 by glucose. To examine the most common target of cAMP, the cAMP-dependent protein kinase (protein kinase A [PKA]), INS-1 cells were pretreated with H89, a PKA inhibitor, before stimulation with glucose or forskolin. H89 had little or no effect on glucose-stimulated ERK1/2 activation even at concentrations well above its half-maximal inhibitory concentration (IC_{50}) for PKA (Fig. 5*A*). Forskolin-stimulated ERK1/2 activation was relatively more sensitive to inhibition by H89, showing clear partial inhibition at 1 μmol/l (Fig. 5*A*). Because H89 is not a highly specific PKA inhibitor, we also tested effects of cAMP analogs that inhibit PKA not by binding to the catalytic subunit, but by stabilizing the PKA holoenzyme (18–20). Neither Rp-8-

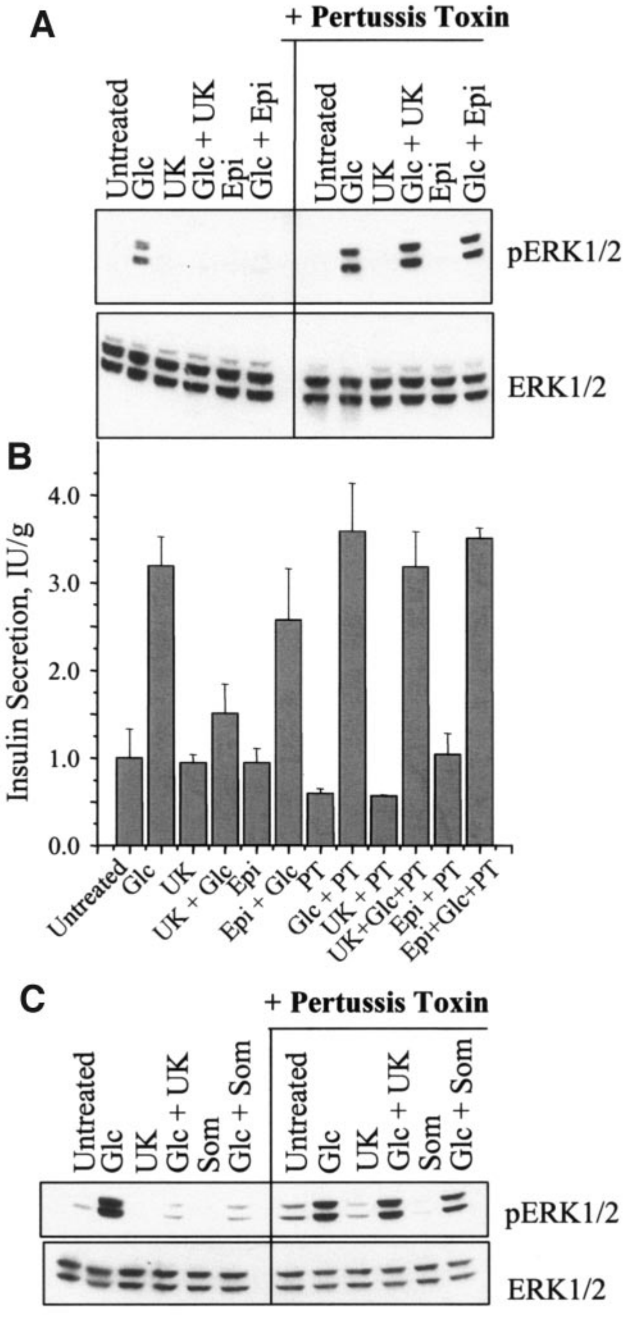

FIG. 3. Effects of pertussis toxin treatment on α_2-adrenergic agonist inhibition of ERK1/2 activity. *A*: Lysates of INS-1 cells treated as indicated were immunoblotted with the indicated antibodies. *Left panel*: thirty-minute treatments with 15 mmol/l glucose (Glc), 10 µmol/l UK14304 (UK), glucose + UK14304, 10 µmol/l epinephrine (Epi), glucose + epinephrine. *Right panel*: pretreatment with 25 ng/ml pertussis toxin for 16 h followed by 30-min treatments with 15 mmol/l glucose, 10 µmol/l UK14304, glucose + UK14304, 10 µmol/l epinephrine, glucose + epinephrine. *B*: Insulin secretion from cells treated as in *A*. *C*: Similar to *A*, except with 10 µmol/l somatostatin (Som). Experiments in *A* were performed three times, and experiments in *B* and *C* were performed twice.

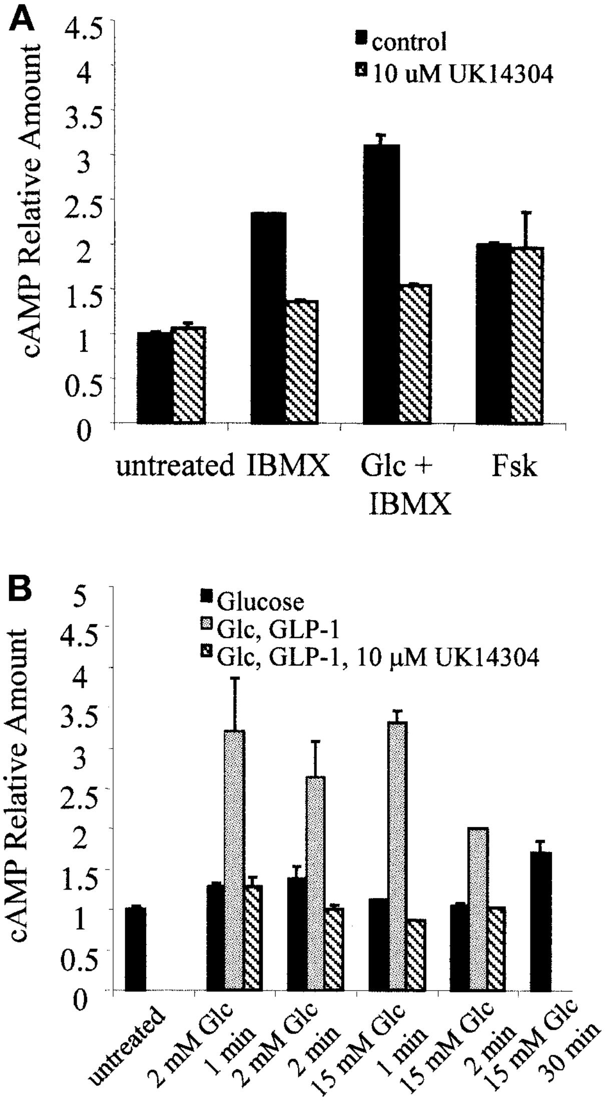

FIG. 4. Production of cAMP in stimulated INS-1 cells. cAMP was measured using a nonenzymatic enzyme-linked immunosorbent assay (ELISA) kit as described in RESEARCH DESIGN AND METHODS. *A*: Fifty micromoles per liter of IBMX, 15 mmol/l glucose (Glc), 10 µmol/l forskolin (Fsk), 10 µmol/l UK14304, 10 µmol/l somatostatin. *B*: Two millimoles per liter glucose + 50 µmol/l IBMX, with 50 nmol/l GLP-1, and with 10 µmol/l UK14304 for 1 or 2 min as indicated. Experiments were performed three times.

CPT-cAMPS nor Rp-8-Br-cAMPS caused a substantial reduction in glucose-stimulated ERK1/2 activity (Fig. 5*B*).

The cAMP analog 8-pCPT-2′-*O*-Me-cAMP has been shown to display selective activation of Epac, a guanine nucleotide exchange factor for the Rap small GTPases (21–23). Compared with glucose, 8-pCPT-2′-*O*-Me-cAMP, as well as the relatively selective PKA activator N^6-benzoyl cAMP (24) (data not shown), had little ability to activate ERK1/2 in INS-1 cells. Below 1 mmol/l, the adenylyl cyclase P site inhibitor 2′,5′-dideoxyadenosine (25) also had little effect on glucose-induced ERK1/2 activation (Fig. 5*C*). These results suggest that the actions of cAMP on two effectors PKA and Epac have little input to glucose stimulation of ERK1/2 in INS-1 cells.

Specificity of α_2-adrenergic signaling in INS-1 cells. To explore the spectrum of agents sensitive to α_2-adrenergic agonists, we tested agents that activate ERK1/2 through different signaling pathways. In contrast to its effects on ERK1/2 activation by glucose, UK14304 did not inhibit activation of ERK1/2 by phorbol ester or nerve growth factor (Fig. 6*A*) (1,26). Likewise, stimulation by insulin was not attenuated by UK14304 (data not shown). On the other hand, UK14304 substantially reduced ERK1/2 activation by KCl at all times examined (Fig. 6*B*). These

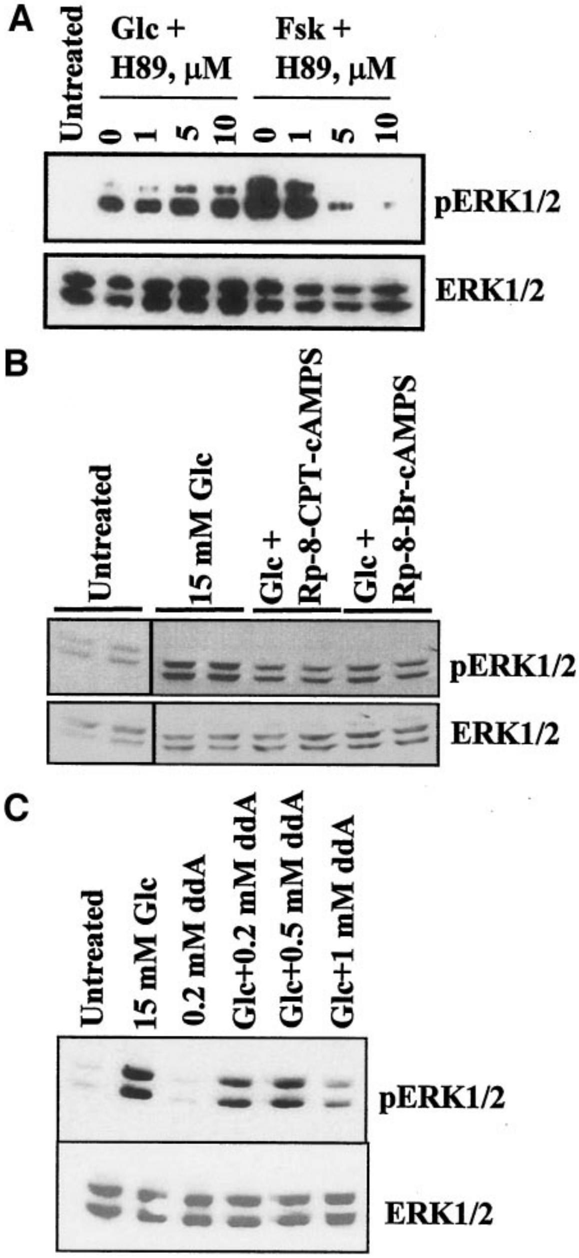

FIG. 5. Effects of PKA inhibition on glucose-stimulated or forskolin-stimulated ERK1/2 activation. Lysates of INS-1 cells treated as indicated were immunoblotted with the indicated antibodies. *A*: Indicated concentrations of H89 for 15 min, followed by 15 mmol/l glucose (Glc) or 10 μmol/l forskolin (Fsk) for 30 min. *B*: Pretreatment with 100 μmol/l Rp-8-CPT-cAMPS or 100 μmol/l Rp-8-Br-cAMPS for 15 min, followed by 15 mmol/l glucose for 30 min. *C*: Thirty-minute preincubation with 2′,5′-dideoxyadenosine (ddA) followed by 30 min with glucose. Experiments were performed four times (*A*) or twice (*B* and *C*).

results indicate that α_2-adrenergic agonists act on a subset of activators of ERK1/2.

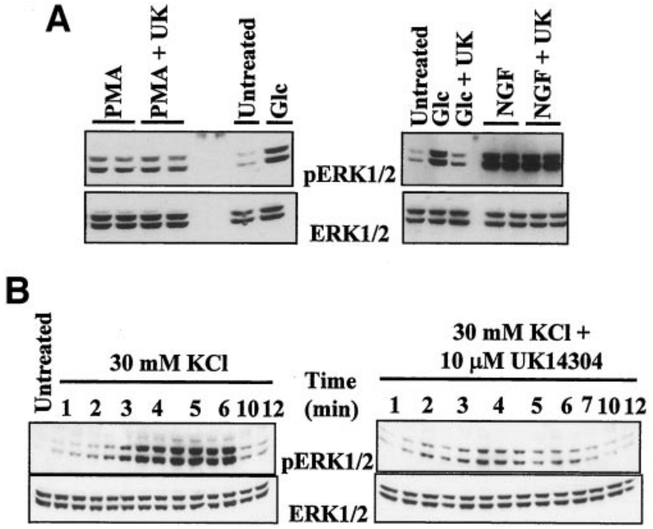

FIG. 6. Effects of α_2-adrenergic agonists on other signal transduction pathways. Lysates of INS-1 cells treated as indicated were immunoblotted with the indicated antibodies. *A*: Fifteen minutes with 100 nmol/l phorbol ester (PMA) +/− 10 μmol/l UK14304 (UK) and 5 min with 500 ng/ml nerve growth factor (NGF), or NGF + 10 μmol/l UK14304. *B*: Time course ERK1/2 phosphorylation stimulated by 30 mmol/l KCl in 2 mmol/l glucose without (*left panel*) or with (*right panel*) 10 μmol/l UK14304. Experiments in *A* were performed twice, and experiments in *B* were performed three times.

Impact of α_2-adrenergic drugs on intracellular calcium signaling. We showed previously that calcium and calcineurin are required for ERK1/2 activation by glucose, although how calcium acts remains sketchy (4). KCl induces calcium influx, and its actions on ERK1/2 are reduced by UK14304. To explore calcium signaling that may be sensitive to UK14304, we monitored changes in intracellular calcium using the cell-permeant, calcium-sensitive fluorescent dye Fluo-4AM. Under control conditions, the fraction of INS-1 cells responding with oscillations in the intracellular calcium concentration was increased when glucose was changed from 2 to 15 mmol/l (Fig. 7*A* and *B*). There was an absolute requirement for extracellular calcium to detect a glucose-induced intracellular calcium change; removal of extracellular calcium abolished intracellular calcium oscillations, while re-addition of calcium resulted in reappearance of oscillations (Fig. 7*A*). Treatment of INS-1 cells with thapsigargin, an inhibitor of the sarcoplasmic reticulum/endoplasmic reticulum ATPase (SERCA), leads to a depletion of intracellular calcium stores and blocks ERK1/2 activation (4,27). Both UK14304 and thapsigargin were associated with small but not statistically significant increases in the fraction of cells in 2 mmol/l glucose responding with calcium oscillations (P = 0.35 and 0.20, respectively). Treatment with UK14304 led to a slight decrease in the fraction of cells responding to 15 mmol/l glucose, whereas treatment with thapsigargin led to a slight increase (P = 0.37 and 0.36 for UK14304 and thapsigargin, respectively) compared with control. Although both treatments led to different changes in the fraction of cells responding, they had a similar effect in reducing the response to increased glucose: with the control cells, addition of 15 mmol/l glucose led to a ~3.8-fold greater fraction of responding cells (0.61 ± 0.10 vs. 0.16 ± 0.08) compared with cells incubated in 2 mmol/l glucose alone, whereas treatment with either UK14304 or thapsigargin reduced this difference (1.8- and 2.5-fold, respectively, Fig. 7*B*). The frequency of calcium oscillations was not changed by any of the treatments (22.6 ± 1.9 mHz) (data not shown).

DISCUSSION

In β-cells ERK1/2 appear to assist in integrating long- and short-term nutrient-sensing information in the nucleus to maintain insulin homeostasis (7,13). The effect of epinephrine to inhibit ERK1/2 activation by glucose in β-cells was mediated specifically by α_2-adrenergic receptors. The ef-

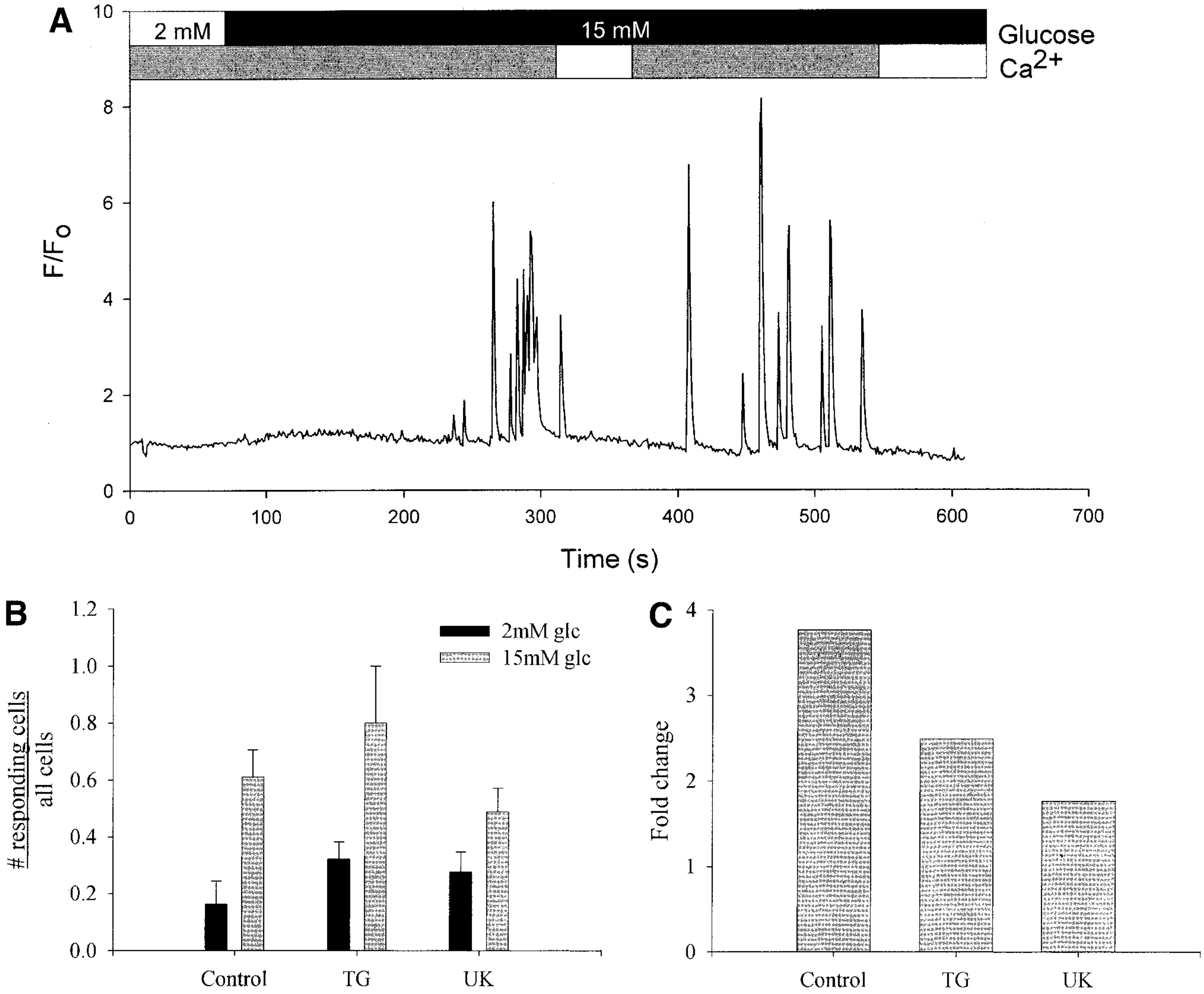

FIG. 7. Effect of UK14304 (UK) on glucose-induced calcium response. ***A*****: Normalized fluorescence trace of an INS-1 cell preincubated under control conditions. Black bar indicates application of 15 mmol/l glucose. Gray bars indicate the presence of extracellular calcium.** ***B*****: Cells preincubated in 0.1% (vol/vol) DMSO, 1 μmol/l thapsigargin (TG) (30 min), or 10 μmol/l UK14304 (15 min) were treated with 2 mmol/l (no change) or 15 mmol/l glucose.** ***C*****: Fold change in fraction of cells responding after increasing glucose from 2 to 15 mmol/l. Experiments were performed three to six times (*****n*** **= 36–129 cells/condition).**

fects of adrenergic agents on ERK1/2 are similar to their actions on insulin secretion. α_2-adrenergic agonists selectively inhibit insulin secretion in cultured cell models and have been shown to inhibit insulin secretion as well as to induce glucagon secretion in isolated perifused rat pancreatic islets (9,15,28). These data further support the idea that ERK1/2 activation generally parallels insulin demand. The inhibitory effect of α_2-adrenergic agonists on ERK1/2 in INS-1 cells is not common to all other cell types. Ligands for adrenergic receptors usually activate ERK1/2 in cells other than β-cells (29–31). α_2-adrenergic receptors are not coupled to ERK1/2 activation in PC12 cells, suggesting that neuroendocrine cells may be exceptions (32).

An unresolved question in the literature is the role of cAMP in glucose activation of ERK1/2. Studies with PKA inhibitors and PKA activators suggest that PKA has little impact on glucose activation of ERK1/2. Less extensive but consistent findings lead to a similar conclusion regarding the Rap exchange factor Epac. Despite that cAMP effectors play important roles in β-cell function (23,33–36), the cumulative results suggest that these cAMP effectors are not direct mediators of glucose signaling to ERK1/2 in β-cells.

Intracellular calcium can increase by influx across the plasma membrane or by release of calcium from intracellular stores (37). Influx can occur through voltage-gated or ligand-gated channels; many of these channels are themselves modulated by intracellular calcium (38). Intracellular calcium stores in the endoplasmic reticulum (ER) are maintained by SERCA. Thapsigargin, an irreversible SERCA inhibitor, prevents calcium uptake into the ER. This has multiple effects on intracellular calcium: *1*) calcium released from the ER (either stimulated or through leak) enters the cytosol but cannot reenter the ER; and *2*) depletion of calcium from intracellular stores activates store-operated channels on the plasma membrane, which promotes a capacitative calcium influx from the extracellular space (37).

INS-1 cells responded to stimulation by glucose with oscillations in the intracellular calcium concentration, consistent with similar observations in the MIN6 β-cell line (13). No calcium response to glucose was detected in calcium-free solution (Fig. 7*A*). Re-addition of calcium caused an immediate increase in intracellular calcium oscillations. These results support the conclusion that there is a requirement for extracellular calcium in the glucose-induced calcium response. Pretreatment of cells with 1 μmol/l thapsigargin led to a slight increase in the fraction of cells responding to stimulation by both 2 and 15 mmol/l glucose. This may be due to activation of store-operated channels in the membrane caused by thapsigargin-induced store depletion, as well as a greater baseline calcium influx, even in low glucose medium. Because more cells still responded to 15 mmol/l glucose than to 2 mmol/l glucose, even in the presence of thapsigargin, thapsigargin-sensitive intracellular calcium stores may play a role in the glucose-induced calcium response of INS-1 cells, but they are not the only factor. Because thapsigargin prevents activation of ERK1/2 by glucose (4), calcium oscillations are apparently not related to the mechanism of regulation of ERK1/2 activity by glucose.

Our results are most consistent with the conclusion that α_2-adrenergic agonists influence some other early event that is required in the mechanism of ERK1/2 activation by glucose. This key step in ERK1/2 activation is shared by secretagogues, but is apparently not a component of the signaling mechanisms used by other agents such as phorbol ester and nerve growth factor. The action of α_2-adrenergic agonists is mediated through the heterotrimeric G protein Gi/o. In contrast to effects of thapsigargin, 10 μmol/l UK14304 led to a slight increase in the fraction of cells responding with calcium oscillations to 2 mmol/l glucose, but a reduction in the fraction of cells responding to 15 mmol/l glucose. Activation of Gαi/o leads to inhibition of adenylate cyclase and a reduction in cytosolic cAMP, which can in turn have effects on L-type calcium channels and store-operated channels. cAMP (via PKA) can enhance calcium entry through L-type calcium channels in hippocampal neurons (39). The fact that α_2-adrenergic agonists blocked the ability of KCl to activate ERK1/2 strongly suggests that Gi/o is affecting the activity of L-type voltage-sensitive Ca^{2+} channels either by decreasing a permissive effect of cAMP or through interactions of Gi with channel proteins.

Inhibition of L-type voltage-sensitive Ca^{2+} channels could be direct or indirect through the ATP-sensitive K^+ channel, which has been proposed as the target of the Gi-mediated effects of galanin (16,40–43). In chromaffin cells, Carbone et al. (44) found that L-type Ca^{2+} channels are tonically inhibited by Gi family members, which are also activated by catecholamines. Several lines of evidence have suggested that the ability of α_2-adrenergic agonists to reduce islet cAMP is probably not the primary mechanism by which glucose-induced insulin secretion is inhibited (45–47). Our results in β-cells suggest that α_2-adrenergic agonists act on Ca^{2+} signaling, in addition to cAMP accumulation. Together these actions may block ERK1/2 activation by glucose.

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