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# Are C-terminal octapeptide of cholecystokinin and [Leu$^{11}$]gastrin-(5–17) different in stimulating acid secretion in isolated rabbit gastric glands?

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**Abstract**

In the present study we compared various $CCK_B$ receptor antagonists and tried to detect a difference in biological activity between the C-terminal octapeptides of cholecystokinin (CCK-8) and [Leu$^{11}$]gastrin-(5–17) in isolated rabbit gastric glands. Binding experiments showed that different $CCK_B$/gastrin receptor agonists bound with high affinity and that antagonists inhibited this binding in accordance with a $CCK_B$/gastrin pharmacological profile. [Leu$^{11}$]Gastrin-(5–17), CCK-8 and cionin were found to induce [$^{14}$C]aminopyrine accumulation to 25% above the basal level. Under the same experimental conditions, histamine induced a response twice as great as the response obtained with [Leu$^{11}$]gastrin-(5–17) or CCK-8. [Leu$^{11}$]Gastrin-(5–17) ($10^{-7}$ M), CCK-8 ($10^{-8}$ M) and cionin ($10^{-8}$ M) appeared to be full agonists. $CCK_B$/gastrin receptor antagonists including L-365,260 (3*R*-(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl)-*N*-(3-methylphenyl) urea), L-364,718 (3*S*-(−)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl)-1*H*-indole-2-carboximide) (a selective $CCK_A$ receptor antagonist), PD-135,158 (4([2-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[[1.7.7-trimethyl-bicyclo[2.2.1]hept-2-yl)oxy]carbonyl]amino] propyl]amino]-1-phenylethyl] amino-4-oxo-[1*S*-1$\alpha$.2$\beta$[*S**(*S**)]4$\alpha$]]-butanoate *N*-methyl-D-glucamine) (bicyclo system 1*S*-endo), YM-022 ((*R*)-1-[2,3-dihydro-1-(2′-methylphenacyl)-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl)urea) and JMV-180 (Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-O-$CH_2$-$CH_2$-$C_6H_5$) exhibited the same profile for inhibition of [Leu$^{11}$]gastrin-(5–17) or CCK-8-induced [$^{14}$C]aminopyrine accumulation in rabbit gastric glands. These results suggested that [Leu$^{11}$]gastrin-(5–17) and CCK-8 induced [$^{14}$C]aminopyrine accumulation by the same mechanism. [Leu$^{11}$]Gastrin-(5–17)- or CCK-8-induced [$^{14}$C]aminopyrine accumulation was inhibited by about 40% by the histamine $H_2$ receptor blocker cimetidine. These results are consistent with there being cooperativity between [Leu$^{11}$]gastrin-(5–17) (or CCK-8) and histamine in the acid secretory pathway. Similarly, the $CCK_B$/gastrin receptor antagonists were tested against histamine-induced [$^{14}$C]aminopyrine accumulation and surprisingly, only compound L-365,260 appeared active and even more potent than cimetidine.

*Keywords:* [$^{14}$C]Aminopyrine accumulation; CCK-8; [Leu$^{11}$]Gastrin-(5–17); CCK receptor antagonist; Gastric gland

## 1. Introduction

Cholecystokinin (CCK) is a brain-gut peptide that was among the first of the discovered gastrointestinal hormones (Ivy and Goldberg, 1928) although its amino acid sequence was determined only in 1968 (Mutt and Jorpes, 1968). In 1964, Gregory and Tracy isolated, sequenced, and pharmacologically characterized gastrin from gastric porcine mucosa (Gregory and Tracy, 1964). Gastrin and CCK possess the same C-terminal pentapeptide sequence (-Gly-Trp-Met-Asp-Phe-$NH_2$) which is crucial for biological activity. Gastrin and the C-terminal octapeptide of CCK (CCK-8) have been shown to stimulate gastric acid secretion in vitro (Magous et al., 1989). However, unlike gastrin which is a strong secretagogue, CCK inhibits in vivo acid secretion (Konturek et al., 1992).

CCK/gastrin receptors are currently classified into

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three types: a gastrin receptor ($CCK_G$) and two CCK receptors ($CCK_A$ and $CCK_B$). $CCK_A$ receptors occur mainly in the periphery but also in localized brain regions (Innis and Snyder, 1980). The majority of CCK receptors in the brain are of the $CCK_B$ subtype (Hill et al., 1987). $CCK_A$ and $CCK_B$ receptors have been cloned from canine parietal cells (Kopin et al., 1992), from rat pancreas and brain (Wank et al., 1992a,b) and from human brain (Pisegna et al., 1992). Considering their nucleotide sequence, $CCK_A$ and $CCK_B$/gastrin receptors are different. On the other hand, $CCK_B$ and gastrin receptors seem to be identical (Song et al., 1993; Miyake et al., 1994).

In the last few years, a remarkable variety of CCK/gastrin receptor antagonists have become available. Among the most potent compounds, PD-134,308 (CI-988) and PD-135,158 exhibit affinity in the nanomolar range for $CCK_B$/gastrin receptors in dispersed guinea pig gastric glands (Hughes et al., 1990). The substituted benzodiazepines, L-364,718 (Chang and Lotti, 1986) and L-365,260 (Lotti and Chang, 1989), were developed from a natural non-peptide compound: asperlicin. L-364,718 is a potent $CCK_A$ receptor antagonist. L-365,260 is a selective antagonist of the brain cortex $CCK_B$ receptor. This last compound has been described to antagonize acid secretion in vivo (Lotti and Chang, 1989; Hayward et al., 1991; Hirst et al., 1991; Kawabata et al., 1991; Nishida et al., 1992; Corwin and Smith, 1993; Pendley et al., 1993) and in vitro in isolated cells (Roche et al., 1991a,b). Recently, two new non-peptide CCK receptor antagonists were described: L-368,935 (Freedman et al., 1994) and YM-022 (Nishida et al., 1994). They were found to antagonize pentagastrin-induced acid secretion in vivo. We have shown that suppression of the C-terminal amide group (Martinez et al., 1986) or modification of the peptide bond between Met and Asp in the C-terminal tetrapeptide (Martinez et al., 1985) leads to gastrin receptor antagonists.

Gastrin, and presumably CCK, which share the same C-terminal pentapeptide, have numerous effects on enterochromaffin-like (ECL) cells, D cells and parietal cells. Two hypotheses for the action of gastrin have been established: (1) gastrin acts directly on the parietal cells to stimulate acid secretion (Soll, 1980; Soll et al., 1984); (2) gastrin acts by stimulating the release of histamine from ECL cells, which then acts on parietal cells via histamine $H_2$ receptor (Sandvik et al., 1987; Lloyd et al., 1992). Today, evidence regarding the regulation of acid secretion appears to support the theory that histamine release from ECL cells and direct effects on parietal cells are both physiologically relevant effects of gastrin (Chuang et al., 1991).

We have previously shown that [Leu$^{11}$]gastrin-(5–17) and CCK-8 were differently coupled to G protein through $CCK_B$ receptors (Lallement et al., 1994, 1995), but no difference in activity between these two peptides was described. We decided to evaluate the effects of [Leu$^{11}$]gastrin-(5–17) and CCK-8 on [$^{14}$C]-aminopyrine accumulation (an index of acid secretion (Berglindh et al., 1976)) and different $CCK_B$ receptor antagonists on [$^{14}$C]aminopyrine accumulation induced by [Leu$^{11}$]gastrin-(5–17) and CCK-8. In this study, we focused on the ability of some of the most important $CCK_B$/gastrin receptor antagonists including PD-135,158, YM-022, L-365,260 and compound JMV-180, a CCK analog which acts as an agonist at the high affinity $CCK_A$ binding site and as an antagonist at the low affinity $CCK_A$ binding site (Galas et al., 1988), to inhibit [$^{125}$I]BHCCK-8 binding, and on their effects on [Leu$^{11}$]gastrin-(5–17)-, CCK-8- or histamine-induced [$^{14}$C]aminopyrine accumulation.

This study was performed with isolated rabbit gastric glands. This model was chosen because it contains the cellular types implicated in the secretory response. The isolated glands represent the functional unit of the gastric mucosa. They allow studies on secretagogues involved in acid secretion integrating intracellular cooperativity and the retro-control phenomenons.

## 2. Materials and methods

### 2.1. Chemicals

Collagenase EC 3.4.24.3 was obtained from Serva (Heidelberg, Germany). The incubation medium used for gastric gland preparations, binding experiments and [$^{14}$C]aminopyrine accumulation contained 132.4 mM NaCl, 5.4 mM KCl, 5 mM $Na_2HPO_4$, 1 mM $NaH_2PO_4$, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$, 2 g/l glucose from Sigma (St Louis, MO, USA), and 2 g/l bovine serum albumin fraction V (Euromedex, France) adjusted to pH 7.4. The sulfated CCK-8 (H-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$) and its radiolabeled analogue [$^{125}$I]BHCCK-8 [*N*-succinimidyl-3-(3-[$^{125}$I]iodo-4′-hydroxyphenyl) propionyl] CCK-8, [Leu$^{11}$]gastrin-(5–17) (H-Leu-$(Glu)_5$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-$NH_2$), cionin (H-Asn-Tyr($SO_3H$)-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$), JMV-180 (Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-O-$CH_2$-$CH_2$-$C_6H_5$) and YM-022 ((*R*)-1-[2,3-dihydro-1-(2′-methylphenacyl)-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl)urea) were synthesized in our laboratory. The $^{125}$I-Bolton Hunter reagent ([$^{125}$I]BH) (*N*-succinimidyl-3-(3-[$^{125}$I]-iodo-4′-hydroxyphenyl) propionate) (2000 Ci/mmol) was purchased from Amersham (Buckinghamshire, UK). Histamine dihydrochloride and cimetidine were from Sigma (St. Louis, MO, USA). L-365,260 (3*R*-(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl)-*N*-(3-methylphenyl) urea) and L-364,718 (MK-329 or devazepide) (3*S*(−)-*N*-(2,3-dihydro-

1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl)-1*H*-indole-2-carboximide) were a gift from Dr. P. Anderson, Merck Sharp and Dohme Research Laboratories (West Point, PA, USA). PD-135,158 (4([2-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[[1.7.7-trimethyl-bicyclo[2.2.1]hept-2-yl)oxy]carbonyl]amino] propyl]-amino]-1-phenylethyl] amino-4-oxo-[1*S*-1$\alpha$.2$\beta$[*S** (*S**)]4$\alpha$]-butanoate *N*-methyl-D-glucamine) (bicyclo system 1*S*-endo) was a gift from Dr. D. Horwell, Parke-Davis Neuroscience Research Centre (Cambridge, UK). A stock solution of each antagonist was prepared in pure dimethyl sulfoxide (DMSO) and stored at −20°C. Dilutions were made with incubation medium (see below). The maximal final concentration of DMSO for [$^{14}$C]aminopyrine accumulation experiments was 0.1%.

### 2.2. Isolated rabbit gastric glands preparation

Gastric glands were isolated according to the method previously described by Berglindh and Öbrink (1976) with some modifications. Young New Zealand White rabbits (2.5 kg, INRA, Montpellier) were killed by cervical dislocation and exsanguination. Stomachs were rapidly removed, the antral portion was cut away and discarded, and the fundus was cut open along the lesser curvature. The stomach contents were emptied out, and the mucosa was rinsed with water at room temperature and wiped with disposable towels to eliminate excess mucus and food particles. The mucosal layer was gently scraped off the muscle layer with a blunt spatula and collected in cold buffer. The mucosal pieces were suspended and decanted in cold buffer several times until the supernatant remained clear (about 3 times). The tissue was rapidly minced into small pieces. As emphasized by Berglindh and Öbrink (1976), this step is crucial and must be carried out very quickly. The minced mucosa was resuspended and allowed to settle in cold buffer; excess buffer was decanted. This operation was repeated 3 times. Mucosal tissue ($x$ grams) was transferred into a round-bottom flask and 2 $x$ ml of collagenase solution in buffer (0.2 mg/ml) was added. Tissue was incubated in a water bath for 50 min at 37°C with gentle agitation and the suspension was continuously bubbled with 95% $O_2$-5% $CO_2$. Incubation was stopped by dilution with 250 ml of cold buffer. Glands were purified by successive decantation/resuspension steps in cold buffer until the desired size was obtained and then the glands were resuspended at the adequate dilution in the incubation medium (about 10 $x$ ml). The protein concentration was evaluated using the Bio-Rad protein assay (Bio-Rad, USA), based on the Bradford dye-binding procedure. In these dilution conditions, the protein concentration was 6 mg/ml.

### 2.3. Binding experiments

$CCK_B$/gastrin receptor binding affinities were determined in binding assays with [$^{125}$I]BHCCK-8 as radioligand. For the displacement experiments, gastric glands (2.4 mg protein) were incubated with 40 pM of radioligand for 50 min at 37°C (concentration equivalent to 0.1 $K_D$) in a final volume of 0.5 ml. Saturation experiments were performed under the same conditions in the presence of various concentrations of [$^{125}$I]BHCCK-8. Non-specific binding was determined in the presence of 1 $\mu$M CCK-8. Incubation was terminated by adding 3 ml of incubation medium at 4°C supplemented with bovine serum albumin (20 g/l). Aliquots were then centrifuged at 4°C for 10 min at 3000 rpm. The supernatants were discarded and the radioactivity bound to the pellet was measured. Data from saturation experiments were analyzed from Scatchard plots by linear and non-linear regression analysis (LIGAND program).

### 2.4. [$^{14}$C]Aminopyrine accumulation

Rabbit gastric glands (6 mg protein) were incubated at 37°C with various secretagogues and 0.1 $\mu$Ci/ml [$^{14}$C]aminopyrine in a final volume of 1.5 ml, under magnetic agitation in 24-well tissue culture plates (Multiwell, Falcon) kept under 95% $O_2$-5% $CO_2$. After 20 min, the incubation was stopped; the samples were transferred to Eppendorf vials and centrifuged at 12 000 rpm for 2 min. The supernatants were discarded and the pellets were dissolved in 100 $\mu$l 10% $HClO_4$ and added to scintillation liquid before counting. Incubations were performed in duplicate and mean values were used for calculations. The background count was obtained after incubation of glands under the same conditions but without any secretagogue and was subtracted from all counts.

## 3. Results

### 3.1. Binding experiments

The ability of [$^{125}$I]BHCCK-8 to bind to isolated rabbit gastric glands was investigated. The binding was time-dependent and reached a steady state within 45 min at 37°C with an apparent pseudo first-order rate constant $k_{+1}$ (app) of 9.8 $10^7$ $M^{-1}$ $min^{-1}$. The plateau remained stable for almost 2 h. However, we found incomplete dissociation. Saturation experiments showed that specific binding was saturable, whereas non-specific binding was a linear function of the radiolabeled peptide concentration. [$^{125}$I]BHCCK-8 interacted apparently with a single class of sites (Fig. 1)

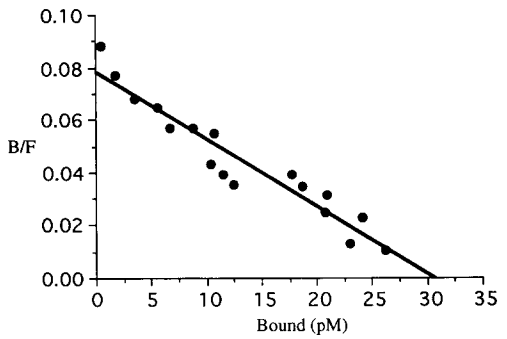


Fig. 1. Scatchard analysis of [$^{125}$I]BHCCK-8 binding to isolated rabbit gastric glands as a function of radiolabeled peptide concentration. Glands (2.4 mg protein) were incubated in a final volume of 0.5 ml at 37°C for 50 min with various concentrations of [$^{125}$I]BHCCK-8 in the presence or in the absence of 1 $\mu$M CCK-8.

characterized by the following parameters (mean ± S.D.): $K_D = 0.6 \pm 0.2$ nM, $B_{max} = 5.5 \pm 0.1$ fmol/mg of protein ($n = 3$ independent experiments).

The effects of some CCK receptor agonists and antagonists on [$^{125}$I]BHCCK-8 binding were investigated. CCK-8, [Leu$^{11}$]gastrin-(5–17), cionin, L-365,260, L-364,718, YM-022, JMV-180 and PD-135,158 were tested for their potency to inhibit the specific binding of the labeled ligand to isolated rabbit gastric glands. The results reported in Table 1 show that CCK-8 and cionin exhibited high affinity for $CCK_B$/gastrin receptor sites ($IC_{50} = 0.5 \pm 0.23$ nM and $0.13 \pm 0.68$ nM respectively) while gastrin had a 10-fold lower affinity ($IC_{50} = 2.92 \pm 2.06$ nM). All the antagonists tested were able to inhibit [$^{125}$I]BHCCK-8 binding with affinities in accordance with a pharmalogical $CCK_B$/gastrin-like profile. L-365,260, YM-022 and PD-135,158 showed a similar $IC_{50}$ value in the nanomolar range whereas JMV-180 and L-364,718 were about 10- and 100-fold less potent, respectively (Table 1).

Table 1
$IC_{50}$ values of various CCK/gastrin agonists and antagonists in isolated rabbit gastric glands, using [$^{125}$I]BHCCK-8 (40 pM) as radiolabeled ligand

| Ligands | $IC_{50}$ (nM) |
|---|---|
| *Agonists* | |
| Cionin | 0.13 ± 0.68 ($n = 3$) |
| CCK-8 | 0.50 ± 0.23 ($n = 6$) |
| [Leu$^{11}$]Gastrin-(5–17) | 2.92 ± 2.06 ($n = 6$) |
| *Antagonists* | |
| YM-022 | 1.73 ± 0.81 ($n = 5$) |
| PD-135,158 | 2.25 ± 1.27 ($n = 6$) |
| L-365,260 | 3.73 ± 1.43 ($n = 5$) |
| JMV-180 | 13.6 ± 5.89 ($n = 5$) |
| L-364,718 | 176.00 ± 80.20 ($n = 5$) |

Each value represents the mean ± S.D. of $n$ experiments performed in duplicate.

### 3.2. [$^{14}$C]Aminopyrine accumulation experiments

[Leu$^{11}$]Gastrin-(5–17), CCK-8 and cionin stimulated [$^{14}$C]aminopyrine accumulation in a dose-dependent manner. Considering the [$^{14}$C]aminopyrine accumulation obtained with $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17) as the reference, CCK-8 and cionin were full agonists with the same maximal response. Maximal stimulation was obtained at about $10^{-7}$ M for [Leu$^{11}$]gastrin-(5–17) and $10^{-8}$ M for CCK-8 and cionin. The apparent effective concentrations producing 50% of the maximal response ($EC_{50}$) are listed in Table 2. Histamine was tested in the same model. This secretagogue was 2-fold more potent than [Leu$^{11}$]gastrin-(5–17). Indeed, $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17) or $10^{-8}$ M CCK-8 produced a 25% increase in [$^{14}$C]aminopyrine accumulation as compared to the basal value, while $10^{-4}$ M histamine produced a 50% increase (Fig. 2) with an $EC_{50}$ of $4.0 \pm 1.1 \times 10^{-6}$ M.

Compounds L-365,260, L-364,718, YM-022, JMV-

Table 2
$EC_{50}$ values of CCK receptor agonists and $IC_{50}$ values of CCK receptor antagonists on $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17)- and $10^{-8}$ M CCK-8-induced [$^{14}$C]aminopyrine accumulation in isolated rabbit gastric glands

| Agonists | $EC_{50}$ (nM) | |
|---|---|---|
| Cionin | 0.35 ± 0.43 ($n = 4$) | |
| CCK-8 | 0.41 ± 0.24 ($n = 4$) | |
| [Leu$^{11}$]Gastrin-(5–17) | 3.20 ± 1.50 ($n = 7$) | |
| Histamine | 4000 ± 1150 ($n = 4$) | |
| Antagonists | $IC_{50}$ (nM) | |
| | [Leu$^{11}$]Gastrin-(5–17)-induced [$^{14}$C]aminopyrine accumulation | CCK-8-induced [$^{14}$C]aminopyrine accumulation |
| PD-135,158 | 23 ± 5 ($n = 4$) | 18 ± 3 ($n = 3$) |
| L-365,260 | 11 ± 6.6 ($n = 7$) | 15 ± 5 ($n = 3$) |
| YM-022 | 210 ± 135 ($n = 8$) | 235 ± 207 ($n = 4$) |
| JMV-180 | 220 ± 170 ($n = 5$) | 183 ± 29 ($n = 3$) |
| L-364,718 | 1580 ± 1230 ($n = 3$) | 867 ± 231 ($n = 3$) |

Each value represents the mean ± S.D. of $n$ separate experiments performed in duplicate.

180 and PD-135,158 were tested for their ability to stimulate [$^{14}$C]aminopyrine accumulation and to inhibit the [$^{14}$C]aminopyrine accumulation induced by $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17) and by $10^{-8}$ M CCK-8. None of these compounds significantly affected the basal [$^{14}$C]aminopyrine accumulation until $10^{-6}$ M. As already described by Roche et al. (1991b), compound L-365,260 inhibited the basal [$^{14}$C]aminopyrine accumulation at a concentration $> 10^{-6}$ M.

These CCK receptor antagonists inhibited the [$^{14}$C]aminopyrine accumulation induced by [Leu$^{11}$]-gastrin-(5–17) and CCK-8 in a dose-dependent manner and displayed the same inhibition profile. The apparent effective concentrations providing 50% inhibition ($IC_{50}$) are reported in Table 2. YM-022 and JMV-180 displayed about the same potency to inhibit the [$^{14}$C]aminopyrine accumulation induced by the two secretagogues; however, L-365,260 and PD-135,158 were 10-fold more potent and about 100-fold more potent than L-364,718 (Table 2).

Some recent reports (Hayward et al., 1991; Kawabata et al., 1991; Corwin and Smith, 1993) showed that L-365,260 inhibits in vivo histamine-induced acid secretion, but no data have been reported concerning the ability of this compound to antagonize in vitro histamine-stimulated acid secretion. Therefore, we examined the ability of the different CCK receptor antagonists to inhibit the [$^{14}$C]aminopyrine accumulation induced by $10^{-4}$ M histamine. L-364,718, YM-022, JMV-180 and PD-135,158 had no effect on histamine-induced [$^{14}$C]aminopyrine accumulation in a concentration range from $10^{-10}$ M to $10^{-6}$ M (not shown). However, compound L-365,260 dose dependently inhibited the [$^{14}$C]aminopyrine accumulation induced by histamine with an $IC_{50}$ of $1.1 \pm 0.6 \times 10^{-7}$ M (mean ± S.D. from four independent experiments performed in duplicate) (Fig. 3).

Histamine is a potent secretagogue in isolated rabbit gastric glands. We tested the potency of the histamine $H_2$ receptor blocker cimetidine to inhibit the [$^{14}$C]aminopyrine accumulation induced by $10^{-4}$ M histamine (Fig. 3). Cimetidine inhibited histamine-induced [$^{14}$C]aminopyrine accumulation with an $IC_{50}$ of $8.0 \pm 2.8 \times 10^{-6}$ M (mean ± S.D. from three independent experiments performed in duplicate). Total inhibition was obtained with $10^{-4}$ M cimetidine. To investigate whether the effects of [Leu$^{11}$]gastrin-(5–17) and CCK-8 were direct or mediated by histamine, we tested the effect of cimetidine on [Leu$^{11}$]gastrin-(5–17)- ($10^{-7}$ M) or CCK-8- ($10^{-8}$ M) induced [$^{14}$C]aminopyrine accumulation. Cimetidine inhibited in a dose-dependent manner the [$^{14}$C]aminopyrine accumulation induced by the two secretagogues (not shown). However, this inhibition was only partial and a significant effect of [Leu$^{11}$]gastrin-(5–17) and CCK-8 remained (about 60% of the total response) even at a high cimetidine concentration ($10^{-4}$ M). In a second experiment, we measured the effects of various doses of [Leu$^{11}$]gastrin-(5–17) and CCK-8 on [$^{14}$C]aminopyrine accumulation in the presence of $10^{-4}$ M histamine (Fig. 4) (or $10^{-4}$ M cimetidine, Fig. 5), concentrations sufficient to saturate the histamine $H_2$ receptor. The $EC_{50}$ values of [Leu$^{11}$]gastrin-(5–17) and CCK-8 were not modified by the presence of $10^{-4}$ M histamine or cimetidine. They

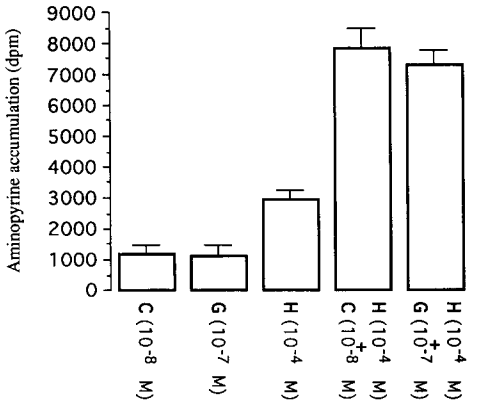


Fig. 2. [$^{14}$C]Aminopyrine accumulation induced by $10^{-8}$ M CCK-8 (C), $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17) (G), $10^{-4}$ M histamine (H), $10^{-8}$ M CCK-8 + $10^{-4}$ M histamine (C+H), $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17) + $10^{-4}$ M histamine (G+H).

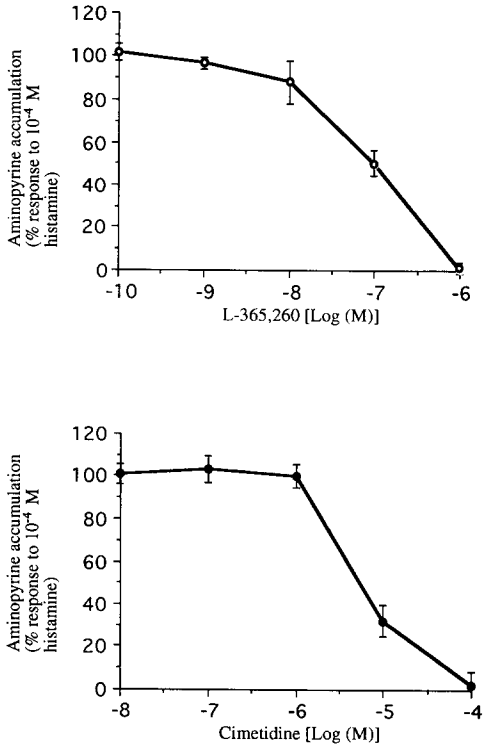


Fig. 3. Effect of L-365,260 (○) and cimetidine (●) on [$^{14}$C]aminopyrine accumulation induced by $10^{-4}$ M histamine in isolated rabbit gastric glands. After subtraction of the basal [$^{14}$C]aminopyrine accumulation, data were expressed as percentages of the response obtained with $10^{-4}$ M histamine.

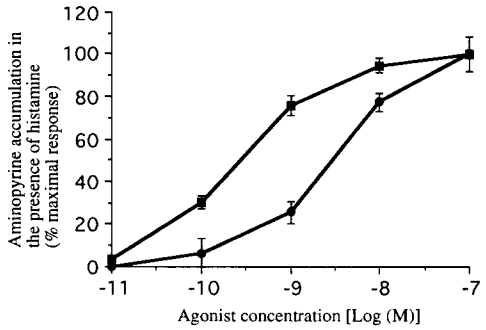


Fig. 4. Dose-response curves for [Leu$^{11}$]gastrin-(5–17) (●) and CCK-8 (■) on [$^{14}$C]aminopyrine accumulation in isolated rabbit gastric glands in the presence of $10^{-4}$ M histamine. After subtraction of the basal [$^{14}$C]aminopyrine accumulation, data were expressed as percentages of the maximal response obtained with $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17) + $10^{-4}$ M histamine, which was about 8 times higher than the $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17) (or the $10^{-8}$ M CCK-8) response.

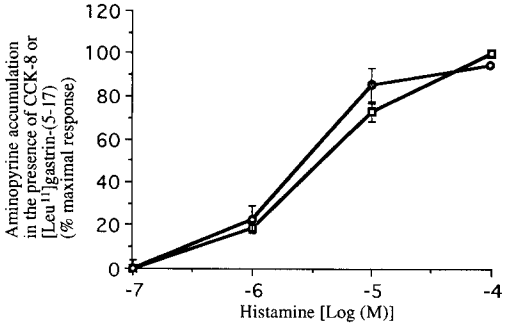


Fig. 6. Dose-response curves for histamine on [$^{14}$C]aminopyrine accumulation in the presence of $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17) (○) or $10^{-8}$ M CCK-8 (□) in isolated rabbit gastric glands. After subtraction of the basal [$^{14}$C]aminopyrine accumulation, data were expressed as percentages of the maximal response obtained with $10^{-4}$ M histamine + $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17), which was about 8 times higher than the $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17) (or the $10^{-8}$ M CCK-8) response.

were $3.2 \pm 1.5$ nM for [Leu$^{11}$]gastrin-(5–17) alone, $3.0 \pm 2.0$ nM in the presence of histamine and $3.0 \pm 2.0$ nM in the presence of cimetidine. Similarly, we found an $EC_{50} = 0.4 \pm 0.2$ nM for CCK-8 alone, $0.4 \pm 0.3$ nM in the presence of histamine and $0.4 \pm 0.3$ nM in the presence of cimetidine. Accordingly, we investigated the effect of histamine on [$^{14}$C]aminopyrine accumulation in the presence of $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17) and $10^{-8}$ M CCK-8, concentrations sufficient to saturate $CCK_B$/gastrin receptor sites (Fig. 6). The $EC_{50}$ value of histamine was not changed by the presence of a saturating concentration of the two secretagogues ($EC_{50} = 4000 \pm 1150$ nM for histamine alone, $3300 \pm 1500$ nM in the presence of [Leu$^{11}$]gastrin-(5–17) and $4000 \pm 1000$ nM in the presence of CCK-8).

However, we observed a potentiation of the CCK-8-($10^{-8}$ M) or [Leu$^{11}$]gastrin-(5–17)- ($10^{-7}$ M) induced [$^{14}$C]aminopyrine accumulation in the presence of $10^{-4}$ M histamine, in accordance with the results obtained by Soll (1978) (Fig. 2). The [$^{14}$C]aminopyrine accumulation was increased by 8-fold as compared to the [$^{14}$C]aminopyrine accumulation induced by $10^{-8}$ M CCK-8 or $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17), and by about 2.5-fold as compared to the [$^{14}$C]aminopyrine accumulation induced by $10^{-4}$ M histamine.

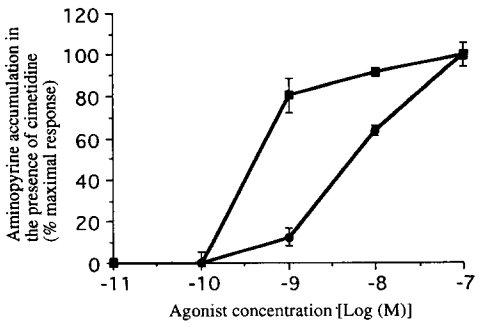


Fig. 5. Dose-response curves for [Leu$^{11}$]gastrin-(5–17) (●) and CCK-8 (■) on [$^{14}$C]aminopyrine accumulation in isolated rabbit gastric glands in the presence of $10^{-4}$ M cimetidine. After subtraction of the basal [$^{14}$C]aminopyrine accumulation, data were expressed as percentages of the maximal response obtained with $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17) + $10^{-4}$ M cimetidine, which was about 2 times lower than the $10^{-7}$ M [Leu$^{11}$]gastrin-(5–17) (or the $10^{-8}$ M CCK-8) response.

## 4. Discussion

With the isolated rabbit gastric gland preparations, binding experiments allowed us to show that CCK-8, cionin, [Leu$^{11}$]gastrin-(5–17), compounds L-365,260, PD-135,158 and YM-022 exhibited a high affinity for $CCK_B$ receptors while compound L-364,718 had a lower affinity, in accordance with some results obtained with dispersed guinea pig gastric glands by Hughes et al. (1990). We also demonstrated a potentiation phenomenon between [Leu$^{11}$]gastrin-(5–17) (or CCK-8) and histamine on the stimulation of [$^{14}$C]aminopyrine accumulation, as described by Soll (1978) or Chew and Hersey (1982).

Acid secretion induced by gastrin from gastric cells or glands has often been considered a controversial subject. Berglindh et al. (1980) found that the glands responded to gastrin when the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX) was included in the incubation medium. These results suggested an indirect action of gastrin. Chew and Hersey (1982) found a discrete response to gastrin with rabbit gastric glands and isolated cells. Addition of dithiothreitol to the incubation medium enhanced the response in both glands and cells.

With our preparation, we obtained a significant [$^{14}$C]aminopyrine accumulation (about 25% higher than the basal [$^{14}$C]aminopyrine accumulation) upon incu-

bation with [Leu$^{11}$]gastrin-(5–17), CCK-8 or cionin, without any additive in the medium.

Considering the [$^{14}$C]aminopyrine accumulation obtained with [Leu$^{11}$]gastrin-(5–17) ($10^{-7}$ M) as the reference, CCK-8 and cionin were full agonists inducing the same maximal response. $EC_{50}$ values were $3.2 \times 10^{-9}$ M, $4.1 \times 10^{-10}$ M and $3.5 \times 10^{-10}$ M for [Leu$^{11}$]gastrin-(5-17), CCK-8 and cionin respectively. These results are in agreement with the binding affinities. We found that the [$^{14}$C]aminopyrine accumulation induced by histamine ($10^{-4}$ M) was 2-fold higher than the [Leu$^{11}$]gastrin-(5–17)- ($10^{-7}$ M), CCK-8- ($10^{-8}$ M) or cionin- ($10^{-8}$ M) induced [$^{14}$C]aminopyrine accumulation. As indicated by Cabero et al. (1993), the lower efficacy of gastrin can be due to the fact that gastrin receptors (in contrast to histamine receptors) might suffer some damage during isolation of the glands.

Compounds L-365,260 and PD-135,158 were the most powerful $CCK_B$/gastrin receptor antagonists and completely inhibited the agonist-induced [$^{14}$C]-aminopyrine accumulation. Compound L-365,260 is often described as an inhibitor of pentagastrin-induced acid secretion in vivo in the anaesthetized rat (Hayward et al., 1991; Hirst et al., 1991; Nishida et al., 1992; Corwin and Smith, 1993; Pendley et al., 1993). Corwin and Smith (1993) also showed that CCK-stimulated acid secretion is not blocked by L-365,260 in the same model. These authors concluded that pentagastrin stimulated acid secretion through a gastrin-type receptor, but not CCK. In contrast, our results seem to indicate that [Leu$^{11}$]gastrin-(5–17) and CCK-8 stimulated acid secretion via the same $CCK_B$/gastrin receptor sites.

Compounds JMV-180 and YM-022 inhibited the [$^{14}$C]aminopyrine accumulation induced by [Leu$^{11}$]-gastrin-(5–17) and CCK-8 with the same order of potency (about $2 \times 10^{-7}$ M), although YM-022 exhibited higher affinity for the $CCK_B$/gastrin receptor. YM-022 has been shown to antagonize pentagastrin-induced acid secretion (Nishida et al., 1994) in the anaesthetized rat.

We tested the different CCK receptor antagonists for their ability to inhibit histamine-induced [$^{14}$C]aminopyrine accumulation in isolated rabbit gastric glands. The inhibitory effects of CCK receptor antagonists have been studied in vivo on histamine-induced acid secretion and the results are controversial. Several authors failed to demonstrate that L-365,260 antagonizes histamine-stimulated acid secretion in the mouse (Lotti and Chang, 1989) or in the rat (Hayward et al., 1991). These observations disagree with other reports (Hirst et al., 1991; Nishida et al., 1992; Pendley et al., 1993) in which L-365,260 significantly inhibited histamine-stimulated acid secretion in the anaesthetized rat. The mechanism by which compound L-365,260 inhibits histamine-stimulated acid secretion is unknown. In a recent report, via in vitro experiments (on the isolated right atrium of guinea pig) and in vivo experiments (on anaesthetized rat), Nishida et al. (1992) indicated that the inhibitory effects of L-365,260 do not result from an interaction of this compound with muscarinic, histamine $H_2$ or benzodiazepine receptors. These authors suggest that the inhibitory effect of compound L-365,260 is mediated by $CCK_B$ receptors in the central nervous system, but the results obtained in vivo by Ishikawa et al. (1985) do not support such a hypothesis. With isolated rabbit gastric glands, we found that compounds L-364,718, YM-022, JMV-180 and PD-135,158 had no effect on [$^{14}$C]aminopyrine accumulation induced by histamine. Surprisingly, compound L-365,260 appeared more potent than cimetidine in inhibiting histamine-induced [$^{14}$C]aminopyrine accumulation. Our results on isolated rabbit gastric glands could suggest a direct effect of compound L-365,260 on histamine-induced [$^{14}$C]aminopyrine accumulation. According to Nishida et al. (1992), L-365,260 inhibits histamine-induced gastric acid secretion through gastrin receptors. Our finding does not support such a hypothesis since PD-135,158 (the best $CCK_B$/gastrin antagonist in this model in our hands) as well as YM-022 failed to inhibit histamine-induced [$^{14}$C]aminopyrine accumulation.

Experiments performed in the presence of histamine (or cimetidine) showed an unmodified $EC_{50}$ for [Leu$^{11}$]gastrin-(5–17) and CCK-8. Similarly, the $EC_{50}$ of histamine was not changed in the presence of [Leu$^{11}$]gastrin-(5–17) (or CCK-8). These results show that there is no interaction between [Leu$^{11}$]gastrin-(5–17) (or CCK-8) and histamine $H_2$ binding sites or between histamine and $CCK_B$/gastrin binding sites. However, the potentiation of [$^{14}$C]aminopyrine accumulation by [Leu$^{11}$]gastrin-(5–17) (or CCK-8) and histamine is not clearly understood. On the basis of the results presented in this paper, we can imagine that this potentiation phenomenon results from post-membrane events, after the interaction of the hormone with its specific receptor sites. It would be of interest to elucidate such a phenomenon.

With isolated rabbit gastric glands, we have shown significant [$^{14}$C]aminopyrine accumulation upon stimulation with [Leu$^{11}$]gastrin-(5–17), CCK-8 and cionin. The [$^{14}$C]aminopyrine accumulation allowed us to evaluate the potency of some CCK receptor antagonists. We have demonstrated that the different CCK receptor antagonists display the same inhibition profile whatever the secretagogue tested ([Leu$^{11}$]gastrin-(5–17) or CCK-8). [Leu$^{11}$]Gastrin-(5–17) and CCK-8 stimulated, with the same maximal response, [$^{14}$C]-aminopyrine accumulation via histamine release which seemed to correspond to 40% of the maximal response. Although we have demonstrated that $CCK_B$ receptors are differently coupled to G protein according to the

ligand which activates the receptor ([Leu$^{11}$]gastrin-(5–17) or CCK-8) (Lallement et al., 1994,1995), we have no evidence of any difference in biological activity between [Leu$^{11}$]gastrin-(5–17) and CCK-8 in isolated rabbit gastric glands.

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