British Journal of Pharmacology (1997) 120, 447–454

Pharmacological and biochemical evidence for the simultaneous expression of CCK_B/gastrin and CCK_A receptors in the pig pancreas

[1]C. Philippe, E.F. Lhoste, †M. Dufresne, #L. Moroder, T. Corring & †D. Fourmy

Laboratoire d'Ecologie et de Physiologie du Système Digestif, INRA, Domaine de Vilvert F-78352 Jouy-en-Josas Cedex, France; †INSERM Unité 151, Institut L. Bugnard, CHU Rangueil, F-31054 Toulouse, France, and #Max Planck Institut für Biochemie, Martinsried, Germany

1 In the pig, the secretory response of the pancreas is not inhibited by the antagonist MK329 suggesting that cholecystokinin$_A$ (CCK_A) receptors are not involved.

2 Membranes were isolated from the pancreas of 6 Large White pigs to characterize their CCK receptors.

3 The binding of [^{125}I]-BH-[Thr, Nle]CCK-9 was dependent on pH, maximal after a 90 min incubation period, saturable and reversible. Saturation analysis of the binding demonstrated a single class of high affinity sites ($K_d = 0.22 \pm 0.02$ nM) and a binding capacity, $B_{max} = 110.64 \pm 12.50$ fmol mg^{-1} protein.

4 Competition binding by agonists and antagonists of CCK_A and CCK_B/gastrin receptors demonstrated the presence of two distinct binding components, sites presenting a high affinity for [Thr, Nle]CCK-9, gastrin, PD 135158, L-365,260 and a low affinity for MK329, SR 27897, and sites presenting a high affinity for [Thr, Nle]CCK-9, MK329, SR 27897 and a low affinity for gastrin, PD 135158, L-365,260.

5 These pharmacological data demonstrate the presence of both CCK_A and CCK_B/gastrin receptors in the pig pancreas, the latter being predominant.

6 Two distinct membrane proteins (50 and 85–100 kDa, respectively) display pharmacological features of CCK_B/gastrin and CCK_A receptors.

7 In pigs, as in calves and humans, CCK_B/gastrin receptors are predominant in the pancreas.

Keywords: Pancreas; CCK_A receptor; CCK_B/gastrin receptor; pig

Introduction

Cholecystokinin (CCK) was initially isolated from the porcine duodenum as a 33 amino acid peptide (Mutt & Jorpes, 1968). A number of biologically-active molecular variants were subsequently described (Rehfeld *et al*., 1982; Eysselein *et al*., 1986). A structural feature of CCK is its homology with gastrin, another gastrointestinal neuropeptide. Indeed, the two peptides share the carboxyterminal amidated pentapeptide Gly-Trp-Met-Asp-Phe-CO-NH_2 which is endowed with the biological activity. CCK is widely distributed within the brain and the gastrointestinal tract while gastrin is more abundant in the gastrointestinal tract (Williams, 1982).

CCK and gastrin exert their physiological functions by interacting with specific receptors. Two different receptor subtypes for the peptides have been identified on the basis of their relative affinity for the natural ligands, their differential distribution and their molecular structure: CCK_A (Wank *et al*., 1992a; Silvente-Poirot *et al*., 1993a; De Weerth *et al*., 1993) and CCK_B/gastrin receptors (Kopin *et al*., 1992; Wank *et al*., 1992b; Pisegna *et al*., 1992). The CCK_A receptor binds sulphated CCK with a 500 to 1000 fold higher affinity than sulphated gastrin or non-sulphated CCK (Silvente-Poirot *et al*., 1993a; Williams & Blevins, 1993). The CCK_B/gastrin receptor binds gastrin and CCK with almost the same affinity, and discriminates poorly between the sulphated and non-sulphated CCK analogues (Saito *et al*., 1980; Kopin *et al*., 1992; Wank *et al*., 1992b; Pisegna *et al*., 1992). The two receptor subtypes can also be distinguished by use of specific non-peptide or peptoïd antagonists such as MK329 or SR 27897 for the CCK_A receptor, and L-365,260 or PD 135158 for the CCK_B/gastrin receptor (Chang *et al*., 1986; Lotti & Chang, 1989; Hughes *et al*., 1990; Gully *et al*., 1993).

The expression of CCK_A and CCK_B/gastrin receptors is tissue-dependent. In laboratory animals, the CCK_B/gastrin receptor is abundant in the central nervous system and several gastric cells, whereas the CCK_A receptor is found predominantly in the gallbladder and the pancreas (Silvente-Poirot *et al*., 1993b; Williams & Blevins, 1993). In the pancreas, CCK stimulates exocrine secretion.

The pharmacological status of the pancreatic CCK receptors seems to be dependent on the species. For instance, the pancreas of rats and mice possesses only CCK_A receptors (Hadjiivanova *et al*., 1992; Povoski *et al*., 1994) whereas both CCK_A and CCK_B/gastrin receptors are present in the pancreas of dogs, guinea-pigs and calves (Fourmy *et al*., 1987; Yu *et al*., 1987; Le Meuth *et al*., 1993). In the latter, CCK_B/gastrin receptors are, by far, predominant over CCK_A receptors (Le Meuth *et al*., 1993). Interestingly, in the human pancreas, only CCK_B/gastrin receptor mRNAs were detected by Northern blot analysis (Kopin *et al*., 1992; Wank *et al*., 1992b; Pisegna *et al*., 1992). However, so far, no clear biological function has been ascribed to the pancreatic CCK_B/gastrin receptor. In the pig, unlike in rodents, CCK is a fairly weak stimulant of pancreatic secretion. This statement is based on the observations that the threshold plasma CCK concentration for stimulating the exocrine pancreatic secretion is 4–5 times higher than the plasma levels encountered after a meal (Cuber *et al*., 1989), and that administration of MK329 only slightly affected the pancreatic exocrine response to a meal and to a co-infusion of CCK and secretin (Lhoste *et al*., 1995).

The identification and characterization of CCK receptors in the pancreas are necessary to understand which peptide, re-

[1] Author for correspondence.

ceptor subtype and mechanism are involved in the regulation of pancreatic functions. With this in mind, we characterized the binding of [^{125}I]-BH-[Thr^{28}, Nle^{31}]CCK-(25–33) ([^{125}I]-BH-[Thr,Nle]CCK-9) to porcine pancreatic membranes by use of specific agonists and antagonists and determined the molecular characteristics of the receptors by photoaffinity labelling.

Methods

Animals

Six male 2.5 month-old Large White pigs were fed a standard diet for 2 weeks. They received two 800 g meals twice daily and were fasted for 16 h before being killed.

Preparation of pancreatic membranes and binding studies with [^{125}I]-BH-[Thr,Nle]CCK-9

After the pigs had been killed, the pancreas was rapidly excised, cooled, dissected from the adipose tissue and weighed. All subsequent preparations were carried out at 4°C. The tissue was first ground into 1–2 mm pieces in 0.7 volume of an ice-cold 0.3 M sucrose solution containing 0.01% STI, 1 mM 2-mercaptoethanol, and 0.1 mM PMSF. It was then homogenized in 6 volumes of the same solution with ten strokes of a large clearance pestle in a Dounce homogenizer (Kontes Co., Vineland, NJ, U.S.A.), filtered through gauze, and again homogenized with ten strokes of a small clearance pestle. The sucrose concentration of the homogenate was brought up to 1.3 M by adding a 2 M sucrose solution containing 0.01% STI, 1 mM 2-mercaptoethanol and 0.1 mM PMSF. This homogenate was overlaid with the 0.3 M sucrose solution and centrifuged at 140,000 *g* and 4°C for 2.5 h. The membranes were collected at the 0.3–1.3 M sucrose interface, washed with 3 volumes of distilled water, and pelleted by centrifugation at 140,000 *g* and 4°C for 20 min. The membrane pellet was resuspended in a HEPES-buffer (50 mM, pH 7.0) containing 115 mM NaCl, 5 mM $MgCl_2$, 0.01% STI, 0.1% bacitracin, 1 mM EGTA, 0.1 mM PMSF and 0.2% BSA (0.7 ml g^{-1} wet tissue). After centrifugation (140,000 *g*, 4°C, 15 min), the pellet was resuspended in the binding HEPES-buffer (0.3 ml g^{-1} wet tissue) and stored at −70°C until use. Membranes retained their binding capacity for at least 4 weeks. The membrane protein concentration was determined by the method of Lowry (Lowry *et al*., 1951).

[Thr,Nle]CCK-9 was conjugated with Bolton-Hunter reagent and the purified resulting product was radio-iodinated as described previously (Fourmy *et al*., 1984). Specific activity was 1500–2000 Ci mmol^{-1}. Preliminary experiments were performed to determine the appropriate conditions for binding: protein content of the membrane preparations, time of incubation and pH of the binding buffer. Rountinely, pancreatic membranes (60 to 160 μg protein) were incubated in the modified HEPES-buffer (pH 6.5, 0.05% BSA) for 90 min at 20°C with 45 pM [^{125}I]-BH-[Thr,Nle]CCK-9 alone or in combination with different concentrations of the CCK-related peptides or antagonists in a final volume of 500 μl. Non-specific binding was determined by incubation of pancreatic membranes in the presence of a 1 μM excesss of [Thr,Nle]CCK-9 under the same conditions as above. The reaction was stopped by adding 500 µl of the ice-cold modified HEPES-buffer. The membrane-associated radioactivity was separated by centrifugation at 10,000 *g* at 4°C for 10 min. The resulting pellets were washed with 500 µl of the ice-cold modified HEPES-buffer before measurement of radioactivity in a gamma-counter.

Photoaffinity labelling

Preparation of the photoaffinity radioligand [^{125}I]-ASA-[Thr,Nle]CCK-9 and the method used for photoaffinity labelling have been previously described in detail (Silvente-Poirot *et al*., 1994). Briefly, binding of 100 pM [^{125}I]-ASA-[Thr,Nle]CCK-9 to plasma membranes (40 µg) was performed in the dark as described above. Pellets of labelled plasma membranes were resuspended in 5 mM HEPES-buffer pH 6.5. Photolysis was induced with a 125 W mercury lamp at 4°C for 10 min. After this time, membranes were pelleted by centrifugation. Labelled membrane proteins were separated by SDS-PAGE in a 10% polyacrylamide gel and visualized by autoradiography. The apparent relative molecular masses of the labelled components were calculated with the standard prestained proteins (Bio-Rad, Richmond): phosphorylase B (111,000), bovine serum albumin (84,000), ovalbumin (47,000), carbonic anhydrase (33,000), STI (24,000), lysozyme (16,000).

Materials

[Thr^{28},Nle^{31}]CCK-(25–33) ([Thr,Nle]CCK-9) and Des-SO_3H-gastrin 1–17 (G17ns) were synthesized as previously described (Fourmy *et al*., 1984). Antagonists were kindly provided by the following manufacturers: 3R(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N′-(3-methylphenyl) urea, (L-365,260) by Merck, Sharp and Dohme Chibret (Rahway, N, U.S.A.), 35(−)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-borzodiazepine-3yl)-1H-indole-2-carboxamide (MK329) by Merck, Sharp and Dohme (West Point, PA, U.S.A.), 4-{[2-[[3-(1H-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-[1S-1α.2β[5*(S*)] 4α]}-butanoate N-methyl-D-glucamine(bicyclo system 15 endo) (PD 13518) by Parke-Davis Research Unit (Cambridge, U.K.), 1-[[2-(4-(2-chlorophenyl)thiazol-2-yl) aminocarbonyl]-indolyl] acetic acid (SR 27897) by Sanofi-Recherche (Toulouse, France). Other compounds were obtained from the following sources: sulphated gastrin 1–17 (G17s) was from Bachem (Bubendorf, Switzerland); [^{125}I]-Na (SA, 137 MBq) was from Amersham International (Les Ulis, France); N-hydroxysuccinimidyl-4-azidosalicylic acid (NHS-ASA) was from Pierce, (Holland). Soybean trypsin inhibitor (STI), bacitracin, phenyl-methylsuphonylfluoride (PMSF), HEPES, ethylene glycolbis(-aminoethylether)N,N,N′,N′-tetraacetic acid (EGTA) and BSA were purchased from Sigma Chemical Co (St Louis, MO, U.S.A.); guanosine 5′-γ-thio-triphosphate (γ[S]) from Boehringer (Mannheim, Germany).

Data processing and statistical analysis

The data from inhibition of binding by [Thr,Nle]CCK-9 were plotted according to the method of Scatchard and analyzed by the LIGAND programme of Munson and Rodbard. The competition binding data were transformed by non-linear regression in order to obtain IC_{50} and Hill slope throughout Graphpad PRISM programme (Graphpad Software Inc.).

Results

Determination of the binding conditions

The binding of [^{125}I]-BH-[Thr,Nle]CCK-9 to porcine pancreatic membranes averaged 10% of the total radioactivity added, and non-specific binding represented 10% of total binding. The binding was dependent on the amount of membrane and increased nearly proportionally from 100 to 400 μg protein ml^{-1} of incubation buffer (Figure 1a). It increased from pH 5, reached a plateau at pH 5.5, remained steady until pH 6.5 and dropped thereafter (Figure 1b). It was maximal after a 90 min incubation and stable up to 180 min (Figure 2a). After a 90 min incubation, the addition of 1 μM [Thr,Nle]CCK-9 led to a slow dissociation of the membrane-bound radioligand reaching 50% after 3 h of incubation (Figure 2b).

Therefore, for further characterization of the CCK receptors from the porcine pancreas, aliquots of membranes

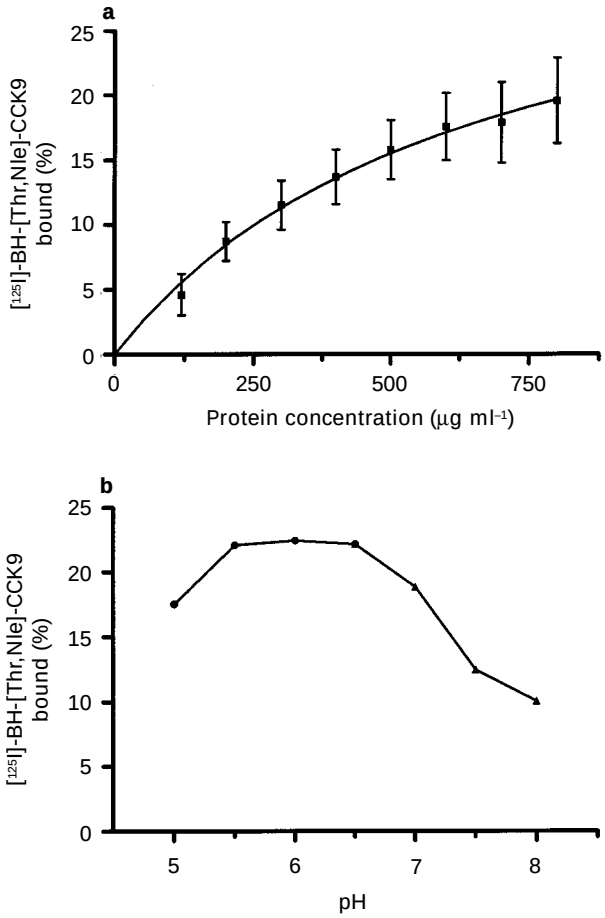

Figure 1 Specific binding of [^{125}I]-BH-[Thr,Nle]CCK-9 to porcine pancreatic membranes. (a) Effect of the quantity of membrane proteins. Membrane were incubated with 45 pM [^{125}I]-BH-[Thr,Nle]CCK-9 in 0.5 ml of the binding buffer (pH 6.5, 0.05% BSA) for 30 min at 20°C. Data are the mean with vertical lines showing s.e.mean of four experiments, performed in duplicate. (b) Effect of pH of the binding buffer. Pancreatic membranes were incubated with [^{125}I]-BH-[Thr,Nle]CCK-9 for 90 min at 20°C, in the buffer at $5 \leqslant$ pH $\leqslant 8$; pH from 5 to 6 were obtained with HEPES-NaOH buffer and pH from 6.5 to 8 with Tris-HCl buffer. Data are the mean of two experiments performed in duplicate.

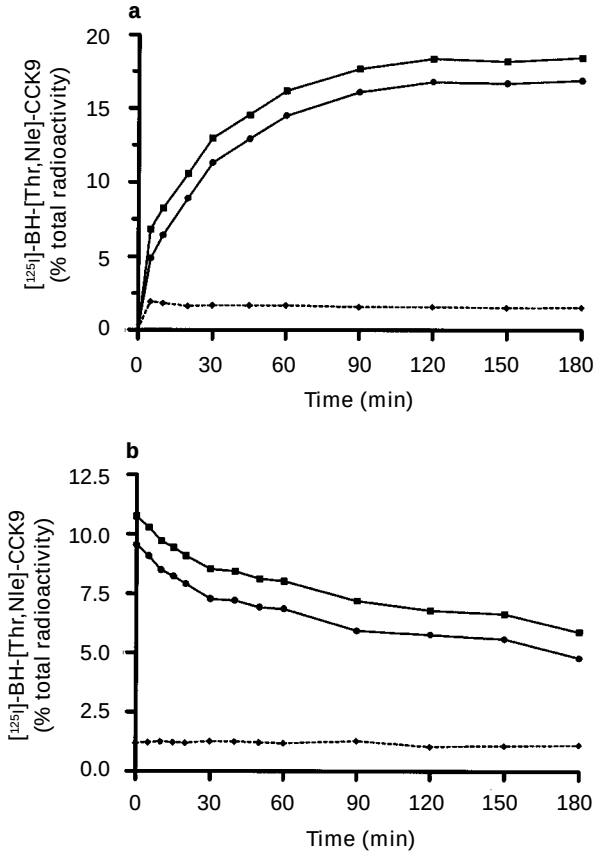

Figure 2 Kinetics of (a) association and (b) dissociation of [^{125}I]-BH-[Thr,Nle]CCK-9 to pancreatic membranes. T membranes (60 to 160 μg) were incubated at 20°C with the radioligand in the absence of [Thr,Nle]CCK-9 (total binding) or presence of 1 μM [Thr,Nle]CCK-9 (non-specific binding). At the indicated times, samples were collected to determine the quantity of membrane-bound [^{125}I]-BH-[Thr,Nle]CCK-9. The dissociation of the membrane-bound [^{125}I]-BH-[Thr,Nle]CCK-9 at steady state (after a 90 min incubation period) was obtained by the addition of 1 μM [Thr,Nle]CCK-9. At the indicated times, samples were collected to determine the remaining membrane-bound [^{125}I]-BH-[Thr,Nle]CCK-9. The specific binding (●) was obtained by subtracting non-specific binding (◆) from total binding (■). Data are the mean of two experiments performed in duplicate.

corresponding to 60–160 μg of protein were incubated with 45 pM [^{125}I]-BH-[Thr,Nle]CCK-9 at 20°C in 0.5 ml HEPES-buffer, pH 6.5 containing 0.05% BSA for 90 min.

Scatchard analysis of [^{125}I]-BH-[Thr,Nle]CCK-9 binding

The saturation analysis of [^{125}I]-BH-[Thr,Nle]CCK-9 binding to pig pancreatic membranes was achieved at a constant concentration of radioligand diluted wtih increasing concentrations of [Thr,Nle]CCK-9. The corresponding Scatchard plot was linear with a Hill slope of -1.03 ± -0.02 ($n = 5$), indicative of a single class of binding sites with a high affinity, $K_d = 0.22 \pm 0.02$ nM, and a binding capacity, $B_{max} = 110.64 \pm 12.50$ fmol mg^{-1} protein (Figure 3).

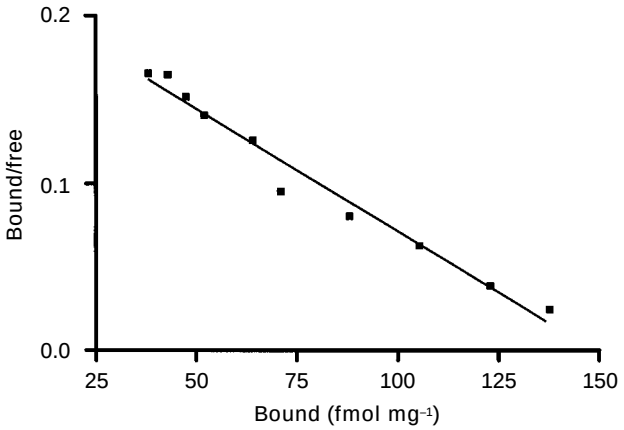

Figure 3 Scatchard plot of the binding of [^{125}I]-BH-[Thr,Nle]CCK-9 to pancreatic membranes in the presence of [Thr,Nle]CCK-9. The results shown are representative of one experiment out of five (mean $K_D = 0.22 \pm 0.02$ nM and $B_{max} = 110.64 \pm 12.50$ fmol mg^{-1} protein).

Inhibition of [^{125}I]-[Thr,Nle]CCK-9 binding by GTPγ[S], CCK agonists and antagonists

The molecular cloning of CCK receptors has shown them to belong to the superfamily of receptors coupled to a guanine nucleotide-binding regulatory protein (Innis & Snyder, 1980; Wank *et al*., 1992a,b; Kopin *et al*., 1992). Therefore, we first evaluated the coupling of pig pancreatic CCK receptors to G-proteins by testing the inhibition of the specific binding of [^{125}I]BH-[Thr,Nle]-CCK9 by the non-hydrolysable analogue of GTP, GTPγ[S]. According to the ternary complex model, activation of the α-subunit of the G-protein induces the dissociation of the G-protein complex from the receptor which in

turn leads to the dissociation of the agonist ligand from the receptor (Taylor, 1990). We observed that the specific binding of [^{125}I]-BH-[Thr,Nle]-CCK9 was inhibited in a dose-dependent manner (IC_{50} = 12.22 ± 1.49 nM) by GTPγ[S] supporting the conclusion that CCK receptors identified in the preparation of membranes are functionally coupled to G proteins (Figure 4a).

We then determined the ability of various competitors to inhibit the binding of [^{125}I]-BH-[Thr,Nle]CCK-9 to porcine pancreatic membranes. The peptide agonists [Thr, Nle],CCK-9, sulphated and unsulphated gastrins and Boc CCK4 had an almost identical potency to inhibit the binding as their IC_{50} values were between 0.34 and 0.87 nM (Figure 4a). The CCK_B/gastrin receptor antagonists PD 135158 and L-365,260 were, respectively, 480 and 60 fold more potent inhibitors of the binding than the CCK_A receptor antagonist MK329, and respectively 4180 and 525 fold more potent than the CCK_A receptor antagonist, SR 27897 (Figure 4b).

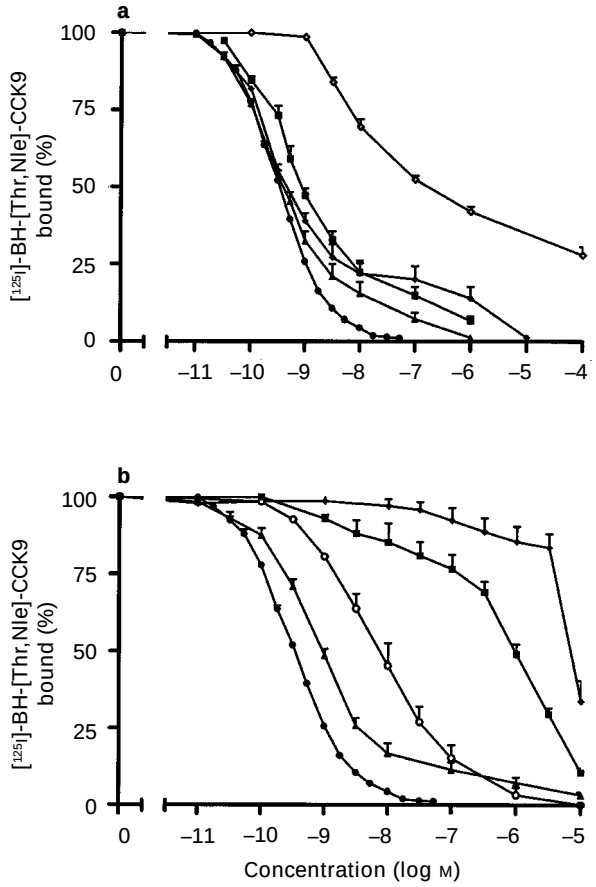

Figure 4 Inhibition of the binding of [^{125}I]-BH-[Thr,Nle]CCK-9 to pancreatic membranes by (a) CCK-9-related compounds: [Thr,Nle]CCK-9 (●), G17s (▲), G17ns (■), Boc CCK-4 (◆), and GTPγ[S] (◇); (b) CCK_B/gastrin receptor antagonists PD 135158 (▲) and L-365,260 (○) and CCK_A receptor antagonists SR 27897 (◆) and MK329 (■). Data are expressed as the percentage of maximal specific binding and are the mean, with vertical lines showing s.e.mean, of three to five experiments performed in duplicate.

This set of results suggests that CCK receptors identified in the porcine pancreatic membranes are of the CCK_B/gastrin subtype. Nevertheless, the competitive inhibition curves were biphasic (Figure 4a and b). For the CCK_B/gastrin ligands, 80% of the radioligand and competition occured at relatively low concentrations (<10 nM for gastrins and PD 135158 and <100 nM for L-365,260) and some further competition (~20%) was seen at much higher concentrations. Conversely, the CCK_A receptor antagonists appeared to displace just ~10–30% of [^{125}I]-BH-[Thr,Nle]CCK-9 at low concentrations, whereas complete competition apparently would require concentrations >10 μM.

Analysis of the competition data yielded Hill slopes which were significantly different from one for most of the competitors and revealed that the best fits were obtained with a two-site model. The two IC_{50} values for sulphated gastrin were 0.27 ± 0.01 nM and 148.41 ± 77.00 nM. The IC_{50} values for the other competitors are presented in Table 1. These results suggest that porcine pancreatic membranes which appear to display a single class of binding sites for the agonist [^{125}I]-BH-[Thr,Nle]-CCK9, in fact possess two receptor subtypes which were revealed by gastrins, PD 135158, L-365,260, MK329 and SR 27897 which are recognized to present a binding selectivity for CCK_A and CCK_B/gastrin receptors. We therefore analysed the binding of [^{125}I]-BH-[Thr,Nle]CCK-9 in the presence of either a CCK_A or a CCK_B/gastrin receptor selective antagonist at a saturating concentration.

Binding of [^{125}I]-BH-[Thr,Nle]CCK-9 in the presence of specific antagonists

In a first set of experiments, we analysed the binding of [^{125}I]-BH-[Thr,Nle]CCK-9 in the presence of 30 nM PD 135158, which should saturate CCK_B/gastrin receptors and therefore reveal putative CCK_A receptor sites (Figure 5a). Under these conditions, the CCK_A antagonist SR 27897 was 730 fold more potent in the presence than in the absence of PD 135158 (IC_{50} = 2.88 ± 0.58 nM, Hill slope = −0.99 ± 0.04 (n = 3), versus 4100.00 ± 180.00 nM (n = 4)). In contrast, sulphated gastrin a selective agonist for the CCK_B/gastrin receptor, inhibited the binding with an IC_{50} = 28.60 ± 2.70 nM, Hill slope = −0.98 ± 0.08 (n = 3) and was 65 fold less potent in the presence of PD 135158 than in the absence (Figure 5a). Thus, preliminary results showed that unsulphated gastrin, another selective agonist for the CCK_B/gastrin receptor, was also less potent in the presence of PD 135158 than in the absence (IC_{50} values from two experiments = 101.30 and 39.40 nM, Hill slope = −0.82 and −1.02).

In a second set of experiments we analysed the binding of [^{125}I]-BH-[Thr,Nle]CCK-9 in the presence of 1 μM SR 27897 (Figure 5b). At this concentration, we expected this CCK_A receptor antagonist to saturate CCK_A receptors (Gully *et al.*,

Table 1 Inhibition of [^{125}I]-BH-[Thr,Nle]CCK-9 binding to porcine pancreatic membranes

Compound	n	*Hill slope*	*IC_{50} 1* (nM)	*IC_{50} 2* (nM)
[Thr,Nle]CCK-9	5	−1.03 ± −0.02	0.25 ± 0.01	–
G17s	5	−0.78 ± −0.08	0.27 ± 0.01	148.41 ± 77.00
G17ns	5	−0.80 ± −0.13	0.75 ± 0.18	814.00 ± 515.00
Boc CCK4	3	−0.61 ± −0.09	0.28 ± 0.03	1432.00 ± 193.00
PD 135158	4	−0.84 ± −0.06	0.71 ± 0.07	1416.00 ± 358.00
L-365,260	3	−0.72 ± −0.07	2.32 ± 0.79	149.90 ± 83.50
MK329	3	−0.60 ± −0.13	1.62 ± 0.44	1073.00 ± 58.60
SR 27897	4	−1.61 ± −0.97	4100.00 ± 180.00	–

IC_{50} and Hill slopes were calculated from inhibition curves shown in Figure 4 after sigmoid non-linear regression by use of GraphPad PRISM software.

1993; Kennedy *et al*., 1995). Therefore, the residual binding would correspond to an homogeneous population of CCK_B/gastrin binding sites. After saturation of the CCK_A receptors, the inhibition curve for sulphated gastrin shifted to the left, was superimposed on that obtained with [Thr,Nle]CCK-9 ($IC_{50} = 0.38 \pm 0.04$ ($n = 3$) compared to 0.44 ± 0.06 nM ($n = 5$)) and a Hill slope value of -1.09 ± -0.01 was obtained. The inhibition curve for unsulphated gastrin was altered in the same way (data not shown). Thus, the residual binding sites were pharmacologically homogeneous and exhibited the same apparent high affinity for CCK and sulphated gastrin, a typical pharmacological feature of CCK_B/gastrin receptors.

Quantification of the CCK_A and CCK_B/gastrin binding sites

To quantify the CCK_B/gastrin receptors, we saturated the CCK_A-type binding sites with the specific CCK_A receptor antagonist SR 27897 and analysed the saturation of [^{125}I]-BH-[Thr,Nle]CCK-9 binding (Figure 6). The Scatchard plot was linear with a Hill slope of -0.99 ± -0.04 and the B_{max} obtained, which represented the number of CCK_B/gastrin binding sites in porcine pancreatic membranes, was 75.35 ± 11.18 fmol mg^{-1} protein. Knowing the total number of binding sites ($B_{max} = 110.64 \pm 12.50$ fmol mg^{-1} protein), we deduced that approximately 70% of these sites were CCK_B/gastrin receptors. Consistent with this result, the saturation analysis of the binding in presence of PD 135158 yielded a population of about 30% CCK_A receptor sites (Figure 6).

Photoaffinity labelling

Photoaffinity labelling of porcine pancreatic membranes by the agonist probe [^{125}I]-ASA-[Thr,Nle]CCK-9 yielded two main labelled components identified at 85–100 and 48–52 kDa which were not labelled in the presence of 1 μM [Thr,Nle]CCK-9, thus suggesting that they represent specific CCK binding proteins (Figure 7). We further evaluated the pharmacological specificity of these two binding proteins. The labelling of the component at 85–100 kDa was abolished in the presence of 500 nM SR 27897 (lane 4) while it was only slightly affected by 50 nM PD 135158 (lane 3). Conversely, the labelling of the component at 48–52 kDa was not affected by 500 nM SR 27897 (lane 4) whereas it was completely inhibited in the pre-

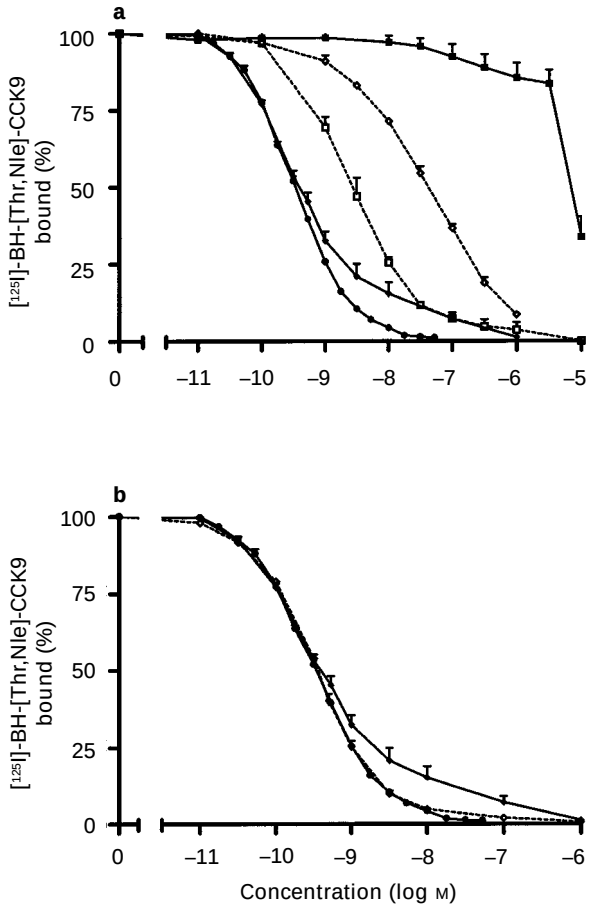

Figure 5 Inhibition of the binding of [^{125}I]-BH-[Thr,Nle]CCK-9 to pancreatic membranes by (a) [Thr,Nle]CCK-9 (●) or G17s (◆) in the absence (closed symbols) or in the presence (open symbols) of the CCK_A receptor antagonist SR 27897 1μM. (b) [Thr,Nle]CCK-9 (●) or G17s (◆) or CCK_A receptor antagonist SR 27897 (■) in the absence (closed symbols) or in the presence (open symbols) of the CCK_B/gastrin receptor antagonist PD 135158 30 nM. Data are expressed as the percentage of maximal binding and are the mean, with vertical lines showing s.e.mean, of three experiments performed in duplicate.

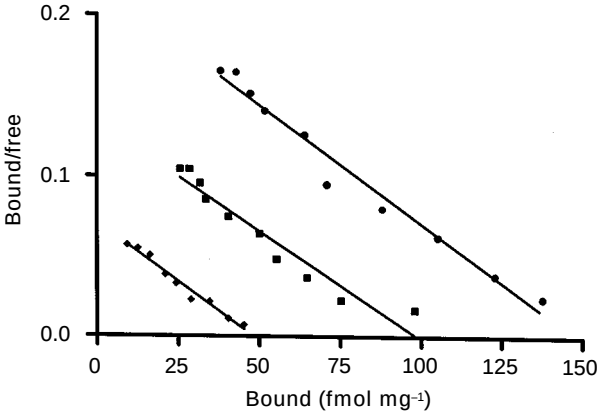

Figure 6 Scatchard plot of the binding of [^{125}I]-BH-[Thr,Nle]CCK-9 to pancreatic membranes before (●) and after saturation with the CCK_A receptor antagonist SR 27897 1μM (■) or with the CCK_B/gastrin receptor antagonist PD 135158 30 nM (◆). Data are expressed as the percentage of maximal binding and are representative of one experiment performed in duplicate ($n = 5$ for ●, $n = 3$ for ■,◆).

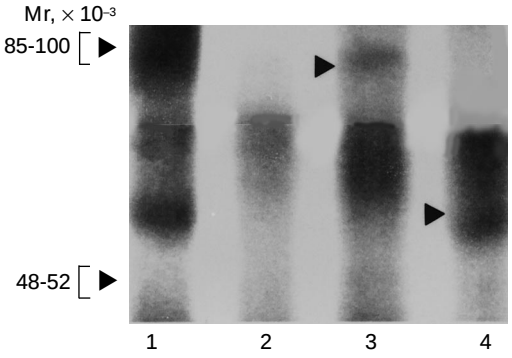

Figure 7 Identification of the molecular components of [^{125}I]-ASA-[Thr,Nle]CCK-9 binding in porcine pancreatic membranes by photoaffinity labelling. After binding of [^{125}I]-ASA-[Thr,Nle]CCK-9, the labelled membrane proteins were photolyzed and then separated by SDS-PAGE in a 10% polyacrylamide gel. Visualization was obtained by autoradiography. Two specific components at 85–100 kDa and 48–52 kDa are shown. Binding was performed in the absence of competitor (lane 1), in the presence of 1μM [Thr,Nle]CCK-9 (lane 2), 50 nM PD 135158 (lane 3) or 50 nM SR 27897 (lane 4) before photolysis under an u.v. source.

sence of 50 nM PD 135158 (lane 3). Taken together, these results suggest that the component identified at 85–100 kDa represents the porcine pancreatic CCK_A receptor and that identified at 48–52 kDa corresponds to the CCK_B/gastrin receptor.

Discussion

For the first time, pancreatic receptors for peptides of the CCK/gastrin family have been characterized in the pig, an animal from which many gastrointestinal regulatory peptides were initially isolated, and which has been proposed as a model for human nutrition (Miller & Ullrey, 1987). The pharmacological characterization was performed with [^{125}I]-BH-[Thr,Nle]CCK-9, a closely related analogue of the natural biologically active peptide CCK-8. This radioligand has been carefully validated as a powerful pharmacological and biochemical probe for the CCK_A and CCK_B/gastrin receptors in various models and species (Fourmy *et al*., 1987; Hadjiivanova *et al*., 1992; Le Meuth *et al*., 1993).

A single class of binding sites presenting a high affinity was demonstrated by the binding studies with [^{125}I]-BH-[Thr,Nle]CCK-9. Indeed, the value for the dissociation constant (0.22 nM) is consistent with that obtained for the high affinity sites of the CCK_A receptors located in the pancreas of rodents (Sankaran *et al*., 1980; 1982; Fourmy *et al*., 1987), dogs (Fourmy *et al*., 1987), and calves (Le Meuth *et al*., 1993), and for the CCK_B/gastrin receptors located in the pancreas of calves (Le Meuth *et al*., 1993) or in the cortex of several species (Innis & Snyder, 1980; Saito *et al*., 1980; Kuwahara *et al*., 1993). Lower affinity values of [^{125}I]-BH-[Thr,Nle]CCK-9 for CCK_B/gastrin receptors in the brain and stomach of guinea-pigs have been demonstrated (Hunter *et al*., 1993). However, the total number of CCK-binding sites in the pancreas of pigs is much smaller than in most rodents (Sankaran *et al*., 1980; Fourmy *et al*., 1987; Silvente-Poirot *et al*., 1993a).

The porcine pancreatic receptor for CCK displays a single affinity for the agonist CCK. Two sites of different affinities have been previously described in the pancreas of rodents, dogs and and calves (Sankaran *et al*., 1980; 1982; Fourmy *et al*., 1987; Le Meuth *et al*., 1993; Silvente-Poirot *et al*., 1993b). In rats, a single affinity class of binding sites was detected in pancreatic membranes with the same agonist radioligand (Hadjiivanova *et al*., 1992; Silvente-Poirot *et al*., 1993a) while both high- and low-affinity sites were found when an antagonist radioligand was used (Silvente-Poirot *et al*., 1993b). These results were explained by considering a model of receptors coupled to guanine-nucleotide-binding regulatory protein(s) which suggests that the balance between high- and low-affinity sites of a receptor is regulated by guanosine 5′-triphosphate (GTP). According to this model, on purified membranes in the absence of GTP, the population of high-affinity sites predominates by far over that of the low-affinity sites. This balance hinders the detection of low-affinity sites, especially when an agonist radioligand of high specific activity is used. Indeed, results showing inhibition of agonist binding in the presence of non-hydrolysable GTP supports the conclusion that CCK receptors identified in the preparation of membranes are functionally coupled to G proteins.

Competition studies revealed a pharmacological heterogenity within the population of binding sites in the porcine pancreas. Indeed, analysis of the binding data yielded Hill slopes different from one for most of the competitors and revealed that the best fits were obtained with a two-site model. Additional competition binding in the presence of selective antagonists of the CCK_A and CCK_B/gastrin receptors demonstrated that both receptor-subtypes are present in the porcine pancreas. Indeed, the binding sites revealed in the presence of the CCK_B/gastrin receptor antagonist PD 135158 retain their affinity for [Thr,Nle]CCK-9 and bind the CCK_A receptor antagonist SR 27897 with a high affinity, while they bound the CCK_B/gastrin agonist, gastrin with a low affinity. Conversely, binding sites revealed in the presence of the CCK_A receptor antagonist, SR 27897 bound CCK and gastrin with the same high affinity.

The apparent affinity of SR 27897 for the porcine CCK_A receptor is similar to that obtained for the cloned human CCK_A receptor (Kennedy *et al*., 1995) but was 5 fold lower than that for the native guinea-pig pancreatic CCK_A receptor (Gully *et al*., 1993). These small differences might be due to either the biological models or to the animal species used or both. The apparent affinity of PD 135158 for the porcine CCK_B/gastrin receptor is similar to that obtained for other native or cloned CCK_B/gastrin receptors (Hughes *et al*., 1990; Pisegna *et al*., 1992; Kopin *et al*., 1992; Wank *et al*., 1992b). The value of the apparent affinity of non-sulphated gastrin for the porcine CCK_A receptor ($IC_{50} = 63.50$ nM) is higher than that (0.1 to 1μM) most often shown for the native and the cloned CCK_A receptors from several species (Jensen *et al*., 1989; Wank *et al*., 1992a; De Weerth *et al*., 1993). In contrast, the affinity of gastrin for the porcine CCK_B/gastrin receptor is identical to that for other native or cloned CCK_B/gastrin receptors (Hughes *et al*., 1990; Pisegna *et al*., 1992; Kopin *et al*., 1992; Wank *et al*., 1992b; Le Meuth *et al*., 1993; Dufresne *et al*., 1996). In the porcine pancreas, gastrin appears to present a 186 fold higher affinity for the CCK_B/gastrin receptor than for the CCK_A receptor. Generally, higher selectivity factors have been found previously for this agonist. However, a recent structure-function study on the cloned rat CCK_A and CCK_B/gastrin receptors demonstrated a selectivity factor of only 263 for gastrin (Silvente-Poirot & Wank, 1996).

Finally, a proportion of 70% CCK_B/gastrin receptors and 30% CCK_A receptors was estimated. Additional evidence for the presence of the two CCK receptors was provided by identification of two distinct membrane proteins of 50 and 85–100 kDa which display pharmacological features of each receptor. The apparent mass of the porcine pancreatic CCK_B/gastrin receptor (50 kDa) is consistent with that previously shown for the porcine brain CCK receptor (Thiele & Fahrenholz, 1993), the pancreatic CCK_B/gastrin receptor in dogs (Fourmy *et al*., 1987) and calves (Le Meuth *et al*., 1993) but differs from that found in the stomach (Matsumoto *et al* 1987; Kopin *et al*., 1992). Glycosylation differences according to the cell where the receptor is expressed could account for these discrepancies. For example, the cloned bovine pancreatic CCK_B/gastrin receptor has an apparent mass of 82 kDa when expressed in COS-7 cells compared to 50 kDa measured in the natural tissue (Le Meuth *et al*., 1993; Dufresne *et al*., 1996). The apparent mass of the porcine pancreatic CCK_A receptor (85–100 kDa) is identical to that described in the pancreas of rats (Powers *et al*., 1988; Silvente-Poirot *et al*., 1994), guinea-pigs (Fourmy *et al*., 1987), and calves (Le Meuth *et al*., 1993). The width of the band is consistent with a high degree of glycosylation as previously suggested (Powers *et al*., 1988).

So far, a predominance of CCK_B/gastrin-receptor sites and mRNAs has been described in the pancreas of large adult mammals such as 3 month-old calves (Le Meuth *et al*., 1993) and has been suggested in man (Dufresne *et al*., 1995). However, in man, the corresponding protein has not been identified. In fact, high-affinity CCK receptors were not detected in preparations of human pancreatic acini (Susini *et al*., 1986). In the canine pancreas only 20% of CCK receptors are of the CCK_B/gastrin subtype (Fourmy *et al*., 1987), whereas in the guinea-pig pancreas, very few CCK_B/gastrin receptors have been described (Yu *et al*., 1987; 1990). On the other hand, the rat and mouse pancreas has been shown to contain only CCK_A receptors (Hadjiivanova *et al*., 1992; Povoski *et al*., 1994).

Further investigation is required to determine the biological functions regulated by porcine pancreatic CCK_A and CCK_B/gastrin receptors. Our previous physiological data indicated that CCK_A receptors might not be involved in the secretory responses induced by a meal or by exogenous CCK (Lhoste *et al*., 1995), and that supra-physiological doses of CCK are required to stimulate enzyme output (Cuber *et al*., 1989). The activation of the CCK_B/gastrin receptor might account for the

biological response to gastrin instead of CCK. Indeed, biological levels of various molecular forms of gastrin stimulate the flow rate of exocrine secretion in the porcine isolated-perfused pancreas (Jensen *et al*., 1980). If this is true, the CCK_B/gastrin receptors which are predominant in the porcine pancreas would be located on the ductal cells which secrete most of the water constituent of pancreatic exocrine secretion. Since we detected the CCK receptors in membrane preparations from the total pancreas, the location of these receptors within the pancreatic tissue is not known. The pancreas typically consists of 90% acinar cells, which synthesize and secrete the digestive enzymes, and 10% ductal and endocrine cells. In the pancreas of rats, CCK_A receptors are present not only in the membranes of acinar cells, but also in those of endocrine cells (mainly β cells) (Verspohl *et al*., 1994), islet blood vessel cells and ductal cells (Sakamoto *et al*., 1985). Previous data suggest that the biological function mediated by the pancreatic CCK receptors may vary according to animal species. In dog and guinea-pig pancreatic acini, which possess both CCK_A and CCK_B/gastrin receptors, enzyme secretion is only stimulated by the activation of the CCK_A receptor (Sankaran *et al*., 1980; 1982; Fourmy *et al*., 1987; Yu *et al*., 1987). In contrast, in the rat pancreatic acinar cell line AR4-2J, CCK-stimulated enzyme exocytosis is mediated by both CCK receptors (Bertrand *et al*., 1994). In man, the pancreatic secretory response to a meal and to exogenous CCK involves a co-ordinated mechanism involving CCK_A receptors and a cholinergic input (Beglinger *et al*., 1992 Soudah *et al*., 1992).

In conclusion, we have presented pharmacological and biochemical data demonstrating that CCK receptors are expressed in the porcine pancreas in a proportion of about 70% CCK_B/gastrin receptors and 30% CCK_A receptors. In this respect, the pancreas of pigs presents strong similarities with that of man and calves. The important species differences between high mammals and rodents concerning the expression of pancreatic CCK receptors should be considered for functional studies and preclinical drug testing.

The authors thank D. Besnard for his careful technical assistance and K. Kennedy for correcting the manuscript.

References

BEGLINGER, C., HILDEBRAND, P., ADLER, G., WERTH, B., LUO, H., DELCO, F. & GYR, K. (1992). Postprandial control of gallbladder contraction and exocrine pancreatic secretion in man. *Eur. J. Clin. Invest*., **22,** 827–834.

BERTRAND, V., BASTIÉ, M.J., BIGAUD, C., PYRONNET, S., VAYSSE, N. & PRADAYROL, L. (1994). Pharmacological study of gastrin-mediated amylase release in pancreatic acinar cells (AR4-2J). *Regul. Peptides*, **54,** 513–525.

CHANG, R.S.L., LOTTI, V.J., CHEN, T.B. & KUNKEL, K.A. (1986). Characterization of the binding of [^{3}H]-(±)-L-364,718: a new potent, nonpeptide cholecystokinin antagonist radioligand selective for peripheral receptors. *Mol. Pharmacol*., **30,** 212–217.

CUBER, JC., CORRING, T., LEVENEZ, F., BERNARD, C. & CHAYVIALLE, J.A. (1989). Effects of cholecystokinin octapeptide on the pancreatic exocrine secretion in the pig. *Can. J. Physiol. Pharmacol*., **67,** 1391–1397.

De WEERTH, A., PISEGNA, J.R., HUPPI, K. & WANK, S.A. (1993). Molecular cloning, functional expression and chromosomal localization of the human cholecystokinin type A receptor. *Biochem. Biophys. Res. Commun*., **194,** 811–818.

DUFRESNE, M., ESCRIEUT, C., CLERC, P., LE HUÉROU-LURON, I., PRATS, H., BERTRAND, V., LE MEUTH, V., GUILLOTEAU, P., VAYSSE, N. & FOURMY, D. (1996). Molecular cloning, developmental expression and pharmacological characterization of the CCK_B/gastrin receptor in the calf pancreas. *Eur. J. Pharmacol*., **297,** 165–179.

DUFRESNE, M., ESCRIEUT, C., CLERC P., SAILLAN C., KENNEDY K., FANJUL, M., MENGOD, G., REAL, F., MORODER, L., VAYSSE, N. & FOURMY, D. (1995). Predominant expression of CCK-B/gastrin receptor in the human pancreas. *Digestion*, **56,** 280.

EYSSELEIN, V.E., REEVE, J.R.J. & EBERLEIN G. (1986). Choleascystokin-gene structure, and molecular forms in tissue and blood. *Z. Gastroenterol*., **24,** 645–659.

FOURMY, D., PRADAYROL, L., VAYSSE N., SUSINI C. & RIBET A. (1984). ^{125}I-(Thr_{34},Nle_{37})-CCK31-39 a non oxidizable tracer for the characterization of CCK receptor on pancreatic acini and radio-immunoassay of C-terminal CCK peptides. *J. Immunoassay*, **5,** 99–120.

FOURMY, D., ZAHIDI A., FABRE, R., GUIDET M., PRADAYROL, L. & RIBET, A. (1987). Receptors for cholecystokinin and gastrin peptides display specific binding properties and are structurally different in guinea-pig and dog pancreas. *Eur. J. Biochem*., **165,** 683–692.

GULLY, D., FRÉHEL, D., MARCY, C., SPINAZZÉ, A., LEPSY, L., NELIAT, G., MAFFRAND, J.P. & LE FUR, G. (1993). Peripheral biological activity of SR 27897: a new potent non-peptide antagonist of CCK_A receptors. *Eur. J. Pharmacol*., **232,** 13–19.

HADJIIVANOVA, C., DUFRESNE, M., POIROT, S., SOZZANI, P., VAYSSE, N., MORODER, L. & FOURMY, D. (1992). Pharmacological and biochemical characterization of cholecystokinin/gastrin receptors in developing rat pancreas. Age-related expression of distinct receptor glycoforms. *Eur. J. Biochem*., **204,** 273–279.

HUGHES, J., BODEN, P., COSTALL, B., DOMENEY, A., KELLY, E., HORWELL, D.C., HUNTER, J.C., PINNOCK, R.D. & WOODRUFF, G.N. (1990). Development of a class of selective cholecystokinin type B receptor antagonists having potent anxiolytic activity. *Proc. Natl. Acad. Sci. U.S.A*., **87,** 6728–6732.

HUNTER, J.C., SUMAN-CHAUHAN, N., MEECHAM, K.G., DISSANAYAKE, V.U.K., HILL, D.R., PRITCHARD, M.C., KNEEN, C.O., HORWELL, D.C. HUGHES, J. & WOODRUFF, G.N. (1993). [^{3}H]PD 140376: a novel and highly selective antagonist radioligand for the cholecystokinin$_B$/gastrin receptor in guinea pig cerebral cortex and gastric mucosa. *Mol. Pharmacol*., **43,** 595–602.

INNIS, R.B. & SNYDER, S.H. (1980). Distinct cholecystokinin receptors in brain and pancreas. *Proc. Natl. Acad. Sci. U.S.A*., **77,** 6917–6921.

JENSEN, R.T., WANK, S.A., ROWLEY, W.H., SATO, S. & GARDNER, J.D. (1989). Interaction of CCK with pancreatic acinar cells. *Trends Pharmacol. Sci*., **10,** 418–423.

JENSEN, S.L., REHFELD, J.F., HOLST, J.J., FAHRENKRUG, J., NIELSEN, O.V. & SCHAFFALITZKY DE MUCKADELL OB. (1980). Secretory effects of gastrins on isolated perfused porcine pancreas. *Am. J. Physiol*., **238,** E186–E192.

KENNEDY,K., ESCRIEUT, C., DUFRESNE, M., CLERC, P., VAYSSE, N. & FOURMY, D. (1995). Identification of a region of the N-terminal of the human CCK-A receptor essential for the high affinity interaction with agonist CCK. *Biochem. Biophys. Res. Commun*., **213,** 845–852.

KOPIN, A.S., LEE, Y.M., MCBRIDE, E.W., MILLER, L.J., LU, M., LIN, H.Y., KOLAKOWSKI, L.F., JR. & BEINBORN, M. (1992). Expression cloning and characterization of the canine parietal cell gastrin receptor. *Proc. Natl. Acad. Sci. U.S.A*., **89,** 3605–3609.

KUWAHARA, T., KUDOH, T., NAKANO, A., YOSHIZAKI, H., TAKAMIYA, M., NAGASE, H. & ARISAWA, M. (1993). Species specificity of pharmacological characteristics of CCK-B receptors. *Neurosci. Lett*., **158,** 1–4.

LE MEUTH, V., PHILOUZE ROME, V., LE HUEROU-LURON, I., FORMAL, M., VAYSSE, N., GESPACH, C., GUILLOTEAU, P. & FOURMY, D. (1993). Differential expression of A- and B-subtypes of cholecystokinin/gastrin receptors in the developing calf pancreas. *Endocrinology*, **133,** 1182–1191.

LHOSTE, E.F., GUEUGNEAU, A.M., GAROFANO, A., PHILIPPE, C., LEVENEZ, F. & CORRING, T. (1995). Role of CCK in the regulation of secretion and adaptation in the pig pancreas. *Pancreas*, **11,** 86–94.

LOTTI, V.J. & CHANG, R.S.L. (1989). A new potent and selective non-peptide gastrin antagonist and brain cholecystokinin receptor (CCK-B) ligand: L-365,260. *Eur. J. Pharmacol*., **162,** 273–280.

LOWRY, O.H., ROSEBROUGH, N.J., LEWIS FARR, A. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol Chem*. **193,** 265–275.

MATSUMOTO, M., PARK, J. & YAMADA, T. (1987). Gastrin receptor characterization: affinity cross-linking of the gastrin receptor on canine gastric parietal cells. *Am. J. Physiol*., **252,** G143–G147.

MILLER, E.R. & ULLREY, D.E. (1987). The pig as a model for human nutrition. *Ann. Rev. Nutr.*, **7,** 361 – 382.

MUTT, V. & JORPES, J.E. (1968). Structure of porcine cholecystokinin-pancreozymin. *Eur. J. Biochem.*, **6,** 156 – 162.

PISEGNA, J.R., DE WEERTH, A., HUPPI, K. & WANK, S.A. (1992). Molecular cloning of the human brain and gastric cholecystokinin receptor: structure, functional expression and chromosomal localization. *Biochem. Biophys. Res. Commun.*, **189,** 296 – 303.

POVOSKI, S.P., ZHOU, W., LONGNECKER, D.S. & BELL, R.H. (1994). Novel expression of gastrin (CCK-B) receptors in pancreatic carcinomas and dysplastic pancreas from transgenic mice. *Am. J. Surg.*, **167,** 120 – 126.

POWERS, S.P., FOURMY, D., GAISANO, H. & MILLER, L.J. (1988). Intrinsic photoaffinity labeling probes for cholecystokinin (CCK)-gastrin family receptors. *J. Biol. Chem.*, **263,** 5295 – 5300.

REHFELD, J.F., HOLST, J.J. & JENSEN, S.L. (1982). The molecular nature of vascularly released cholecystokinin from the isolated perfused porcine duodenum *Regul. Pept.*, **3,** 15 – 28.

SAITO, A., SANKARAN, H., GOLDFINE, I.D. & WILLIAMS, J.A. (1980). Cholecystokinin receptors in the brain: Characterization and distribution. *Science*, **208,** 1155 – 1156.

SAKAMOTO, C., GOLDFINE, I.D., ROACH, F. & WILLIAMS, J.A. (1985). Localization of saturable CCK binding sites in rat pancreatic islets by light and electron microscope autoradiography. *Diabetes*, **34,** 390 – 394.

SANKARAN, H., GOLDFINE, I., DEVENEY, C., WONG, K., & WILLIAMS, J. (1980). Binding of cholecystokinin to high affinity receptors on isolated rat pancreatic acini. *J. Biol. Chem.*, **255,** 1849 – 1853.

SANKARAN, H., GOLDFINE, I.D., BAILEY, A., LICKO, V. & WILLIAMS, J. (1982). Relationship of cholecystokinin receptor binding to regulation of biological functions in pancreatic acini. *Am.J. Physiol.*, **242,** G250 – G257.

SILVENTE-POIROT, S., DUFRESNE, M., VAYSSE, N. & FOURMY, D. (1993a). The peripheral cholecystokinin receptors. *Eur. J. Biochem.*, **215,** 513 – 529.

SILVENTE-POIROT, S., HADJIIVANOVA, C., ESCRIEUT, C., DUFRESNE, M., MARTINEZ, J., VAYSSE, N. & FOURMY, D. (1993b). Study of the states and populations of the rat pancreatic cholecystokinin receptor using the full peptide antagonist JMV 179. *Eur. J. Biochem.*, **212,** 529 – 538.

SILVENTE-POIROT, S., ESCRIEUT, C., DUFRESNE, M., MARTINEZ, J., BOUISSON, M., VAYSSE, N. & FOURMY, D. (1994). Photoaffinity labeling of rat pancreatic cholecystokinin type A receptor antagonist binding sites demonstrates the presence of a truncated cholecystokinin type A receptor. *Mol. Pharmacol.*, **45,** 599 – 607.

SILVENTE-POIROT, S. & WANK, S.A. (1996). A segment of five amino acids in the second extracellular loop of the cholecystokinin B receptor is essential for selectivity of the peptide agonist gastrin. *J. Biol. Chem.*, **271,** 14698 – 14706.

SOUDAH, H.C., LU, Y., HASLER, W.L. & OWYANG, C. (1992). Cholecystokinin at physiological levels evokes pancreatic enzyme secretion via a cholinergic pathway. *Am. J. Physiol.*, **263,** G102 – G107.

SUSINI, C., ESTIVAL, A., SCEMAMA, J.L., RUELLAN, C., VAYSSE, N., CLEMENTE, F., ESTEVE, J.P., FOURMY, D. & RIBET. A. (1986). Studies on human pancreatic acini: action of secretagogues on amylase release and cellular cyclic AMP accumulation. *Pancreas* **1,** 124 – 129.

TAYLOR, C.W. (1990). The role of G proteins in transmembrane signalling. *Biochem. J.*, **272,** 1 – 13.

THIELE, C. & FAHRENHOLZ, F. (1993). Photoaffinity labeling of central cholecystokinin receptors with high efficiency. *Biochemistry*, **32,** 2741 – 2746.

VERSPOHL, E.J., HAFNER, B., HE, X. & KNITTEL, J.J. (1994). Evidence for cholecystokinin receptor subtype in endocrine pancreas. *Peptides,* **15,** 1353 – 1360.

WANK, S.A., HARKINS, R., JENSEN, R.T., SHAPIRA, H., DE WEERTH, A. & SLATTERY, T. (1992a). Purification, molecular cloning, and functional expression of the cholecystokinin receptor from rat pancreas. *Proc. Natl. Acad. Sci. U.S.A.*, **89,** 3125 – 3129.

WANK, S.A., PISEGNA, J.R. & DE WEERTH, A. (1992b). Brain and gastrointestinal cholecystokinin receptor family: structure and functional expression. *Proc. Natl. Acad. Sci. U.S.A.*, **89,** 8691 – 8695.

WILLIAMS, J.A. (1982). Cholecystokinin: a hormone and a neurotransmitter. *Biomed. Res.*, **3,** 107 – 121.

WILLIAMS, J.A. & BLEVINS, G.T.Jr. (1993). Cholecystokinin and regulation of pancreatic acinar cell function. *Physiol. Rev.*, **73,** 701 – 723

YU, D.H., HUANG, S.C., WANK, S.A., MANTEY, S., GARDNER, J.D. & JENSEN, R.T. (1990). Pancreatic receptors for cholecystokinin: evidence for three receptor classes. *Am. J. Physiol.*, **258,** G86 – G95.

YU, D., NOGUCHI, M., ZHOU, Z., VILLANUEVA, M.L., GARDNER, J.D. & JENSEN, R.T. (1987). Characterization of gastrin receptors on guinea pig pancreatic acini. *Am. J. Physiol.*, **253,** G793 – G801.

(Received April 22, 1996
Revised October 20, 1996
Accepted October 31, 1996)