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A Role for the p38 Mitogen-activated Protein Kinase/Hsp 27 Pathway in Cholecystokinin-induced Changes in the Actin Cytoskeleton in Rat Pancreatic Acini*

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Cholecystokinin (CCK) and other pancreatic secretagogues have recently been shown to activate signaling kinase cascades in pancreatic acinar cells, leading to the activation of extracellular signal-regulated kinases and Jun N-terminal kinases. We now show the presence of a third kinase cascade activating p38 mitogen-activated protein (MAP) kinase in isolated rat pancreatic acini. CCK and osmotic stress induced by sorbitol activated p38 MAP kinase within minutes; their effects were dose-dependent, with maximal activation of 2.8- and 4.4-fold, respectively. The effects of carbachol and bombesin on p38 MAP kinase activity were similar to those of CCK, whereas phorbol ester, epidermal growth factor, and vasoactive intestinal polypeptide stimulated p38 MAP kinase by 2-fold or less. Both CCK and sorbitol also increased the tyrosyl phosphorylation of p38 MAP kinase. Using the specific inhibitor of p38 MAP kinase, SB 203580, we found that p38 MAP kinase activity was required for MAP kinase-activated protein kinase-2 activation in pancreatic acini. SB 203580 reduced the level of basal phosphorylation and blocked the increased phosphorylation of Hsp 27 after stimulation with either CCK or sorbitol. CCK treatment induced an initial rapid decrease in total F-actin content of acini, followed by an increase after 40 min. Preincubation with SB 203580 significantly inhibited these changes in F-actin content. Staining of the actin cytoskeleton with rhodamine-conjugated phalloidin and analysis by confocal fluorescence microscopy showed disruption of the actin cytoskeleton after 10 and 40 min of CCK stimulation. Pretreatment with SB 203580 reduced these changes. These findings demonstrate that the activation of p38 MAP kinase is involved not only in response to stress, but also in physiological signaling by gastrointestinal hormones such as CCK, where activation of G_q-coupled receptors stimulates a cascade in which p38 MAP kinase activates MAP kinase-activated protein kinase-2, resulting in Hsp 27 phosphorylation. Activation of p38 MAP kinase, most likely through phosphorylation of Hsp 27, plays a role in the organization of the actin cytoskeleton in pancreatic acini.

CCK[1] regulates a variety of pancreatic functions, including secretion of pancreatic enzymes (1), stimulation of pancreatic growth (2, 3), and synthesis of digestive enzymes (4). It is thought that some of these nonsecretory effects are a result of the ability of CCK to regulate expression of transcriptional factors such as c-Myc, c-Jun, and c-Fos (5). In previous studies with isolated rat pancreatic acini, we found that CCK activates ERKs and JNKs, as well as other upstream components of the mitogen-activated protein kinase signaling cascades such as MEK and Ras. CCK also stimulates downstream components such as MAPKAP kinase-1 (6–8).

The mitogen-activated protein kinase signaling pathways are ubiquitous cascades that regulate cellular growth, differentiation, and responses to environmental stress (9–11). In mammalian cells, at least three parallel pathways are differentially regulated by a number of extracellular signals that act via different cell-surface receptor types. Central to these signaling pathways are the MAP kinases themselves: ERKs, JNKs, and p38 MAP kinase. The p38 MAP kinases (p38/CSBP/RK) are mammalian homologues of the HOG-1 MAP kinase of *Saccharomyces cerevisiae* (12–14). p38 MAP kinase is activated by physical and chemical stresses including UV irradiation, heat, and osmotic stress, as well as by bacterial lipopolysaccharide and the pro-inflammatory cytokines tumor necrosis factor-α and interleukin-1 (12, 13, 15, 16). More recently, it was also reported that hematopoietic growth factors such as granulocyte/macrophage colony-stimulating factor, steel locus factor, interleukin-3, and colony-stimulating factor-1, but not interleukin-4, activate the p38 MAP kinase pathway (17). Another study reported that p38 MAP kinase was activated by the chemotactic peptide *N*-formyl-Met-Leu-Phe and that this process involved phosphatidylinositol 3-kinase, protein kinase C, and calcium (18). Experiments with dominant-negative or active mutant proteins have demonstrated that p38 MAP kinase lies downstream of Rac, Cdc42 (19–21), and three kinases, MKK3, MKK4, and MKK6 (22–27). Activation of p38 MAP kinase involves phosphorylation on threonine and tyrosine residues present in a TGY amino acid motif (15, 28), resulting in increased enzyme activity.

p38 MAP kinase has been demonstrated to play a role in the phosphorylation and activation of transcription factors including CHOP, Elk-1, and ATF-2 (29–31). In addition, p38 MAP kinase was shown to phosphorylate and activate two protein kinases, MAPKAP kinase-2 and MAPKAP kinase-3, which

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[1] The abbreviations used are: CCK, cholecystokinin; ERK, extracellular signal-regulated kinase; JNK, Jun N-terminal kinase; MAP, mitogen-activated protein; MAPKAP, MAP kinase-activated protein; Hsp, heat shock protein; EGF, epidermal growth factor; GST, glutathione *S*-transferase; PBS, phosphate-buffered saline.

share ~75% amino acid sequence identity (13, 32). Further experiments indicate that the small heat shock protein (Hsp) 25/27 is a physiological substrate for MAPKAP kinase-2/MAPKAP kinase-3 (13, 16, 32). The phosphorylation of Hsp 27 appears to enhance the polymerization of actin (33) and is proposed to play a role in repairing the actin microfilament network, which becomes disrupted during cellular stress (34). In contrast to effects of hematopoietic growth factors, little is known about activation of p38 MAP kinase via G_q-coupled receptors. Here we demonstrate that CCK and other pancreatic secretagogues that activate secretion via the G_q-coupled CCK-A receptor can induce tyrosyl phosphorylation and activate p38 MAP kinase at physiological concentrations in rat pancreatic acinar cells. Furthermore, this activation leads to the phosphorylation of Hsp 27. We also present data showing that CCK affects the actin cytoskeleton due to an activation of the p38 MAP kinase/Hsp 27 pathway.

EXPERIMENTAL PROCEDURES

Materials—CCK octapeptide (CCK-8) was a gift from Squibb Research Institute (Princeton, NJ) or was purchased from Research Plus, Inc. (Bayonne, NJ). Natural mouse EGF was purchased from Collaborative Biomedical Products (Bedford, MA); 12-*O*-tetradecanoylphorbol-13-acetate was from LC Laboratories (Woburn, MA); and chromatographically purified collagenase was from Worthington. Bacteria transformed with the expression plasmid for GST-ATF-2-(1–109) were a gift from Dr. J. Han (Scripps Research Institute, La Jolla, CA). Aprotinin and leupeptin were from Boehringer Mannheim Co. (Mannheim, Germany); prestained molecular mass standards were from Bio-Rad; and nitrocellulose membranes were from Schleicher & Schuell. [γ-^{32}P]ATP (3000 Ci/mmol) was from NEN Life Science Products. The enhanced chemiluminescence (ECL) detection system, horseradish peroxidase-conjugated protein A, and x-ray film were from Amersham Pharmacia Biotech. Protein A-agarose was from Pierce. Rhodamine-conjugated phalloidin was from Molecular Probes, Inc. (Eugene, OR). SB 203580 was a gift from Dr. John Lee (SmithKline Beecham). Rabbit polyclonal p38 (C-20) antibody and anti-p90$^{rsk\text{-}1}$ (C-21) antibody were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-specific p38 MAP kinase antibody raised against a peptide corresponding to residues 171–186 of human p38 MAP kinase, which detects p38 MAP kinase only when activated by phosphorylation at Tyr-182, was from New England Biolabs Inc. (Beverly, MA). Antibodies to p70 S6 kinase (catalog No. 06-321), MAPKAP kinase-2 (catalog No. 06534), and the MAPKAP kinase-2 peptide substrate (amino acid sequence KKLNRTLSVA) were from Upstate Biotechnology, Inc. (Lake Placid, NY).

The monoclonal anti-mouse Hsp 27 antibody was a gift from Dr. Michael Welsh (University of Michigan). All other reagents were obtained from Sigma.

Preparation of Pancreatic Acini and Cell-free Extract—The preparation of pancreatic acini from Sprague-Dawley rats by means of collagenase digestion was according to Williams and co-workers (7, 8, 35). Acini were preincubated at 37 °C for 180 min, followed by stimulation with different agonists for the indicated times. When used, SB 203580 was included for the last 60 min of preincubation and in the incubation solution. Following stimulation, acini were pelleted; washed once with 1 ml of ice-cold PBS containing 1 mM Na_3VO_4 (pH 7.4); and sonicated for 5 s in 0.5 ml of ice-cold lysis buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 5 mM EDTA, 1 mM dithiothreitol, 0.2 mM Na_3VO_4, 25 mM NaF, 10 mM sodium pyrophosphate, 25 mM β-glycerophosphate, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride. The lysates were then centrifuged in a microcentrifuge at 4 °C for 15 min, and the amount of protein in the cell extracts was assayed with the Bio-Rad protein assay reagent.

Immunoprecipitation and Western Blotting—Immunoprecipitation and Western blotting were carried out as described earlier (7, 8). For Western blotting, immunoprecipitates were boiled for 5 min in SDS sample buffer and subjected to SDS-polyacrylamide gel electrophoresis, followed by Western blot analysis with the indicated antibody using the ECL detection system.

Kinase Assays—To measure p38 MAP kinase activity, immunoprecipitated p38 MAP kinase was used to phosphorylate 5 μg of GST-ATF-2-(1–109) in 20 μl of kinase buffer (18 mM HEPES (pH 7.4), 10 mM magnesium acetate, 50 μM ATP, and 2.5 μCi/sample [γ-^{32}P]ATP). The reaction mixture was incubated at 30 °C for 30 min with shaking. Reactions were terminated by addition of 4× SDS sample buffer, and samples were then subjected to SDS gel electrophoresis. Labeled phosphoprotein was visualized by autoradiography and quantitated using a phosphoimager system (Bio-Rad GS-250). Two major GST-ATF-2 purification products were observed by autoradiography after ^{32}P incorporation and SDS gel electrophoresis, likely full-length GST-ATF-2 and a shorter degradation product. For quantitation of kinase assays, the upper band was used. In control experiments, GST alone was not phosphorylated by immunoprecipitated p38 MAP kinase. To measure MAPKAP kinase-2 activity, immunoprecipitated MAPKAP kinase-2 was used to phosphorylate a peptide from Hsp 27 (250 μM) (36) in 20 μl of kinase buffer (50 mM β-glycerophosphate (pH 7.0), 0.1 mM EDTA, 10 mM magnesium acetate, 50 μM ATP, and 5 μCi/sample [γ-^{32}P]ATP). The reaction mixture was incubated at 30 °C for 30 min with shaking and terminated by transferring 25 μl of reaction products onto Whatman P-81 paper. The P-81 paper was then washed three times with 0.75% phosphoric acid and once with acetone. Radioactivity was determined by liquid scintillation counting. MAPKAP kinase-1 and p70 S6 kinase assays were performed as described previously (8, 37).

Measurement of F-actin Content—Changes in F-actin content after hormone stimulation were measured by the method of Condeelis and Hall (38), as modified by Ding *et al.* (39). Acini were fixed for 15 min with 3.7% formaldehyde in PIPES buffer (40 mM KPO_4, 10 mM PIPES, 5 mM EGTA, and 2 mM $MgSO_4$ (pH 6.8)) and centrifuged for 1 min at 12,000 × *g*. The supernatants were discarded, and pellets were resuspended in PIPES buffer containing 0.1% saponin and incubated with 0.7 μM rhodamine-conjugated phalloidin for 60 min in darkness on a rotator. This concentration of rhodamine-conjugated phalloidin saturated the F-actin in the acini as determined by using concentrations of rhodamine-conjugated phalloidin in a range from 0.2 to 1 μM. Stained pellets were washed three times with 0.5 ml of saponin buffer. Rhodamine-conjugated phalloidin was extracted from cell pellets with methanol, and protein was measured in each sample with the Bio-Rad protein assay. The fluorescence of extracts was measured using excitation at 541 nm and emission at 565 nm. The relative F-actin content was calculated as the ratio of the fluorescence emission per microgram of protein of the hormone-stimulated sample divided by the fluorescence emission per microgram of protein of the control sample. The contribution of endogenous fluorescence to the fluorescence of the methanol extract at these wavelengths was negligible (<1%), as determined with extracts from unlabeled tissues. The extent of nonspecific binding of rhodamine-conjugated phalloidin was determined by the simultaneous addition of excess unlabeled phalloidin. At 100-fold excess of unlabeled phalloidin, nonspecific binding was 3% for both control and treated samples.

Immunocytochemistry and Confocal Fluorescence Microscopy—Pancreatic acini were incubated as indicated above for fluorometric determination of rhodamine-conjugated phalloidin binding to actin. At the time points indicated, 100-μl aliquots were transferred to slides previously coated with polylysine (17–30 kDa). The acini were allowed to attach spontaneously to the polylysine substrate during a 1-min incubation. Subsequently, acini were fixed for 30 min at room temperature with 4% formaldehyde, prepared from paraformaldehyde. After rinsing in PBS, fixed acini were incubated sequentially with 1 mg/ml sodium borohydride in PBS for 10 min, with PBS containing 0.2% Triton X-100 and 5% normal goat serum for 30 min, and then with 5 μg/ml rhodamine-conjugated phalloidin in PBS containing 0.2% Triton X-100 and 2% normal goat serum for 60 min. Acini were rinsed thoroughly with PBS and covered with a drop of mounting medium (3:1 mixture of glycerol and PBS containing 4 mg/ml *p*-phenylenediamine) and a glass coverslip. Actin staining was analyzed by conventional epifluorescence microscopy and by confocal fluorescence microscopy (Noran OZ). With the confocal microscope, the distribution of actin was examined in a series of digitized optical sections (1-μm increments in the Z plane) that encompassed the thickness of the acinus being viewed. A contiguous series of three to four optical sections was sufficient to capture most of the actin staining associated with the luminal region of the acinus. For presentation of the data, this series of individual sections, for each incubation condition, was projected as a stack using Noran Intervision software and was processed using Adobe Photoshop 4.0.

RESULTS

Activation of p38 MAP Kinase by Cholecystokinin—The presence of p38 MAP kinase in rat pancreatic acini was demonstrated by Western blotting with a specific antibody that revealed a strong signal from a single protein band at an apparent molecular mass of 38–40 kDa (Fig. 1). This protein could be quantita-

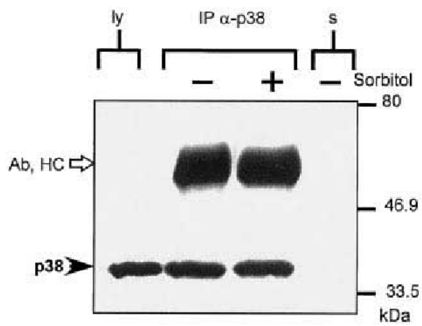

FIG. 1. **Identification of p38 MAP kinase in rat pancreatic acinar cells.** Acinar lysates (40 μg), immunoprecipitates (*IP*) of p38 MAP kinase from 200 μg of protein lysate of untreated cells and cells treated with 0.3 M sorbitol before lysis, and 40 μg of supernatant after immunoprecipitation were subjected to SDS-polyacrylamide gel electrophoresis and Western-blotted with the anti-p38 antibody. *Bars* on the right indicate the positions of prestained, low range molecular mass markers. The *arrowhead* indicates p38 MAP kinase. The *arrow* marks the heavy chain (*HC*) of the antibody (*Ab*). *ly*, cell lysate; *s*, supernatant.

tively immunoprecipitated from both control and stimulated acinar cell lysates, as shown by its absence in the supernatant after immunoprecipitation (Fig. 1). The immunoprecipitates contained kinase activity for the p38 substrate GST-ATF-2-(1–109) (Fig. 2*A*). When acini were stimulated with CCK for varying times, the immunoprecipitated p38 MAP kinase activity was increased by 1 min and reached a maximum after 10 min, when a 2.8 ± 0.1-fold increase was observed (Fig. 2*A*). The p38 MAP kinase activity remained elevated for at least 30 min. After incubation for 10 min with different doses of CCK, the minimal response of p38 MAP kinase to CCK stimulation was observed at a 10 pM concentration of the hormone, whereas maximal responses were observed between 300 pM and 1 nM (Fig. 2*B*). Higher concentrations led to a decrease in p38 MAP kinase activity.

Effect of Hyperosmolarity on p38 MAP Kinase Activity—Addition of 0.3 M sorbitol to the incubation medium to increase osmolarity induced a rapid activation of p38 MAP kinase that was significant at 2.5 min and maximal at 10 min and that remained elevated for at least 20 min (Fig. 3*A*). The activation of p38 MAP kinase in pancreatic acini was dependent on the concentration of sorbitol (Fig. 3*B*). Addition of 0.1 M sorbitol led to an ~2.2-fold increase in p38 MAP kinase, whereas maximal activation (4.4 ± 0.4-fold) was observed with addition of 0.3 M sorbitol. Higher concentrations of sorbitol led to decreased activation. To distinguish whether activation of p38 MAP kinase was specific for sorbitol or whether hyperosmolarity was the cause of the activation, we also stimulated the acini by addition of either mannitol or sucrose. The 4–5-fold activation of p38 MAP kinase observed for each agent was of the same magnitude as that obtained after sorbitol stimulation (data not shown). These data indicate that hyperosmotic stress can activate p38 MAP kinase in isolated rat pancreatic acini.

Effect of Different Stimuli on p38 MAP Kinase Activity—Stimulation of pancreatic acini with 1 nM CCK, 100 μM carbachol, or 100 nM bombesin for 10 min led to a 2.5–3-fold increase in p38 MAP kinase activity (Fig. 4). 1 μM 12-*O*-tetradecanoylphorbol-13-acetate, a potent stimulator of protein kinase C, activated p38 MAP kinase by ~2-fold. The Ca^{2+}-ATPase inhibitor cyclopiazonic acid (30 μM), EGF (0.1 μM), and vasoactive intestinal polypeptide (1 μM) induced only a minimal increase, whereas anisomycin (50 μg/ml) activated p38 MAP kinase ~2-fold. Incubation of acini with a combination of cyclopiazonic acid and 12-*O*-tetradecanoylphorbol-13-acetate resulted in additive effects on p38 MAP kinase activity. The strongest p38 MAP kinase activation (~4.4-fold increase) was observed with hyperosmolarity induced by addition of 0.3 M sorbitol (Fig. 4). The combination of sorbitol and CCK or sorbitol and carbachol showed no additive effect (data not shown).

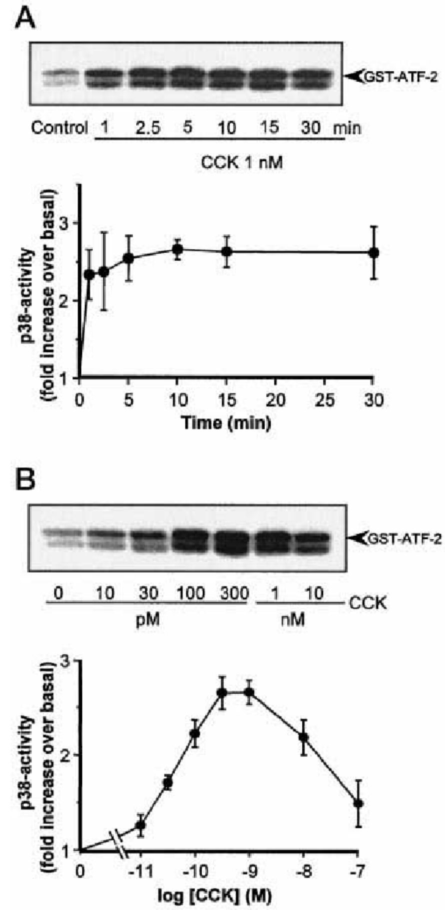

FIG. 2. **Time course and concentration-dependent effect of CCK-induced activation of p38 MAP kinase in rat pancreatic acini.** Acini were incubated with or without 1 nM CCK for the indicated times (*A*) or with CCK at various concentrations for 10 min and then lysed (*B*). Samples were immunoprecipitated overnight with p38 MAP kinase antibody, and the recovered protein was used in a kinase reaction with GST-ATF-2 as substrate. A representative experiment for each condition is shown at the top of the graphs. The intensity of phosphorylation was measured by a phosphoimager and is expressed as a -fold increase of the value at time 0. Each point represents the mean ± S.E. of three to five independent experiments, each performed in duplicate.

Tyrosyl Phosphorylation of p38 MAP Kinase—Using an antibody that recognizes only the activated form of p38 MAP kinase by detecting its phosphorylation at tyrosine 182, we were able to measure the tyrosyl phosphorylation of p38 MAP kinase as an alternative way of assessing activation (Fig. 5*A*). Densitometric analysis of phosphorylated p38 MAP kinase following Western blotting showed a 2–3-fold increase in tyrosyl phosphorylation after CCK and sorbitol stimulation (Fig. 5*B*).

Activation of MAPKAP Kinase-2 by CCK and Sorbitol Involves p38 MAP Kinase—MAPKAP kinase-2 has been reported to be a substrate of p38 MAP kinase (15, 16) and to be activated in cells stimulated with granulocyte/macrophage colony-stimulating factor, interleukin-3 (40), or hematopoietic growth factors (17). To determine whether the activation of MAPKAP kinase-2 by CCK was due to activation of p38 MAP kinase, we immunoprecipitated MAPKAP kinase-2 from isolated acini that had been stimulated with 1 nM CCK or 0.3 M sorbitol and assessed its activity using a peptide from Hsp 27 as substrate. Stimulation with CCK led to an ~2-fold increase in MAPKAP kinase-2 activity (Fig. 6*A*), which correlated with the ability of the hormone to activate p38 MAP kinase. Stimulation with sorbitol produced a 3–4-fold increase in MAPKAP kinase-2 activity, similar to the activation of p38 MAP kinase.

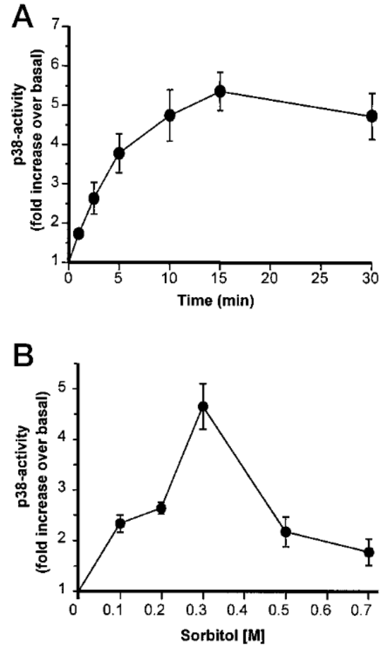

FIG. 3. **Time course and concentration-dependent effect of sorbitol on p38 MAP kinase activity in rat pancreatic acini.** Acini were incubated in HEPES-Ringer solution with or without 0.3 M sorbitol for the indicated times (*A*) or with various concentrations of sorbitol for 10 min (*B*). p38 MAP kinase activation was determined by immunoprecipitation and kinase assay. The data are expressed as a -fold increase of control. Each point represents the mean ± S.E. of three to five independent experiments, each performed in duplicate.

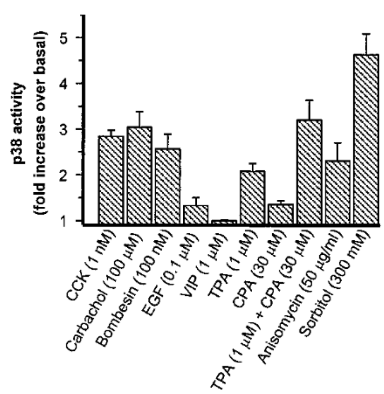

FIG. 4. **Effects of various agonists on p38 MAP kinase activity in isolated rat pancreatic acini.** Acini were stimulated for 10 min with the concentrations of the indicated agonists. The results are expressed as a -fold increase of control values for acini incubated without any stimulator. The data presented are the means ± S.E. of 3 to 11 independent experiments, each performed in duplicate. *VIP*, vasoactive intestinal peptide; *TPA*, 12-*O*-tetradecanoylphorbol-13-acetate; *CPA*, cyclopiazonic acid.

MAPKAP kinase-2 has also been reported to be activated by the ERK MAP kinase family (41). To investigate which of these kinases was responsible for the activation of MAPKAP kinase-2 by CCK or sorbitol, we used SB 203580, a pyridinyl imidazole derivative that is a highly specific inhibitor of p38 MAP kinase activity (42). SB 203580 inhibits p38 MAP kinase activity competitively by binding to the ATP-binding domain of the kinase (43). Therefore, to assess the inhibitory effect of SB 203580 on the activity of p38 MAP kinase in the acinar cell, we measured the activity of a downstream target, MAPKAP kinase-2, after incubation of the acini with the inhibitor for 60 min. The p38 MAP kinase inhibitor SB 203580 (20 μM) lowered the basal MAPKAP kinase-2 activity by 50% and blocked the CCK- and sorbitol-induced MAPKAP kinase-2 activity completely (Fig. 6*A*).

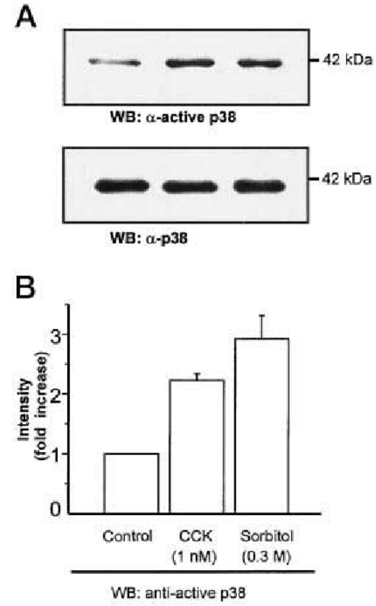

FIG. 5. **Tyrosyl phosphorylation of p38 MAP kinase in rat pancreatic acinar cells.** Acinar cells were treated with 1 nM CCK or 0.3 M sorbitol for 10 min or left untreated and then sonicated in lysis buffer, subjected to SDS-polyacrylamide gel electrophoresis, and Western-blotted (*WB*) with the anti-phospho-p38 MAP kinase antibody. The blots were stripped and reprobed with the anti-p38 MAP kinase antibody (*A*). A densitometric analysis of three independent experiments, each performed in duplicate, after Western blotting with the anti-phospho-p38 MAP kinase antibody is shown (*B*).

To confirm that SB 203580 did not affect the activity of the ERK MAP kinases, we investigated the effects of the compound on the activation of MAPKAP kinase-1, known to be downstream of the ERKs (37). Pretreatment of the cells with 20 μM SB 203580 alone or in combination with CCK did not affect the activation of MAPKAP kinase-1 (Fig. 6*B*), implying that the ERK pathway was not affected by SB 203580 and that p38 MAP kinase did not activate MAPKAP kinase-1. A similar lack of effect was observed when we evaluated CCK stimulation of p70 S6 kinase activity after pretreatment of the cells with 20 μM SB 203580 (data not shown).

Hsp 27 Phosphorylation and Its Inhibition through SB 203580—Recently, we demonstrated, by use of immunoblotting after two-dimensional gel electrophoresis, that Hsp 27 exists in three isoforms in rat pancreatic acini, one nonphosphorylated and two phosphorylated, and that Hsp 27 phosphorylation is stimulated by cholecystokinin, both *in vivo* and *in vitro* (44). Isoelectric focusing electrophoresis followed by Western blotting also demonstrated that Hsp 27 exists in three isoforms (1, 2, and 3) that represent nonphosphorylated, monophosphorylated, and diphosphorylated isoforms (Fig. 7*A*). In untreated acini, all three isoforms were found in nearly equal amounts, indicating a high basal level of phosphorylation. Nevertheless, treating the acini with 1 nM CCK or 0.3 M sorbitol for 10 min led to an acidic shift, indicating an increase in the more phosphorylated isoforms (Fig. 7, *A* and *B*). Pretreatment of the acini with 20 μM SB 203580 for 60 min reduced the high basal level of Hsp 27 phosphorylation by eliciting a 54% increase in the amount of the nonphosphorylated isoform and a 24% decrease in the amount of the diphosphorylated isoform (Fig. 7, *A* and *B*, *bar graphs*). The p38 MAP kinase inhibitor was also able to block the Hsp 27 phosphorylation induced by CCK and sorbitol. The inhibitor decreased the amount of the diphosphorylated form by 36% for both CCK- and sorbitol-stimulated acini. These data indicate that p38 MAP kinase and MAPKAP kinase-2 are directly involved in phosphorylation of Hsp 27 in rat pancreatic acini.

Effect of CCK on Relative F-actin Content in Pancreatic Acinar Cells—Since Hsp 27 phosphorylation has been shown to affect F-actin polymerization (33), and CCK is known to alter the acinar cell cytoskeleton (45, 46), we quantitated changes in F-actin content after CCK stimulation using a rhodamine-conjugated phalloidin binding assay. CCK (1 nM) induced a rapid decrease in total F-actin content, which was maximal by 1 min (Fig. 8). After 5 min, total F-actin increased, and by 10 min, returned to prestimulation levels. After 40 min, a significant increase in total F-actin content was detected. To determine whether p38 MAP kinase/Hsp 27 phosphorylation plays a role in these changes, acini were pretreated with SB 203580. Incubation with SB 203580 alone showed no effects on F-actin content. After pretreatment with SB 203580 and stimulation with CCK (1 nM) for different times, the changes in total F-actin content were considerably reduced, indicating that activation of p38 MAP kinase/Hsp 27 phosphorylation most likely plays a role in actin dynamics after CCK treatment.

Effect of CCK on the Actin Cytoskeleton—After we found changes in total F-actin content with a biochemical assay (Fig. 8), we investigated the cellular localization of actin microfilaments in acini in response to CCK. Untreated acini incubated with and without SB 203580 and cells treated for different times with 0.3 M sorbitol or 1 nM CCK, alone or in combination with 20 μM SB 203580, were fixed, stained for F-actin with rhodamine-conjugated phalloidin, and examined by confocal fluorescence microscopy. In control cells, actin was primarily localized as an intense fluorescent band just beneath the luminal membrane. Weak staining was associated with the basolateral plasmalemma, and the cytoplasm was largely unlabeled (Figs. 9*A* and 10*A*). After a 1-min treatment with 1 nM CCK, the intensity of subapical membrane staining was reduced and appeared more diffuse (Fig. 9*B*). After 10 min of CCK treatment, the intensity of luminal actin staining was greatly reduced (Fig. 9*C*) and, in some acini, difficult to resolve. An increase in diffuse cytoplasmic fluorescence was often apparent in these acini. Punctate staining not seen in control acini was also resolved to a varying extent at or near the basolateral membrane when these surfaces were viewed en face at points of contact with the glass slide (Fig. 9*D*). These effects seen at 10 min were more severe after 40 min of CCK treatment (Fig. 9*E*). In contrast, treatment with 0.3 M sorbitol for 1 or 10 min showed no effects on the actin cytoskeleton. Treatment for 40 min revealed minor changes in the actin cytoskeleton in some acini, with slightly increased cytoplasmic and basolateral membrane staining (Fig. 9*F*). Overall, however, effects of sorbitol were minor compared with those of CCK. We then investigated the effects of SB 203580 on the actin cytoskeleton.

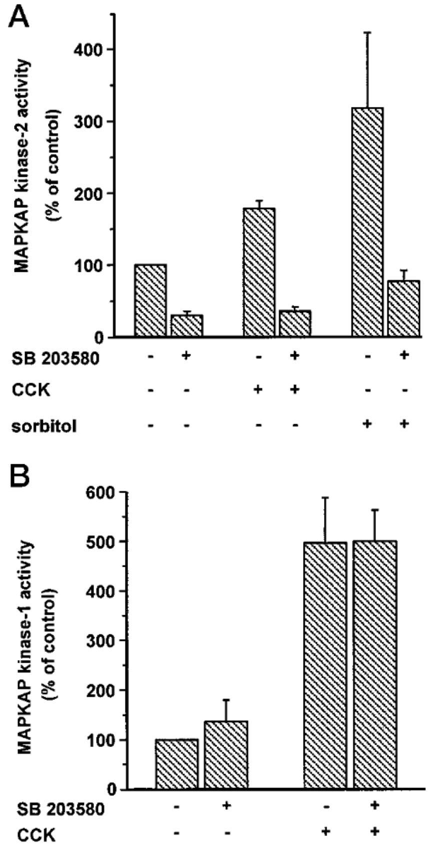

FIG. 6. **Effect of SB 203580 on MAPKAP kinase-2 and MAPKAP kinase-1 activity in pancreatic acini.** Acinar cells were incubated at 37 °C with or without 20 μM SB 203580 for 60 min prior to stimulation. The cells were then left untreated or were stimulated with 1 nM CCK or 0.3 M sorbitol for 10 min. MAPKAP kinase-2 (*A*) or MAPKAP kinase-1 (*B*) was immunoprecipitated from aliquots of cell lysates, and their activities were assessed in immune complex kinase assays using a peptide from Hsp 27 or S6 phosphate acceptor peptide in the presence of [γ-^{32}P]ATP as described under "Experimental Procedures." Mixtures were then spotted on Whatman P-81 paper, and peptide-bound radioactivity was determined. Data are expressed as a percentage of the value at time 0. Results are the means ± S.E. of three to four independent experiments, each performed in duplicate.

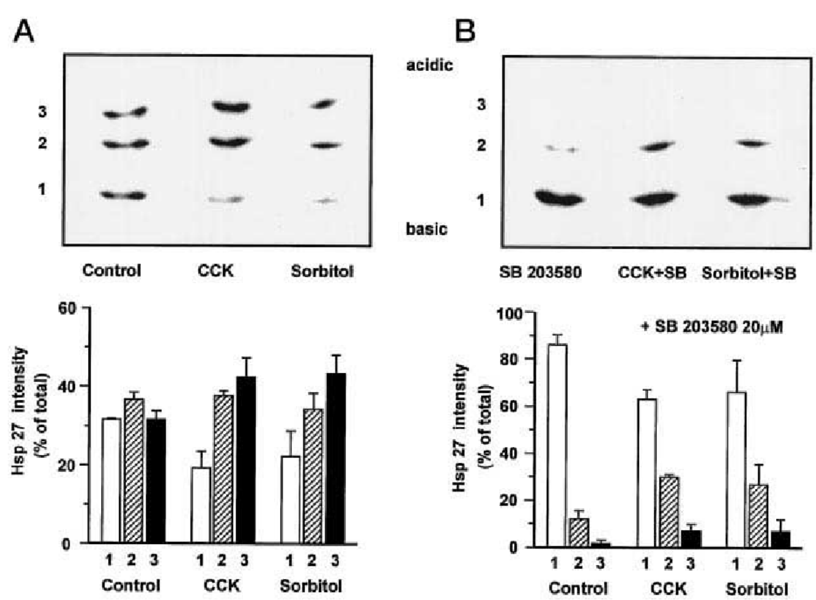

FIG. 7. **SB 203580 inhibits Hsp 27 phosphorylation in pancreatic acinar cells.** Acinar cells were incubated at 37 °C without (*A*) or with (*B*) 20 μM SB 203580 for 60 min prior to stimulation. The cells were then left untreated or were stimulated with 1 nM CCK or 0.3 M sorbitol for 10 min and sonicated in urea lysis buffer. 40 μg of cell lysate protein for each lane was subjected to isoelectric focusing and Western-blotted with the anti-Hsp 27 antibody. Hsp 27 was quantitated by expressing the intensity of each isoform of the protein as a percentage of the total intensity of all three isoforms. *Isoform 1*, nonphosphorylated; *isoform 2*, monophosphorylated; *isoform 3*, diphosphorylated. The *bar graphs* show the means ± S.E. of three separate experiments, each performed in duplicate, with a single representative Western blot above each graph.

Treatment with SB 203580 alone showed no effect (Fig. 10, *B–D*). Preincubation with SB 203580, however, reduced the extent of loss of subapical actin membrane staining, particularly after 10 and 40 min of CCK treatment (Fig. 10, *F–H*; data for 40 min not shown). SB 203580 also partially inhibited the increased cytoplasmic and punctate basolateral membrane staining observed with treatment with CCK alone. The extent of this effect was variable between acini. These results suggest that the actin reorganization and the disassembly of the apical membrane structure triggered by CCK are mediated in part by activation of the p38 MAP kinase/Hsp 27 pathway.

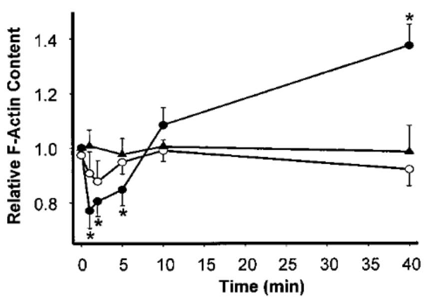

FIG. 8. **Effect of CCK, sorbitol, and SB 203580 on relative F-actin content of pancreatic acinar cells.** Acinar cells were incubated at 37 °C with (○) or without (●) 20 μM SB 203580 for 60 min prior to stimulation with 1 nM CCK. ▲, effects of 0.3 M sorbitol. Each experimental point represents the mean ± S.E. of four to five paired determinations. *Asterisks* indicate significant reduction or increase compared with paired control ($p < 0.05$).

DISCUSSION

We recently reported that treatment of isolated rat pancreatic acini with CCK activates ERKs and JNKs, as well as other upstream components of the MAP kinase signaling cascade, including Ras and MEK1/MEK2 (6, 7, 47, 48). In the present study, we have demonstrated that CCK activates p38 MAP kinase and that this activation leads to activation of MAPKAP kinase-2, resulting in an increase in Hsp 27 phosphorylation. In response to CCK, p38 MAP kinase was rapidly activated in pancreatic acini, with maximal activation occurring after 5–10 min. These results demonstrate that p38 MAP kinase activation by CCK more closely resembles that of ERKs (p42 MAP kinase and p44 MAP kinase), whose activation is maximal 5–10 min following treatment (6, 47), as compared with CCK-induced activation of JNKs ($p46^{jnk}$ and $p55^{jnk}$), whose activity was maximal after 30 min (47). Furthermore, the activities of p38 MAP kinase and ERKs show a similar dependence on CCK concentration, which is distinct from that of JNKs. The minimal CCK concentration that activated p38 MAP kinase or ERKs was in the picomolar range, whereas maximal activation was observed at 300 pM and 1 nM, respectively (6, 47). In contrast, the CCK concentration necessary to induce the maximal response of JNKs in pancreatic acini was 100 times greater (47).

Since JNKs and ERKs are known to be activated by distinct signaling cascades in acini, it was of interest to compare the activation of these kinases and p38 by different secretagogues and intracellular messengers. In acini, CCK, carbachol, and bombesin receptors are all known to interact with heterotri-

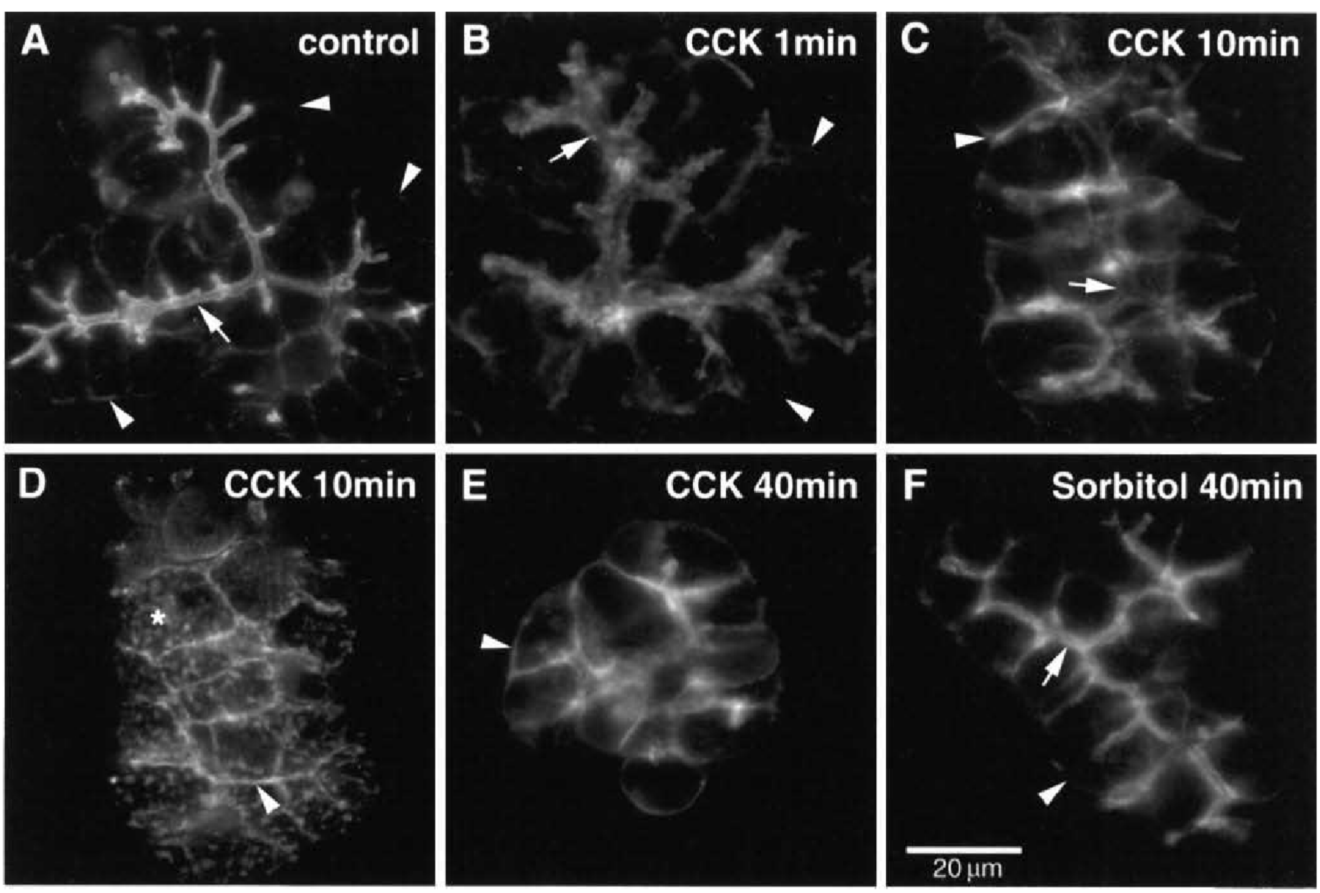

FIG. 9. **Effect of CCK and sorbitol on the distribution of filamentous actin in isolated rat pancreatic acini.** Acini were left untreated (*A*) or were incubated with 1 nM CCK or 0.3 M sorbitol for various times (*B–F*), fixed in 4% paraformaldehyde as described under "Experimental Procedures," and stained with rhodamine-conjugated phalloidin. Each *micrograph* is derived from stacking three to four adjacent optical sections (1-μm increments) as described under "Experimental Procedures," taken through the center of the acinus, except for *D*, which is presented as a single confocal image of the region of the acinar basolateral membrane where it is in contact with the glass slide. *Arrows* indicate the actin distribution in the subapical region; *arrowheads* point to the basolateral membrane; and the *asterisk* marks the punctate staining of the basolateral region. Images are representative of at least three to four experiments, in each of which multiple acini were examined.

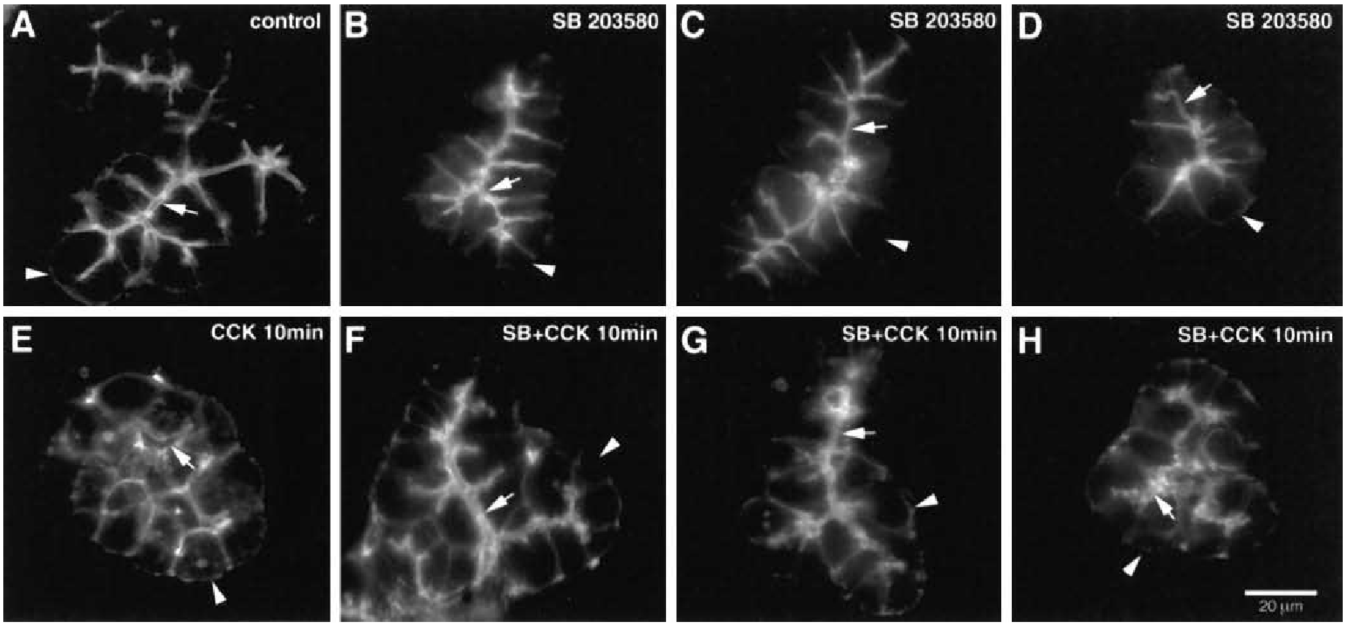

FIG. 10. **Effect of SB 203580 on CCK-induced changes in the actin cytoskeleton.** A representative image of untreated acini after a 45-min preincubation is shown in *A,* whereas in *B–D,* acini were preincubated for 45 min with SB 203580. When the effects of CCK were investigated, CCK in the absence (*E*) and presence (*F–H*) of SB 203580 was added for and additional 10 min. *Arrows* indicate the actin distribution in the subapical region, and *arrowheads* point to the basolateral membrane. Images are representative of three to five experiments.

meric G proteins and thereby activate a phospholipase C that hydrolyzes phosphatidylinositol bisphosphate, generating inositol 1,4,5-triphosphate and diacylglycerol. These messengers, in turn, mobilize intracellular Ca^{2+} and activate protein kinase C, respectively (49). CCK, carbachol, and bombesin all increased p38 MAP kinase activity ~2.6–3.1-fold, compared with an ~3.5–4.5-fold increase in p42 ERK activity. CCK also induced a ~4-fold increase in $p55^{jnk}$, whereas bombesin and carbachol caused lesser activation, ~1.8- and 2.0-fold, respectively. Comparison of the effects of CCK and EGF on the activation of the ERK pathway indicates that the major mechanism of ERK activation by CCK involves protein kinase C-mediated activation of multiple forms of Raf. This is distinct from the action of EGF, which activates Ras and is protein kinase C-independent (48). Activation of protein kinase C with active phorbol ester, which is known to stimulate ERK activity in acini (6), also activates p38 MAP kinase. However, stimulation with EGF showed a minimal effect on p38 MAP kinase, similar to the effects on JNKs (47). Increasing intracellular Ca^{2+} with cyclopiazonic acid induced only a small increase in p38 MAP kinase activity, but the combination of 12-*O*-tetradecanoylphorbol-13-acetate and cyclopiazonic acid mimicked the effects of CCK on p38 MAP kinase activity. Although cAMP is stimulated by high concentrations of CCK, this second messenger does not appear to be important in activating p38 MAP kinase because vasoactive intestinal polypeptide, which dramatically increases cAMP, had no effect. Even if the upstream regulators of p38 MAP kinase in pancreatic acini are not known, these results suggest that protein kinase C and Ca^{2+} play roles in p38 MAP kinase activation and that the pathway leading to p38 MAP kinase activation is different from that leading to ERK activation.

Interestingly, the strongest activation of p38 MAP kinase was observed when acinar cells were stressed with hyperosmolarity induced by addition of sorbitol, mannitol, or sucrose. The kinetics for p38 MAP kinase activation after stimulation with sorbitol are slower compared with CCK stimulation. Hyperosmolarity, however, is not a specific activator of p38 MAP kinase as addition of sorbitol also activates ERKs. Anisomycin, known as a JNK activator, also increased p38 MAP kinase activity ~2.3-fold. Although the specific biological role of p38 MAP kinase in cell signaling is not known, evidence exists that activation of the p38 MAP kinase pathway plays a key role in cell cycle regulation, apoptosis, and cytoskeletal dynamics (50–52). Since CCK, carbachol, and bombesin all activate heterotrimeric G proteins, it seems likely that activation of the p38 MAP kinase cascade involves G proteins and diverges at that level from pathways activating enzyme secretion. This is also consistent with recently published data showing that $G\beta\gamma$ mediates the signal from m2 muscarinic and β-adrenergic receptors to p38 MAP kinase, whereas the signal from the m1 muscarinic receptor is mediated by both $G\beta\gamma$ and $G\alpha_{q/11}$ (53). The specific G protein subtype that is involved in the activation of p38 MAP kinase in pancreatic acini remains to be determined.

To investigate whether p38 MAP kinase was involved in the phosphorylation of Hsp 27, we looked at the effects of CCK on MAPKAP kinase-2 and its substrate, Hsp 27. The data obtained from the MAPKAP kinase-2 assays showed similar -fold activation of MAPKAP kinase-2 compared with the activation of p38 MAP kinase after stimulation with CCK. This is consistent with our hypothesis that MAPKAP kinase-2 is a physiological substrate for p38 MAP kinase in pancreatic acini. To examine this hypothesis further, we used the specific p38 MAP kinase inhibitor SB 203580. The specificity of SB 203580 has been characterized by its failure to inhibit 12 other protein kinases *in vitro* and by its lack of effect on the activation of kinases upstream of p38 MAP kinase and other MAP kinase cascades *in vivo* (42). We demonstrated the specificity of this inhibitor in our system by examining the activity of MAPKAP kinase-1, known to be downstream of the ERKs, and p70 S6 kinase. SB 203580 inhibited the CCK-induced activation of MAPKAP kinase-2, but not MAPKAP kinase-1 or p70 S6 kinase, indicating that MAPKAP kinase-2 is a physiological substrate for p38 MAP kinase in pancreatic acini. It is known that activation of MAPKAP kinase-2 and MAPKAP kinase-3 leads to the phosphorylation of Hsp 27 (13, 16, 29). Recently, we also reported that CCK stimulates Hsp 27 phosphorylation in rat pancreas, both *in vivo* and *in vitro,* using Western analysis after two-dimensional electrophoresis (44). Other groups have shown that Hsp 27 *in vitro* exerts a phosphorylation-modulated inhibitory function on F-actin polymerization and influences

actin dynamics in response to stress and growth factors (52, 54). We therefore focused on the regulation of Hsp 27 in rat pancreatic acini and showed that activation of p38 MAP kinase by CCK or hyperosmotic stress is responsible for Hsp 27 phosphorylation since this phosphorylation can be blocked by inhibiting p38 MAP kinase with SB 203580. Changes in the apical cytoskeleton of intact and permeabilized pancreatic acinar cells after treatment with CCK have been reported earlier (45, 46, 55). Our data are consistent with these findings. Furthermore, we demonstrated that CCK-mediated effects on the actin cytoskeleton occurred within 1 min; increased actin associated with the basolateral membrane was also observed after 10 min. When compared with the biochemical assay, the loss of total F-actin after 1 min corresponds temporally to the loss of subapical staining and changes in the subapical area as seen by confocal microscopy. After pretreatment with SB 203580, it was difficult to determine if the cytoskeletal changes induced by 1 nM CCK for 1 min were reduced in the majority of examined cells. The secondary increase in total F-actin to prestimulation levels after 10 min and to levels higher than control after 40 min of CCK treatment may correspond, at least in part, to increased staining in the basolateral area. Some of the increase in total F-actin content could be also due a loss of secretory proteins. However, if we estimate that 10% of acinar protein is secretory protein and that 25–30% of this is secreted, it is unlikely that this loss of 2–3% of cell protein is responsible for the changes in total F-actin content seen after CCK stimulation.

The minor changes in the actin cytoskeleton seen after 40 min of treatment with sorbitol are also consistent with data obtained from the actin binding assay. Only after 40 min of treatment with 0.3 M sorbitol did we observe slightly enhanced F-actin levels (data not shown). Other reports have shown that p125 focal adhesion kinase ($p125^{FAK}$) and paxillin are also involved in cytoskeletal changes (56, 57), and activation of $p125^{FAK}$ by CCK has recently been reported in pancreatic acini (58). These observations, together with our data that activation of p38 MAP kinase by sorbitol is greater than that by CCK, whereas the effects on the actin cytoskeleton are less, indicate that more than one pathway is involved in actin cytoskeletal changes. Because there is some evidence that the cytoskeleton is involved in secretion, we looked for effects on amylase secretion. Inhibition of p38 MAP kinase/Hsp 27 phosphorylation by SB 203580, however, did not affect amylase secretion after stimulation with CCK for 5 and 30 min (data not shown).

In summary, this study demonstrates that activation of the p38 MAP kinase/Hsp 27 pathway is involved not only in response to stress, but also in physiological signaling by gastrointestinal hormones via G_q-coupled receptors. Using two different approaches, confocal microscopy and a biochemical actin binding assay, we were able to demonstrate a role for p38 MAP kinase activation and Hsp 27 phosphorylation in regulating the amount and cellular localization of F-actin in pancreatic acini.

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REFERENCES

1. Solomon, T. E. (1994) in *Physiology of the Gastrointestinal Tract* (Johnson, L. R., ed) 3rd. Ed., Vol. 2, pp. 1499–1529, Raven Press, New York
2. Solomon, T. E., Vanier, N., and Morisset, J. (1983) *Am. J. Physiol.* **245,** G99–G105
3. Logsdon, C. D., and Williams, J. A. (1986) *Am. J. Physiol.* **250,** G440–G447
4. Schick, J., Kern, H., and Scheele, G. (1984) *J. Cell Biol.* **99,** 1559–1564
5. Lu, L., and Logsdon, C. D. (1992) *Am. J. Physiol.* **263,** G327–G332
6. Duan, R.-D., and Williams, J. A. (1994) *Am. J. Physiol.* **267,** G401–G408
7. Duan, R.-D., Zheng, C.-F., Guan, K.-L., and Williams, J. A. (1995) *Am. J. Physiol.* **268,** G1060–G1065
8. Bragado, M. J., Dabrowski, A., Groblewski, G. E., and Williams, J. A. (1997) *Am. J. Physiol.* **272,** G401–G407
9. Seger, R., and Krebs, E. G. (1995) *FASEB J.* **9,** 726–735
10. Karin, M. (1995) *J. Biol. Chem.* **270,** 16483–16486
11. Pages, G., Lenormand, P., L'Allemain, G., Chambard, J. C., Meloche, S., and Poyssegur, J. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 8319–8323
12. Han, J., Lee, J.-D., Bibbs, L., and Ulevitch, R. J. (1994) *Science* **265,** 808–811
13. Rouse, J., Cohen, P., Trigon, S., Morange, M., Alonso-Llamazares, A., Zamanillo, D., Hunt, T., and Nebreda, A. R. (1994) *Cell* **78,** 1027–1034
14. Lee, J. C., Laydon, J. T., McDonnell, P. C., Gallagher, T. F., Kumar, S., Green, D., McNulty, D., Blumenthal, M. J., Heys, J. R., Landvatter, S. W., Strickler, J. E., McLaughlin, M. M., Siemens, I. R., Fisher, S. M., Livi, G. P., White, J. R., Adams, J. L., and Young, P. R. (1994) *Nature* **372,** 739–746
15. Raingeaud, J., Gupta, S., Rogers, J. S., Dickens, M., Han, J., Ulevitch, R. J., and Davis, R. J. (1995) *J. Biol. Chem.* **270,** 7420–7426
16. Freshney, N., Rawlinson, L., Guesdon, F., Jones, E., Cowley, S., Hsuan, J., and Saklatvala, J. (1994) *Cell* **78,** 1039–1049
17. Foltz, I. N., Lee, J. C., Young, P. R., and Schrader, J. W. (1997) *J. Biol. Chem.* **272,** 3296–3301
18. Krump, E., Sanghera, J. S., Pelech, S. L., Furuya, W., and Grinstein, S. (1997) *J. Biol. Chem.* **272,** 937–944
19. Minden, A., Lin, A., Claret, F.-X., Abo, A., and Karin, M. (1995) *Cell* **81,** 1147–1157
20. Zhang, S., Han, J., Sells, M. A., Chernoff, J., Knaus, U. G., Ulevitch, R. J., and Bokoch, G. M. (1995) *J. Biol. Chem.* **270,** 23934–23936
21. Bagrodia, S., Derijard, B., Davis, R. J., and Cerione, R. A. (1995) *J. Biol. Chem.* **270,** 27995–27998
22. Derijard, B., Raingeaud, J., Barrett, T., Wu, I.-H., Han, J., Ulevitch, R. J., and Davis, R. J. (1995) *Science* **267,** 682–685
23. Lin, A., Minden, A., Martinetto, H., Claret, F.-X., Lange-Carter, C., Mercurio, F., Johnson, G. L., and Karin, M. (1995) *Science* **268,** 286–290
24. Han, J., Lee, J.-D., Jiang, Y., Li, Z., Feng, L., and Ulevitch, R. J. (1996) *J. Biol. Chem.* **271,** 2886–2891
25. Raingeaud, J., Whitmarsh, A, J., Barrett, T., Derijard, B., and Davis, R. J. (1996) *Mol. Cell. Biol.* **16,** 1247–1255
26. Stein, B., Brady, H., Yang, M. X., Young, D. B., and Barbosa, M. S. (1996) *J. Biol. Chem.* **271,** 11427–11433
27. Moriguchi, T., Kuroyanagi, N., Yamaguchi, K., Gotoh, Y., Irie, K., Kano, T., Shirakabe, K., Muro, Y., Shibuya, H., Matsumoto, K., Nishida, E., and Hagiwara, M. (1996) *J. Biol. Chem.* **271,** 13675–13679
28. Doza, Y. N., Cuenda, A., Thomas, G. M., Cohen, P., and Nebreda, A. R. (1995) *FEBS Lett.* **364,** 223–228
29. Wang, X., and Ron, D. (1996) *Science* **272,** 1347–1349
30. Cavigelli, M., Dolfi, F., Claret, F.-X., and Karin, M. (1995) *EMBO J.* **14,** 5957–5964
31. Gupta, S., Campbell, D., Derijard, B., and Davis, R. J. (1995) *Science* **267,** 389–393
32. McLaughlin, M. M., Kumar, S., McDonnell, P. C., Van Horn, S., Lee, J. C., Livi, G. P., and Young, P. R. (1996) *J. Biol. Chem.* **271,** 8488–8492
33. Piotrowicz, R. S., and Levin, E. G. (1997) *J. Biol. Chem.* **272,** 25920–25927
34. Lavoie, J. N., Lambert, H., Hickey, E., Weber, L. A., and Laundry, J. (1995) *Mol. Cell. Biol.* **15,** 505–516
35. Williams, J. A., Korc, M., and Dormer, R. L. (1978) *Am. J. Physiol.* **253,** E517–E524
36. Stokoe, D., Caudwell, B., Cohen, P. T. W., and Cohen, P. (1993) *Biochem. J.* **296,** 843–849
37. Bragado, M. J., Groblewski, G. E., and Williams, J. A. (1997) *Am. J. Physiol.* **273,** C101–C109
38. Condeelis, J., and Hall, A. L. (1991) *Methods Enzymol.* **196,** 486–495
39. Ding, G., Franki, N., Condeelis, J., and Hays, R. M. (1991) *Am. J. Physiol.* **260,** C9–C16
40. Ahlers, A., Engel, K., Scott, C., Gaestel, M., Herrmann, F., and Brach, M. A. (1994) *Blood* **83,** 1791–1798
41. Stokoe, D., Campbell, D. G., Nakielny, S., Hidaka, H., Leevers, S. J., Marshall, C., and Cohen, P. (1992) *EMBO J.* **11,** 3985–3994
42. Cuenda, A., Rouse, J., Doza, Y. N., Meier, R., Cohen, P., Gallagher, T. F., Young, P. R., and Lee, J. C. (1995) *FEBS Lett.* **364,** 229–233
43. Young, P. R., McLaughlin, M. M., Kumar, S., Kassis, S., Doyle, M. L., McNulty, D., Gallagher, T. F., Fisher, S., McDonnell, P. C., Carr, S. A., Huddleston, M. J., Seibel, G., Porter, T. G., Livi, G. P., Adams, J. L., and Lee, J. C. (1997) *J. Biol. Chem.* **272,** 12116–12121
44. Groblewski, G. E., Grady, T., Mehta, N., Lambert, H., Logsdon, C. D., Landry, J., and Williams, J. A. (1997) *Gastroenterology* **112,** 1354–1361
45. O'Konski, M. S., and Pandol, S. J. (1993) *Pancreas* **8,** 638–646
46. O'Konski, M. S., and Pandol, S. J. (1990) *J. Clin. Invest.* **86,** 1649–1657
47. Dabrowski, A., Grady, T., Logsdon, C. D., and Williams, J. A. (1996) *J. Biol. Chem.* **271,** 5686–5690
48. Dabrowski, A., Groblewski, G. E., Schäfer, C., Guan, K.-L., and Williams, J. A. (1997) *Am. J. Physiol.* **273,** C1472–C1479
49. Williams, J. A., and Yule, D. I. (1993) *Exocrine Pancreas,* 2nd Ed., pp. 167–189, Raven Press, New York
50. Molnar, A., Theodoras, A. M., Zon, L. I., and Kyriakis, J. M. (1997) *J. Biol. Chem.* **272,** 13229–13235
51. Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J., and Greenberg, M. E. (1995) *Science* **270,** 1326–1331
52. Huot, J., Houle, F., Marceau, F., and Landry, J. (1997) *Circ. Res.* **80,** 383–392
53. Yamauchi, J., Nagao, M., Kaziro, Y., and Itoh, H. (1997) *J. Biol. Chem.* **272,** 27771–27777
54. Benndorf, R., Hayes, K., Ryazantsev, S., Wieske, M., Behlke, J., and Lutsch, G. (1994) *J. Biol. Chem.* **269,** 20780–20784
55. Muallem, S., Kwiatkowska, K., Xu, X., and Yin, H. L. (1995) *J. Cell Biol.* **128,** 589–598
56. Seufferlein, T., and Rozengurt, E. (1994) *J. Biol. Chem.* **269,** 27610–27617
57. Seufferlein, T., and Rozengurt, E. (1995) *J. Biol. Chem.* **270,** 24343–24351
58. Garcia, L. J., Rosado, J. A., Tsuda, T., and Jensen, R. T. (1997) *Biochim. Biophys. Acta* **1358,** 189–199