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Peptones Stimulate Cholecystokinin Secretion and Gene Transcription in the Intestinal Cell Line STC-1*

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ABSTRACT

In rats, protein hydrolysates (peptones) stimulate cholecystokinin (CCK) release both *in vivo* and in a model of isolated vascularly perfused duodeno-jejunum. However, the mechanisms involved in peptone-induced stimulation of CCK cells are not well understood. In particular, the possibility that peptones may directly interact with CCK-producing cells to stimulate CCK release and gene transcription has not yet been examined. To test this hypothesis, we used the enteroendocrine cell line STC-1. Incubation of STC-1 cells for 2 h with albumin egg hydrolysate over the concentration range 0.01–1% (wt/vol) caused a dose-dependent release of CCK, with a maximal increase at 1420% of the control value. In contrast, BSA (1%, wt/vol) or a mixture of amino acids (1%, wt/vol) induced a modest rise in CCK secretion. A dose-dependent, hydrolysate-specific, increase in the CCK steady state RNA level was also observed. It was detectable by 2–4 h of peptone treatment and sustained until 24–48 h. Peptones did not increase the CCK RNA level in the colonic CCK-producing cell line GLUTag or in nonintestinal CCK-expressing cell lines, namely the pancreatic cell line RINm5F and the medullar thyroid carcinoma cell line CA77. The peptone-induced increase in the CCK RNA level resulted from enhanced gene transcription, because labeled CCK transcripts from nuclear run-on incubations increased 3-fold when cells were incubated with peptones, whereas the level of β-actin transcripts was not modified. Finally, peptones dose-dependently stimulated the transcriptional activity of an 800-bp fragment of CCK gene promoter transfected in STC-1 cells. These studies indicate that peptones specifically stimulate CCK secretion and gene transcription in the intestinal cell line STC-1, and that *cis*-acting elements conferring peptone inducibility are located in the first 800 bp of the 5′-flanking region of the CCK gene. (*Endocrinology* **138:** 1137–1144, 1997)

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THE BRAIN-GUT peptide cholecystokinin (CCK) displays a broad spectrum of biological effects, especially with regard to postprandial pancreatic secretion and gallbladder contraction. Moreover, endogenous CCK may play an important role in the control of appetite and food intake (1).

Many physiological factors modulating CCK release *in vivo* and *ex vivo* have been described, including nutrients, hormones, and neuropeptides. In the rat, dietary proteins are the major stimulus of CCK release (2, 3). Little, however, is known about the mechanisms regulating CCK secretion. The hypothesis that intact proteins stimulate CCK release by protecting CCK-releasing peptides from proteolytic inactivation by pancreatic enzymes in the intestinal lumen has recently been substantiated by the purification and sequencing of three distinct protease-sensitive CCK-releasing peptides, namely the monitor peptide from rat pancreatic juice (4), the luminal CCK-releasing factor from rat intestinal secretion (5), and finally, a peptide identical to the porcine diazepam binding inhibitor, which was purified and characterized from porcine intestinal mucosa (6). The release of the latter peptide was inhibited by the cholinergic muscarinic receptor antagonist atropine, implying that CCK secretion was under the indirect control of a cholinergic nervous pathway. Noteworthy, a recent *in vivo* study conducted in rats indeed showed that peptone-induced CCK secretion was dependent on the activation of intestinal submucosal cholinergic neurons (7). Alternatively, peptones induced a marked CCK release in an isolated vascularly perfused rat duodeno-jejunum preparation (8). In this case, the underlying mechanisms did not involve the participation of intramural nerves, as neither the axonal blocker tetrodotoxin nor atropine altered peptone-induced CCK release (8).

Overall, these results suggest that peptones may stimulate CCK release via both indirect and direct mechanisms. The latter hypothesis was tested in the present study. Ideally, these experiments should have been performed with purified intestinal CCK-producing cells. However, the isolation of native intestinal I cells remains a tedious process and produces fairly low yields (9). Hence, the intestinal CCK-producing cell line STC-1 was instead selected, as these cells harbor many features of native intestinal CCK-producing cells (10, 11). We here show that peptones stimulate CCK secretion and gene transcription, and that peptones dose-dependently increase the transcriptional activity of an 800-bp fragment of the CCK gene promoter transfected in STC-1 cells.

Materials and Methods

Materials

RPMI medium, DMEM, additives, and FCS were obtained from Life Technologies (Cergy-Pontoise, France). Peptones were protein enzymatic digests of various origins: albumin egg hydrolysate (AEH), meat hydrolysate type I (MH), and soybean hydrolysate type III (SH) from Sigma (Saint-Quentin-Fallavier, France); and casein pancreatic digest (CH) from Life Technologies. The relative proportions (percentages) of

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free amino acids and the peptide mol wt distribution of the various types of peptones were previously determined (8). BSA was purchased from Sigma, and Hyperamine 25 (B. Braun Medical, Boulogne, France), a mixture of amino acids, had a total amino acid content of 153 g/liter mixture. It contains: L-isoleucine, 6.4 g; L-leucine, 11.2 g; L-lysine, 8.8 g; L-methionine, 4 g; L-phenylalanine, 9.05 g; L-threonine, 6.4 g; L-tryptophan, 1.6 g; L-valine, 8.0 g; L-arginine, 27.65 g; L-histidine, 7.2 g; L-alanine, 9.6 g; L-aspartic acid, 15.05 g; L-cysteine, 1.6 g; L-glutamic acid, 15.05 g; glycine, 12.8 g; Lproline, 5.3 g; L-serine, 2.55 g; and L-tyrosine, 0.55 g. Actinomycin D (Sigma) was dissolved in dimethylsulfoxide (B. Bruneau, Boulogne-Billancourt, France). α-Amanitin (Sigma) was dissolved in distilled water. [α-^{32}P]Deoxy-CTP, [α-^{32}P]UTP, and the random priming labeling kit were purchased from Amersham (Les Ulis, France). Agarose, positively charged nylon membranes, and restriction endonucleases were obtained from Appligene-Oncor (Illkirch, France). Other chemicals and reagents, of analytical grade, were purchased from Sigma.

Probes and plasmids

A 575-bp rat CCK complementary DNA (cDNA) fragment (12) and an 800-bp fragment from the 5′-flanking region of the rat CCK gene (13) were provided by Dr. Dixon (Michigan Medical School, Ann Harbor, MI). A 2-kb human β-actin cDNA fragment was obtained from Clontech (Ozyme, Montigny-le-Bretonneux, France). cDNA inserts used as probes were excised from plasmids by digestion with restriction endonucleases, and purified from agarose after electrophoresis gel separation. pGL2, pGL3, pTK-RN plasmids, and luciferase reporter assay systems were obtained from Promega (Charbonnières, France).

Cell lines and culture conditions

Several CCK-expressing cell lines were used in the present study. The STC-1 plurihormonal cell line is derived from an endocrine tumor that developed in the small intestine of a double transgenic mouse expressing the rat insulin promoter linked to the simian virus 40 large T antigen and to the polyoma virus small t antigen, respectively (14). It was provided by Dr. Andrew B. Leiter (Department of Medicine, New England Medicine Center, Boston, MA). The GLUTag cell line is derived from a large bowel tumor developed in a transgenic mouse carrying the glucagon/simian virus 40 large T antigen transgene (15) and was provided by Dr. Daniel J. Drucker (Clinical Biochemistry and Molecular Genetics, Toronto Hospital, Toronto, Canada). The CA77 cell line is derived from a rat medullary thyroid carcinoma (16) and was kindly provided by Dr. A. Kervran (INSERM U-376, Montpellier, France). The RINm5F cell line has been established from a nude mouse heterotransplant of a rat radioinduced pancreatic cell tumor described previously (17). It was provided by Dr. M. Laburthe (INSERM U-410, Paris, France).

STC-1 and RINm5F cell lines were grown in RPMI 1640 medium supplemented with 5% FCS, 2 mM glutamine, penicillin (100 IU/ml), and streptomycin (50 μM). CA77 cells were grown in medium containing Ham's F-10 nutrient mixture and DMEM (1:1, vol/vol) with 4.5 g/liter glucose, supplemented with 10% FCS plus penicillin (100 IU/ml), 2 mM glutamine, and streptomycin (50 μM). GLUTag cells were grown in DMEM containing 10% FCS plus 2 mM glutamine, penicillin (100 IU/ml), and streptomycin (50 μM).

Secretion studies

Two days before the experiments, cells were seeded into 24-well culture plates. When cells reached 85% confluence, the supernatant was replaced by Krebs-Ringer bicarbonate buffer containing 0.1% (wt/vol) BSA, with or without the tested agents or peptones. These were directly prepared at the desired concentrations in the Krebs-Ringer buffer, and pH was adjusted to 7.2. Cells were then incubated for 2 h at 37 C before the supernatants were collected and frozen at −20 C for subsequent RIA of CCK as previously described (18). Briefly, CCK immunoreactivity was measured using antiserum 39A that cross-reacts at 100% with CCK-33 and CCK-8, at 12% with sulfated gastrin-17, at 5% with unsulfated gastrin, and at less than 0.1% with unsulfated CCK-8, unsulfated CCK-7-Gly, and gastrin-34.

RNA isolation and quantitation

A total of 15×10^6 cells were seeded in 100-mm culture plates in the corresponding culture medium, as described above. After 24 h, the growth medium was replaced by fresh medium containing the agents to be tested. Peptones were directly dissolved at the desired concentration in RPMI 1640 medium without FCS, containing 0.3% (wt/vol) BSA, glutamine, penicillin, and streptomycin. The pH was adjusted to 7.2, and the mixture was filtered before use. Cells were then incubated at 37 C for 18 h (except in time-course experiments), then washed once in PBS buffer and harvested in the same buffer. Dry cell pellets were stored at −70 C before RNA extraction. RNA was extracted from dry cell pellets using the rapid mini-prep method described by Wilkinson (19). Briefly, cells were resuspended in 400 μl Tris-saline [25 mM Tris (pH 7.4), 130 mM NaCl, and 5 mM KCl] plus 100 μl ice-cold NDD buffer, containing 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.01% dextran sulfate, in Tris-saline. The nuclear pellet was discarded, and the supernatant, containing RNA, was extracted twice with phenol-chloroform before ethanol precipitation. Ten micrograms of RNA per assay were separated on a 1% agarose-formaldehyde denaturing gel and then blotted onto nylon-positive membrane. Blots were prehybridized in 1 M NaCl, 1% SDS, and 10% dextran sulfate at 60 C for 4 h and then hybridized overnight with 10^6 cpm/ml [α-^{32}P]deoxy-CTP-labeled probe at 60 C in prehybridization solution. Blots were then washed in 2 × SSC (0.3 M NaCl and 0.03 M sodium citrate)-2% SDS and exposed to Kodak X-Omat AR films (Sigma) for 6–24 h at −70 C with intensifying screens. Quantitative analysis of autoradiograms was performed using a Bio-Rad scanning densitometer (Ivry sur Seine, France) coupled with the Bio-Rad PC molecular analyst program.

Nuclear run-on experiments

Gene transcription rates were determined by quantification of nascent CCK and β-actin transcripts in nuclei from control and peptone-treated cells, as described previously (20). STC-1 cells were first incubated with peptone (MH; 1%, wt/vol) for 18 h. Nuclei were isolated by disrupting the cells in Nonidet P-40 buffer and stored at −70 C until nuclear transcripts were labeled. Each assay was performed on 2.5×10^7 nuclei in the presence of 100 μCi [α-^{32}P]UTP in a classical run-on reaction mixture for 30 min at 37 C. RNA extraction was then performed. In parallel, α-amanitin was added at a concentration of 5 μg/ml (a concentration that preferentially inhibits polymerase II) in one set of nuclei. RNA was hybridized to 10 μg linearized plasmid containing (or not) CCK or β-actin cDNA fragments bound to nylon-positive membrane using a slot-blot apparatus. Filters were hybridized for 48 h at 60 C in the same hybridization solution as that used for Northern blot analysis, then washed twice in 2 × SSC-0.1% SDS at 60 C for 15 min, once in 2 × SSC for 5 min at 60 C, once in 2 × SSC containing 10 μg/ml of ribonuclease A at 37 C for 30 min, and finally once in 2 × SSC during 30 min at 37 C. Filters were exposed to Kodak X-Omat AR for 7–10 days at −70 C with intensifying screens. Quantitative analysis of autoradiograms was performed as described above.

Transfection experiments and luciferase activity measurement

STC-1 cells were transfected by electroporation. Briefly, exponentially growing cells were trypsinated and washed twice in PBS before being electroporated in RPMI 1640 medium without FCS. A total of 15×10^6 cells per assay were submitted to a 250 V/960 μF pulse in a Bio-Rad Gene Pulser in the presence of 20 μg plasmid pGL3*CCK800*, 10 μg sonicated salmon sperm DNA, and 2.5 μg plasmid pRL-TK. pGL3*CCK800* was obtained by subcloning an 800-bp *Kpn*I/*Sac*I CCK promoter fragment into the corresponding restriction sites of PGL3basic vector. Transfected cells were maintained overnight at 37 C in the presence of RPMI 1640 medium containing 5% FCS to allow cell attachment. Then, the medium was replaced by RPMI 1640 without FCS but containing 0.3% BSA and increasing concentrations of peptones. After 24 h, cells were collected in PBS. Luciferase activities were measured using the dual luciferase reporter assay system and a Dynatech luminometer (Elvetec, Vénissieux, France). Similar to firefly luciferase, the luminescence reaction catalyzed by *Renilla* luciferase provides high sensitivity and a linear range extending over 7 orders of magnitude of enzyme concentration.

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Calculations and statistics

Data in figures are presented as the mean ± SEM. Statistical significance was assessed using one-way ANOVA, followed by multiple comparison tests. Differences were considered significant when $P < 0.05\%$.

Results

CCK release is stimulated by peptones in STC-1 cell line

We first determined if peptones could induce CCK release from STC-1 cells by measuring CCK-like immunoreactivity in the culture supernatant of STC-1 cells after a 2-h incubation without (control) or with increasing concentrations (0.01–1%, wt/vol) of AEH. As shown in Fig. 1, AEH induced a dose-dependent increase in CCK release, reaching 1420 ± 191% of the control value (n = 16; $P < 0.01$) upon treatment with AEH (1%, wt/vol). AEH treatment did not increase CCK release after 2-h incubation at 4 C, when exocytosis was inhibited (data not shown).

Incubation of STC-1 cells for 2 h at 37 C with MH or CH (1%, wt/vol) also resulted in increased CCK release [793 ± 29% (n = 6) and 595 ± 104% (n = 6) of the control, respectively; $P < 0.05$]. For comparison, incubation of STC-1 cells with 10^{-4} M forskolin or 10^{-7} M bombesin (Fig. 1) induced CCK release that was approximately half (or less) of that obtained with the highest doses of AEH [647 ± 107% (n = 6) and 349 ± 27% (n = 8) of the control, respectively; $P < 0.05$]. In contrast, incubation of STC-1 cells with the amino acid mixture HA25 (1%, wt/vol) or with BSA (1%, wt/vol) had only a modest effect on CCK release, representing 132 ± 9% ($P < 0.05$) and 227 ± 17% ($P < 0.05$) of the control value, respectively (n = 6 for each; Fig. 1).

Peptones dose-dependently increase CCK steady state RNA level

The effect of peptones on CCK gene expression was examined at the RNA level by Northern blot analysis. The single 750-bp CCK transcript and the 2.1-kilobase β-actin transcript that were previously described in intestinal extracts (21) are shown in Fig. 2. Incubation of STC-1 cells with various peptones (1%, wt/vol) induced an increase in the CCK steady state RNA level, representing 339 ± 54% of the control with AEH, 512 ± 41% with MH, 334 ± 77% with SH, and 235 ± 62% with CH (n = 3 for each). The level of β-actin RNA always remained constant in all of these experiments.

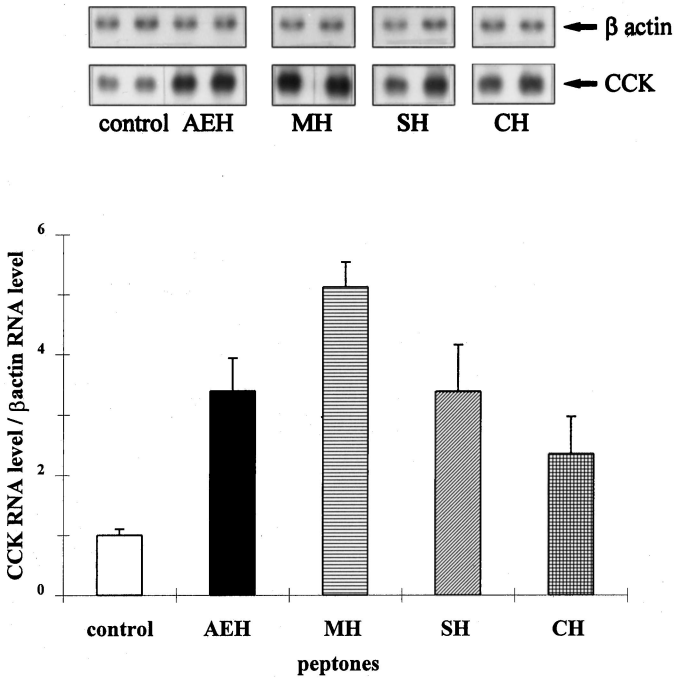

FIG. 2. Effects of different types of peptones on CCK RNA level. *Top*, Northern blot analysis. STC-1 cells were incubated for 18 h with peptones of various origin (described in *Materials and Methods*) at a concentration of 1% (wt/vol), namely AEH, MH, SH, and CH. Northern blot analysis was performed as described in *Materials and Methods*. Duplicates on the blot represent the results of two individual experiments. The same membrane was hybridized with CCK and β-actin cDNA probes. Unique CCK and β-actin transcripts were detected with a size of 0.75 and 2.1 kilobases, respectively. *Bottom*, Quantitative analysis. The autoradiograms were quantified by scanning densitometry. The blots were exposed to films only for 6 h due to the intensity of the signal in the experiments with peptones. Results are expressed as the CCK RNA level/β-actin RNA level ratio and represent the mean ± SEM of three individual experiments.

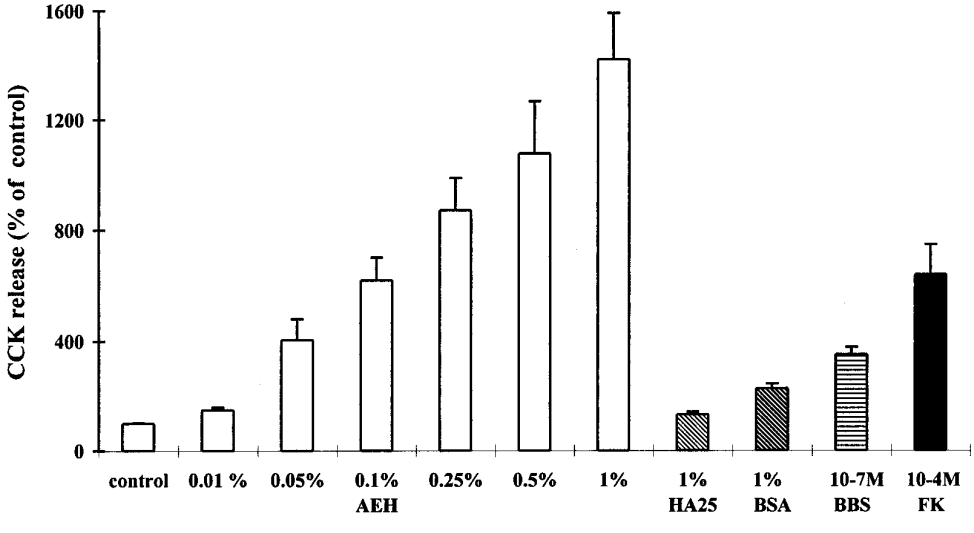

FIG. 1. CCK immunoreactivity released in the culture supernatant of STC-1 cells incubated with peptones and other reagents. STC-1 cells were maintained for 2 h in the absence (control) or presence of increasing doses (0.01–1%, wt/vol) of AEH. Parallel experiments were performed with a 1% amino acid mixture (HA25), 1% BSA, 10^{-7} M bombesin (BBS), or 10^{-4} M forskolin (FK). CCK immunoreactivity released in the supernatant was measured by RIA. Results are expressed as a percentage of the control value and represent the mean ± SEM of at least six individual experiments.

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Moreover, incubation of STC-1 cells with HA25 or BSA (1%, wt/vol) did not elicit any detectable increase in CCK RNA level (data not shown).

We observed that incubation of STC-1 cells for 24 h with either AEH or MH over the concentration range 0.25–3% dose-dependently increased the CCK steady state RNA level (Fig. 3). The lowest active concentration was 0.25% (wt/vol) for MH and 1% (wt/vol) for AEH. The maximal response obtained was 499 ± 49% of the control value with AEH (n = 6) and 866 ± 136% of the control value with MH (n = 6; $P < 0.01$). Because of its potency, MH was selected for subsequent experiments.

Time-course study of peptone effect on CCK RNA level

STC-1 cells were incubated with MH (1%) for various time intervals, from 1–48 h, before Northern blot analysis. As shown in Fig. 4, the increase in the CCK RNA level was significant from 4 h of MH treatment (269 ± 18% of control; n = 3). A plateau value was then observed between 6 h (333 ± 53% of control; n = 3) and 48 h (307 ± 17% of control; n = 4).

Peptone effect on CCK steady state RNA in other cell lines

The CCK gene has been reported to be transcribed not only in STC-1 cells, but also in GLUTag enteroendocrine cells, CA-77 medullary thyroid carcinoma cells, and RINm5F pancreatic endocrine cells. Indeed, we detected in these cells a single 750-bp CCK transcript (data not shown). The relative proportions of CCK RNA levels in these cell lines were 1, 0.5, 0.2, and 0.05 for STC-1, GLUTag, CA-77, and RINm5F cells, respectively. In comparison to STC-1 cells, treatment for 24 h with increasing concentrations of either AEH (1–2%) or MH (0.5–3%) had no effect on the CCK RNA level in nonintestinal cell lines CA77 and RINm5F or in the colonic cell line GLUTag (Fig. 5).

Peptones increase the CCK gene transcription rate

Whether the observed peptone-induced increase in the CCK RNA level was mediated at the transcriptional level was tested using actinomycin D and performing nuclear run-on experiments.

STC-1 cells were first incubated for 2–24 h with MH (1%, wt/vol) in the presence or absence of actinomycin D (5 μg/ml), which was added 30 min before MH and maintained during the entire incubation. Figure 6 shows that the time-dependent increase in CCK RNA that appeared when STC-1 cells were incubated with MH alone was clearly abolished by actinomycin D. After 24 h, the CCK RNA level was 378 ± 34% of the control value (n = 6) with MH alone and 52 ± 7% of the control value (n = 6) with MH plus actinomycin D. The observed decrease in β-actin RNA level upon incubation

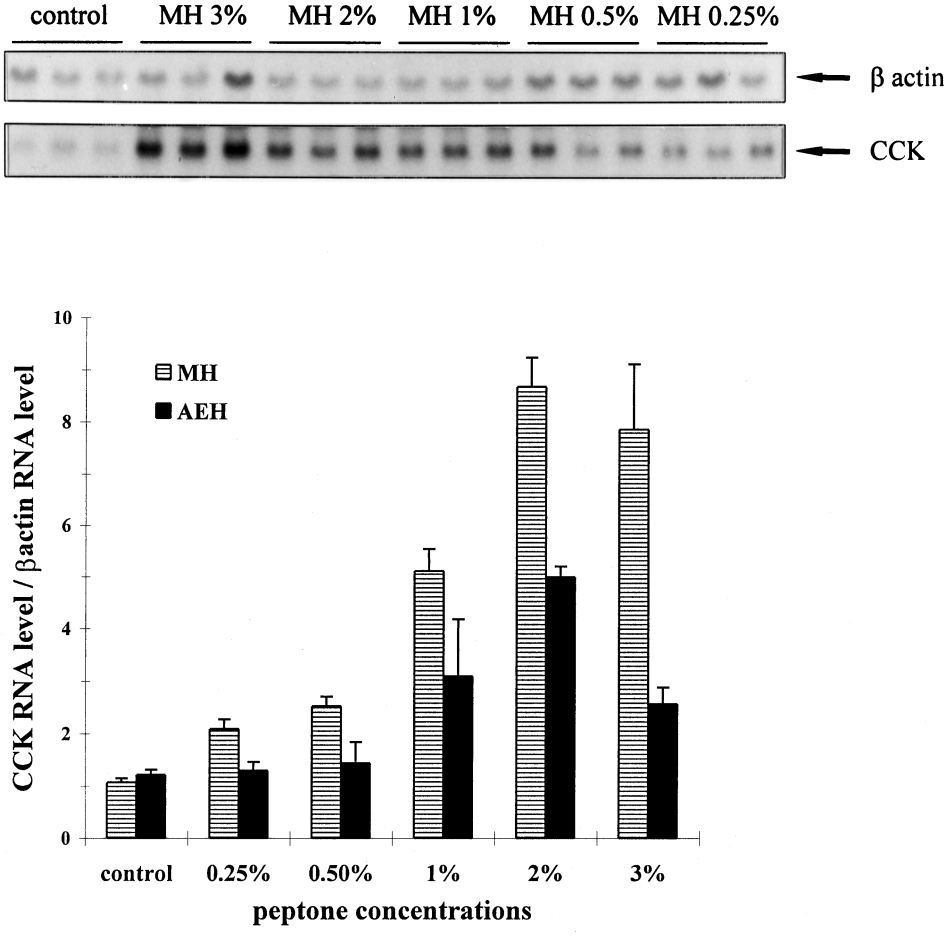

FIG. 3. Effect of increasing concentrations of peptones on the CCK RNA level. *Top*, Northern blot analysis. STC-1 cells were incubated for 18 h with increasing concentrations (0.25–3%, wt/vol) of MH. CCK and β-actin RNA were detected by Northern blot analysis as described in *Materials and Methods*. For each concentration, triplicates on the blot represent the results of three individual experiments. *Bottom*, Quantitative analysis. CCK RNA levels in STC-1 cells treated with increasing concentrations of MH or AEH. Blots were exposed to films for 6 h. The autoradiograms were quantified by scanning densitometry. Results are expressed as the CCK RNA level/β-actin RNA level ratio and represent the mean ± SEM of six individual experiments.

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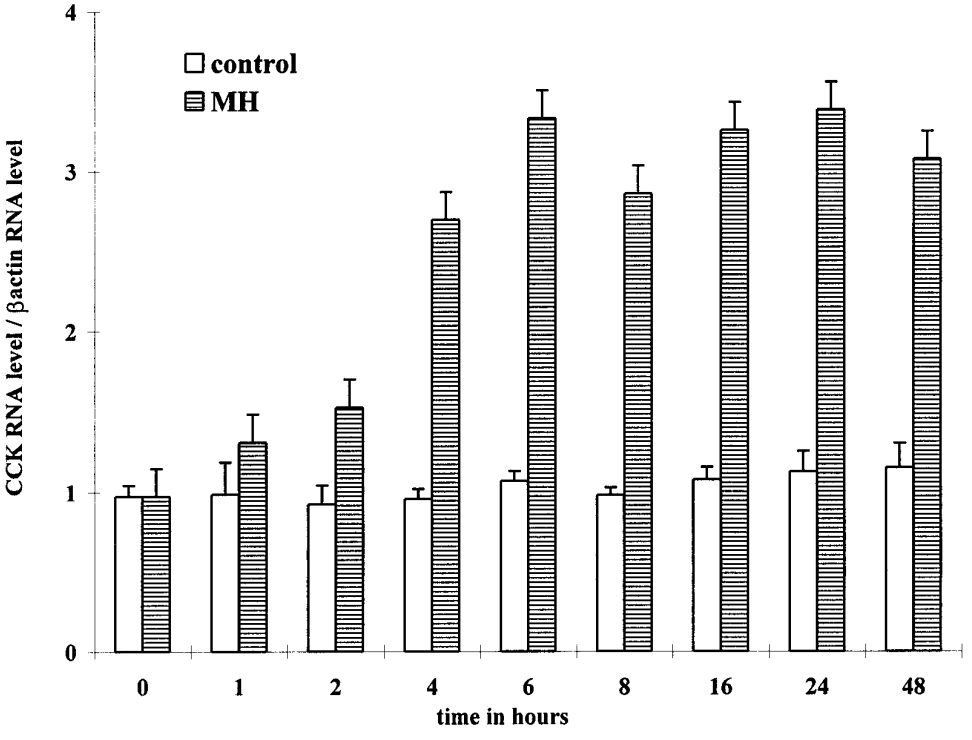

FIG. 4. Time-course study of the effect of peptone on CCK RNA levels. STC-1 cells were incubated with MH (1%, wt/vol) for increasing time periods (1–48 h) before CCK and β-actin RNA were detected by Northern blot analysis as described in *Materials and Methods*. The autoradiograms were quantified by scanning densitometry. Results are expressed as the CCK RNA level/β-actin RNA level ratio and represent the mean ± SEM of at least three individual experiments.

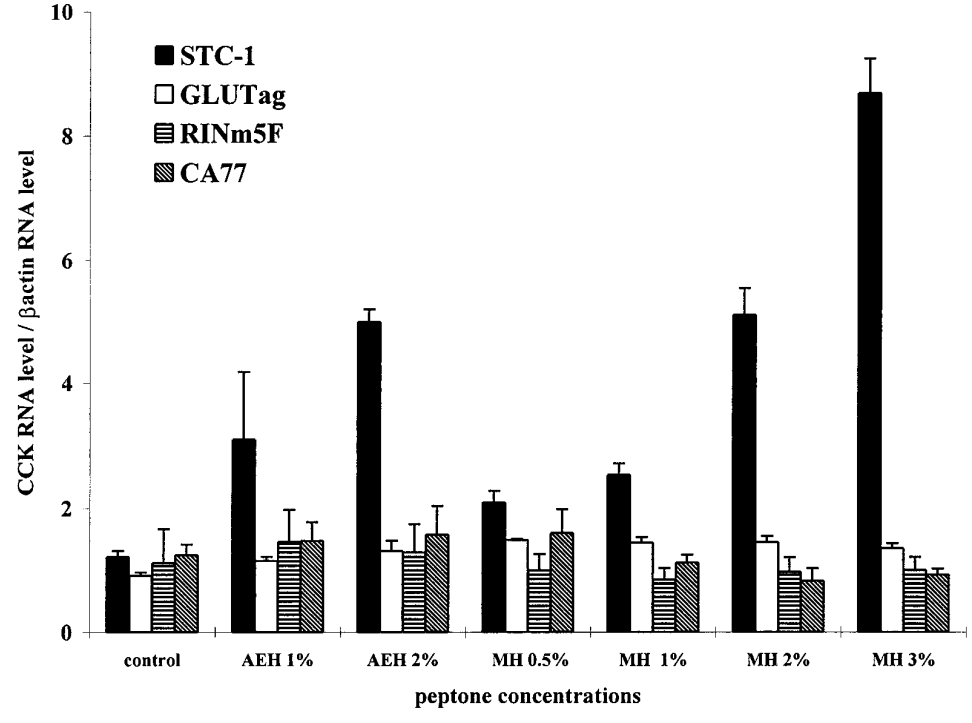

FIG. 5. Peptone effect on CCK RNA levels in various CCK-expressing cell lines. STC-1, GLUTag, CA77, and RINm5F cells were maintained for 18 h in the presence of increasing concentrations of AEH (1–2%, wt/vol) or MH (0.5–3%, wt/vol) before CCK and β-actin RNA were detected as described in *Materials and Methods*. The autoradiograms were then quantified by scanning densitometry. Results are expressed as the CCK RNA level/β-actin RNA level ratio and represent the mean ± SEM of six individual experiments.

with actinomycin D reflected adequate inhibition of transcription.

The rate of nascent CCK transcripts was then measured in comparison with that of β-actin in isolated nuclei from STC-1 cells treated or not with MH (1%, wt/vol). The results in Fig. 7 show that the CCK hybridization signal obtained with MH-treated nuclei was clearly increased over the CCK signal obtained with MH-untreated nuclei (48 ± 6% *vs.* 15 ± 5%, respectively; n = 2). In comparison, the β-actin signal remained unchanged. The addition of 5 μg/ml α-amanitin (a concentration that preferentially inhibits RNA polymerase II) abolished the hybridization signals, indicating that the observed CCK signal resulted from RNA polymerase II activity.

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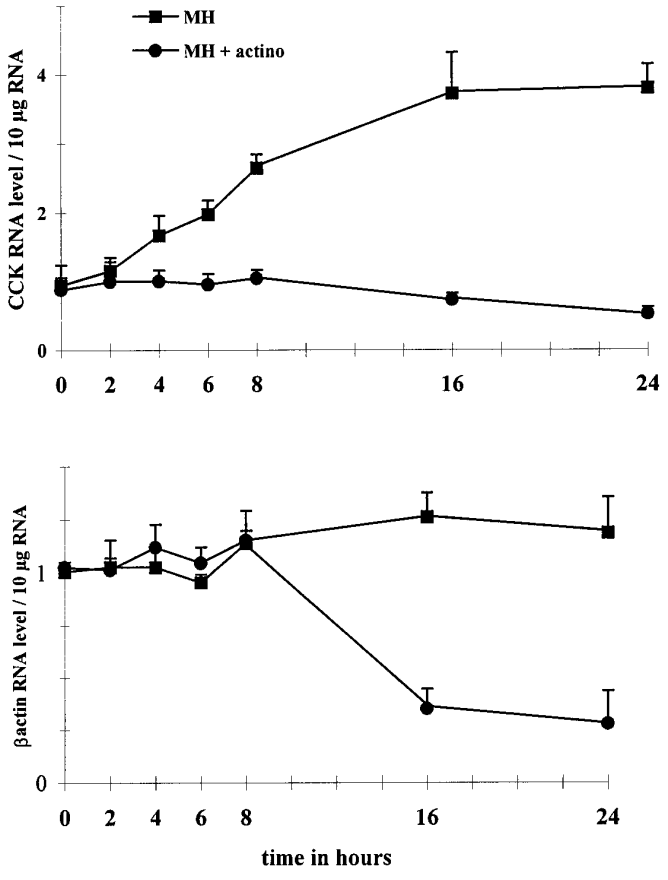

FIG. 6. Peptone effect in the presence of actinomycin D. STC-1 cells were incubated with MH (1%, wt/vol) in the absence or presence of actinomycin D (5 μg/ml) added 30 min before MH and maintained during the entire experiment. Cells were harvested at the indicated times, between 2–24 h. Northern blot analysis was performed as described in *Materials and Methods*. The autoradiograms were quantified by scanning densitometry. Results are expressed as the ratio of CCK RNA level/β-actin RNA level 10 μg RNA and represent the mean ± SEM of six individual experiments.

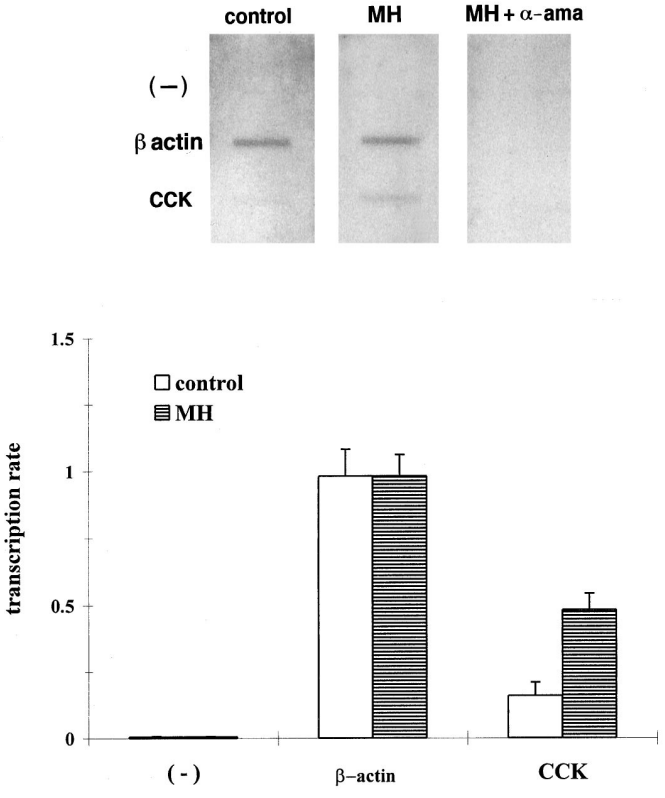

FIG. 7. Run-on assays on peptone-treated STC-1 cells. *Top*, Run-on analysis. STC-1 cells were incubated for 18 h with MH (1%, wt/vol) before cells were collected, and nuclei were isolated as described in *Materials and Methods*. Radioactive RNA obtained from run-on reactions with nuclei from MH-treated (MH) and untreated (control) cells were then hybridized to β-actin and CCK cDNA-containing plasmids as well as to plasmidic vector without cDNA insert (−) bound to nylon membrane as described in *Materials and Methods*. The results of hybridization with radioactive RNA obtained from run-on reactions in the presence of α-amanitin and with MH-treated nuclei (MH + α-ama) are also shown. *Bottom*, The autoradiograms were quantified by scanning densitometry. Results are expressed as the ratio of CCK hybridization signal/β-actin hybridization and represent the mean ± SEM of two individual experiments.

Peptones increase CCK gene promoter activity

We then tested whether peptones could also stimulate the transcription of an isolated CCK promoter sequence that was transfected into these cells. For this purpose, an 800-bp fragment from the 5′-nontranscribed region of CCK gene, containing the potential transcription initiation site and the putative TATA box, was subcloned upstream of a *Firefly* luciferase encoding reporter gene (pGL3*CCK800)*. This plasmid was then cotransfected into STC-1 cells with a plasmid (pRL-TK) containing the viral herpes simplex thymidine kinase gene upstream of a *Renilla* luciferase reporter gene, which served as an internal standard. After 18 h, transfected STC-1 cells were incubated with increasing concentrations of MH (0.5–3%).

The results reported in Fig. 8 show that MH dose-dependently stimulated the transcriptional activity of the 800-bp CCK promoter fragment from 163,445 ± 10,309 relative light units (RLU)/OD unit with 0.25% peptone to 507,688 ± 26,825 RLU/OD unit with 3% MH (n = 3), whereas herpes simplex thymidine kinase promotor activity was not changed (57,022 ± 2,808 to 58,927 ± 8,096 RLU/OD unit, respectively; n = 3).

Discussion

Digestion of proteins in the intestinal lumen mainly yields amino acids and oligopeptides. Peptones used in the present study are fair counterparts of the protein fraction of intestinal chyme. Several studies indicate that protein hydrolysates are strong stimulants of CCK release in some species, including rat, man, and pig (2, 22, 23). However, the underlying mechanisms are poorly understood. In particular, the question of whether oligopeptides, when in contact with the apical pole of intestinal CCK-producing cells, stimulate CCK release and possibly gene transcription has not been documented. *In vivo* and *ex vivo* models as well as perifusion systems of dispersed mucosal cells are inadequate to answer this question, as CCK-producing cells in these preparations are in contact with cells of other types that can, in turn, indirectly affect CCK secretion. A model of enriched rat intestinal CCK cells,

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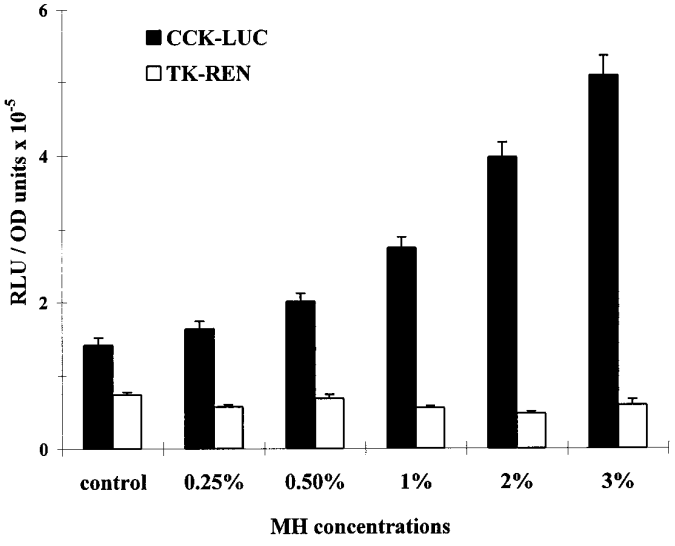

FIG. 8. Peptone activation of the rat CCK gene promoter in STC-1 cells. STC-1 cells were cotransfected by electroporation with the plasmid pGL3*CCK800* containing a 800-bp CCK promoter fragment and the plasmid pRL-TK containing herpes simplex thymidine kinase promoter. Cells were maintained with increasing concentrations (0.25–3%, wt/vol) of MH for 18 h before cell extracts were prepared, and luciferase activities were measured as described in *Materials and Methods*. Transcriptional activity was normalized to the quantity of proteins. All transfection experiments were carried out in triplicate, and the results shown represent the mean ± SEM of three individual experiments. Results were expressed as the ratio of relative light units/OD_{590nm} units.

identified and sorted spectrofluorometrically, was developed (9). In this model, CCK secretion was enhanced by potassium depolarization, calcium ionophore, $(Bu)_2$cAMP, or monitor peptide (9). The obvious advantage of this model is that direct effects of secretagogues can be determined in a homogeneous population of CCK cells (>90%). Unfortunately, the preparation and isolation of native intestinal CCK cells produced low yields in a tedious process. Additionally, only short term studies (120 min at most) have been described, so that parallel studies on CCK secretion and gene transcription could not be designed; longer incubation periods are required to allow for a detectable increase in the CCK RNA level in intestinal tissue, at least *in vivo* (21). We thus selected the STC-1 intestinal cell line to study the direct effect of peptones on CCK secretion and gene transcription. In STC-1 cells, as in native I cells, CCK release is increased by agents that induce membrane depolarization and increase the cytosolic calcium or cAMP level (10, 11). Owing to the high degree of cellular homogeneity of the STC-1 cell line, these cells represent a valuable model for studying CCK secretion and gene transcription.

We previously showed that CCK release was potently stimulated by AEH in the isolated perfused rat duodenojejunum preparation (8). Arterial infusion of tetrodotoxin or atropine had no significant effect on this peptone-induced CCK release, thus excluding any major involvement of intramural nerves in CCK secretion. The present study indicates that incubation of STC-1 cells with the same peptones for 2 h elicited a dose-dependent release of CCK over the concentration range 0.05–3%. Additionally, incubation of STC-1 cells with peptones at 4 C had no effect on CCK secretion. These experiments indicate that the peptone-induced CCK response cannot be related to any toxic effect on STC-1 cells. The profile of the STC-1 cell response to various protein-derived agents was highly consistent with the pattern obtained using the isolated rat duodeno-jejunum preparation. AEH potently stimulated CCK release as well as the other peptones tested, namely meat, casein, and soybean hydrolysates (8). In contrast, a mixture of free amino acids or undigested proteins were weak stimulants of CCK release. These results are in good agreement with those reported in two separate studies that showed a weak stimulatory effect of aromatic amino acids on CCK release from STC-1 cells (11, 24). Overall, it is suggested that the products of protein hydrolysis are capable of directly triggering peptide release from CCK-producing cells. Peptones mainly consist of oligopeptides with a mol wt below 1200 (8). The sequence specificity of CCK-releasing oligopeptide remains to be established. It should be noted that proteins and protein digests did not stimulate CCK release in a perifusion system containing isolated mucosa cells from the rat duodeno-jejunum (25). However, due to the heterogeneity of such cell preparations, a direct stimulatory effect of peptones could not be assessed.

The possible effect of peptones on the regulation of CCK gene transcription had not been documented. It was previously shown that infusion of soybean trypsin inhibitor into the duodenum of anesthetized rats for 12–24 h induced a 3- to 4-fold increase in the CCK RNA level that was the result of an enhanced transcriptional activity of the CCK gene (21). The rationale of the experiment, however, was stimulation of CCK release through interruption of the negative feedback with luminal trypsin, not the effect of peptones. In man, ingestion of mixed nutrients was followed by an increase in the CCK messenger RNA level in extracts of mucosal biopsies (26), but the contribution of protein-derived nutrients was not specifically assessed. We show that various peptones elicit a dose-dependent increase in the CCK RNA level in the STC-1 cell line. This effect appears highly specific, as no parallel change in the β-actin RNA level was observed. Additionally, RNA levels of two other intestinal peptides secreted by STC-1 cells, namely glucose-dependent insulinotropic peptide and secretin, were not modified upon peptone incubation (our unpublished observations). The peptone-induced increase in the CCK RNA level was remarkably tissue and cell specific. Indeed, no variation in the CCK transcript level was recorded when using three additional cell lines (GLUTag, Ca77, and RINm5F), respectively, of colonic, thyroid (C cell), and pancreatic origin. Among the four CCK-expressing cell lines tested, only the STC-1 cell line, established from an endocrine tumor of the small bowel, displayed a response pattern that would fit the alleged reactivity of intestinal I cells to luminal nutrients. The suppression of the peptone-induced CCK RNA increase by actinomycin D as well as the results of run-on experiments both indicated that transcription of CCK gene was stimulated when STC-1 cells were incubated with peptones. Whether RNA stabilization was additionally involved cannot be ruled out. Further experiments are required to test this hypothesis.

The transcriptional mechanisms regulating CCK gene ex-

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pression in response to physiological stimuli such as nutrients are unknown. We here obtained evidence that the transcriptional activity of a 800-bp fragment of the CCK gene promoter region (located immediately upstream of the transcriptional start site) was markedly increased in peptone-treated STC-1 cells. Experiments aimed at determining the precise location of the promoter elements that confer peptone inducibility are currently underway.

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