Caerulein and gastrin(2–17 ds) regulate differently synthesis of secretory enzymes, mRNA levels and cell proliferation in pancreatic acinar cells (AR4-2J)

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In order to characterize the biological functions coupled to cholecystokinin (CCK) A and B receptors, the effects of gastrin(2–17 ds) and caerulein were compared. An isolated cell model, the pancreatic acinar cell line AR4-2J, was used and the experiments were carried out in serum-free media. Caerulein was found to evoke no mitogenic effects either alone or in the presence of the CCK antagonists L364,718 and CR1409. Gastrin(2–17 ds) increased cell proliferation by 2-fold with an IC_{50} of 150 pM, corresponding to the occupancy of the CCK B receptors. CR1409, at concentrations that fully occupied CCK B receptors, inhibited the gastrin(2–17 ds) effects. Caerulein enhanced chymotrypsinogen biosynthesis by 100%

and the corresponding mRNA level by 75%; amylase biosynthesis and mRNA level were enhanced by 40% only. Half-maximal increases in chymotrypsin activity and mRNA level were recorded in response to caerulein at concentrations of 100 pM and 50 pM respectively. Gastrin(2–17 ds) at 100 nM enhanced chymotrypsinogen biosynthesis by 26% and its mRNA level by 35%; these responses were lower than those evoked by 0.1 nM caerulein. Furthermore, CR1409 completely inhibited caerulein- and gastrin(2–17 ds)-stimulated chymotrypsinogen synthesis, with similar IC_{50} (4 μ M). These results suggest that both peptides induced the synthesis of the secretory enzyme after occupancy of CCK A receptors.

INTRODUCTION

Cholecystokinin (CCK) and gastrin exert important regulatory effects on digestive cells including pancreatic acinar cells [1,2]. Pancreatic acinar cells from dog and guinea pig possess two classes of CCK receptors [3,4]. CCK A receptors have a high affinity for CCK and a low affinity for gastrin peptides, whereas CCK B receptors do not discriminate between CCK and gastrin peptides.

In vivo studies have shown that CCK and caerulein, a decapeptide structurally related to the C-terminal sequence of CCK, control the synthesis and secretion of digestive enzymes [5,6]. These peptides modulate the relative amounts of the secretory enzymes, acting preferentially on the biosynthesis of serine proteinases rather than that of amylase [5]. After repeated injections, they also induce pancreatic hypertrophy and hyperplasy. Although gastrin, at high concentration only, has been reported to stimulate the secretion of pancreatic enzymes in dog and guinea pig [3,4], its effects on enzyme synthesis are not yet known. Therefore the CCK receptor subtype involved in the induction of secretory-enzyme biosynthesis is not clearly established.

Since it is difficult to define the functions specific to each class of CCK receptor from studies performed *in vivo*, isolated systems such as the rat pancreatic acinar cell line AR4-2J [7–10] have recently been used. This cell line possesses both CCK A and CCK B receptor subtypes [9,10]. CCK-9 and caerulein interact with CCK A receptors with high affinity, in the nanomolar range [10], whereas desulphated gastrin(2–17), namely gastrin(2–17ds), interacts with CCK A receptors with a 1000-fold lower affinity. On the other hand, CCK-9, caerulein and gastrin(2–17ds) have

comparably high affinities for CCK B receptors (K_d in the nanomolar range) [10]. Therefore CCK and caerulein do not discriminate between the two receptor subtypes. It has been established in our laboratory that the occupancy of CCK B receptors by gastrin stimulates ornithine decarboxylase activity in AR4-2J cells, an early event associated with cell proliferation [10,11].

In the present study, in order to characterize the CCK receptors involved in pancreatic enzyme induction, we compared the effects of caerulein and gastrin(2–17ds). AR4-2J cells were cultured in serum-free medium, in order to prevent interference by serum factors, and in 4.5 g/l glucose since, in preliminary studies, enzyme inductions were found to be higher than in 1 g/l glucose. Our results suggest that the occupancy of CCK A receptors by caerulein or gastrin(2–17ds) may be linked to secretory-enzyme biosynthesis.

MATERIALS AND METHODS

Materials

Gastrin(2–17ds) (fragment 2–17 of desulphated gastrin) was obtained from Bachem (Bubendorf, Switzerland). Caerulein was a gift from Dr. De Castiglione (Farmitalia Carlo Erba, Milan, Italy). CR1409 and CR1505 were from Dr. Rovatti (Rotta Research Laboratory, Milan, Italy) and L364,718 was from Merck, Sharp and Dohme (Rahway, NJ, U.S.A.). *N*-Benzoyl-L-tyrosine ethyl ester (BTEE), bacitracin and albumin were from Sigma (St. Louis, MO, U.S.A.). Dulbecco's modified Eagle's medium (DMEM) (4.5 g/l glucose), glutamine, penicillin, streptomycin and fetal calf serum (FCS) were from Gibco Laboratories (Grand Island, NY, U.S.A.). 125 I-labelled

Abbreviations used: Gastrin(2–17 ds), desulphated gastrin(2–17); CCK, cholecystokinin; BTEE, *N*-benzoyl-L-tyrosine ethyl ester; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; 1 $\times$ SSC, 0.15 M NaCl+0.015 M-sodium citrate.

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Bolton–Hunter reagent [*N*-succinimidyl-3-(4-hydroxy)-5- 125 I]-iodophenylpropionate; specific radioactivity 2000 Ci/mmol} was from Amersham (Les Ullis, France).

Cell culture

AR4-2J cells, generously provided by Dr. C. Logsdon (University of California, San Francisco, CA, U.S.A.), were routinely grown in DMEM (4.5 g glucose/l) containing 10% (v/v) FCS, penicillin (100 i.u./ml) and streptomycin (50 μ g/ml). Cells were plated at 30000 cells/cm² on 60 mm dishes. The medium was changed every 2 days. For the experiments, cells were plated in the complete medium, and 18 h later, they were washed and the medium was replaced by DMEM buffered with 15 mM Hepes, pH 7.2 (zero time). The cells were then treated daily with various concentrations of gastrin(2–17ds) or caerulein for 48 h. The cells were then washed in PBS, mechanically harvested and the cells and culture media frozen at -30°C until the enzyme assay.

Binding studies

CCK-9 and gastrin(2–17ds) were radioiodinated by conjugation with 125 I-labelled Bolton–Hunter reagent and purified using h.p.l.c. as previously described [10–12]. Specific radioactivities for both peptides were about 1.8–2.0 Ci/ μ mol. Binding assays were carried out on intact cells harvested with 0.025% EDTA as previously reported [9]. Briefly, AR4-2J cells at a concentration of 3×10^5 – 4×10^5 cells/ml, were incubated in Krebs–Hepes buffer containing 0.5% BSA, 30 mg/ml soya-bean trypsin inhibitor and 100 mg/ml bacitracin. Either 60 pM 125 I-CCK-9 or 60 pM 125 I-gastrin(2–17ds) and increasing concentrations of unlabelled caerulein or gastrin(2–17ds) were added to the incubation mixture. Binding was measured in a total volume of 0.5 ml, for 30 min at 25°C . Total binding for 125 I-gastrin(2–17ds) was about 2% of total radioactivity and that for 125 I-CCK-9 was about 8%. Specific binding was defined as the excess binding over that in samples containing 1 μ M unlabelled peptide. Non-specific binding was less than 20% of total binding. Binding parameters (B_{max} and K_d) were determined by computer analysis using a non-linear least-squares curve-fitting program (LIGAND).

Enzyme assays

Cells were disrupted by freezing and thawing, and finally homogenized with a Turrax homogenizer (3 $\times$ 15 s, at 2000 rev./min at 4°C). Amylase activity was assayed by Bernfeld's method [13]. Chymotrypsin activity was measured spectrophotometrically as described by Hummel [14], after activation of the zymogen by trypsin (10 μ g/ml at 4°C for 2 h). BTEE was used as substrate at a final concentration of 0.5 mM. The molar absorption coefficient used for BTEE was $964 \text{ M}^{-1} \cdot \text{cm}^{-1}$ [14]. Activities are expressed as μ mol of substrate hydrolysed/min per mg of cell DNA present in the same culture dish.

DNA concentration

DNA was measured as described by Burton with calf thymus DNA as reference [15].

Quantification of mRNA

Total RNA was prepared using the guanidinium thiocyanate method [16] and further purified by sedimentation through a

layer of 5.7 M CsCl [17]. The concentration of individual mRNAs was determined by slot-blot hybridizations. For this purpose, RNA samples were denatured and spotted at five different concentrations (1–5 μ g) on to a nitrocellulose filter [17]. The filters were baked at 80°C for 2 h, and prehybridized at 42°C for 3 h in a $6 \times \text{SSC}$, 0.5% SDS, $5 \times \text{Denhardt's}$ solution and with 100 μ g/l salmon sperm DNA previously heated at 100°C for 10 min. The filters were then hybridized at 42°C for 16 h with ^{32}P nick-translated cDNA probes (specific radioactivities about 2×10^7 c.p.m./ μ g). A 702 bp cDNA clone encoding rat pancreatic amylase mRNA [18] and a 621 bp cDNA clone encoding rat pancreatic chymotrypsinogen mRNA [19] were used. After hybridization, the filters were washed and exposed to X-ray films. The radioactivity of each spot was quantified by Cerenkov counting using a Packard Tri-Carb scintillation unit.

Statistics

Statistical analyses were carried out using Student's *t* test.

RESULTS

Binding studies

The existence of CCK A and B binding sites has been previously demonstrated on AR4-2J cells cultured in 1 g/l glucose [9,10]. We checked the presence of the two CCK-binding subtypes on cells cultured in 4.5 g/l glucose. Inhibition of 125 I-CCK-9 binding by increasing concentrations of caerulein was monophasic (Figure 1a). Scatchard analysis revealed a single class of binding sites with a K_d value of 4 nM and a B_{max} of about 345 fmol/ 10^6 cells. In contrast, gastrin(2–17ds) inhibited the binding of 125 I-CCK-9 with a biphasic pattern (Figure 1a); Scatchard analysis showed that the K_d of the first site was 7 nM and that of the second site 0.5 μ M. Thus labelled CCK-9 occupied two binding sites. Caerulein had the same high affinity for both sites whereas gastrin(2–17ds) was able to discriminate between them. The first site, exhibiting high affinity for caerulein and gastrin(2–17ds), corresponds to the previously described CCK B receptor [10], and the second one, with high affinity for caerulein and low affinity for gastrin(2–17ds), corresponds to CCK A receptor [10].

In contrast, caerulein and gastrin(2–17ds) inhibited 125 I-gastrin(2–17ds) binding with comparable potencies (Figure 1b),

Figure 1 Displacement of 125 I-CCK-9 (a) and 125 I-gastrin(2–17ds) (b) by caerulein and gastrin(2–17ds)

The iodinated peptides at a concentration of 60 pM were incubated with 3×10^5 – 4×10^5 cells/ml for 30 min at 25°C in the presence of increasing concentrations of unlabelled caerulein (○) or gastrin(2–17ds) (▽). Each point represents the mean of three independent experiments.

Figure 2 Effects of increasing concentrations of caerulein (◇) and gastrin(2-17ds) (●) on cell proliferation

AR4-2J cells were plated at 30 000 cells/cm² in 10% FCS. After 18 h, the medium was removed, the cells were washed and grown in Hepes-buffered serum-free medium for 48 h in the presence of peptides added at 24 h intervals. Values are means $\pm$ S.D. ($n = 6$). *** $P < 0.001$. C, control (i.e. no peptide added).

Figure 3 Effects of increasing concentrations of caerulein (◇) and gastrin(2-17ds) (●) on (a) chymotrypsin and (b) amylase activities

AR4-2J cells were plated at 30 000 cells/cm² in 10% FCS. After 18 h, the medium was removed, the cells were washed and grown in Hepes-buffered serum-free medium for 48 h in the presence of peptides added daily. Results are expressed as the percentage increase in enzyme activity compared with controls (100%). Values are means $\pm$ S.D. ($n = 6$). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. C, control (i.e. no peptide added).

as reported for CCK B sites [10]. Scatchard analysis of the data reported in Figure 1(b) revealed a single class of binding sites with a K_d of 1 nM for both caerulein and gastrin(2-17ds) and a B_{max} of only 120 fmol/10⁶ cells. As found at low glucose concentrations, the CCK A and B sites respectively represented about 60% and 40% of the total CCK-binding sites.

A comparison, between the present data and those previously obtained at lower glucose concentration [9,10] indicated that culturing AR4-2J cells at 4.5 g/l glucose did not alter either the levels of CCK A- and B-binding sites or the binding parameters.

Effects of caerulein and gastrin(2-17ds) on cell proliferation and enzyme induction

Growth effects

AR4-2J cells grown in 4.5 g/l glucose and serum-free media were treated for 48 h with increasing concentrations of gastrin(2-17ds) or caerulein. Gastrin(2-17ds) stimulated cell growth, as reflected by the DNA contents of the culture dishes (Figure 2). This effect was detectable at concentrations above 10 pM and the half-

maximal response occurred at 100 pM. At a concentration of 150 nM, however, the effects on growth decreased considerably. This inhibition cannot be attributed to a toxic effect of the peptide, in view of the fact that enzymic responses were maintained (see below) and no cell detachment was noticed.

In contrast, caerulein up to 100 nM elicited no mitogenic effects; high caerulein concentrations reduced the cell proliferation slightly but not significantly. Therefore these results show that caerulein and gastrin(2-17ds) had different effects on cell growth.

Enzyme induction

Enzyme secretion in serum-free culture media is linear for 48 h in both unstimulated and stimulated cells [20]. No chymotrypsin activity was detected in either the cells or the secretion before the addition of activating trypsin. The possibility that the secreted enzymes may have been degraded by proteolysis was checked by adding known amounts of amylase and chymotrypsinogen to the culture media, and then incubating the media in the presence or absence of AR4-2J cells, as under the growth conditions. No decrease in enzyme activity was observed during the 48 h incubation period.

Chymotrypsin activity was measured in cells and secretion after 48 h treatment with either caerulein or gastrin(2-17ds). Caerulein induced a concentration-dependent increase in chymotrypsin activity in the secretion medium (Figure 3a) but not in the cells (not shown), indicating that synthesis of the enzyme was enhanced without any change in the cell storage compartment. The amount of secreted enzyme accounted for more than 90% of the total enzyme activity (medium plus cell content) in whatever conditions. The effects on enzyme synthesis were detectable at a caerulein concentration of 10 pM and the half-maximal response occurred at 100 pM. The maximal response observed for chymotrypsinogen induction was 100% (6.36 ± 0.22 units/mg of DNA and 3.18 ± 0.31 units/mg of DNA with caerulein-stimulated cells and controls respectively) (Figure 3a), whereas gastrin(2-17ds) at the maximal concentration used (100 nM) led only to a small increase (26% increase).

Caerulein stimulated amylase synthesis is a dose-dependent manner, with a maximal effect of 40% as compared with the control values (2446 ± 66 units/mg of DNA in caerulein-treated cells and 1653 ± 165 units/mg of DNA in controls) (Figure 3b). As in the case of proteolytic enzyme, the increase in amylase activity was found to occur only in the secretion and not in the cells and the amount of amylase secreted corresponded to more than 90% of the total activity. Amylase synthesis at the gastrin(2-17ds) concentration of 100 nM did not differ statistically from that of unstimulated cells (1983 ± 115 units/mg of DNA and 1653 ± 165 units/mg of DNA respectively in gastrin-stimulated cells and controls).

These data show that caerulein stimulated biosynthesis of the secretory enzymes at lower concentrations than did gastrin(2-17ds) and that the increase in amylase synthesis was less important than that of chymotrypsinogen.

Effects of caerulein and gastrin(2-17ds) on the mRNA levels of the secretory enzymes

The differences observed in the enzyme levels induced by the two peptides might simply be due to a translational control of enzyme synthesis. In order to test this hypothesis, variations in the amount of the transcript concentrations of both enzymes were analysed.

Figure 4 Effects of various concentrations of caerulein (◇) and gastrin(2-17ds) (●) on chymotrypsinogen (a) and amylase (b) mRNA levels

AR4-2J cells were inoculated at 30 000 cells/cm² in 10% FCS. After removal of the cell-attachment medium, the cells were cultured for 48 h in serum-free medium, in the presence of peptides added at 24 h intervals. Total RNAs were slotted and hybridized with ³²P-labelled cDNA probes encoding rat pancreatic amylase or chymotrypsinogen. Values are means $\pm$ S.D. ($n = 3$). C, control (i.e. no peptide added).

Chymotrypsinogen mRNA

The effects of various concentrations of caerulein and gastrin(2-17ds) on chymotrypsinogen mRNA levels are given in Figure 4(a). At the concentrations tested, caerulein was more effective than gastrin(2-17ds). Chymotrypsinogen mRNA level was enhanced by caerulein at concentrations as low as 10 pM, and the IC₅₀ was 50 pM. The maximal increase in mRNA level observed was 75% of that found in unstimulated cells. Gastrin(2-17ds) at the high concentration of 100 nM increased the mRNA level by only 35%.

Amylase mRNA

Caerulein and gastrin(2-17ds) increased the cell contents of amylase mRNA (Figure 4b) at lower amounts than those found for the chymotrypsinogen mRNA, as in the case of enzyme activity. The increase in amylase mRNA occurred at higher gastrin(2-17ds) than caerulein concentrations. At 100 nM, caerulein and gastrin(2-17ds) enhanced the transcript levels by 40% and 32% respectively (Figure 4b).

These data show that the different effects of the two peptides on enzyme induction did not take place at the translational level. On the other hand, as observed in the case of enzyme synthesis, caerulein was more potent than gastrin(2-17ds).

Effects of CCK antagonists on cell proliferation and enzyme induction

Inhibition of gastrin(2-17ds)-stimulated cell growth

In order to define the CCK receptor subtype involved in cell proliferation more precisely, three CCK antagonists were tested: L364,718, CR1409 and CR1505. The last one (CR1505) was found to be toxic to AR4-2J cells during chronic treatment. L364,718, was used at a concentration of 1 nM, previously shown to fully occupy CCK A receptors and only slightly occupy CCK B receptors [10]. CR1409, used at a concentration of 100 μ M, interacting with both CCK A and B receptors [11,21]. These two antagonists when tested alone had no effect on cell proliferation. For growth-inhibition studies, cells were stimulated

Table 1 Effect of antagonists on gastrin(2-17ds)-induced cell growth

AR4-2J cells were seeded at 30 000 cells/cm² in 10% FCS. After 18 h, medium was removed and the cells were cultured for 48 h in a serum-free medium, buffered with 15 mM Hepes at pH 7.2, in the presence of L364,718 (1 nM) or CR1409 (100 μ M). Gastrin(2-17ds) was added at 24 h intervals. Results are expressed as percentage of control values [absence of gastrin(2-17ds)], and are means of three experiments, repeated three times.

[Gastrin (2-17ds)] (nM)	Cell growth (% of control)		
	No antagonist	L364,718	CR1409
0.1	160	158	110
1.0	196	190	105
10.0	240	236	113

Figure 5 Inhibitory effects of CR1409 on caerulein- or gastrin(2-17ds)-stimulated chymotrypsinogen synthesis

After an 18 h attachment period, the medium was removed, the cells were washed and grown in Hepes-buffered serum-free medium for 48 h, with increasing amounts of antagonist in the presence of a fixed concentration of caerulein (10 nM, ●) or gastrin(2-17 ds) (200 nM, ▽). Chymotrypsin activity was then measured in the secretion. Results are expressed as % of controls (without CR1409). Values are means $\pm$ S.D. ($n = 6$). At any CR1409 concentration there was a statistically significant difference between the data of the two curves.

for 48 h with increasing concentrations of caerulein or gastrin(2-17ds), from 0.1 nM to 10 nM, in the presence of a fixed amount of antagonist. L364,718 did not modify either the level of the growth-promoting effect of gastrin(2-17ds) or the pattern of the dose-response curve (Table 1). In contrast, CR1409 completely inhibited the effects of gastrin(2-17ds) (Table 1). Caerulein had no effect on cell growth even in the presence of antagonists. These results, which are comparable with those previously found for AR4-2J cells cultured at 1 g/l glucose [11], suggest the involvement of CCK B receptors in the gastrin(2-17ds)-induced proliferation.

Inhibition of caerulein- and gastrin(2–17 ds)-induced chymotrypsinogen synthesis

The two CCK antagonists, L364,718 and CR1409, were also used to confirm whether the CCK A receptors were involved in the enzyme induction. L364,718 used alone at a concentration of 1 nM was found to induce enzyme synthesis. Chymotrypsinogen was increased by 66% compared with the controls, and amylase by 10%. CR1409 alone did not modify the enzyme synthesis. In the presence of caerulein (10 nM) and increasing amounts of CR1409, a concentration-dependent inhibition of caerulein-stimulated chymotrypsin activity was observed during a 48 h treatment of AR4-2J cells (Figure 5). A near-total inhibition was found to occur at a CR1409 concentration of 30 μ M. In the presence of gastrin(2–17 ds) at a concentration of 200 nM which induces a 30% increase in chymotrypsinogen biosynthesis, the antagonist inhibited the enzyme synthesis with a dose-response curve close to that obtained with caerulein (Figure 5).

DISCUSSION

The results of *in vivo* experiments have suggested that caerulein and gastrin may regulate pancreatic acinar cell functions. However, the specific effects of these peptides and the receptors involved have not so far been clearly established. The present results, obtained in isolated cells, suggest that these peptides have different regulatory effects on acinar cells.

We compared the effects of caerulein and gastrin(2–17ds) on AR4-2J cells cultured in 4.5 g/l glucose. Binding studies demonstrated the presence of CCK A and CCK B sites with affinities comparable with those previously observed at 1 g/l glucose [9,10]. Indeed, caerulein did not discriminate between CCK A- and B-binding sites, interacting with both with the same affinity, in the nanomolar range. In contrast, gastrin(2–17ds) discriminated between the two sites by interacting with CCK B-binding sites with a K_d of 7 nM and with those of CCK A at higher concentrations (K_d of 0.5 μ M). In addition, the respective percentages of CCK A and B sites were not modified in AR4-2J cells cultured at high glucose concentration (about 60% CCK A and 40% CCK B).

Our results show that gastrin(2–17ds) increased AR4-2J cell proliferation. The concentration found to evoke the mitogenic response corresponded to the occupancy of CCK B receptors (half-maximal response at 0.1 nM). Concentrations of gastrin(2–17ds) above 10 nM decreased cell proliferation. A similar inhibitory effect of high concentrations of gastrin-17 was also reported for seleniomethionine uptake at AR4-2J cells [22] and for growth promotion of colon cancer cells [23]. Caerulein, which also interacts with CCK B receptors, did not induce any mitogenic effect. To define the CCK receptor subtype involved in the gastrin(2–17 ds) action more precisely, three antagonists were tested. However, one of them, CR1505, was found to be toxic during chronic cell treatment and was not further analysed. L364,718 is one of the most efficient discriminating agents [24–26]: its affinity for CCK A receptors on AR4-2J cells is 1000 times higher than that for CCK B receptors (IC_{50} 0.2 nM for CCK A and 0.3 μ M for CCK B [10]). At a concentration of 1 nM, at which CCK A receptors are fully occupied [10], L364,718 did not modify gastrin(2–17 ds)-stimulated growth. In contrast, CR1409, at a concentration of 100 μ M at which it interacts with both CCK receptors [11], abolished the effects of gastrin(2–17 ds). The lack of caerulein effect on cell proliferation was not modified by the two antagonists. These results suggest that the proliferative response was evoked by activation of CCK B receptors. Similar conclusions have been recently reached

for the same cell line by using the CCK B antagonist L365,260 [22]. The mechanisms involved in the lack of caerulein action on cell growth and on ornithine decarboxylase activity [11] have not yet been established. The intracellular events evoked by gastrin and caerulein acting on their specific receptors seem to differ. A tyrosine kinase activity has been reported to be coupled to the gastrin receptor [27], whereas phosphatidylinositol breakdown, Ca^{2+} release and protein kinase C activation mediate the effects produced by the occupancy by CCK peptides of the CCK A receptor [28]. The intracellular second messengers induced by the concomitant activation of the two receptors, as should happen during caerulein stimulation of AR4-2J cells, have not yet been characterized. Analysis of the signal-transduction pathways stimulated in AR4-2J cells by the occupancy of CCK A and B receptors by caerulein and gastrin will shed some light on the different effects of these peptides. It will be of interest to know whether, when CCK A and B receptors are simultaneously occupied, the CCK B-related signal-transduction pathway is found to be uncoupled.

Caerulein, in a dose-dependent manner, increased the biosynthesis of chymotrypsinogen and amylase, as well as the levels of their respective mRNAs, preferentially inducing the synthesis of the proteolytic enzyme. As seen in a previous study [20], spontaneous activation of chymotrypsinogen was never found and inactivation of the secreted enzymes did not occur. The levels of the enzymes secreted were found to reflect their rates of biosynthesis, as their intracellular storage did not increase in response to caerulein [20] or gastrin(2–17ds). Owing to its greater induction, the chymotrypsinogen response could be analysed more closely than that of amylase. The caerulein concentrations producing half-maximal responses in chymotrypsinogen synthesis and mRNA level (100 and 50 pM respectively) were lower than the K_d of caerulein for CCK A and B sites (700 pM), as already reported for other biological effects of CCK on acinar cells [29]. The 2-fold enhancement of the proteolytic enzyme caused by caerulein was comparable with that observed *in vivo* during chronic caerulein stimulation [30,31]. Amylase was found to be less sensitive to caerulein (40% increase), in agreement with *in vivo* data (29%) obtained during long-term stimulation [31]. In AR4-2J cells, the increase in chymotrypsinogen mRNA (75%) was only slightly lower than that of the corresponding protein (100%), suggesting that the translational control or chymotrypsinogen synthesis by caerulein plays a less important role *in vitro* than *in vivo* [30].

High gastrin(2–17 ds) concentrations were necessary to cause an increase in enzyme synthesis. Chymotrypsin activity was increased by only 26% at 100 nM, a concentration at which CCK B but not CCK A receptors were completely saturated by gastrin(2–17ds). Since similar patterns were observed for mRNA levels, enzyme synthesis caused by occupancy of the CCK B receptors by gastrin(2–17ds) did not appear to result from control of mRNA translation. These data do not favour the hypothesis that induction of the secretory enzymes is mediated by CCK B receptors. In order to confirm this, the inhibitory effects of CCK antagonists on enzyme induction were analysed. L364,718, a CCK A receptor antagonist [24–26], stimulated chymotrypsinogen synthesis in the absence of caerulein. CR1409, another antagonist that discriminates between CCK A and B receptor subtypes [26], did not modify the enzyme synthesis by itself. In the presence of 10 nM caerulein or 200 nM gastrin(2–17 ds), it inhibited chymotrypsinogen synthesis induced by these peptides with a comparable IC_{50} of 4 μ M, indicating that the enzyme induction is mediated by CCK A receptors.

The data presented here, taken as a whole, suggest that gastrin(2–17ds) stimulates cell proliferation by occupancy of

CCK B receptors, whereas caerulein and gastrin(2–17ds) stimulate enzyme synthesis by occupancy of CCK A receptors.

One of the most interesting post-receptor events induced by caerulein *in vivo*, as in AR4-2J cells, is the increase in levels of secretory-enzyme mRNA. At present, two points need to be analysed in AR4-2J cells: the first one deals with the molecular mechanisms involved in the action of CCK, the second one with the CCK-regulated regions of the pancreatic genes.

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