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# Control of c-*fos* expression in STC-1 cells by peptidomimetic stimuli

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**Abstract**

Enteroendocrine cells respond to nutrient and non-nutrient stimuli in the gut lumen. The intestinal hormone cholecystokinin (CCK) is secreted in response to luminal fatty acids, amino acids, peptides and proteins. The peptidomimetic cephalosporins have been reported to provide model, stable, compounds with similar secretagogue activity to peptide. Putative luminal stimuli also influence transcriptional activity in enteroendocrine cells, but the mechanisms are uncertain. In the present study we have investigated the control of c-*fos* expression in STC-1 cells (an enteroendocrine cell line). Peptidomimetics stimulated calcium-dependent release of CCK, and increased intracellular calcium, phosphorylation of p42/44 mitogen-activated protein kinase (MAP kinase) and c-*fos* mRNA abundance. Hypotonic stress also increased p42/44 MAP kinase phosphorylation and c-*fos* mRNA, but not CCK release. The increase in c-*fos* mRNA was strikingly potentiated by peptidomimetics in hypotonic medium. Increased c-*fos* expression, but not CCK release, was suppressed by the MAP kinase (MEK) inhibitor PD98059, and by the tyrosine kinase inhibitor genistein. We conclude that in STC-1 cells, peptidomimetics act through the p42/44 MAP kinase pathway to increase c-*fos* expression but not exocytosis. Moreover, a putative non-nutritive stimulus, hypotonic stress, may interact with this pathway to enhance c-*fos* expression, independently of hormone release. 

*Keywords:* c-*fos*; c-*jun*; Cholecystokinin; MAP (mitogen-activated protein), kinase; Hypotonic stress

## 1. Introduction

Enteroendocrine cells secrete humoral factors in response to stimuli in the gut lumen, and cytokines, growth factors or neurohumoral agents acting at their basolateral membrane. In recent years, it has become clear that stimuli acting at both luminal and basolateral membranes also change gene expression in gut endocrine cells. For example, expression of the gastric hormone, gastrin, is increased by food and epidermal growth factor, and inhibited by luminal acid and somatostatin (Brand and Stone, 1988; Karnik et al., 1989; Wu et al., 1991); similarly, expression of the gene for the structurally related intestinal hormone, cholecystokinin (CCK), is increased by luminal trypsin inhibitor and bombesin, and inhibited by somatostatin (Liddle et al., 1988; Kanayama and Liddle, 1990,1991). Some progress has been made in elucidating the signalling pathways activated by stimuli acting at the basolateral membrane of gut endocrine cells (Marks et al., 1996; Hocker et al., 1997), but the transduction mechanisms mediating the action of luminal stimuli are largely unexplored.

Expression of the immediate early gene, c-*fos*, is an early event in mediating transcriptional responses in many cell types including enteroendocrine cells and gut neurons (Kirchgessner et al., 1992; Dimaline et al., 1995; Marks et al., 1996; Hocker et al., 1997). In the present study we have examined the expression of c-*fos* in response to peptidomimetic secretagogues in a CCK-secreting cell line. Peptidomimetic cephalosporins have previously been shown to stimulate CCK release in the same way as peptone, which is commonly employed for gut hormone studies in vivo (CordierBussat et al., 1997; Nemoz-Gaillard et al., 1998a). However, peptone is a crude mixture of many potential secretagogues of unknown identity and concentration, and its use in cellular studies is limited. In contrast, peptidomimetics are attractive model compounds for activation of luminal-protein stimulated pathways in cell culture, since their chemical identity is unambiguous and unlike both peptone and synthetic peptides these com-

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pounds are relatively stable in experimental conditions (Nemoz-Gaillard et al., 1998a). For these studies we used STC-1 cells, which are derived from an intestinal endocrine cell tumour in a mouse doubly transgenic for SV40 early region antigen and the polyoma small T antigen, both under control of the insulin promoter (Rindi et al., 1990). These cells have been used as a model for studies of enteroendocrine cell differentiation (Ratineau et al., 1997), gene expression (CordierBussat et al., 1997), prohormone processing (Yoon and Beinfeld, 1997) and secretory mechanisms. They synthesise and store CCK, secretin and gastric inhibitory polypeptide, and release these peptides on stimulation by amino acids, gastrin releasing peptide (GRP), and cephalosporin peptidomimetics (Snow et al., 1994; Mangel et al., 1995; CordierBussat et al., 1997; Nemoz-Gaillard et al., 1998a,b). We report here that peptidomimetic CCK secretagogues increase c-*fos* expression in STC-1 cells via the p42/44 mitogen-activated protein (MAP) kinase pathway, and that there is an unexpectedly strong potentiation of this pathway by hypotonic stress. The results suggest interactions between nutrient and non-nutritive stimuli to control gene expression in gut endocrine cells that have so far been largely neglected.

## 2. Materials and methods

### 2.1. Chemicals

Routine chemicals, cephalosporins, nifedipine, rapamycin and reagents for sodium dodecyl sulphate–polacrylamide gel electrophoresis (SDS–PAGE) and Western blotting were obtained from Sigma (Poole, Dorset, UK); Phe-Ala-Asn was obtained from Bachem (Essex, UK), other synthetic peptides were obtained from Sigma; PD98059, SB203580, wortmannin and genistein were obtained from Calbiochem-Novabiochem (Nottingham, UK). Culture media, horse and fetal bovine serum were obtained from Gibco (Paisley, UK). Antibodies to phosphorylated p42/44 MAP kinase and to total p42/44 MAP kinase were obtained from New England Biolabs.

### 2.2. Cell culture

STC-1 cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% horse serum, 5% foetal bovine serum, 100 $\mu g\ ml^{-1}$ streptomycin, 100 IU $ml^{-1}$ penicillin, 5 $\mu g\ ml^{-1}$ ascorbate and 4.5 $gl^{-1}$ glucose. Cells were maintained in 75 $cm^3$ flasks and were incubated at 37°C under 95% air–5% $CO_2$. Experiments were performed on cells grown in either 60 mm dishes treated with poly-L-lysine, or four-well 16 mm dishes, plated at $2 \times 10^6$ or $0.1 \times 10^6$ cells $dish^{-1}$, respectively. Experiments were performed after 48–72 h, when cells were approximately 90% confluent.

### 2.3. Experimental conditions

Cells were washed once with 5 ml Earle's balanced salt solution (EBSS, 290, mosM $l^{-1}$) and preincubated with the same solution for 1 h at 37°C. They were then exposed to peptidomimetics in concentrations up to 10 mM. In studies of c-*fos* expression a comparison was made of the effects of peptidomimetics and osmotic stress, by incubating cells in 205 or 250 mosM $l^{-1}$ media (prepared by reducing NaCl) or 400 mosM $l^{-1}$ (prepared by addition of NaCl). After incubation for up to 2 h, medium was removed and centrifuged at 4°C and the supernatant taken for radioimmunoassay of CCK. Cell pellets were extracted to recover RNA for Northern analysis using QuickPrep Total RNA extraction kits (Pharmacia Biotech, Herts, UK).

### 2.4. Northern analysis

Samples of total RNA (10 $\mu g$) were electrophoresed in 1% agarose gels, transferred to nylon membranes and hybridized with cRNA probes for c-*fos*, c-*jun* and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA as previously described (Dimaline et al., 1995).

### 2.5. Radioimmunoassay

The secretion of CCK was determined by radioimmunoassay using antibody L48 which reacts with COOH-terminally amidated forms of CCK and [$^{125}$I]G17 as label employing assay conditions described previously (Dockray, 1980). Synthetic sulphated CCK8 was used as standard.

### 2.6. Intracellular $Ca^{2+}$

Changes in intracellular calcium were studied in cells plated at a density of $0.5 \times 10^6$ on 25 mm coverslips in 6-well dishes, 24–48 h before experiments. Cells were loaded with the cell permeant acetoxymethyl ester form of Indo-1 (5 $\mu M$) in HEPES-buffered salt solution (HBSS) (in mM): NaCl 145, KCl 5, $MgCl_2$ 1.3, $NaH_2PO_4$ 1.2, HEPES 20, $CaCl_2$ 2 for 1 h at 22°C. Cells were washed with HBSS and coverslips mounted in a perfusion chamber on the stage of an inverted microscope. Indo-1 fluorescence was excited at 340 nm and emitted fluorescence recorded from individual cells at 400 and 500 nm and the ratio used as an index of internal calcium. Cells were continuously perfused with HBSS and exposed to 5 mM cephalothin in HBSS or hypotonic medium (through reduction of NaCl concentration), or both.

### 2.7. Reporter assays

One million STC-1 cells per well were plated into six well plates 24 h prior to transfection. Cells were trans-

fected using Transfast reagent (Promega, UK), and 0.7 μg pAP1-LUC plasmid (Stratagene) which is a *cis*-reporting activator protein-1 (AP-1) system upstream of a luciferase reporter gene, together with a second reporter for transfection efficacy, pRL-TK (Promega) containing the *Renilla* luciferase gene downstream of the constitutive thymidine kinase promoter. Cells were transfected in DMEM without supplement for one hour and then returned to full media for 18 h prior to stimulation with hypotonic medium (205 mosM $l^{-1}$, 2 h) in 1 mM cefaclor, followed by 1 mM cefaclor in normal medium (4 h). Cells were then harvested for assay using the dual luciferase assay system (Promega).

### 2.8. Western analysis

Proteins separated by SDS–PAGE were electroblotted to nitrocellulose membranes for 1 h at 4°C at 100 V in transfer buffer (Tris 20 mM, glycine150 mM, methanol 20% v/v ). Membranes were blocked with 5% non-fat dry milk in 20 mM Tris, NaCl 137 mM, 0.1% Tween-20 (TBS-T), incubated in the primary antiserum (1:1000, 18 h, 4°C), washed three times in TBS-T (22°C, 10 min), incubated in secondary antibody, anti-rabbit immunoglobulin G conjugated to horseradish peroxidase, diluted 1:2000, then washed again three times in TBS-T, and then incubated in enhanced chemoluminescence reagents (Photope®-HRP Western detection system, NEB) for subsequent autoradiography.

### 2.9. Statistics

Results are presented as means ± S.E.; comparisons were made by analysis of variance (ANOVA) and by *t*-test, and were considered significant when $P < 0.05$.

## 3. Results

### 3.1. CCK releases by cephalosporin peptidomimetics and atripeptide

The secretion of CCK was stimulated by a variety of peptidomimetics and the tripeptide Phe-Ala-Asn (FAN) over the concentration range 0.1–10 mM (Fig.1, Table 1). The largest CCK responses were evoked by cefaclor and cephalothin; at 1 mM, both cefaclor and FAN produced half-maximal CCK release, but the maximal response to cefaclor was about three-fold higher than that to FAN (Fig. 1). The rank order of potency of four cephalosporins for CCK release was cefaclor ≥ cephalothin > cephradine ≥ cephamandole (Table 1). At a concentration of 1 mM several synthetic dipeptides (Gly-Sar, Ala-Asp, Phe-Gly, Gly-Gln) and the tripeptide Gly–Gly–Ile, were inactive.

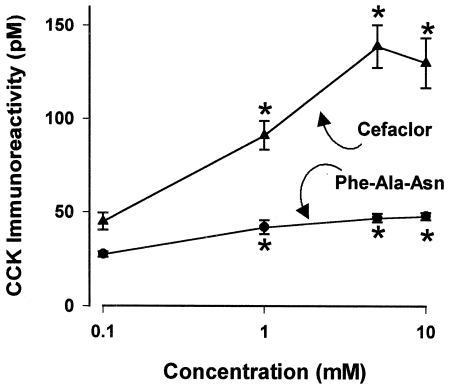


Fig. 1. Release of CCK measured by radioimmunoassay in response to graded concentrations of cefaclor and the tripeptide FAN for 2 h. Results are means ± S.E., $n = 4–8$; $^{*}P < 0.05$, compared with 0.1 mM.

### 3.2. Peptidomimetics increase c-fos mRNA in STC-1 cells

The abundance of c-*fos* mRNA was then examined in cells after exposure to peptidomimetic secretagogues. There was no change in c-*fos* mRNA after incubation with peptidomimetics for 30 min, but by 2 h there was an approximately three-fold increase in c-*fos* mRNA abundance (Figs. 2 and 3). The rank order of potency of four cephalosporin peptidomimetics for induction of c-*fos* mRNA was similar to that for CCK-release, i.e. cefaclor = cephalothin > cephradine = cephamandole (Table 1). There was no increase in c-*jun* mRNA in responses to peptidomimetics, and the abundance of GAPDH mRNA was unchanged by these compounds, providing evidence of the specificity of the responses (Fig. 2 and 3).

### 3.3. Potentiation of the peptidomimetic increase in c-fos mRNA in hypotonic media

For the purposes of comparison we examined c-*fos* mRNA abundance in response to osmotic stress, since the latter increases immediate early gene expression in many cell types (Sadoshima et al., 1996; Sinning et al., 1997; Crepel et al., 1998), and since variations in osmotic pressure occur in the gastrointestinal lumen. Exposure to hypertonic media (30 min, 400 mosM $l^{-1}$) produced a small increase in c-*fos* mRNA in STC-1 cells (134 ± 7%, relative to isotonic, 100%). Hypotonic medium of 250 mosM $l^{-1}$ had no significant effect (101 ± 1%, relative to isotonic), but hypotonic medium of 205 mosM $l^{-1}$ produced a significant three-fold increase $P < 0.05$ in c-*fos* mRNA, i.e., comparable in magnitude to that evoked by peptidomimetic stimulation (Figs. 2 and 3). However, unlike the response to peptidomimetics, the increase in hypotonic medium was maximal after 30 min, and declined slightly over 2 h. Moreover, hypotonic medium also increased c-*jun* mRNA 50% after 30 min, and 250% after 2 h ($P < 0.05$).

Table 1
Relative potency of cephalosporins on release of CCK, c-*fos* mRNA abundance and phosphorylation of p42/44 MAP kinase[a]

| Compound | CCK-release | c-*fos* mRNA | c-*fos* mRNA (hypotonic stress) | p42/44 MAP kinase phosphorylation |
|---|---|---|---|---|
| Cefaclor | 100 ± 3.4 | 100 ± 6.4 | 100 ± 2.7 | 100 |
| Cephalothin | 88.5 ± 0.4 | 114 ± 17.4 | 81.0 ± 7.7 | 57 |
| Cephradine | 33.2 ± 0.5 | 39.5 ± 3.9 | 38.3 ± 4.2 | 4 |
| Cephamandole | 34.9 ± 2.8 | 20.8 ± 2.3 | 20.6 ± 2.5 | ND |

[a] Potency expressed relative to the response to cefaclor (100%) in each case. Data are derived from responses after 2-h stimulation by the relevant cephalosporin (1–5 mM) in isotonic or hypotonic media. CCK release was determined by radioimmunoassay, c-*fos* mRNA by Northern blot and p42/44 MAP kinase phosphorylation by Western blot. means ± S.E., for $n = 3$ experiments.

We examined possible interactions between peptidomimetics and hypotonic stress in controlling c-*fos* mRNA abundance and, interestingly, found a striking synergy between the two. Thus, after 2 h incubation in cefaclor in hypotonic medium, c-*fos* mRNA abundance was over 10-fold higher than control (Figs. 2 and 3). By comparison there was no synergistic interaction for control of c-*jun* mRNA abundance. Moreover, the changes in c-*fos* mRNA abundance in STC-1 cells could be dissociated from the release of CCK, since hypotonic medium did not induce a significant increase in CCK secretion (*control*: 29.4 ± 3.6 pM; *hypotonic*: 33.2 ± 4.6 pM, after 2 h), and there was no indication of an interaction between hypotonic stress and peptidomimetic stimulation of CCK release (*cefaclor* 1 mM and *hypotonic stress*: 108.9 ± 10.9% relative to cefaclor alone).

### 3.4. Role of calcium

Stimulated secretion in STC-1 cells is inhibited by calcium channel blockers including nifedipine(Snow et al., 1994; Nemoz-Gaillard et al., 1998a), and in the present study we found the release of CCK in response to cefaclor was substantially reduced by nifedipine (Fig. 4). The increase in c-*fos* mRNA produced by peptidomimetic compounds was also significantly ($P < 0.05$) reduced by exposure of cells to nifedipine, suggesting an involvement of voltage-sensitive calcium channels (Fig. 4). In order to define the role of calcium more precisely we examined changes in intracellular calcium using the fluorescent indicator Indo-1. We found that cefaclor exhibited endogenous fluorescence which precluded studies with Indo-1. However, cephalothin, which in other respects evoked similar

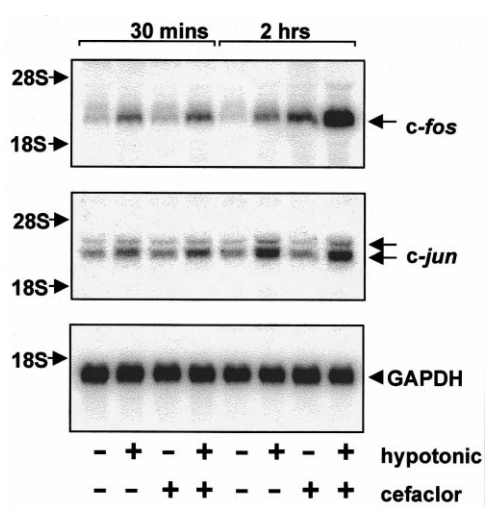


Fig. 2. Effects of cefaclor (1 mM), hypotonic stress (205 mosM $l^{-1}$), and cefaclor in hypotonic medium, on mRNA abundance in STC-1 cells. Representative Northern blots are shown for c-*fos* mRNA (*upper panel*), c-*jun* mRNA (*centre*) and GAPDH mRNA (*lower panel*) after 30 min (left) and 2 h (right) treatment.

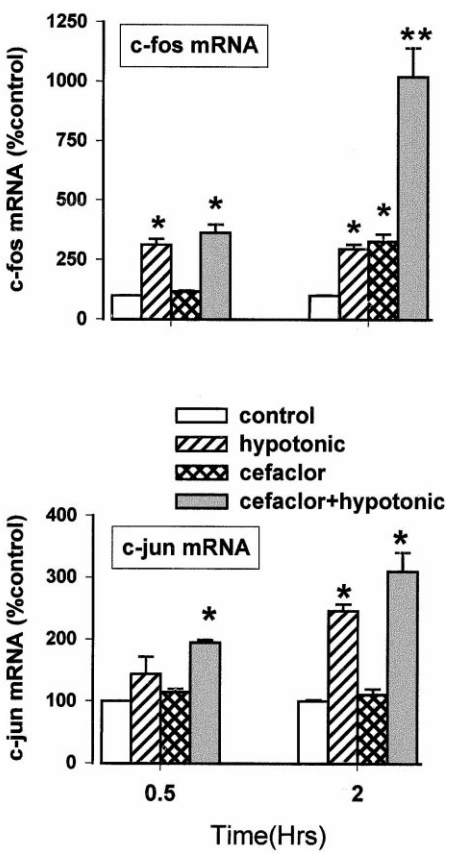


Fig. 3. Quantification of the changes in c-*fos* and c-*jun* mRNA abundance in response to cefaclor, hypotonic stress and the combination (see also Fig. 2 for further details). Results are means ± S.E., $n = 3$–16; $^{*}P < 0.05$ vs. control; $^{**}P < 0.05$ vs. other groups.

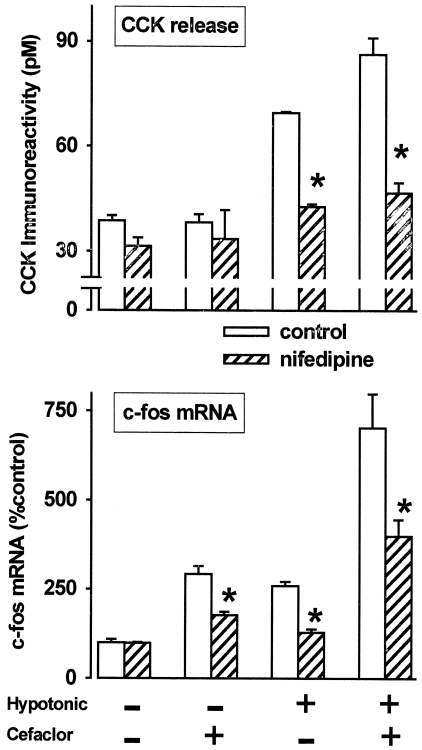


Fig. 4. Effect of nifedipine (10 μM) on CCK release (top) and c-*fos* mRNA abundance (bottom) in response to cefaclor (1 mM), hypotonic stress (205 mosL $l^{-1}$) and the combination. Means ± S.E., $P < 0.05$ vs. vehicle.

responses to cefaclor (Table 1), did not exhibit endogenous fluorescence. Incubation of STC-1 cells in cephalothin produced increased intracellular calcium concentrations (Fig. 5), that declined to resting levels on removing the stimulus. In view of the data described above on the effect of hypotonic stress, it is interesting to note that the latter did not significantly increase intracellular calcium (not shown), and did not change in the response to cephalosporins (Fig. 5).

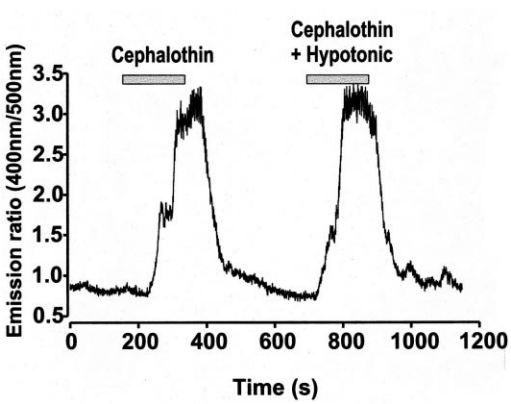


Fig. 5. Changes in intracellular calcium monitored by Indo-1 in a single STC-1 cell, during perfusion with 5 mM cephalothin in isotonic medium, followed by cephalothin in hypotonic medium. Note the peptidomimetic compound increased intracellular calcium; the response was similar when cells were exposed to cephalothin in hypotonic medium. A representative trace is shown; similar observations were made in 18 different experiments.

### 3.5. Role of p42 / 44 MAP kinase

Because c-*fos* expression is increased by activation of the MAP kinase pathway in many cell types, we examined expression in the presence of the MEK inhibitor, PD98059. The latter significantly inhibited the increase in c-*fos* mRNA in response to peptidomimetic stimulation both alone and in hypotonic medium (Fig. 6). In order to determine the specificity of this response, we also examined the effect of an inhibitor of the p38 stress kinase pathway, SB203580; the latter had no effect on the increase in c *fos* mRNA abundance in response to peptidomimetic stimulation, or hypotonic medium in STC-1 cells (Fig. 6).

Since it seemed that the mechanisms controlling secretion and c-*fos* mRNA in response to peptidomimetics exhibited some similarities, we also examined the action of PD98059 on peptidomimetic-evoked CCK secretion. We found PD98059 had no effect on basal secretion (*control*: 100 ± 5%; *PD98059:* 98.0 ± 7%), or on peptidomimetic-stimulated CCK release (*cefaclor* 1 mM: 220 ± 16%, relative to control; cefaclor and *PD98059:* 189 ± 28%). The pathway by which peptidomimetics activate exocytosis and c-*fos* expression in STC-1 cells therefore diverge upstream of the activation of MEK.

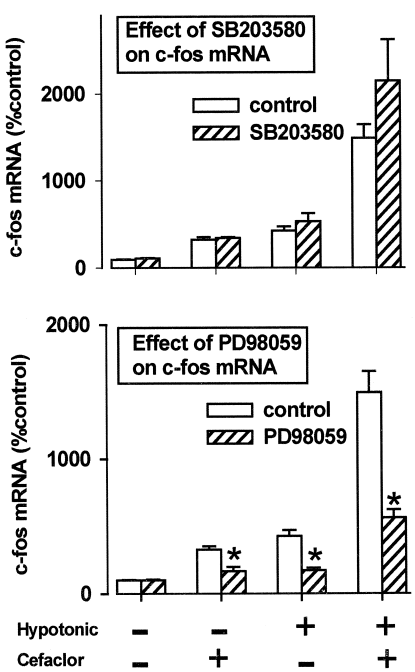


Fig. 6. Effect of PD98059 (20 μM; *bottom*) and SB203580 (10 μM, *top*) on c-*fos* mRNA abundance in response to cefaclor and hypotonic stress (see also Figs. 2 and 3, for details). Results are means ± S.E. of three to six independent experiments, $P < 0.05$ vs. vehicle.

In order to address the possible activation of p42/44 MAP kinase, we examined by Western blot whether phosphorylated p42/44 MAP kinase changed in these experiments, using for this purpose antibodies that are specific for phosphorylated forms of the enzyme. Peptidomimetics progressively increased phosphorylation of p42/44 MAP kinase from 10 min to 2 h. This time course was distinct from that in response to hypotonic medium, which produced a transient increase in phosphorylated p42/44 MAP kinase, that was already in decline after 10 min (Fig.7). However, the combination of hypotonic medium and peptidomimetics suggested a synergistic interaction after 2 h, although not earlier (Fig. 7). There was no change in total p42/44 MAP kinase abundance as shown by Western blots using an antibody that detects the unphosphorylated and phosphorylated enzyme (not shown). Of several peptidomimetics examined, cefaclor was again the most potent activator of p42/44 phosphorylation (Table 1).

The possible involvement of a tyrosine kinase up-stream of p42/44 MAP kinase, was explored using the tyrosine kinase inhibitor genistein. There was a substantial inhibition of p42/44 MAP kinase phosphorylation in the presence of genistein (Fig. 8). In addition genistein abolished the increase in c-*fos* mRNA in response to cefaclor and hypotonic stress, and inhibited by over 90% the response to the combination of the two ($P < 0.05$).

In parallel experiments, we were unable to influence the change in c-*fos* mRNA in response to peptidomimetic stimuli by incubation with pertussis toxin, or with the protein kinase C inhibitor GF109203X, the $p70^{s6k}$ inhibitor rapamycin, and the phosphoinositide-3-kinase (PI-3-kinase) inhibitor, wortmannin (not shown). These observations do not, therefore, support a role for $G_i$, protein kinase C, $p70^{s6k}$, PI-3-kinase or p38 kinase in the c-*fos* mRNA responses to peptidomimetic CCK-secretagogues.

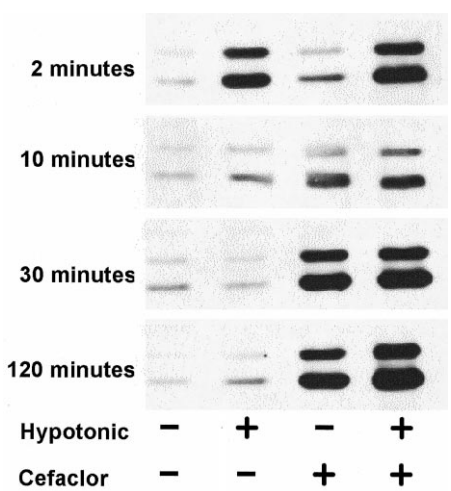


Fig. 7. Activation of p42/44 MAP kinase by cefaclor and hypotonic medium. Western blots are shown using antibody specific to phosphorylated p42/44 MAP kinase. STC-1 cells were incubated in normal and hypotonic EBSS in the presence and absence of 1 mM cefaclor for up to 120 min. Hypotonic medium stimulated a transient increase in p42/44 MAPK phosphorylation. In 1 mM cefaclor there was a progressive increase in p42/44 MAPK phosphorylation which was potentiated in hypotonic media.

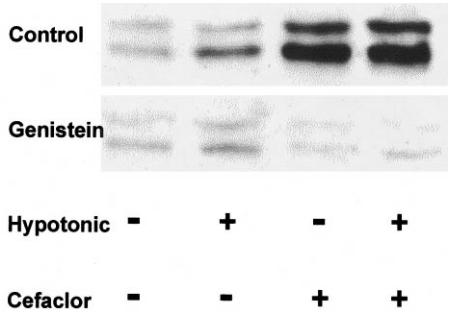


Fig. 8. Inhibition of the cefaclor-induced phosphorylation of p42/44 MAP kinase by genistein. See Fig. 7 for further details. STC-1 cells were incubated for 2 h in 1 mM cefaclor either with or without genistein (100 μM).

### 3.6. AP-1 reporter expression

In order to determine the functional significance of the increase in c-*fos* mRNA we examined expression of a luciferase reporter construct with AP-1 enhancer element. After exposure of STC-1 cells to 1 mM cefaclor in hypotonic medium there was a statistically significant increase in luciferase expression ($100 \pm 13$ vs. $158 \pm 19\%$, $P < 0.05$) indicating the functional capacity of increased Fos.

## 4. Discussion

The results of the present study indicate that in STC-1 cells, peptidomimetic CCK-secretagogues also stimulated c-*fos* expression. Peptidomimetic stimulation was associated with increased intracellular calcium, and the calcium channel blocker nifedipine inhibited both secretory responses and the induction of c-*fos* mRNA. In addition, however, stimulation of c-*fos* mRNA was shown to depend on tyrosinekinase mediated activation of the p42/44 MAP kinase pathway. Unexpectedly, the stimulation of MAP kinase and induction of c-*fos* by peptidomimetics was found to be strongly potentiated by hypotonic stress. However, osmotic stress did not influence CCK release, and activation of the MAP kinase pathway was not required for secretory responses to peptidomimetic compounds. We suggest, therefore, that control of immediate early gene expression in enteroendocrine cells can be mediated by putative luminal secretagogues via the MAP kinase pathway, independent of hormone release. The finding that there are interactions between peptidomimetic secretagogues and osmotic stress in controlling immediate early gene expression in STC-1 cells has implications for understanding how enteroendocrine cell function is regulated in vivo. In particular, factors such as variations in osmotic

pressure that are encountered in the gut lumen, but do not evoke hormone release, may nevertheless influence endocrine cell responses via changes in gene expression.

Previous work has shown that STC-1 cells secrete CCK in response to phenylalanine (Mangel et al., 1995), bombesin (Snow et al., 1994; Nemoz-Gaillard et al., 1998b), fatty acids (McLaughlin et al., 1999), PACAP (Chang et al., 1996), as well as protein extracts and peptidomimetics (CordierBussat et al., 1997; Nemoz-Gaillard et al., 1998a). Peptidomimetics are attractive model compounds for analysis of enteroendocrine cell responses to stimulation by protein and peptides, since they are more stable, and more potent than native proteins or hydrolysates. Moreover in the case of the latter, the structural identity of the active compound is often uncertain, whereas the chemical identity of peptidomimetics is unambiguous. Nemoz-Gaillard et al. (1998a) reported that the secretion of CCK by STC-1 cells in response to cephalosporin peptidomimetics depended on external calcium and was sensitive to pertussis toxin suggesting a $G_i$-mediated effect. Our data extend these observations by showing directly that peptidomimetics increase intracellular calcium; in addition we show that they stimulate tyrosine kinase mediated activation of the p42/44 MAP kinase pathway resulting in c-*fos* expression. Although it has been reported that the secretory response to bombesin in STC-1 cells is in part due to activation of p42/44 MAP kinase (Nemoz-Gaillard et al., 1998b), our data do not suggest that this pathway is important in mediating secretory responses to peptidomimetics. Moreover, we found that while hypotonic stress activated the p42/44 MAP kinase pathway this was not associated with CCK release. The calcium channel blocker nifedipine inhibited both CCK secretion and the increase in c-*fos* in response to peptidomimetic stimulation. It seems then, that increased intracellular calcium mediates both secretory and transcriptional responses to peptidomimetics: the transcriptional response also requires activation of p42/44 MAP kinase but this step is not essential for CCK-release. These findings demonstrate, for the first time, a specific pathway by which luminal stimuli might influence gene expression in enteroendocrine cells.

Mechanical stimulation of the mucosal surface has been reported to increase Fos-immunoreactivity in myenteric neurons in guinea-pig ileum, and gastric distension increased c-*fos* mRNA detected by Northern blot and in situ hybridization in rat gastric myenteric neurons (Kirchgessner et al., 1992; Dimaline et al., 1995). The possible transduction mechanisms were not examined. In recent years, rapid progress has been made in the characterization of multiple kinase pathways activated by cellular stressors and mechanical deformation; the same pathways may also be activated by growth factors and cytokines (Kyriakis and Avruch, 1996; Cohen, 1997). In a number of different cell types including a human intestinal cell line, hepatocytes and astrocytes, exposure to hypotonic media increased tyrosine kinase activity and stimulated MEK activity (Tilly et al., 1993; Noe et al., 1996 Crepel et al., 1998). Moreover, osmotic and shear stresses have been shown to increase expression of the immediate early genes, c-*fos* and c-*jun* in endothelial cells, cardiac myocytes, hepatocytes, and glial cells (Finkenzeller et al., 1994; Sadoshima et al., 1996; Jo et al., 1997; Li et al., 1997; Sinning et al., 1997). The present data suggest that similar mechanisms are activated in enteroendocrine cells in response to hypotonic stress. Enteroendocrine cell responses appear to be distinctive, however, in that both putative nutrient and non-nutrient stimuli work via MAP kinase activation to increase c-*fos* expression.

The increased c-*fos* expression in STC-1 cells stimulated by peptidomimetics in the present study is not a general property of endocrine cell lines, since in other experiments we have found that cefaclor does not increase c-*fos* mRNA in the pheochromocytoma cell line, PC12, or in the islet cell line, HIT-T15, although both cell lines exhibited increased c-*fos* mRNA in hypotonic medium (unpublished observations). Plainly further work is needed to determine whether the responses of STC-1 cells are indeed representative of native gut endocrine cells. It is, however, noteworthy that cefaclor in low millimolar concentrations has been reported to delay gastric emptying in the rat, by a mechanism that was sensitive to a CCK-A receptor antagonist, and compatible with CCK-release from the upper gastrointestinal tract (Bozkurt et al., 1999). The present findings indicate that a detailed examination of this system will be useful in understanding how nutrients influence transcriptional events in gut endocrine cells.

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