Gastrin: An Acid-Releasing, Proliferative and Immunomodulatory Peptide?

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Abstract: Gastrin release is affected by gastric inflammatory conditions. Antral G cells respond to inflammatory mediators by increasing gastrin secretion. Accumulating experimental evidence suggests that gastrin exerts immunomodulatory and proinflammatory effects. Gastrin could be a contributing factor to these pathologies, which may constitute a new justification for pharmacological blockade of gastrin action.

INTRODUCTION

Gastrin has long been recognized as a peptidic hormone. It was discovered as far back as 1905 by Edkins [1, 2], and was isolated and sequenced in 1964 by Gregory and Tracy [3]. The first role assigned to gastrin was the stimulation of gastric acid secretion [4], which motivated the pharmacy industry to develop drugs that, by blocking the gastrin's action, could be used to treat gastrointestinal pathologies in which gastric acid is an important contributing factor. However, the limited ability of these drugs to reduce acid secretion and the antisecretory efficacy demonstrated by histamine-2 receptor antagonists and proton pump inhibitors (PPI) have undermined the relevance of gastrin antagonists. The second described effect of gastrin is its ability to promote cell proliferation [5, 6], which justifies the interest of these drugs to cancer chemotherapy. A third therapeutic target would seem to be psychiatric diseases such as anxiety and panic disorders in which the chemically related hormone CCK plays a significant role [7, 8] (not covered in the present review).

The present review compiles the evidence which suggests that gastrin may influence the function of the inflammatory / immune systems, with a proinflammatory profile having been observed in functional studies. We aim to highlight that, despite the long history of gastrin, there are aspects of this peptide that need to be clarified. The scope of gastrin may be much broader than initially suspected, as previously pointed out by others [9], an idea that provides a stimulus for continuing basic research into its physiological and pathological role, and which should be considered in the clinical trials that are currently analyzing the therapeutic value of new drugs designed to impede its actions.

GASTRIN PEPTIDES

The human gastrin gene is located in chromosome 17 and contains two introns and three exons that encode the gastrin precursor. This initial product of the gastrin gene expression is processed into multiple alternative active peptides (Fig. **1**):

Preprogastrin: The precursor. This is a peptide of 101 amino-acid residues including a 21-amino-acid signal peptide, a 37-amino-acid amino terminal "spacer", the gastrin-34 sequence, and a 9-amino-acid C-terminal extension. It is co-translationally cleaved between Ala21 and Ser22, which leads it to yield progastrin. Mice preprogastrin also contains 101 amino-acid residues, while that of the pig, cow, rat and dog has 104 residues.

Progastrin: A peptide that is produced as a result of the elimination of the signal peptide in preprogastrin. It contains 80 amino-acid residues with three pairs of basic residues that are sites for cleavage by prohormone convertases (PC) and carboxypeptidase E, by which Gly-gastrins are generated. Progastrin is also modified by sulphation of Tyr residues (Tyr-66) by the tyrosil-protein sulphotransferase.

Gly-gastrins (G34-Gly and G17-Gly): Peptides of 35 or 18 amino-acid residues with COOH-terminal glycine.

Amidated gastrins (G34 and G17): Peptides that are generated by amidation of the COOH-terminal in G34-Gly and G17-Gly by the peptidyl-alpha-amidating mono-oxygenase. Both molecules may be sulphated at the tyrosine in the sixth position from the C-terminus. The C-terminal tetrapeptide amide sequence of both gastrins is common to that present in cholecystokinin (CCK) and is essential for the activation of their two shared receptors (see below).

The main sites of gastrin synthesis are the endocrine G-cells in the antroduodenal region of the stomach. In these cells, the progastrin formed after translation of gastrin mRNA in the endoplasmic reticulum is transported to the Golgi apparatus. The sequential transformation of this precursor takes place as it progresses from the trans-Golgi network to the immature secretory vesicles and, finally, to the secretory granules in the basal part of the G-cells, where gastrin peptides are stored.

This elaborate biosynthetic process constitutes a regulated secretory pathway that is characteristic of endocrine cells and leads to the release of a variety of progastrin prod-

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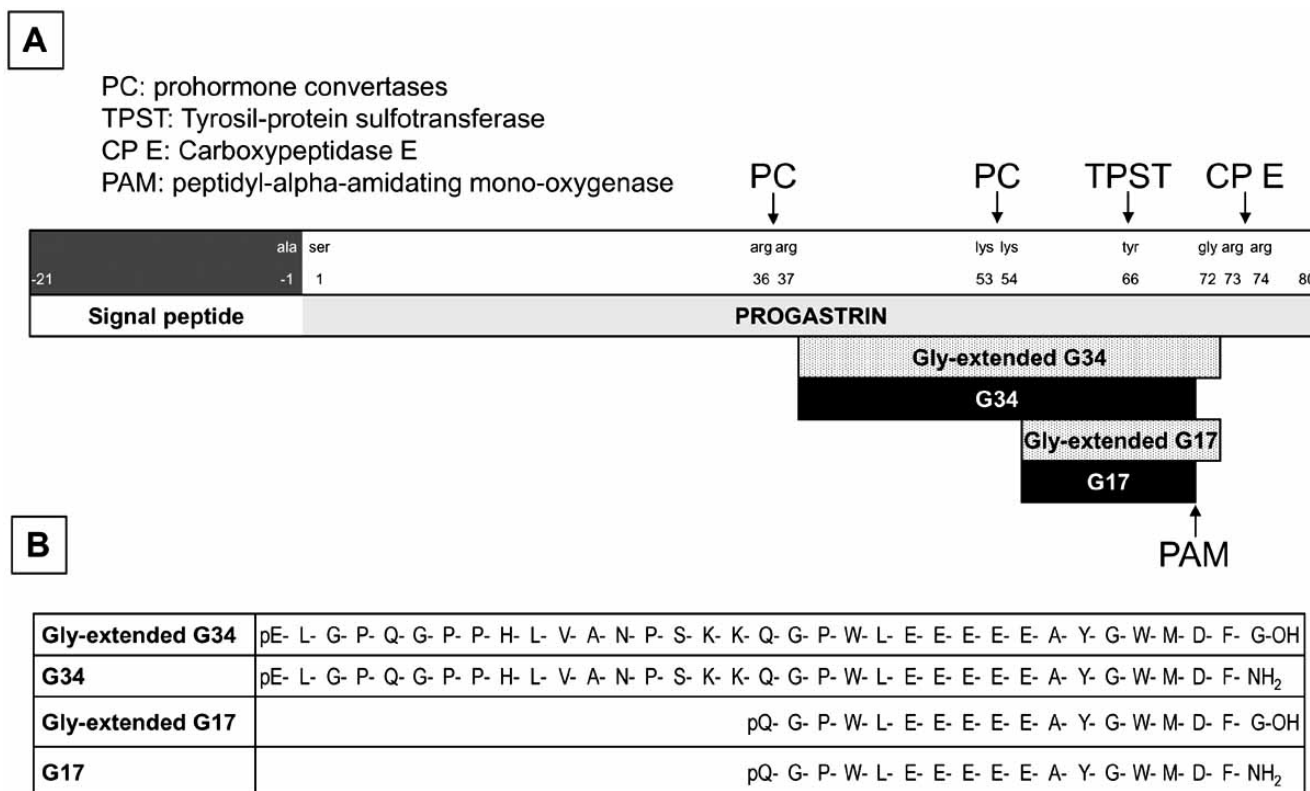Fax: 34-96-3864625; E-mail: sara.calatayud@uv.es

Fig. (1). Diagram of the structure of the initial peptide obtained after gastrin gene expression (preprogastrin), its main products and its processing enzymes (**A**). Aminoacid sequences corresponding to the amidated and glycin-extended gastrin peptides (**B**).

ucts into the blood. In humans, it is estimated that more than 95 % of secreted gastrins are found in the amidated form and approximately half are tyrosyl-sulphated [4]. Around 85% of these amidated peptides are gastrin-17, 5-10% are gastrin-34 and the rest are a mixture of bigger and smaller peptides. However, due to the metabolism of gastrin-17 being faster than that of gastrin-34, similar levels of both forms of gastrin are encountered in the blood of normal fasting subjects. For the same reason, and although the initial rise in gastrin levels after a meal is attributable mainly to gastrin-17, the presence of gastrin-34 prevails [10]. The relative concentration of the different gastrin peptides found in blood may change in circumstances of hypo- or hypergastrinemia [10]. The gastrin gene can also be expressed in non-endocrine cells (such as some tumour cells), where secretory vesicles may be absent and processing enzymes are scarcely expressed. Thus, the main products of secretion tend to be unprocessed peptides (e.g. progastrin and Gly-gastrins) [11]. Although these partially processed peptides were initially viewed as mere inactive precursors, it is now recognized that they have their own biological properties whose clearest effect is the stimulation of tumour cell growth [11].

RECEPTORS

The receptors for gastrin and CCK were named according to the territories where they were initially identified: CCK-A in the alimentary tract and CCK-B in the brain. Now, however, the terms CCK-1 and CCK-2, respectively, are more generally applied. The CCK-1 receptor (CCK1R)

has sulphated CCK as the main activating ligand, and shows much lower affinity for gastrin or non-sulphated CCK. However, the CCK-2 receptor (CCK2R) binds gastrin and CCK with very similar affinities and discriminates poorly between the sulphated and non-sulphated forms. CCK2R are capable of binding non-amidated gastrins, but with an affinity several orders of magnitude lower than that observed for the amidated peptides. The identity of the receptor(s) mediating the effects of these partially processed gastrins remains elusive [12].

The genes of CCK1R and CCK2R are each organized into five exons. Both receptors present the seven transmembrane hydrophobic segments characteristic of G protein-coupled receptors. CCK2R are considered the gastrin receptors because they mediate all the established effects of amidated gastrins [13], and so they will centre our attention. CCK2R activation can elicit multiple signalling pathways, which are listed in Table **1** (see Dufresne *et al.* for an extensive review, [13]). Several isoforms of CCK2R have been detected, although their physiological relevance is not entirely clear. Alternative splicing of exon 4 of the human CCK2 receptor gene results in a CCK2 receptor transcript that lacks a block of five aminoacids within the third intracellular loop [14]. A variant of exon-1 that generates a truncated isoform of CCK2R without the extracellular N-terminal has also been reported in the human stomach and in a human gastric cancer cell line [15]. Finally, a novel splice variant of CCK-BR with its fourth intron retained (CCK-

Table 1. Signalling Pathways Evoked After CCK2R Activation

- Phospholipases: PLC (PLC-β and PLC-γ1 isoforms)
- Calcium
- Protein Kinases C (-α, -δ, -ε, and -η), D and D2
- Mitogen-Activated Kinases: ERK1/2, JNK, p38-MAPK, and ERK5
- Tyrosine kinases: Src family
- Transactivation of the epidermal growth factor (EGF) receptor.
- Lipid kinases: PI 3-kinase/AKT pathway
- Focal Adhesion Kinase: p125-FAK
- Janus kinases (JAKs): JAK2/STAT 3 Pathway
- Small GTPases: Ras, Rho, Rac, and Cdc42
- Transcription factors: NFκB, AP-1

BRi4sv) and exhibiting constitutive (ligand independent) activation has been detected in colorectal and pancreatic cancers but not in normal tissue [16, 17].

CCK2R ANTAGONISTS

CCK1 and CCK2 receptors have been a target for the medicinal chemical industry for the last four decades. These investigations have discovered a significant number of chemical entities capable of blocking the effects of gastrin and CCK, which has greatly expanded the knowledge of the role played by both peptides in physiology and pathology. The distance covered during the search for useful drugs has been analyzed in numerous reviews [18-20]. This section of the present review briefly describes the main lines of chemical development and their most important findings. Table **2** summarizes the principal characteristics of the CCK2R antagonists mentioned below, while Fig. (**2**) reveals their chemical structures.

The first drug reported to inhibit the effects of gastrin was the aminoacid-based non-selective CCKR antagonist proglumide (benzoyl-DL-glutamic acid dipropylamide) (**1**) (Fig. **2**) [21], the only gastrin antagonist to have reached the commercial market. Although a weak inhibitor, it constituted a starting point for the development of the most potent and selective antagonists for both CCK1R and CCK2R. Regarding CCK2R, the most selective aminoacid derivatives obtained are spiroglumide (**2**) [22] and itriglumide (**3**) [23]. Spiroglumide shows an excellent oral bioavailability but low affinity (micromolar range) and selectivity for CCK2R. Itriglumide maintains an adequate oral bioavailability, together with nanomolar affinity and excellent CCK2R selectivity, which justifies ongoing clinical studies.

Another strategy considered the modification of the active part of gastrin and CCK, namely the C-terminal tetrapeptide sequence. However, despite the considerable potency and selectivity of some of these peptidic antagonists, their poor oral availability, low metabolic stability and, in some cases, mixed antagonist/agonist character have ham-

pered their therapeutic development. James Black Foundation' researchers succeeded to mimic the topography of this tetrapeptide with a dibenzobicyclo[2.2.2.]octane structure that was used as a scaffold to develop selective antagonists. The most significant contribution of this line of research has been the compound JB95008 (gastrazole) (**9**), a potent and CCK2R-selective benzimidazole antagonist that suffers the handicap of a very low oral bioavailability [24]. The problems encountered with peptidic compounds motivated what was called the "dipeptoid strategy" rational by Parke-Daves' scientists. This strategy assumes that a dipeptide extracted from a large biologically active peptide may retain some affinity for the receptor, which can be modulated by manipulation of the side chains. The principal fruit of this line of research was compound CI-988 (**10**), a highly selective and orally active CCK2R antagonist [25].

The discovery by researchers at Merck of the natural non-peptide compound asperlicin, which shows a weak affinity for the CCK1 receptor, represented a breakthrough in the search for gastrin antagonists [26]. This finding led to the development of the 1,4-benzodiazepine L-365,260 (**4**), the first highly potent and selective non-peptide CCK2R antagonist [27]. The main problem encountered with this drug is its low aqueous solubility, which could explain its limited oral bioavailability. Further manipulation of this structure led to the development of several antagonists with higher selectivity for the CCK2R, amongst which YM022 (**5**) and YF-476 (**6**) were shown to have a significant oral potency [28, 29].

In Glaxo, a series of 1,5-benzodiazepine derivatives with affinity for CCK receptors was discovered by screening. Some of these compounds exhibit CCK2R antagonist activity, which is optimized by introducing substituents into the 1,5-benzodiazepine skeleton similar to those found to be effective in the Merck 1,4-benzodiazepine antagonists. This chemical group includes the compounds GV150013X (**7**) [30, 31] and Z-360 (**8**) [32], which are both currently under clinical evaluation. The research concerning these structures has continued to analyze the pharmacological profile of 1,3,4- and 1,3,5- benzotriazepines [20].

Finally, the ureidoacetamide-based CCK2 receptor antagonists may be considered acyclic analogues of 1,4-benzodiazepine-derived antagonists. Among the compounds of this series, S-0509 (**11**) affords the optimum balance between receptor affinity and selectivity and *in vivo* potency [33].

PHYSIOLOGICAL EFFECTS OF GASTRIN

The principal role of those assigned to gastrin is the stimulation of gastric acid secretion. Physiologically, gastrin is released from G cells in response to food intake, and more specifically to dietary amino-acids and amines present in the gastric lumen. Gastrin release can also be induced by the neurotransmitter gastrin-releasing peptide (GRP) acting on its basolateral receptors in the G cell. Once released, it is transported in an endocrine manner to the oxyntic mucosa, where it encounters the CCK2R in parietal and enterochromaffin-like (ECL) cells [34, 35]. Gastric binding to these receptors can directly activate the parietal cells to secrete acid, and induces the release of histamine from ECL cells. Histamine reaches the parietal cells by paracrine diffusion
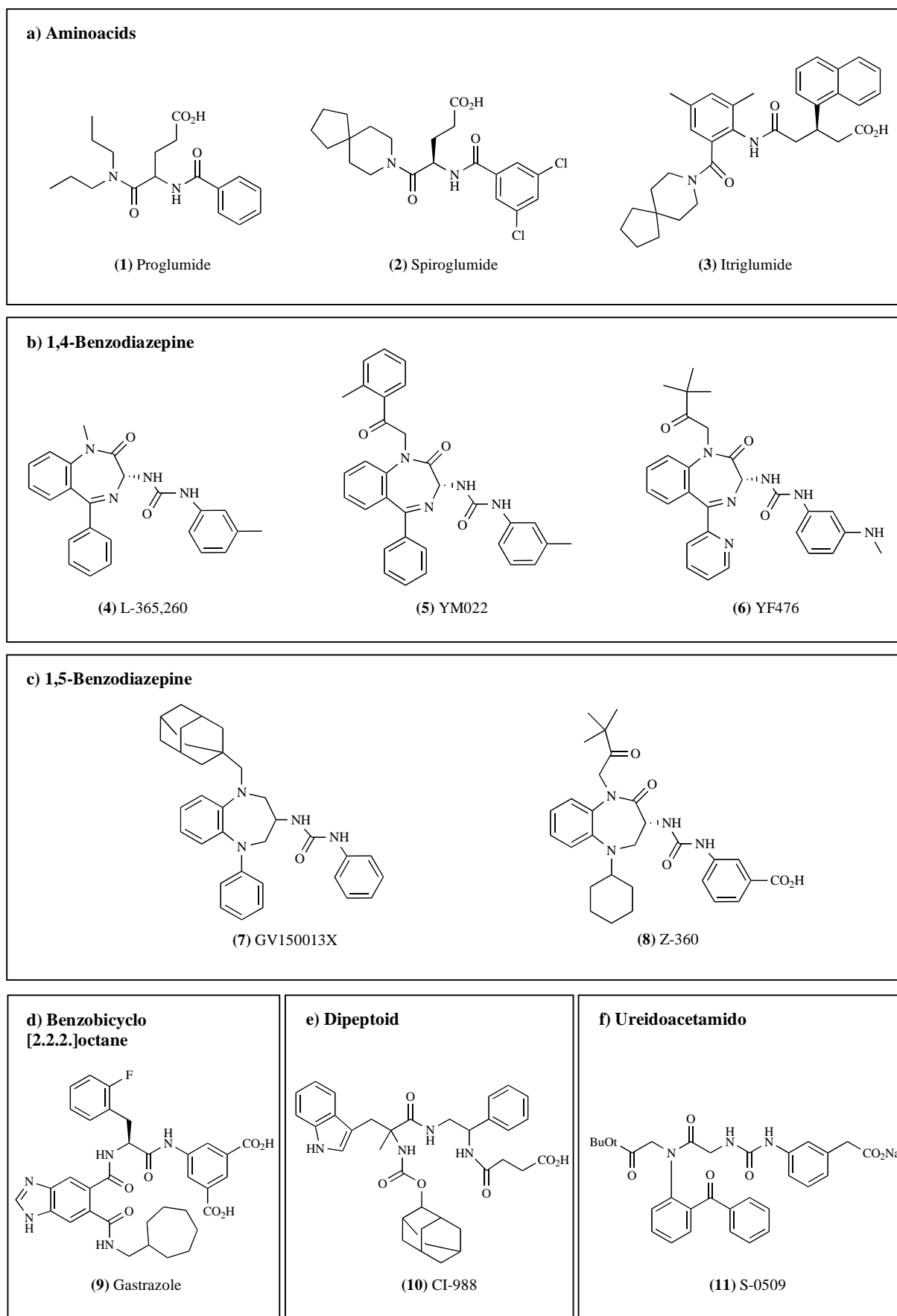
a) Aminoacids

(**1**) Proglumide   (**2**) Spiroglumide   (**3**) Itriglumide

b) 1,4-Benzodiazepine

(**4**) L-365,260   (**5**) YM022   (**6**) YF476

c) 1,5-Benzodiazepine

(**7**) GV150013X   (**8**) Z-360

d) Benzobicyclo [2.2.2.]octane

(**9**) Gastrazole

e) Dipeptoid

(**10**) CI-988

f) Ureidoacetamido

(**11**) S-0509

Fig. (2). CCK2R antagonists used in human studies classified according to their basic structure.

Table 2. CCK2R Antagonists Used in Human Studies

Basic structure	Compound	IC50 (μM)		Selectivity CCK2/CCK1	Activity tested	Furthest Development Phase Reached	Reason for Discontinuation
		CCK1	CCK2				
Aminoacids	Proglumide	6100	11000	0.54	GAS [109, 110]	Marketed (Rotta Laboratories)	Low efficacy
	Spiroglumide	13.5	1.4	9.6	GAS [111]	I	Low affinity / selectivity
	Itriglumide	20.7	0.0023	9000	GAS Anxiety	II II (I.P., Web Rottapharm)	
1,4-Benzodiazepine	L-365,260	0.28	0.002	140	GAS [106] Anxiety [112]	II	Low efficacy Low oral bioavailability
	YM022	0.15	0.00011	1364	GAS [18]	I n.a.	
	YF476	0.502	0.00011	5020	GAS [107, 108]	I	Loss of efficacy
1,5-Benzodiazepine	GV150013X	pKi 6.15	pKi 8.64	309	Anxiety [18]	II n.a.	
	Z-360	0.316	0.00047	672	GAS Cancer	I II (I.P., Web Zeria)	
Ureidoacetamido	S-0509	3.4	0.042	81	GAS [18]	I (n.a.)	
Dipeptoid	CI-988	4.3	0.0017	2529	Anxiety [113-116]	II	Low efficacy
Benzobicyclo [2.2.2.]octane	Gastrazole (JB 95008)	4	0.001	4000	Cancer [24]	Pilot trials	

IC_{50} and selectivity data taken from references [18, 19, 117]. GAS: gastric acid secretion or related pathologies, n.a.: information non-available, I.P.: in progress.

and stimulates acid secretion by activating their H2 receptors. The luminal acid exerts a counterregulatory effect on gastrin release, probably mediated by the liberation of the paracrine inhibitor somatostatin from D cells [4].

The other long-known effect of gastrin is its trophic action in the stomach [36]. Studies carried out in gastrin or CCK2R-deficient mice have demonstrated the importance of gastrin in maintaining the normal histology and function of the gastric mucosa. These mice showed a reduced acid secretion and remarkable atrophy due to a decrease in parietal and ECL cells [37-39]. Although gastrin deficient mice also present a reduced proliferation rate in the colonic mucosa [37], amidated gastrins do not seem to affect proliferation in human colon, where CCK2R are not present [13]. This contrasts with the growth promoting action that has been established for partially processed gastrins in this tissue [5].

CCK2R have also been detected in other tissues of the gastrointestinal tract, such as the pancreas and the smooth muscle, although the relevance of gastrin in their functional modulation is not clear [13]. CCK2R are also present in adipocytes, where gastrin seems to regulate leptin expression and secretion [40], and in human and rat adrenal zona glomerulosa cells, where they could stimulate aldosterone secretion [13]. Finally, CCK2R have been found in several elements of the immune system (see below).

ALTERED PATTERNS OF GASTRIN SECRETION

Increased gastrin secretion is observed in patients with gastrinoma, who present gastric hyperacidity as part of Zollinger-Ellison syndrome. However, this is a relatively uncommon disease and hypergastrinemia is more usually related to conditions of gastric hypoacidity in which the

negative feedback of gastrin secretion induced by luminal acid does not occur and G cells secrete gastrin unrestrained. This mechanism explains the increased serum levels of gastrin observed when acid secretion is inhibited pharmacologically with proton pump inhibitors or with antagonists of the histamine-2 receptor, and in patients with chronic atrophy of the corpus mucosa. Gastrin hypersecretion is also observed in *Helicobacter pylori*-infected patients, where it is associated with reduced or increased acid output, depending on the pattern of mucosal inflammation caused by the germ. Low acid secretion is observed when the infection causes corpus-predominant gastritis or pangastritis, while the contrary is true when the inflammation only affects the antrum [41]. Either way, the increase in gastrin induced by *Helicobacter* occurs in parallel with a decrease in somatostatin secretion [42, 43]. Both effects may be due to a direct action of the germ on G and D cells respectively. However, in recent years, it has become clear that these secretions are influenced by the local inflammatory process that is triggered in response to the germ, which is the main cause of non-autoimmune gastritis. In this respect, gastrin release from primary cultures of animal antral G cells is stimulated by proinflammatory cytokines and activated monocytes [44-46]. In fact, some studies have found a correlation between gastrinemia and the severity of gastritis [47-49], and gastrin hypersecretion is known to occur in animals with gastritis in the absence of bacterial overgrowth and normal gastric acidity [50].

As in the case of normal gastrin secretion, gastrin hypersecretion in response to the abovementioned circumstances requires an adequate population of functional G cells in the antrum, which may be undermined when chronic gastritis causes atrophy in the antral mucosa. The consequences for gastrin secretion of all these pathological alterations of the gastric mucosa are the focus of a line of research that explores the viability of employing serum levels of gastrin, pepsinogen I and II and antibodies to *H. pylori* as a non-invasive method to diagnose gastric mucosal atrophy [51-54]. In this sense, a high level of gastrin in the serum may point to corpus atrophic gastritis without antral involvement, while low levels might suggest atrophic gastritis in the antrum, with or without corpus atrophy. However, it must be said that the sensitivity of this method is still a matter of debate [53, 54].

Abnormal secretion of gastrin is also linked to cancer. The expression of gastrin and its precursors, as well as that of CCK2R and its variants, has been detected in multiple cancer cell types both within and outside the gastrointestinal system [11, 55].

RELEVANCE OF GASTRIN IN PATHOLOGY

Apart from the Zollinger-Ellison syndrome, the main pathological entity linked to gastrin is cancer. The basis for this association is the proliferative effect of gastrin. However, there is a great deal of controversy surrounding the role played by the peptide in this disease, and although numerous reviews analyze this matter, it is difficult to draw a clear and simple picture that summarizes all the evidence.

As said before, gastrin and its receptor have been observed in some tumoral tissues. However, their detection may be influenced by many factors that complicate the extraction of general conclusions: techniques used (detection in tissue homogenates, e.g. by RT-PCR, vs cell localizing techniques); difficulties distinguishing whether the detected receptors are present in the cells in which it is normally expressed or in transformed cells; presence of amidated and non-amidated peptides, whose receptors and pattern of effects differ; presence of different CCK2R isoforms; and/or the complexity of the disease. These complications probably justify the lack of agreement around the types and proportion of tumours expressing the hormone and its receptor [56].

Since the spatial coincidence of gastrin and CCK2R in the tumoral tissue does not necessarily imply a functional role of this pathway in the progress of the disease, this issue has been approached by studying the effects of gastrin on tumour growth *in vitro* and in xenotransplants *in vivo* and, interestingly, a proliferative effect of gastrin was observed [57-60]. In fact, pilot studies have obtained positive effects with CCK2R antagonists or gastrin-neutralizing agents in patients with advanced pancreatic, colon or gastric cancers [61]. These evidences support the idea that some neoplasic lesions produce gastrin and are responsive to its growth-promoting effect.

Assuming that gastrin can promote the growth of certain tumours once the neoplasic process is established, the main concern is whether or not hypergastrinemia contributes to the initiation of cancer, as increased gastrin secretion is observed in cancer-predisposing circumstances such as atrophic gastritis and *Helicobacter pylori* infection. This preoccupation has been extended to patients treated with PPI, especially those with gastroesophageal reflux disease (GERD), as the therapy that they undergo is often prolonged and does not include systematic treatment to eradicate *Helicobacter pylori*. This latter point is important because anti-secretory drugs alter the pattern of atrophic gastritis induced by *Helicobacter* in the antrum into the corpus, and corpus affectation seems to be related to an increased risk of cancer development [62]. The question is whether hypergastrinemia is a secondary effect or if, on the contrary, it contributes to the development of cancer. A causal relationship between the two elements has been established exclusively in rodents. Continuous hypergastrinemia in gastrin-transgenic mice caused thickening of the fundic mucosa and multifocal hyperplasia with increased proliferation index. This initial hyperproliferative state gave way to a gradual parietal cell loss, gastric atrophy, foveolar hyperplasia, gastric metaplasia and gastric carcinoma in older animals, processes that were accelerated by *Helicobacter* [63]. The relevance of these findings to human disease is still a matter of debate [62, 64].

A final consideration is the evidence provided by recent studies which suggests that gastrin, as well as increasing cell proliferation, can modify other cellular functions relevant to cancer. Gastrin seems to modulate migration [65], invasion [66] and apoptosis [67-70] in epithelial cells. Moreover, amidated and glycine-extended gastrin promote angiogenesis *in vitro* and in tumorigenic processes *in vivo* [71, 72]. The following section analyzes how gastrin may modulate the

inflammatory / immune reactions, which may be relevant in both the genesis of cancer and the fight against it.

LINKS BETWEEN GASTRIN AND INFLAMMATION / IMMUNITY

CCK2R are Present in Key Elements for the Immune / Inflammatory Responses

The first evidence of a connection between gastrin and cells of the immune system was reported more than twenty years ago. Okahata *et al.* [73] demonstrated gastrin immunoreactivity in pure populations of polymorphonuclear leukocytes (PMN) and mononuclear cells from human peripheral blood.

More recent studies have detected the mRNA of CCK2R in PMN [74], and that of CCK1R, CCK2R and the splice variant CCK-BRi4sv in mononuclear cells [75] isolated from human peripheral blood. Two other studies have analyzed the presence of CCK2R in mononuclear leukocytes trying to distinguish between monocytes and lymphocytes, with contradictory results. Binding experiments performed by Sacerdote *et al.* [76] demonstrated the presence of a "brain-like" CCK receptor in human monocytes but not in lymphocytes. However, Iwata *et al.* [74] did not find the mRNA for CCK2R in either lymphocytes or monocytes/macrophages. This discrepancy may be due to differences in methodology. In the first study, monocytes and lymphocytes were separated by centrifugal elutriation, while in the second, mononuclear cells were separated from lymphocytes by their adhesivity to culture dishes, a process that may have induced changes in the pattern of mRNA expression. Taken as a whole, it seems probable that monocytes express CCK2R while the opposite is true for lymphocytes. Additionally, the expression of this receptor is a common feature in leukaemia cell lines from both myeloid and lymphocytic lineages [74, 77, 78].

The gastrin receptor has also been detected in tissue-infiltrating leukocytes. Its mRNA was detected in immune cells of the gastric lamina propria [79] and lung macrophages [80] in rats, while the protein was observed in macrophages of the rat mesenteric tissue [81] and in PMN within the stroma of human colorectal tumours [82]. In addition, an alternative molecular target for gastrin was revealed in eosinophils of the lamina propria in both rats [83] and humans [84].

Finally, CCK2R are also present in the cellular interface between blood and tissues, namely endothelial cells. Lefranc *et al.* [71] demonstrated the expression of CCK2R mRNA in human umbilical vein endothelial cell (HUVEC) primocultures and in microvessels dissected from a human glioblastoma.

Does Gastrin Modulate the Function of the Immune System?

Over the last two decades, a series of observations have been made regarding the ability of gastrin to modulate the function of cells involved in immune/inflammatory responses. The summary below suggests that gastrin is impli-

cated in this system while demonstrating that we are still far off from establishing the exact nature of the role it plays.

A. In vitro Studies – Cellular Effects

In one study, CCK induced a chemotactic effect on human mononuclear cells isolated from peripheral blood, which was reversed by the CCK1R/CCK2R antagonists benzotript and CR-1369 [85]. A similar gastrin-induced effect was reported by De la Fuente *et al.* [86, 87], who observed this chemotaxis together with an increased adherence and a reduced fagocytic capacity in murine macrophages and human PMN treated with the peptide, although they did not analyse the involvement of CCK2R in said responses.

Some studies have described the ability of gastrin to modulate lymphocyte proliferation and activity, but results are conflicting. Gastrin seems to increase the secretion of the factor responsible for clonal expansion (IL-2) and stimulate cell growth in resting peripheral blood lymphocytes [88], and a similar response was observed in several lymphocytic cell lines, in some of which an autocrine growth-promoting effect of gastrin was detected [74]. Gastrin also intensified the stimulation of Jurkat T cells in response to phytohaemagglutinin [89, 90], but counteracted this process in normal lymphocytes [75, 88]. The significance of these complex effects of gastrin on lymphocytic cells is as yet unclear, and is obscured even more so if we assume the absence of CCK2R in normal lymphocytes (see above).

In addition to these direct effects on blood cells, gastrin may modulate the function of leukocytes by acting on stromal or tissue cells. Lefranc *et al.* [71] reported that gastrin induced the release of the chemokine IL-8 from human endothelial cells (HUVEC), and we observed that gastrin activated these cells to synthesise the adhesion molecules VCAM-1 and P-selectin, an effect that was mediated by CCK-2 receptors and which caused an increased adhesivity for human mononuclear leukocytes [91]. Gastrin directly induced the expression of proinflammatory molecules such as the chemokines IL-8 and CINC-1 and the enzyme COX-2 in gastric epithelial cells transfected with CCK2R, while significantly increasing the cellular activation induced by *Helicobacter pylori* [92, 93].

Some of the above mentioned studies have analyzed the downstream events after CCK2R activation and demonstrated the implication of pathways typically involved in immune / inflammatory responses as responsible for the reported effects, which suggests that other immunomodulatory effects of gastrin can be expected.

B. *In vivo* Studies

B.1. *Immune / Inflammatory Responses*

Some experiments performed in mice lead us to consider the possibility that gastrin influences the immune response to *Helicobacter*. Zavros *et al.* [94] established that the immune/inflammatory response to *Helicobacter* infection can be modified considerably by somatostatin. Of particular interest, they demonstrated that the Th1 immune response and consequent secretion of IFNγ induced by *Helicobacter felis* reduced somatostatin release, and that the reestablishment of this secretion in response to treatment with a Th2 cytokine

(IL-4) was sufficient to reverse the immune/inflammatory response induced by the germ. In this way, they established the capacity of somatostatin to modify the host response to *Helicobacter* in a way that resolves inflammation. Gastrin was situated in the other part of the process; its levels were elevated in the presence of the pathology and lowered when the IL-4/somatostatin pathway exerted its therapeutic effect. Although the role of gastrin was not analysed in that study, later evidence suggests that alterations in gastrin secretion may not be merely a secondary aspect in pathology. In this sense, a shift towards a Th2 response, reflected by an increased IgG1:IgG2a ratio, was observed in hypergastrinemic INS-GAS mice infected with *Helicobacter felis* when a CCK2R antagonist was administered. This effect was accompanied by a significant reduction in gastric inflammation and a tendency to exhibit lower levels of IFN and TNF and higher amounts of IL4. These actions of the CCK2R antagonist occurred in addition to similar effects induced by the H2-antagonist loxitidine, and both drugs synergistically inhibited the development of gastric atrophy and cancer induced by *Helicobacter felis* in these hypergastrinemic mice [95].

Several experimental studies endorse a role for gastrin as a contributing factor of the inflammatory process. Mast cells from human cutaneous and rat intestinal tissues seem to be activated by gastrin or CCK acting on CCK2R, which provoke cell degranulation and histamine release [96, 97]. Sacerdote *et al.* [85] reported that CCK caused a receptor-mediated chemotaxis of rat monocytes when injected into the peritoneal cavity in both acute and chronic experiments, while we observed that gastrin induced leukocyte-endothelial cell interactions in the rat mesenteric venules through the activation of CCK-2 receptors. This effect was independent of histamine release and unaffected by somatostatin, and seemed to contribute to the inflammation induced by *Helicobacter pylori* [81] and the antisecretory drugs omeprazole and famotidine [98]. Similarly, Takaishi *et al.* [95] reported that the aggravating effect of omeprazole on the gastritis induced by *Helicobacter felis* in INS-GAS mice was prevented by blocking CCK2R.

B.2. Gene Expression

The influence of gastrin on the pattern of gene expression in the gastric mucosa has been analyzed in several experimental studies and, in all of which, interestingly, changes in the expression of genes involved in inflammatory / immune responses have been observed. The first study assessed the effects of prolonged hypergastrinemia in rats treated with high doses of omeprazole for 10 weeks, reporting the upregulation of several immunoregulatory genes [99]. These results indicate that potent acid inhibition causes a proinflammatory state in the gastric mucosa which, according to findings of our group [98], could be partially dependent on gastrin hypersecretion. However, the former study was performed in rats treated over a longer period and with higher doses of omeprazole than those used in our study. The potent acid inhibition and sustained achlorhidria caused by this treatment often causes bacterial overgrowth [100], and so it is possible that overexpressed immunomodulatory genes merely reflect the natural immune response to these germs.

Adopting an alternative approach, Friis-Hansen *et al.* [101] compared the gene expression in gastric tissues from wild-type and gastrin-knockout mice. Once again, the expression of several genes implicated in immune-defences was observed to have increased, although in this case, it was due to the lack of gastrin. As in the previous study [99], it was difficult to discern whether or not these genes were directly regulated by gastrin, as bacterial overgrowth and lymphocyte infiltration in response to achlorhydria was detected in gastrin-KO animals. Moreover, some of the genes that were overexpressed in gastrin-KO animals were also elevated in hypergastrinemic wild-type animals treated with omeprazole, in which achlorhydria without bacterial colonization or lymphocyte infiltration was detected.

Jain *et al.* [102] designed a study to distinguish the contribution of each of the functional elements concurring in gastrin-KO mice to the changes in gene expression that characterize these animals. They chose to work with young gastrin-KO mice in order to minimize the gastritis that occurs with age, and observed that, despite a lack of any obvious histological signs of inflammation, the expression of several inflammatory genes was increased in these animals. Among these genes, three were identified whose expression was only affected by the lack of gastrin and not by the accompanying hypochlorhydria. The authors compared the pattern of expression in GAS-KO and in gastrin-CCK-KO, which present a normal level of gastric acid secretion in the absence of gastrin. The affected genes were defensin beta 3, the polymeric immunoglobulin receptor and member 6 of the interleukin 1 family. The first two genes are clearly involved in antimicrobial responses, while the third appears to have a proinflammatory character [103-105]. This may indicate that gastrin exerts a tonic inhibitory effect on the expression of these genes and, although the role played by gastrin in immune / inflammatory responses is difficult to deduce from these results, this evidence clearly calls for future investigation into the matter.

PERSPECTIVES OF CCK2R ANTAGONISTS

The therapeutic interest of CCK2R antagonists has been centred in gastrointestinal pathologies in which acid secretion is a contributing factor, in cancer chemotherapy, and in psychiatric diseases such as anxiety and panic disorders, where CCK and central CCK2R play a significant role.

The development of CCK2R antagonists as antisecretory agents may have been hampered by the good efficacy/security profile of PPI, current pivotal elements in the therapy of acid-related gastrointestinal diseases such as peptic ulcers or GERD. However, it is important to note that studies published concerning this issue have not directly analyzed the therapeutic value of these drugs, but instead were designed to determine their ability to inhibit acid secretion [106-108]. In this sense, a disadvantage of CCK2R antagonists vs PPI is to be expected, as the former only inhibits a stimulatory pathway while PPI blocks the last step of the secretory process independently of the activating stimulus. In fact, the study of several CCK2R antagonists was aborted on this basis (see Table **2**). Although the importance of gastric acid in the abovementioned pathologies is unquestionable, these diseases are characterized by several other ethiopatho-

genic factors amongst which inflammation is preeminent. Mucosal inflammation is also relevant to the progression of disease, and has been identified as a predisposing factor to neoplasic transformation. This is especially important in *Helicobacter pylori*-positive patients where, additionally, PPI seem to induce a worsening effect on corpus gastritis. It is remarkable that gastrin hypersecretion is commonly encountered in the inflamed mucosa, and this effect is also enhanced by antisecretory drugs. If future studies confirm that the effects of gastrin go beyond acid secretion and cell growth, as suggested by the evidence compiled in this and previous reviews [9], then CCK2R antagonists could modify the disease and its progression in many ways.

The pharmacokinetic (low oral bioavailability) and pharmacodynamic (low potency or selectivity) problems encountered in the developmental process have now been overcome, and years of research have led to the appearance of drugs with what would seem to be an appropriate pharmacological profile. We believe that these CCK2R antagonists should be studied with a broader view taking into account that gastrin may have other targets beyond the acid-secretory machinery. Blockade of these as yet unknown roles of gastrin with CCK2R antagonists may significantly influence the therapeutic value of these compounds, which could be complementary to that of the established anti-secretory drugs. The results of clinical trials that are currently underway (Table **2**) will provide a better idea of the real efficacy of CCK2R antagonists in these crucial gastrointestinal pathologies. In the meantime, preclinical studies should pursue a better knowledge of the scope of gastrin's functions.

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