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 acinar 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 Gs-coupled receptors stimulates a cascade in which p38 MAP kinase and p38 MAP kinase-activated protein kinase-2 result in phosphorylation of Hsp 27. 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.

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CCK1 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

¶ 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\textsubscript{i}-coupled receptors. Here we demonstrate that CCK and other pancreatic secretagogues that activate secretion via the G\textsubscript{i}-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 purchased from Research Plus, Inc. (Burlington, MA). Bovine serum albumin and calf thymus DNA were from Sigma. Acetylated BSA was from Bio-Rad. RTLSVA) were from Upstate Biotechnology, Inc. (Lake Placid, NY). MAPKAP kinase-1 (catalog No. 06-321), MAPKAP kinase-2 (catalog No. 06534), and the kinase only when activated by phosphorylation at Tyr-182, was from Research Plus, Inc. (Burlington, MA). 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. [\gamma-32P]ATP (300 Ci/mmol) was from NEN Life Science Products. The enhanced chemiluminescence (ECL) detection system, horseradish peroxidase-conjugated antibody, 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\textsubsuperscript{rsk-1} (C-21) antibody were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-specific p38 MAP kinase antibody was 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 KKLNLRTLSVA) were from Upstate Biotechnology, Inc. (Lake Placid, NY).

The monoclonal anti-mouse Hsp 27 antibody was a gift from Dr. Michael Welch (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). The preparative protocol was as follows: the supernatants 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 μM 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 acini, and likely due to nonspecific interactions of the anti-phospho-specific p38 MAP kinase antibody with endogenous phosphoproteins. A strong signal from a single protein band at an apparent molecular mass of 38–40 kDa (Fig. 1). This protein could be quantitated 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).

**RESULTS**

**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 KPO4, 10 mM PIPES, 5 mM EGTA, and 2 mM MgSO4 (pH 6.8)) and centrifuged for 1 min at 300 g. Fixed acini were then centrifuged for 1 min at 12,000 g in 0.7 M sucrose to pellet acini. Sucrose 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. The 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 μM 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 acini, and likely due to nonspecific interactions of the anti-phospho-specific p38 MAP kinase antibody with endogenous phosphoproteins. A strong signal from a single protein band at an apparent molecular mass of 38–40 kDa (Fig. 1). This protein could be quantitated 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).

**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). The protein could be quantitated 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 quantitated 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 quantitated 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 quantitated 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 quantitated 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 quantitated 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 quantitated 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 quantitated 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 quantitated 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 quantitated 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 quantitated 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 quantitated 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 quantitated 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).
The combination of sorbitol and CCK or sorbitol and carbachol
to an additive effect 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).

Effect of Different Stimuli on p38 MAP Kinase Activity—
Stimulation of pancreatic acini with 1 nM CCK, 100 μM carbachol,
or 100 μM bombesin for 10 min led to a 2.5- to 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 Ca2+-ATPase inhibitor
cyclosporine A (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 cyclosporine 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).

Tyrosyl Phosphorylation of p38 MAP Kinase—Using an
antibody that recognizes only the activated form of p38 MAP
kinase by detecting the tyrosyl phosphorylation at tyrosine 182, we
were able to measure the tyrosyl phosphorylation of p38 MAP
kinase in rat pancreatic acini. As shown in Fig. 5A, we
immunoprecipitates 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.

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.

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.
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. 6A).

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. 6B), 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 and B). 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. 9A and 10A). After a 1-min treatment with 1 nM CCK, the intensity of subapical membrane staining was reduced and appeared more diffuse (Fig. 9B). After 10 min of CCK treatment, the intensity of luminal actin staining was greatly reduced (Fig. 9C) 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. 9D). After a 10-min treatment with 1 nM CCK, the intensity of subapical membrane staining was reduced and appeared more diffuse (Fig. 9E). After 10 min of CCK treatment, the intensity of luminal actin staining was greatly reduced (Fig. 9C) 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. 9D). These effects seen at 10 min were more severe after 40 min of CCK treatment (Fig. 9E).

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. 9F). Overall, however, effects of sorbitol were minor compared with those of CCK. We then investigated the effects of SB 203580 on the actin cytoskeleton.
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.

**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 (p46jnk and p55jnk), 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-
mamic 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 (48). 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 Gβγ 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 Gβγ and Gαq/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

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.
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 content could also be due to a loss of secretory vesicles. The secondary increase in total F-actin to prestimulation with CCK for 5 and 30 min (data not shown).

In summary, this study demonstrates that activation of the p38 MAP kinase by CCK is greater than that by CCK, whereas the effects on the actin cytoskeleton are less, indicating 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 Gα-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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