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Different actions of CCK on pancreatic and gastric growth in the rat: effect of CCK_A receptor blockade

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1 It is now well established that cholecystokinin (CCK) has a major physiological role in the regulation of pancreatic secretion and gastro-intestinal (GI) motility. Both these actions are mediated by stimulation of CCK_A-receptors located on pancreatic acini and GI smooth muscle cells. While chronic administration of CCK-like peptides invariably causes pancreatic hypertrophy and hyperplasia, their action on gastric growth remains controversial.

2 In the present investigation the action of exogenous and endogenous CCK on both pancreatic and gastric growth was studied in the same animal. In addition, the ability of dexloxiglumide, a new potent and selective CCK_A-receptor antagonist, to counteract CCK-mediated effects was evaluated.

3 The amphibian peptide caerulein (1 μg kg^{-1} intraperitoneally three times daily) was used as a CCK agonist, while camostate (200 mg kg^{-1} intragastrically once daily), a synthetic protease inhibitor, was used to release endogenous CCK. They were administered to rats for seven days with or without dexloxiglumide (25 mg kg^{-1} subcutaneously 15 min before the stimulus). On the eighth day, animals were killed, the pancreas and stomach excised, weighed, homogenized and their protein and DNA content measured.

4 Both exogenous and endogenous CCK increased the weight of the pancreas as well as the total pancreatic protein and DNA content. Dexloxiglumide, which alone did not affect pancreatic size and composition, was able to counteract both caerulein- and camostate-induced pancreatic changes. Neither stimuli affected gastric growth in respect of weight and composition of the oxyntic gland area and the antrum.

5 These results show different effects of CCK on pancreatic and gastric growth. The CCK-induced pancreatic hypertrophy and hyperplasia are blocked by the potent and specific CCK_A-receptor antagonist, dexloxiglumide. This compound therefore represents a useful tool to investigate CCK-receptor interactions in peripheral organs.

Keywords: CCK; CCK-receptors; CCK-antagonists; dexloxiglumide; pancreatic growth; gastric growth

Introduction

It is now well established that cholecystokinin (CCK) exerts a major role in the physiological regulation of pancreatic enzyme secretion (for review see Chey, 1993). *In vivo* experiments have shown that both exogenous and endogenous CCK increase enzyme output from the pancreas of different animal species, including man (for review see Liddle, 1994). Along with its stimulant action on pancreatic secretion, the peptide is able to produce both hypertrophy and hyperplasia of the exocrine pancreas (Fölsch, 1984). Indeed, it increases pancreatic weight without affecting the endocrine component (i.e. islets of Langerhans) of the gland (Petersen *et al*., 1978).

CCK is also one of the most important hormones involved in the regulation of gastro-intestinal (GI) motility. The fact that minute amounts of CCK are sufficient to affect GI motility under different *in vivo* and *in vitro* conditions would indicate that its action on the gut is one of physiological actions of the peptide (Scarpignato *et al*., 1993; Grider, 1994). Indeed, the peptide and its synthetic derivatives (e.g. CCK-8 and its amphibian counterpart, the decapeptide caerulein) significantly delay emptying of gastric contents in both animals and man. The fact that CCK, in doses corresponding to postprandial plasma levels, strongly affects emptying rate suggests that the peptide is a physiological regulator of gastric emptying. Despite the large number of studies dealing with gastric motor actions of CCK-like peptides, few data (Hoang *et al*., 1988; Axelson *et al*., 1990; Dembinski *et al*., 1990) are available on the peptide effect on gastric growth. Although endogenous CCK (released by bombesin) was found to increase stomach weight and gastric DNA content (Dembinski *et al*., 1990), other studies with either exogenous (Hoang *et al*., 1988; Axelson *et al*., 1990) or endogenous CCK (Axelson *et al*., 1990) were unable to confirm this finding.

It is now clear that specific receptors mediate the biological actions of CCK on either exocrine pancreas (Liddle, 1994) and GI smooth muscle (Scarpignato *et al*., 1993) and they both belong to the CCK_A-subtype. The availability of potent and selective CCK-receptor antagonists (Woodruff & Hughes, 1991; Scarpignato, 1992; D'Amato *et al*., 1994) represents a useful tool to investigate CCK-receptor interaction in peripheral organs and has therefore stimulated a broad array of investigations into the physiological actions of the peptide.

At least eight classes of CCK-receptor antagonists are available (Scarpignato, 1992; D'Amato *et al*., 1994). Amongst the amino acid derivatives, proglumide, which was discovered more than 20 years ago, has been considered as the prototype CCK antagonist (Hahne *et al*., 1981). Its low potency and specificity (the compound effectively also binds gastrin

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receptors) stimulated the synthesis of glutaramic acid derivatives, the most interesting of which are lorglumide and loxiglumide. These are potent, specific and competitive antagonists of CCK_A-receptors (Scarpignato *et al*., 1989; Varga *et al*., 1989). They are active after oral administration and able to antagonize the effects of both endogenous and exogenous CCK (Rovati & Makovec, 1988). Since loxiglumide is a racemic mixture, both isomeric forms can be obtained. The dextro isomer, dexloxiglumide, is about twice as potent as the parent compound because the anti-CCK activity is specific for the **R**-form, whereas the **S**-isomer is almost ineffective (Makovec *et al*., 1989). Although about 30 times less potent than devazepide, a benzodiazepine derivative, dexloxiglumide is endowed with a better selectivity towards CCK_A-receptors. *In vitro* binding studies have shown that the CCK_B IC_{50}/CCK_A IC_{50} ratio is 169.2 and 43.6 for dexloxiglumide and devazepide, respectively (Makovec *et al*., 1989; D'Amato *et al*., unpublished data). Results from our laboratories have recently confirmed the selectivity of this new compound for CCK_A receptors *in vivo* (Scarpignato *et al*., 1996).

The aim of this investigation was: (1) to study the action of exogenous and endogenous CCK on both pancreatic and gastric growth in the same animal and (2) to evaluate the ability of dexloxiglumide to counteract CCK-mediated effects.

In these experiments the CCK analogue caerulein was used as the exogenous CCK agonist. Caerulein is an amphibian decapeptide in which the C-terminal octapeptide sequence differs only in one amino acid residue from CCK. As a consequence, these two peptides express the same spectrum of biological actions (Bertaccini, 1976). From an experimental point of view, caerulein has the advantage of being more resistant to enzymatic cleavage than CCK (Tokiwa *et al*., 1975). Camostate, a synthetic protease inhibitor, was employed to release endogenous CCK (Göke *et al*., 1986; Douglas *et al*., 1990; Varga & Scarpignato, 1996).

Preliminary results of the present investigation have been presented at the 4th Congress of the International Brain-Gut Society (Pécs, September 1996) and published in abstract form (Varga *et al*., 1997).

Methods

Animals

Male Sprague-Dawley rats, purchased from Lati Ltd. (Gödöllô, Hungary), were housed for at least one week before use, at constant temperature (24°C), under a 12–12 h light/dark cycle, and fed standard rat chow *ad libitum*.

Surgery

Under pentobarbitone (40 mg kg^{-1} intraperitoneally) anaesthesia, a Gregory-type gastric cannula was implanted in the forestomach of some animals. Experiments were started after at least 1 week to allow for recovery.

Experimental design

To study the effect of caerulein on pancreatic and gastric growth as well as its interaction with dexloxiglumide, four different groups of animals (weight range 130–160 g) were used. They were treated with the following compounds given three times daily, for seven days: group 1, saline: group 2, dexloxiglumide (25 mg kg^{-1}); group 3, caerulein (1 μg kg^{-1}); group 4, caerulein + dexloxiglumide (doses as above). We (Scarpignato *et al*., 1996) have previously shown that at the dose used in these experiments (i.e. 25 mg kg^{-1}), dexloxiglumide is able to counteract CCK-8-induced delay in gastric emptying, affecting only slightly and transiently pentagastrin-induced acid hypersecretion in the rat. Both caerulein and the CCK_A-antagonist were administered subcutaneously, with the antagonist being injected 15 min before the agonist. The last dose of the peptide was injected 12 h before the rats were killed after which the entire pancreas and stomach were excised.

Four additional groups of rats (weight range 320–370 g) were studied in order to investigate the effect of camostate. They were equipped with a gastric cannula to allow direct intragastric administration of camostate, a protease inhibitor which releases endogenous CCK (Göke *et al*., 1986; Douglas *et al*., 1990; Varga & Scarpignato, 1996). They were treated with the following compounds given once daily, for seven days: group 1, saline; group 2, dexloxiglumide (25 mg kg^{-1}); group 3, camostate (200 mg kg^{-1}); group 4, camostate plus dexloxiglumide (doses as above). Dexloxiglumide was administered subcutaneously 15 min before the administration of the protease inhibitor.

Tissue assays

In both trophic studies aninals were killed on the eighth day after overnight fasting. The pancreas was excised, carefully trimmed free of fat, mesentery and lymph nodes, weighed and homogenized in buffer (pH 8.0) of the following composition (mM): Tris-HCl 100, KCl 100 and $CaCl_2$ 20. The stomach was also removed, opened along the greater curvature and gently rinsed with water. The oxyntic gland area was carefully separated from the pyloric gland area (antrum) and forestomach. The oxyntic gland area and the antrum were homogenized in distilled water.

Protein and DNA concentrations were then measured in the pancreatic and gastric tissues. Protein was determined according to the method of Lowry *et al*. (1951) with bovine albumin (Sigma St. Louis, MO, U.S.A.) used as a standard. Tissue DNA content was extracted according to Munro & Fleck (1966) and measured by Burtons method (1956) with calf thymus DNA used as a standard.

Evaluation of data

All the values are presented as a mean ± s.e.mean. Tissue data (i.e. pancreatic and gastric weight, protein and DNA content) were normalized to final body weight and expressed as mg kg^{-1}. Comparisons between the groups were performed by analysis of variance (ANOVA) and Dunn's multiple range test (1961). All the calculations were made by using InStat program (GraphPad Software Inc., San Diego, California, U.S.A.).

Drugs

Dexloxiglumide (compound coded CR-2017, molecular weight 461.4, batch PP 9200) was a generous gift of Dr Lucio Rovati (Rotta Research Laboratorium, Monza, Italy). The compound was dissolved daily in saline and pH adjusted to 7.5–8.0 by addition of 0.1 N NaOH under continuous stirring. Caerulein (Farmitalia-Carlo Erba SpA, Milan, Italy) was dissolved in 0.9 M NaCl containing 0.2% BSA (Sigma St. Louis MO) whereas camostate (compound marked FOY-305, Ono Pharmaceuticals Co., Osaka, Japan) was dissolved in warmed saline. All the other chemicals were from Sigma (St. Louis, MO, U.S.A.).

Results

Effect of exogenous CCK on pancreatic and gastric growth

Final body weight was similar in each of the four groups of animals studied (data not shown). As previously shown (Varga *et al*., 1985; 1989; Scarpignato *et al*., 1989) administration of caerulein increased the weight of the pancreas and the total pancreatic protein and DNA contents (Figure 1). Dexloxiglumide, administered alone did not significantly affect pancreatic size and composition. However, it did almost counteract the caerulein-induced increase in pancreatic weight, protein and DNA content (Figure 1).

Caerulein or dexloxiglumide, given alone or in combination, had no statistically significant effect on gastric growth in respect of weight, DNA or protein content of either the oxyntic gland area or the antrum (Figures 2 and 3).

Effect of endogenous CCK on pancreatic and gastric growth

Body weight was not affected by any of the treatments (data not shown). When camostate was administered, it also increased the weight of the pancreas and the total pancreatic protein and DNA content (Figure 4). The administration of dexloxiglumide, again, abolished the growth promoting effect of this endogenous CCK releaser (Figure 4).

Camostate given alone or in combination with dexloxiglumide did not significantly affect the weight, DNA or protein content of either the oxyntic gland area or the antrum (Figures 5 and 6).

Discussion

Rothman & Wells (1967) were the first to show that treatment of rats with CCK caused an increase in pancreatic weight and enzyme content as well as increased acinar cell size, suggesting acinar cell hypertrophy. Subsequent studies in rats (for review see Fölsch, 1984) have confirmed these observations and, in addition, have shown an increased content and rate of synthesis of DNA, suggesting hyperplasia to be a constant

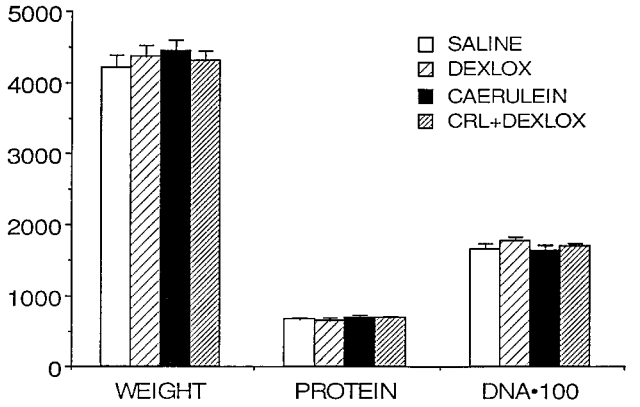

Figure 2 Oxyntic gland area weight, protein and DNA content (expressed as mg kg^{-1} of final body weight) in rats after short-term treatment (7 days) with saline or caerulein (CRL; 1 μg kg^{-1} three times daily) with and without dexloxiglumide (DEXLOX; 25 mg kg^{-1} three times daily). Each column refers to the mean of the values obtained from 10 animals. Vertical lines show s.e.mean.

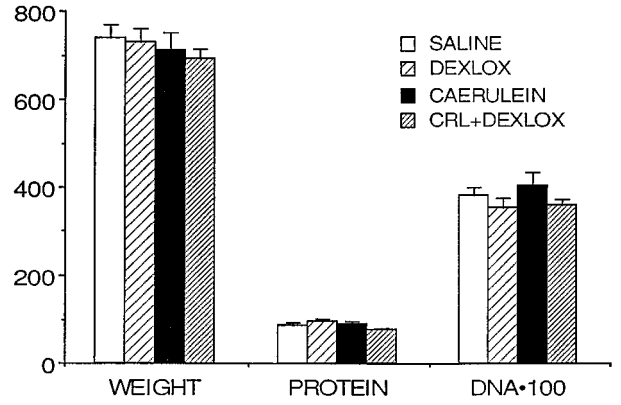

Figure 3 Gastric antrum weight, protein and DNA content (expressed as mg kg^{-1} of final body weight) in rats after short-term treatment (7 days) with saline or caerulein (CRL; 1 μg kg^{-1} three times daily) with and without dexloxiglumide (DEXLOX; 25 mg kg^{-1} three times daily). Each column refers to the mean of the values obtained from 10 animals. Vertical lines show s.e.mean.

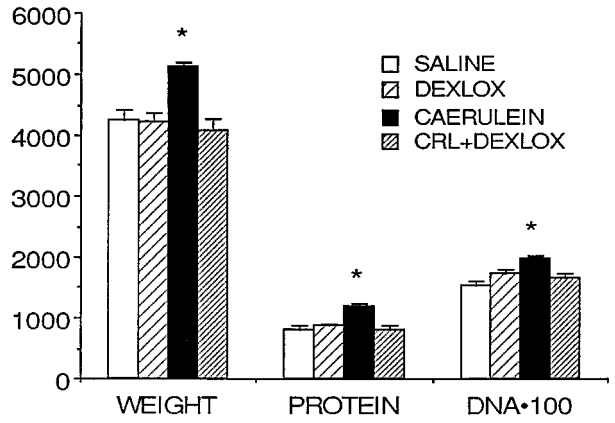

Figure 1 Pancreatic weight, protein and DNA content (expressed as mg kg^{-1} of a final body weight) in rats after short-term treatment (7 days) with saline or caerulein (CRL; 1 μg kg^{-1} three times daily) with and without dexloxiglumide (DEXLOX; 25 mg kg^{-1} three times daily). Each column refers to the mean of the values obtained from 10 animals. Vertical lines show s.e.mean. *$P<0.01$ *versus* saline value.

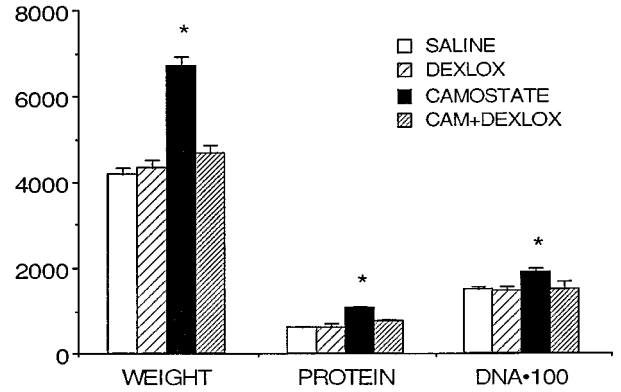

Figure 4 Pancreatic weight, protein and DNA content (expressed as mg kg^{-1} of final body weight) in rats after short-term treatment (7 days) with saline or camostate (CAM; 200 mg kg^{-1} once daily) with and without dexloxiglumide (DEXLOX; 25 mg kg^{-1} three times daily). Each column refers to the mean of the values obtained from 10 animals. Vertical lines show s.e.mean. *$P<0.01$ *versus* saline value.

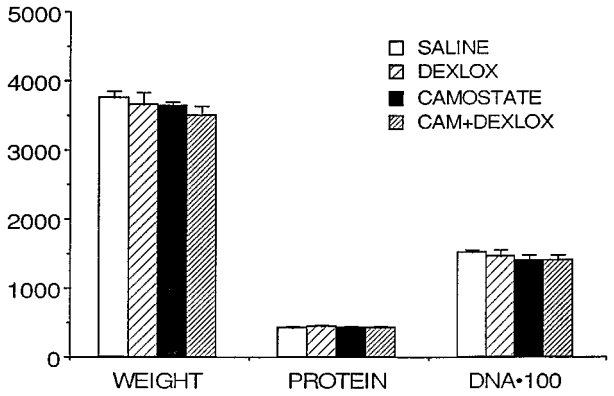

Figure 5 Oxyntic gland area weight, protein and DNA content (expressed as mg kg^{-1} of final body weight) in rats after short-term treatment (7 days) with saline or camostate (CAM; 200 mg kg^{-1} once daily) with and without dexloxiglumide (DEXLOX; 25 mg kg^{-1} three times daily). Each column refers to the mean of the values obtained from 10 animals. Vertical lines show s.e.mean.

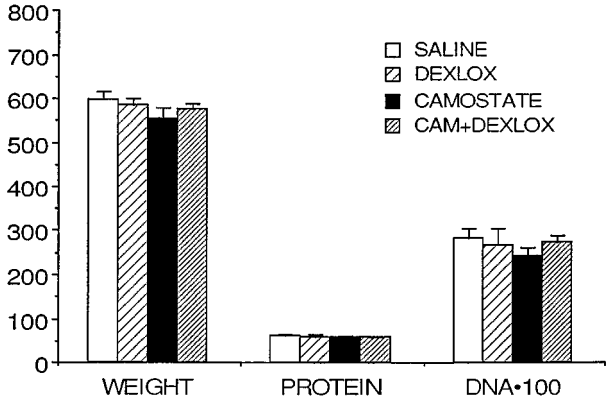

Figure 6 Gastric antrum weight, protein and DNA content (expressed as mg kg^{-1} of final body weight) in rats after short-term treatment (7 days) with saline or camostate (CAM; 200 mg kg^{-1} once daily) with and without dexloxiglumide (DEXLOX; 25 mg kg^{-1} three times daily). Each column refers to the mean of the values obtained from 10 animals. Vertical lines show s.e.mean.

finding after chronic CCK treatment. The ability of caerulein to mimic this biological effect of CCK was first demonstrated by Solomon *et al.* (1978) and was later confirmed by us (Varga *et al.*, 1985; 1989; Scarpignato *et al.*, 1989). In addition, Göke *et al.* (1986) showed that chronic stimulation of endogenous CCK release by camostate also induces pancreatic hypertrophy and hyperplasia. Subsequent studies (Niederau *et al.*, 1986; Wisner *et al.*, 1988; Schmidt *et al.*, 1989; Douglas *et al.*, 1990; Konturek *et al.*, 1991) have confirmed the initial observation and have shown that this effect is mediated through stimulation of CCK_A-receptors.

The results presented here confirm once more the ability of caerulein and camostate to increase pancreatic growth in rats. The ability of dexloxiglumide, a new potent and selective CCK_A-receptor antagonist, to reduce the trophic effect of both the amphibian peptide and of the protease inhibitor is in line with previous results from our laboratories (Scarpignato *et al.*, 1989) obtained with lorglumide, another CCK_A-antagonist belonging to the glutaramic acid derivatives. In accordance with data from the present study, other investigators have found that CCK_A-antagonists (namely devazepide and lorglumide) are able to counteract pancreatic growth induced by exogenous or endogenous CCK in rodents (Niederau *et al.*, 1986; Wisner *et al.*, 1988; Scarpignato *et al.*, 1989; Schmidt *et al.*, 1989; Axelson *et al.*, 1990; Douglas *et al.*, 1990; Konturek *et al.*, 1991).

Besides affecting motility of the stomach, CCK could also have a trophic effect on gastric musculature. However, in only one study (Axelson *et al.*, 1990) has this possibility been explored specifically, whereas in other investigations (Hoang *et al.*, 1988; Dembinski *et al.*, 1990) the peptide effect on gastric growth was marginally studied. As a consequence, data available are few and sparse. However, with the exception of the study of Dembinski *et al.* (1990), they all showed a lack of effect of CCK on GI smooth muscle. In accordance with these findings, results of the present investigation did show that CCK does not affect gastric weight and composition in both the oxyntic gland area and antrum. Taking into account the strong stimulant action of CCK on gastric smooth muscle, the lack of effect of chronic peptide administration on muscle weight is a bit surprising. However, as well as acting on CCK_A-receptors of the smooth muscle to induce contraction, CCK also binds the same receptor subtype located on the gastric D cells to stimulate somatostatin release (Lloyd *et al.*, 1994). The released somatostatin, by acting on specific receptors of the smooth muscle cells (Gu *et al.*, 1992), inhibits in a paracrine manner gastric contraction and may also counterbalance CCK-induced muscle growth. The peptide was, indeed, found to be endowed with an antitrophic action on the fundic and antral mucosa (Lehy *et al.*, 1979).

Since gastrin is able to stimulate gastric growth (for review see Baldwin, 1995) and gastrin and CCK are almost equipotent at the CCK_B/gastrin receptor (Scarpignato, 1992), one might expect that – under CCK_A-receptor blockade – CCK would exert a 'gastrin-like' trophic effect on the stomach via CCK_B-receptor stimulation. Again, this was not the case. Several experimental findings could help explain this lack of effect. Firstly, the trophic effect of gastrin is confined to the gastric mucosa, which represents only 35% of the gastric tissue (Axelson *et al.*, 1990). Secondly, the doses of the exogenous peptide needed to achieve such an effect are much higher than the doses of CCK required to stimulate pancreatic growth (Dembiski *et al.*, 1990) and employed in the present investigation. Thirdly, compared to gastrin, CCK is more potent at CCK_A-receptors than at CCK_B/gastrin receptors (Scarpignato, 1992). Finally, gastrin might exert its trophic effect via stimulation of a third subtype of receptor (like, for instance, the so-called CCK_C-receptor) for which CCK has low affinity. However, the existence of this new gastrin receptor has been recently questioned (Monstein *et al.*, 1997).

The molecular mechanisms involved in the trophic actions of CCK are beginning to be understood and they can offer alternative explanation for the different effects of CCK on pancreatic and gastric growth. Recent studies (Bianchi *et al.*, 1994; Rivard *et al.*, 1994; Tsunoda & Owyang, 1995) indicate that the CCK_A-receptor exists in two (i.e. high and low) affinity states, and CCK occupancy of high and low affinity sites is thought to be related to the initiation of different intracellular events and consequent biological responses. Binding of the peptide to high affinity CCK_A-receptors leads to synchronized activation of tyrosine kinase, phosphatidylinositol 3-kinase and phospholipase D (Rivard *et al.*, 1994; Tsunoda & Owyang, 1995), while occupation of low affinity state receptors induces activation of phospholipase C and an enhanced phosphoinositide breakdown (Bianchi *et al.*, 1994; Tsunoda *et al.*, 1996).

Although the nucleotide sequence of cloned cDNAs is identical (de Weerth *et al*., 1993), affinity states of CCK_A-receptors on pancreas and GI smooth muscle (e.g. gallbladder) are different and can be distinguished by the CCK-analogue, JMV-180 (Maubach *et al*., 1991; Taniguchi *et al*., 1995). Indeed, whereas CCK_A-receptors in the pancreas are present in both high and low affinity states, those on GI smooth muscle only exist in the low affinity state (Maubach *et al*., 1991; Taniguchi *et al*., 1995) and mediate CCK-induced contraction. It has been shown that stimulation of high affinity CCK_A-receptors is invariably followed by a trophic response (Dawra *et al*., 1993; Hoshi & Logsdon, 1993; Rivard *et al*., 1994). Occupation of low affinity CCK_A-receptors could also be involved in pancreatic growth, provided high affinity receptors are concomitantly activated (Hoshi & Logsdon, 1993; Rivard *et al*., 1994). These studies clearly show that the presence and activation of high affinity CCK_A-receptors are mandatory for growth in response to CCK. Therefore, in the pancreas, CCK stimulates high affinity CCK_A-receptors the occupation of which is followed by the sequence of intracellular events leading to growth (Rivard *et al*., 1994). In contrast, occupation of low affinity receptors in the GI smooth muscle does not lead to cell proliferation (Maubach *et al*., 1991; Taniguchi *et al*., 1995).

In conclusion, results of the present investigation show that the effects of CCK on pancreatic and gastric growth are different. The CCK-induced pancreatic hypertrophy and hyperplasia are blocked by the potent and specific CCK_A-receptor antagonist, dexloxiglumide. This compound, which is also active after oral administration, therefore represents a useful tool to investigate CCK-receptor interactions in peripheral organs.

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