

The Regulation of Histidine Decarboxylase Gene Expression

Michael Höcker, Zhensheng Zhang, Theodore J. Koh and Timothy C. Wang^a

*Gastrointestinal Unit and Department of Medicine,
Massachusetts General Hospital, Boston, Massachusetts*

(Received January 17, 1996; accepted June 4, 1996)

Histamine is a biogenic amine, which is involved in a variety of biologic processes comprising inflammation, allergic responses, neurotransmission and regulation of gastric acid secretion. The key enzyme for the generation of histamine is histidine decarboxylase (HDC), which converts the amino acid L-histidine to histamine. In this article, we review the history, biochemistry and molecular biology of this enzyme. Northern blot studies in rats demonstrated that HDC gene expression in the stomach and liver are developmentally regulated with highest levels of expression in the late fetal state, indicating a role of the gene in growth and development. In the stomach of adult rats, HDC mRNA levels are elevated after omeprazole-induced hypergastrinemia, and *in situ* hybridization showed that expression of HDC is restricted to the glandular area in which ECL cells are located. Since no permanent ECL cell line is at hand for *in vitro* studies, we established a suitable cell system by stable transfection of a human gastric adenocarcinoma cell line (AGS) with the CCK-B/gastrin receptor. Transfection of this AGS-B cell line with reporter gene constructs comprising 5'-flanking DNA sequence of the HDC gene joined to the firefly luciferase gene revealed transcriptional regulation of the HDC promoter by gastrin through a protein-kinase C-dependent pathway. Taken together, these studies are consistent with the concept of HDC transcriptional regulation as at least one phase of the overall response to gastrin.

I. INTRODUCTION TO HISTAMINE AND HISTIDINE DECARBOXYLASE

Since the first description of its physiologic actions by Dale and Laidlaw in 1910 [1], histamine has been shown to play a number of biologic functions, including roles in inflammation, allergic responses, neurotransmission, and growth and repair [2]. However, particular attention has been given to its role as a gastric secretagogue. An important breakthrough occurred with the discovery by Black and co-workers that histamine-2 receptor antagonists potently inhibit acid secretion [3]. More recent studies have now confirmed that histamine represents the final common pathway in the stimulation of acid secretion. The secretion of histamine by the enterochromaffin-like (ECL)^b cell underscores the importance of this gastric endocrine cell in the overall physiology of the stomach, and has generated greater interest in the molecular mechanisms of histamine production. Histamine is generated in the stomach through the action of the enzyme histidine decarboxylase (HDC), which decarboxylates the amino acid L-histidine (Figure 1), leading to the production and release of CO₂ that can be readily measured. The enzyme histidine decarboxylase is present in a variety of cell types, including mast cell, skin, platelets and basophils, but in the adult mammal (e.g., rat) it is found at high levels in gastric ECL cells [2]. This article will review the discovery of histidine decarboxylase, the cloning of

^aTo whom correspondence should be addressed: Timothy C. Wang, Gastrointestinal Unit and Department of Medicine, Massachusetts General Hospital, Boston, MA 02114. Tel: (617) 726-9228; Fax: (617) 726-3673; E-mail: wang@helix.mgh.harvard.edu.

^bAbbreviations: ECL, enterochromaffin-like (cell); HDC, histidine decarboxylase; CCK, cholecystokinin; α -FMH, α -fluoro-methylhistidine; ODC, ornithine decarboxylase.

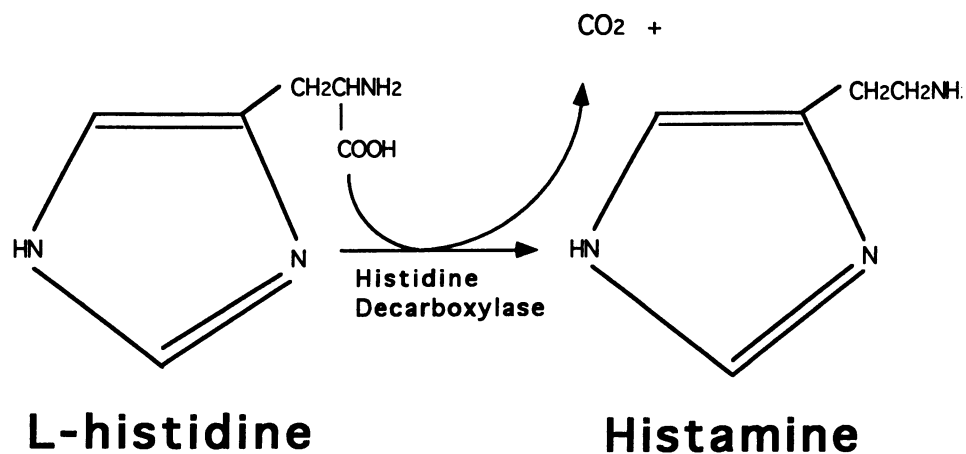


Figure 1. Histidine decarboxylase converts L-histidine to L-histamine by decarboxylation.

the cDNAs and genes for HDC and recent work that has explored the regulation of HDC gene expression.

II. HISTORY OF HISTIDINE DECARBOXYLASE

The role of histamine as a stimulant of acid secretion has been known since the initial work by Popielsky dating back to 1920 [4]. However, the importance of histamine as the key physiologic mediator was not widely appreciated until more recently, when studies by Kahlson and Code in the 1960s [5, 6], the discovery of H_2 receptor antagonists by Black in the 1970's [3] and more recent *in vivo* and *in vitro* models over the last decade have highlighted the importance of histamine secretion as the final common pathway of acid secretion. Studies in amphibians [7], rabbits [8], rats [9] and humans [10, 11] have now demonstrated that gastrin stimulates gastric acid secretion mainly through the release of histamine. In isolated perfused rat stomachs, gastrin is able to stimulate histamine release followed later by acid secretion [12], and the response is completely inhibited by H_2 blockers or diamine oxidase [13].

The source of histamine production in the stomach was a point of contention for many years. Although the possibility of mast cell-derived histamine as a source was considered initially, attention eventually shifted to an endocrine cell located in the glandular region of the gastric fundus. The ECL cells were first recognized in the stomach using light microscopy and argyrophilic staining techniques (Grimelius and Sevier-Munger) that included exogenous reducing agents, which stained not only ECL cells, but other endocrine cells (EC) cells as well [14]. Evidence that the ECL cell synthesizes and stores histamine was first reported by Håkanson and et al. [15], and further studies identified ECL cells in the oxyntic mucosa of a broad range of vertebrate species [15]. In response to acute gastrin stimulation, ECL cells respond with histamine release, leading to a depletion of histamine stores. With chronic gastrin stimulation, proliferation of ECL cells occurs, leading to ECL cell hyperplasia and eventually ECL cell carcinoid tumors [16]. However, an intermediate response was also recognized, which was characterized by an increase in HDC activity leading to repletion of histamine stores.

The presence of a histidine decarboxylating enzyme in mammalian tissue was first reported by Werle when he discovered that slices of rabbit kidney formed histamine from histidine [17]. The stimulation of HDC enzymatic activity in response to feeding and

gastrin stimulation was first described by Kahlson et al. in 1964 [5]. Subsequently, a number of studies carried out in rodents by Håkanson and others demonstrated good correlations between increases in plasma gastrin levels and gastric HDC activity [16]. These increases were reversed by reductions in serum gastrin levels accomplished through surgical antrectomy. In humans, elevations of HDC activity and fundic histamine content have been reported in patients with atrophic gastritis and hypergastrinemia [18, 19]. *In vitro* studies demonstrated that gastrin is able to induce increases in HDC activity in highly purified ECL cell preparations derived from rabbits [20] or rats [21]. The induction of HDC enzymatic activity in these preparations is dose-dependent and occurs through direct activation of the CCK-B/gastrin receptor. Since maximal responses (10^{-8} M gastrin) in both preparations were observed within 30-60 min, the rapidity of the response raised the question as to whether the increase in HDC activity was due to the synthesis of new enzyme or activation of stored forms. Histidine decarboxylase in the rat hypothalamus and lung have been shown to be activated by effects of protein kinase A [22]. In addition, one study demonstrated that HDC activity can be altered by phosphorylation, which could provide a regulatory mechanisms for activation of cellular stores of the HDC enzyme [23]. However, the effect of gastrin on HDC synthesis and gene expression awaited further advances in molecular biology and the cloning of the HDC gene.

III. ISOLATION AND CHARACTERIZATION OF THE HDC GENE

A number of groups were successful early on in the partial purification of the histidine decarboxylase protein. Watanabe and Taguchi purified HDC from fetal rat liver and reported that the enzyme was a dimer of 54 kDa subunits with a pI of 5.1 [24, 25]. Several other groups were also successful in partially purifying the enzyme from rat gastric mucosa [26], rat basophilic leukemia cells [27] and mouse mastocytoma cell lines [28]. However, Joseph et al. were the first group to successfully clone a cDNA for histidine decarboxylase [29]. During their studies on androgen binding proteins, they isolated the rat HDC cDNA from a fetal liver library. The cDNA clone was found to encode a 655 amino acid protein that was 50 percent homologous to rat dopa decarboxylase. The cDNA predicted a protein with two potential glycosylation sites and two consensus sites for phosphorylation. The molecular weight of the deduced protein (73 kDa) was significantly larger than the two 55 kDa subunits believed to form the dimer, suggesting post-translational processing [29]. Subsequently, the cDNA's for HDC were cloned from mouse mastocytoma cells [30], human basophilic leukemia KU-812-F cells [31] and human erythroleukemia cells [32]. The human and mouse clones were found to encode proteins of 662 amino acids, and the sequences were 85 percent homologous with rat liver HDC. In addition, the human HDC gene has been mapped to a gene locus on chromosome 15 [32]. Northern blot analysis showed two types of HDC transcripts (2.7 and 3.5 kb) in rat liver and rat corpus [29, 33] and two types of HDC transcripts (2.4 kb and 3.4 kb) in KU-812-F human basophilic leukemia cells [3]. Studies carried out with human basophilic leukemia cells demonstrated that the different sized transcripts arose from alternative splicing of the human HDC gene and that only the 2.4 kb mRNA encodes for functional HDC [34].

IV. TISSUE SPECIFIC EXPRESSION OF HDC

Early studies by Kahlson et al. demonstrated that the fetal rat contained high concentrations of HDC activity, with large increases seen at the 15th day of pregnancy and climbing to a peak in the last one to two days before term, followed by a precipitous decline at the time of birth [5]. Interestingly, despite the high rate of histamine production in the rat

fetus, the actual concentration of histamine was lower in the rat fetus than in the adult, a factor that was attributed to decreased histamine binding and rapid turnover. Most of the fetal HDC activity was localized to the liver, which overall accounted for 80 percent of the HDC activity in the fetus. The fetal stomach and lung demonstrated lower levels of HDC activity compared to the liver, but in general, all fetal tissues exhibited HDC activity that was quite high when compared to that observed in neonatal and adult animals. In the adult rat, HDC activity was lower with a somewhat different distribution. Significant activity was detected in the adult stomach, with lower levels seen in the lung, liver and kidney [5]. Other investigators have confirmed that the most abundant source of HDC and HDC mRNA is in the fetal rat liver, the tissue from which the cDNA sequence was originally detected. Taguchi et al., for example, noted that the fetal rat liver contains about 75 percent of the total level of HDC [25].

Apart from the stomach, the identity of the cells that express HDC remain unclear. Most mammalian tissues that have been studied have been found to express some HDC activity, albeit at low levels. Although mast cells express HDC and are present in many organs, histamine is apparently synthesized by cells other than mast cells. With respect to the liver, it was initially felt that the HDC-expressing cell was of non-mast-cell origin [5]. However, studies by Watanabe et al. showed that embryos from genetically mast cell deficient W/W^v mice lacked the usual increase in hepatic HDC activity demonstrated by normal fetuses at the time of birth [35]. A more recent study by Ohmuri et al. suggests that the HDC-expressing cells in the fetal liver may be mast cell precursors but not mast cells [36]. In addition, the study by Ohmuri et al. provided evidence for possible regulation of fetal hepatic HDC by the plasma glucocorticoid level through the pituitary-adrenal system. This glucocorticoid regulatory mechanism may apply as well to mouse mastocytoma P-815 cells [37] and the rat glandular stomach [38].

In order to investigate the tissue specific expression of the HDC gene in the rat, we carried out a series of Northern blots, using a panel of total RNA prepared from day-19 rat fetuses, as well as from adult (> 6 weeks) rats. In fetal rats, the liver could be identified as the organ with highest levels of HDC expression, the stomach and pancreas were also positive, but displayed lower levels of HDC gene expression (Figure 2). In adult animals, liver, corpus and pancreas were the only organs in which HDC expression could be detected (Figure 2); the highest levels of HDC mRNA abundance were found in the liver, lower expression was found in the gastric corpus and the pancreas. These findings are in contrast to early reports of Kahlson et al. [5] who found in the stomach of adult rats higher HDC enzymatic activity than in the liver.

To analyze the developmental regulation of the HDC gene in liver and stomach, total RNA was isolated from unborn rats at day 19 of gestation and from newborn rats at various time points after delivery. In both organs an identical pattern of HDC expression could be found: HDC mRNA could be detected at day 19 of fetal development while no expression of the gene was detectable at days one, three and five after birth. HDC mRNA was seen again at day seven after delivery, and reached maximal levels after additional 21 days (Figure 3).

V. POSSIBLE ROLE OF HDC EXPRESSION IN GROWTH AND REGENERATION

The role of HDC and histamine in the fetus, and in adult tissues aside from ECL and mast cells, is not well understood. The possibility that histamine may play a significant role in cellular proliferation and tumor growth was first suggested over 30 years ago by Kahlson et al. [39]. These investigators initially observed that, using a combination of pyridoxine deficiency and a small dose of semicarbazole, they were able to lower HDC activity to 20 percent of normal. As a consequence, fetal growth was arrested, and the rat

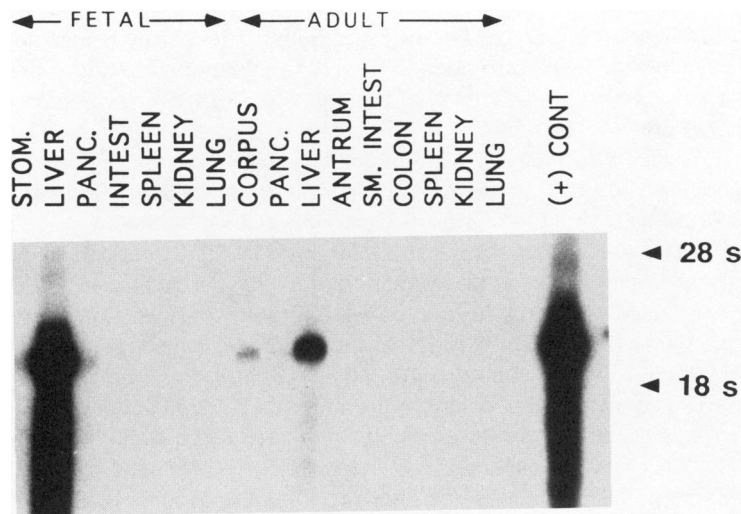


Figure 2: Northern blot analysis of HDC expression in various tissues of fetal and adult rats. Total RNA was prepared as previously described and 20 µg total RNA/lane were electrophoresed in an one percent formaldehyde/agarose gel under denaturing conditions. Following Northern transfer, filters were hybridized with a radiolabeled 354 bp antisense RNA probe complementary to the 3' end of the rat HDC cDNA and autoradiography was performed for 24 hr. 20 µg of total RNA prepared from fetal rat liver was used as a positive control. Positions of 28s and 18s ribosomal RNA's are indicated by arrows.

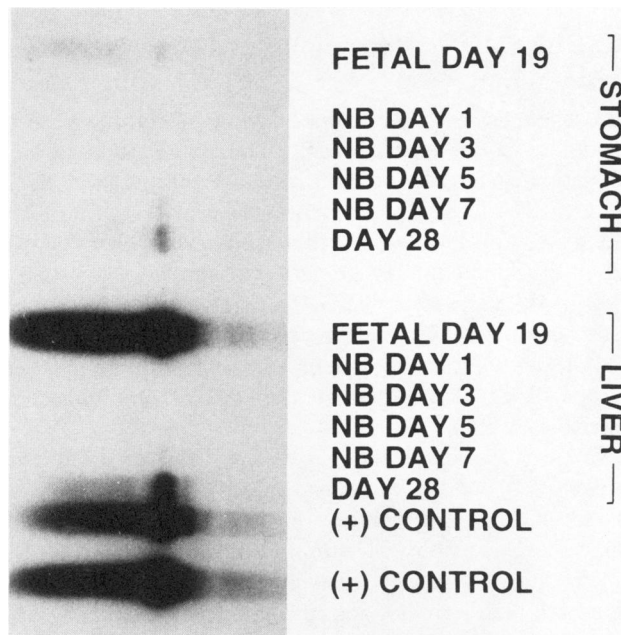


Figure 3. Northern blot analysis of HDC expression in stomach (left) and liver (right) of fetal rats and at various time points after birth. Total RNA was prepared from unborn rats at day 19 of pregnancy and at day 1, day 3, day 5, day 7 and day 28 after delivery. Twenty µg of total RNA per lane were electrophoresed under denaturing conditions in an formaldehyde/agarose gel, transferred to nylon membrane and hybridized with a radiolabeled 354 bp antisense RNA probe, complementary to the 3' end of the rat HDC cDNA. Twenty µg of total RNA prepared fetal rat liver was used as a positive control. Positions of 28s and 18s ribosomal RNA's are indicated by arrows.

fetus died [40]. In addition, rat liver tissue regenerating after partial hepatectomy displays a substantially elevated HDC activity [41, 42]. HDC activity is also raised in the tissue of healing skin wounds [41], mouse skin after application of tumor promoters [43] and in experimental tumors [44, 45]. Exposure of rats to restraint and cold-induced stress results in formation of gastric ulcerations and simultaneous elevations of gastric and hypothalamic HDC activity. The extent of mucosal lesions was diminished by 70 percent after application of an H₂ blocker or by inhibition of HDC activity by treatment with α -flouromethyl histidine (α -FMH) — a specific irreversible inhibitor of HDC, indicating that increased HDC activity is playing a role in the formation of lesions in the rat [46]. The same compound has been used to assess the role of HDC in tumor growth in different systems. Hosoda et al. showed that when α -FMH was given (by i.p. injection or continuous subcutaneous infusion) to an African rodent (*Mastomys natalensis*) bearing transplanted gastric carcinoids, it resulted in a decrease in histamine production and a reduction in tumor size [47]. Thus, in addition to being markedly stimulated in the setting of growth and proliferation, HDC activity may actually contribute to the growth state. The mechanism by which histamine or HDC stimulate cellular proliferation remains unclear, although the possibility has recently been raised that histamine may be acting through intracellular receptors [2, 48]. In addition, Fujimoto et al. have shown that HDC, and ODC, activity increase during mucosal repair in the small intestine after ischemia-reperfusion. Based on studies using α -FMH, they concluded that enzymatically active HDC is necessary for restoration of mucosal function and that this is mediated in part by stimulation of ODC activity [49]. A relationship between stimulation of HDC and ODC activity is also supported by earlier studies showing that both HDC and ODC activity in the colon are stimulated by phorbol esters such as 12-o-tetradecanoylphorbol-13-acetate [50].

VI. REGULATION OF HDC mRNA ABUNDANCE IN THE STOMACH BY GASTRIN — *IN VIVO* MODELS

Early studies carried out in rats established the concept that gastrin acts to control HDC mRNA abundance. Initial experiments by Dimaline et al. showed that fasting rats for 48 hr led to reductions in circulating gastrin concentrations and reductions (by 70 percent) in corpus HDC mRNA levels [33]. Omeprazole treatment of fasted rats led to hypergastrinemia (eight to ten-fold) and significant (three-fold) elevations in HDC mRNA abundance compared to fasted controls. The effect of omeprazole on HDC mRNA abundance could be blocked by specific CCK-B/gastrin receptor antagonists, such as PD 134308. In contrast, omeprazole treatment of fed rats did not result in significant stimulation of HDC mRNA levels [33]. A subsequent study in rats by Sandvik et al. showed that infusion of exogenous gastrin also led to increases in HDC activity and mRNA levels at 2 hr, although the increases were small [51].

However, these early studies did not analyze the time-course for changes in HDC expression after elevation of endogenous plasma gastrin levels. Further, they did not localize these changes in HDC gene expression to specific regions of the gastric mucosa. Therefore, to address these questions, we administered omeprazole (400 mmol/kg) to fasted rats, and analyzed plasma gastrin levels by radioimmune assay and corpus HDC mRNA levels using both Northern blot and *in situ* hybridization analysis [52]. Within 12 hr after a single injection of omeprazole, serum gastrin levels increased 5.5-fold and HDC mRNA levels increased by 4.4-fold. Since earlier studies showed that hypergastrinemia occurs within two to eight hr after a single i.p. injection of omeprazole, this implies a relatively short time span (4 to 10 hr) for the activation of HDC gene expression in response to gastrin. Finally, the stimulation of HDC mRNA abundance could be localized by *in situ* hybridization studies to the basal third of the oxyntic glands, consistent with the known location of ECL cells. The signal was much greater in omeprazole treated rats, compared

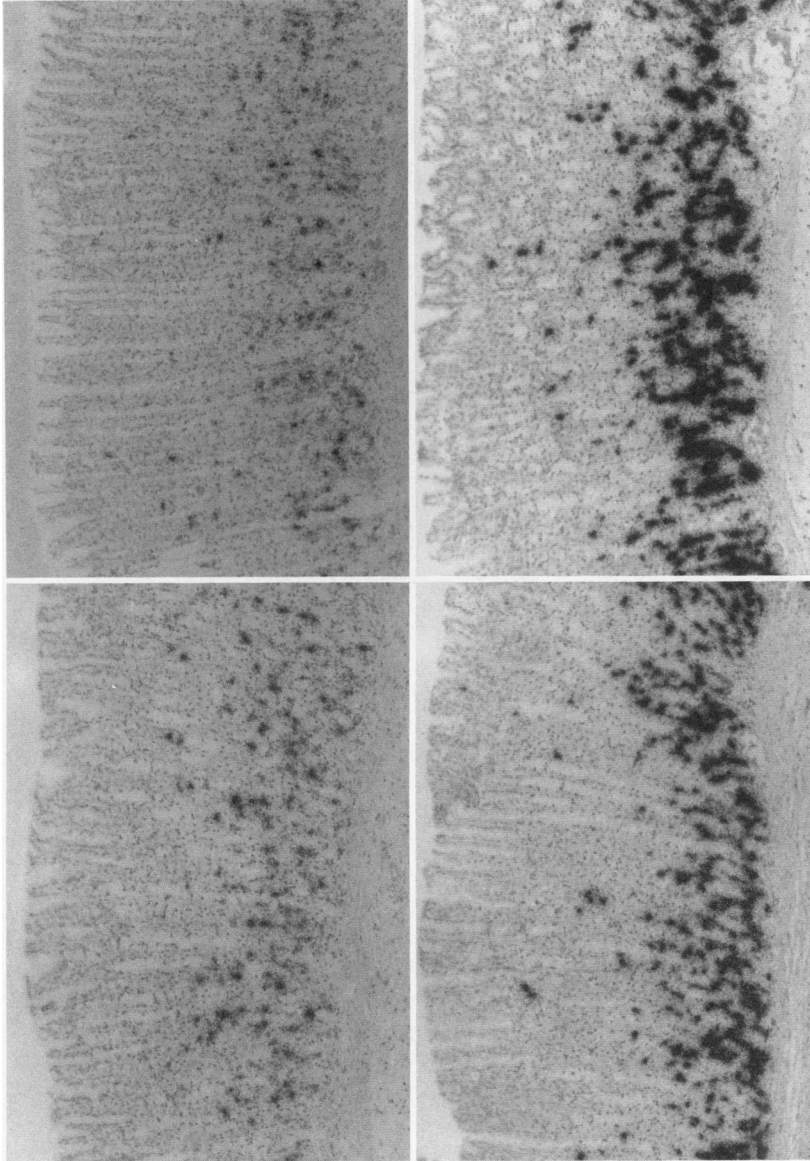


Figure 4. *In situ* hybridization, demonstrating localization of HDC mRNA in the rat gastric corpus. Fasting rats received daily intraperitoneal injections of either methylcellulose (upper panels) or omeprazole (400 mmol/kg) (lower panels) for two days and were sacrificed 48 hr after the first injection. Tissue samples from the corpus regions were fixed and embedded in paraffin. Sections were cut, placed on slides and hybridized with an (32p)-UTP-labeled antisense RNA-probe complementary to the 3' end of the rat HDC cDNA, stained with hematoxylin and eosin and exposed for 14 days. Original photographs were taken at a magnification of x400.

to controls (Figure 4). No stimulation of HDC expression could be detected in the lamina propria, where mucosal mast cells are typically located [52]. Thus, these studies support the concept of a rapid, gastrin-dependent increase in HDC gene expression that occurs specifically in gastric epithelial cells — presumably ECL cells — that are located in the basal third of the oxyntic glands.

VII. REGULATION OF HDC ACTIVITY AND mRNA ABUNDANCE IN MAST CELL/BASOPHILIC LEUKEMIA CELLS IN VITRO

Initial work examining HDC activity and mRNA abundance *in vitro* was carried out in cell lines derived from mastocytomas or basophilic leukemia cells. Rat basophilic leukemia (RBL-2H3) cells were shown to exhibit histidine decarboxylase activity, which was stimulated by higher oligomeric IgE or phorbol myristate acetate [53]. The histidine decarboxylase activity in the human basophilic leukemia cell line (KU-812-F) was also found to be high, could be stimulated by phorbol ester, and its induction was blocked by the protein kinase C inhibitor staurosporine [54]. The mouse mastocytoma cell line (P-815) was shown to contain HDC activity and Northern blot analysis also demonstrated expression of HDC mRNA. Levels of HDC mRNA in P-815 cells were increased by dexamethasone, and TPA treatment had a synergistic effect in combination with dexamethasone [55]. HDC mRNA levels in P-815 cells were also increased by a combination of cyclic AMP and the Ca^{2+} ionophore A23187 [56]. Finally, phorbol ester treatment of P-815 cells stimulated binding to a phorbol ester-response element by a nuclear factor extracted from the mastocytoma cells, which most likely represents transcription factor AP-1 activity [57].

VIII. REGULATION OF HDC TRANSCRIPTION IN GASTRIC CELLS BY GASTRIN — *IN VITRO* MODELS

In order to study the molecular mechanism governing the HDC transcriptional response to gastrin, we turned to an *in vitro* cell culture system. Since no permanent ECL cell line is yet available, a gastric cancer cell line (AGS) was stably transfected with the human CCK-B/gastrin receptor to generate the AGS-B cell line. The presence of functionally intact CCK-B/gastrin receptors in AGS-B cells was confirmed by binding studies, Northern blot analysis, and $[\text{Ca}^{2+}]_i$ measurements after gastrin stimulation [52]. A reporter gene construct was created, which consisted of 1.2 kb of rat HDC 5'-flanking DNA (plus 67 nucleotides of the non-coding portion of exon I), joined to the firefly luciferase reporter gene in the plasmid pXP2. When transfected into plain AGS cells (lacking CCK-B/gastrin receptors), no response to gastrin stimulation was seen. When the rat 1.2 kb HDC-luciferase construct was transfected into AGS-B cells, a dose-dependent increase in reporter gene (luciferase) activity was seen in response to stimulation with the CCK-B/gastrin receptor-specific agonists gastrin or CCK-8. Maximal increases (three to four-fold) were seen with 10^{-7} M gastrin or CCK-8. The effects of gastrin and CCK-8 could be blocked by the CCK-B/gastrin receptor-specific antagonist L 365,260 and the CCK-A receptor-specific antagonist L 364,718 with typical difference in their IC_{50} concentrations [52]. Time-course studies demonstrated that in continuous presence of 5×10^{-7} M gastrin maximal stimulation occurred after 12 hr. After a 4-hr gastrin pulse-exposure to gastrin, maximal activation of the HDC promoter was seen 6 to 8 hr after the end of the gastrin treatment. Finally, deletion analysis indicated that the gastrin response element was located in the rat HDC gene within 201 nucleotides of the translational start site, and thus very close to the basal rat HDC promoter [52]. Overall, these *in vitro* studies with the AGS-B cell line transfected with the rat HDC-luciferase constructs are consistent with our *in vivo* data, supporting the concept of a gastrin-induced transcriptional response of the HDC gene being mediated by specific DNA sequences located in the rat HDC promoter.

IX. SIGNAL TRANSDUCTION PATHWAYS REGULATING HDC GENE EXPRESSION

Using the AGS-B cell system, it has been possible to begin to examine the signal transduction pathways that are activated in response to gastrin stimulation, and which may be mediating transcriptional responses such as activation of the HDC gene. The responses to gastrin are mediated by the CCK-B/gastrin receptor, which was recently cloned and shown to be a seven transmembrane domain, G-protein coupled receptor [58]. Previous studies using transfected COS-7 cells or chinese hamster ovary cells demonstrated that stimulation of the recombinant human CCK-B/gastrin receptor leads to elevation of $[Ca^{2+}]_i$ and induction of protein kinase C translocation, consistent with activation of phospholipase C pathways [58-60].

Using our system of AGS-B cells transfected with rat HDC-luciferase constructs, we have been able to show that gastrin activates HDC transcription through a protein kinase C-dependent pathway. Using the phorbol ester PMA, we showed that direct activation of protein kinase C also stimulated rat HDC promoter activity in a dose-dependent fashion [52]. Stimulation of rat HDC promoter activity by either gastrin or PMA could be blocked by downregulation of protein kinase C (using prolonged phorbol ester pretreatment) or through the use of non-toxic doses of a protein kinase C-specific protein kinase antagonist (H-7). Induction of Ca^{2+} -dependent signaling pathways with thapsigargin did not significantly

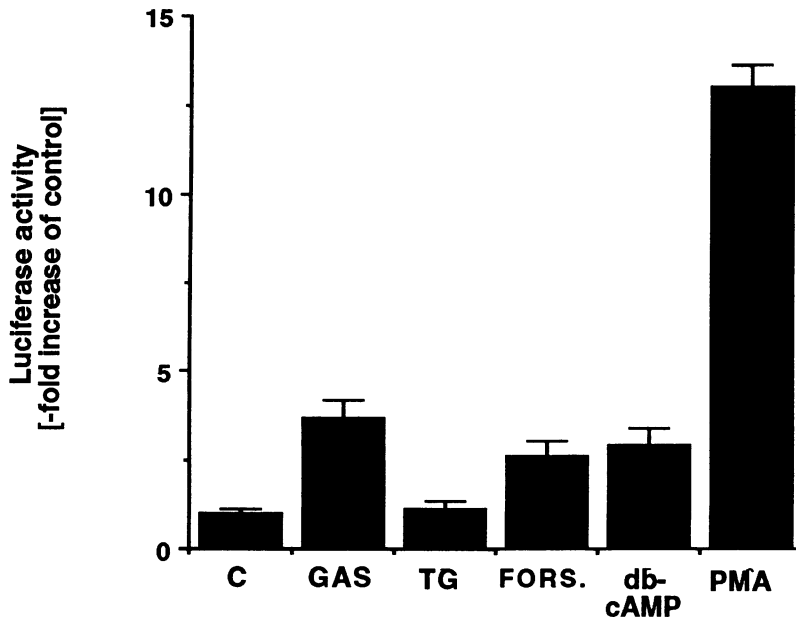


Figure 5. Stimulation of rat HDC promoter activity by gastrin, thapsigargin, forskolin, dibutyryl-cAMP or the phorbol ester PMA. AGS-B cells transiently transfected with the 1.2 kb rHDC-luc reporter construct were incubated with gastrin (5×10^{-8} M), thapsigargin (10^{-6} M), forskolin (10^{-4} M), db-cAMP (10^{-3} M) or PMA (10^{-8} M). Transfected cells were incubated with stimuli for appropriate time spans and cells lysates were assayed for luciferase activity. Results were expressed as increase of luciferase activity detected in unstimulated controls. Values represent means \pm SD of four independent experiments.

induce HDC promoter activity (Figure 5). We also found that the rat HDC promoter could be activated by stimulation of protein kinase A-dependent pathways, using dibutyryl-cAMP or forskolin (Figure 5) [52]. As yet, however, there has been no clear link between the CCK-B/gastrin receptor and protein kinase A pathways, although this stimulatory pathway may represent a potential regulatory mechanism for some other receptor/ligand system.

X. OTHER POTENTIAL REGULATORS OF HDC GENE EXPRESSION

ECL cells play a central role in the peripheral regulation of acid secretion. In addition to being regulated by gastrin, ECL cells respond to a number of other hormones and neurotransmitters. Studies using highly enriched and purified rat ECL cells in culture have demonstrated that ECL cells respond not only to gastrin but also to carbachol, somatostatin, and histamine stimulation [20, 21]. Carbachol, like gastrin, stimulates ECL cells to release histamine and elevates HDC enzymatic activity. Somatostatin has been shown to inhibit gastrin-mediated histamine release. Histamine may exert a negative feedback control on the secretion of histamine by ECL cells, since a specific agonist for the H₃ receptor subtype (R-methyl-histamine) inhibits, and the H₃ receptor antagonist thioperamide potentiates gastrin stimulated histamine release [21].

Although the role of carbachol, somatostatin, and histamine in regulating HDC gene expression has not yet been examined directly, it is likely that they may play a role in this regard. HDC mRNA abundance is clearly regulated by food stimulation; fasting in the rat leads to a reduction in HDC mRNA abundance, while refeeding induces a rapid increase in HDC mRNA, often within 30 min of feeding, which is not reduced by neutralizing gastrin antibodies [51]. The possible role of direct vagal stimulation in regulating HDC activity was raised initially in early studies by Lundell et al. [61]. More recently, it has been shown that HDC mRNA is also increased in the rat oxyntic mucosa by passive distention of the stomach [62]. Thus, at present, there is supportive data which suggest that the response of HDC mRNA to feeding may be mediated by a cholinergic muscarinic reflex.

XI. CONCLUSIONS AND FUTURE STUDIES

Although the studies by our group and those of other labs clearly demonstrate stimulation of HDC gene expression by gastrin, these observations do not exclude the possibility that other levels of HDC activation may be operating *in vivo*, including that of direct activation of HDC enzymatic activity by post-translational modification of pre-formed protein. Experimental evidence for this mechanism was provided by a study using purified ECL cells from rabbits, which suggested that both gastrin and carbachol may increase HDC activity in a manner independent from increases in transcription or translation [20]. However, our data have now clearly established a role of transcriptional control of HDC by gastrin. In addition, our AGS-B cell model now provides the opportunity for a detailed examination of the molecular mechanisms regulating HDC gene expression in the stomach. Using this system, it may be possible to delineate the signal transduction pathways and transcription factors activated by gastrin which regulate HDC gene expression. These studies may also provide insights into the gastrin-dependent pathways involved in other processes, including the regulation of cellular proliferation.

ACKNOWLEDGEMENTS: *This study was supported by a grant from the National Institutes of Health (RO I DK 48077) to TCW. MH was supported by a grant of the German Research Council (DFG), Bonn, Germany (Ho 1288/2-1). TJK is recipient of a NIH Training grant (NIDDK 5T3 DK07191 D21) and a Merck/AGA Senior Fellowship Award.*

REFERENCES

1. Dale, H.H. and Laidlaw, P.P. The physiological action of b-aminazoly-ethylamine. *J. Physiol. Lond.* 41:318-344, 1910.
2. Rangachari, P.K. Histamine: mercurial messenger in the gut. *Am. J. Physiol.* 262 (Gastrointest. Liver Physiol. 25):G1-G13, 1992.
3. Black, J.W., Duncan, W.A.M., Durant, C.J., Ganellin, C.R., and Parsons, E.M. Definition and antagonism of histamine H₂-receptors. *Nature* 236:385-390, 1972.
4. Popielski, L. b-Imidazoläthylamin und die Organextrakte. b-Imidazolyläthylamin als mächtiger Erreger der Magendrüsen. *Pflügers Archiv* 178:214-236, 1920.
5. Kahlson, G., Rosengren, E., Svahn, D., and Thunberg, R. Mobilization and formation of histamine in the gastric mucosa as related to acid secretion. *J. Physiol.* 174:400-416, 1964.
6. Code, C.F. Histamine and gastric secretion: a later look, 1955-1965. *Federation Proceedings* 24:1311-1312, 1965.
7. Ekblad E.B.M. Histamine: the sole mediator of pentagastrin-stimulated acid secretion. *Acta. Physiol. Scand.* 125:135-143, 1985.
8. Bergqvist, E. and Obrink, K.J. Gastrin-histamine as a normal sequence in gastric acid stimulation in the rabbit. *Ups. J. Med. Sci.* 84:145-154, 1979.
9. Chew, C. and Hersey, S.J. Gastrin stimulation of isolated gastric glands. *Am. J. Physiol.* 250:G504-G512, 1982.
10. Lönroth, H., Rosengren, E., Olbe, L., and Lundell, L. Histamine metabolism in human gastric mucosa. Effect of pentagastrin stimulation. *Gastroenterology* 98:921-928, 1990.
11. Man, W.K., Ingoldby, H.J.G., and Spencer, J. Is pentagastrin-stimulated secretion mediated by histamine. *Gut* 25:965-970, 1984.
12. Sandvik, A.K., Waldum, H.L., Kleveland, P.M., and Sognen, B.S. Gastrin produces an immediate and dose-dependent histamine release preceding acid secretion in the totally isolated, vascularly perfused rat stomach. *Scand. J. Gastroenterol.* 22:803-808, 1987.
13. Chew, C. Cholecystokinin, carbachol, gastrin, histamine and forskolin increase [Ca²⁺]_i in gastric glands. *Am. J. Physiol.* 250:G912-G917, 1982.
14. Solcia, E., Capella, C., Vassallo, G., and Buffa, R. Endocrine cells of the gastric mucosa. *Int. Rev. Cytol.* 42:223-286, 1975.
15. Håkanson, R., Börrhwe, F., Ekblad, E., Panula, P., Simonsson, M., Dohlsten, M., Hallberg, T., and Sundler, F. Histamine in endocrine cells in the stomach: a survey of several species using a panel of histamine antibodies. *Histochemistry* 86:5-17, 1986.
16. Håkanson, R. and Sundler, F. The trophic effects of gastrin. *Scand. J. Gastroenterol.* 26(Suppl. 180): 130-136, 1991.
17. Werle, E. Über die Bildung von Histamin aus Histidin durch tierisches Gewebe. *Biochem. Z.* 288:292-293, 1936.
18. Miyazaki, Y., Imamura, I., Shinomura, Y., Wada, H., and Tarui, S. Marked increase in gastric histidine decarboxylase in patients with hypergastrinemia. *Life Sci.* 48:51-58, 1991.
19. Cattani, D., Roucayrol, A.-M., Launary, J.-M., Callebort, J., Charasz, N., Nurit, Y., Belaiche, J., and Kalifat, R. Circulating gastrin, endocrine cells, histamine content, and histidine decarboxylase activity in atrophic gastritis. *Gastroenterology* 97:586-96, 1989.
20. Hollande, F., Bali, J.P., and Magous, R. Neurohormonal regulation of histamine synthesis in isolated rabbit fundic mucosal cells. *Am. J. Physiol.* 266:G395-G402, 1994.
21. Prinz, C., Scott, D.R., Hurwitz, D., Helander, J.F., and Sachs, G. Gastrin effects on isolated rat enterochromaffin-like cells in primary culture. *Am. J. Physiol.* 267 (Gastrointest. Liver Physiol. 30):G663-G675, 1994.
22. Huszti, Z., Magyar, K., and Keleti, J. Possible regulation of hypothalamus and lung histidine decarboxylase activity by cAMP-dependent protein kinase. *Eur. J. Biochem.* 197:191-196, 1991.
23. Savany, A. and Cronenberg, L. Relationship between the multiple forms of rat gastric histidine decarboxylase: Effects of conditions favouring phosphorylation and dephosphorylation. *Biochem. Int.* 20:363-374, 1990.
24. Watanabe, T., Nakamura, H., Liang, L.Y., Yamatodani, A., and Wada, H. Partial purification and characterization of L-histidine decarboxylase from fetal rats. *Biochem. Pharmacol.* 28:1149-1155, 1979.
25. Taguchi, Y., Watanabe, T., Kubota, H., Hayashi, H., and Wada, H. Purification of histidine decarboxylase from the liver of fetal rats and its immunochemical and immunohistochemical characterization. *J. Biol. Chem.* 259:5214-5221, 1984.
26. Savany, A. and Cronenberger, L. Isolation and properties of multiple forms of histidine decarboxylase from rat gastric mucosa. *Biochem. J.* 205:405-412, 1982.

27. Grzanna, R. Histidine decarboxylase: isolation and molecular characteristics. *Neurochem. Res.* 9:993-1009, 1984.
28. Hammar, L. and Hjerten, S. Mammalian histidine decarboxylase; change in molecular properties induced by oxidation and reduction. *Agents Actions* 10:93-8, 1980.
29. Joseph, D.R., Sullivan, P.M., Wang, Y.M., Kozac, M., Fenstermacher, D.A., Behrendsen, M.E., and Zahnov, C.A. Characterization and expression of the complementary DNA encoding rat histidine decarboxylase. *Proc. Natl. Acad. Sci. U.S.A.* 87:733-737, 1990 (correction *Proc. Natl. Acad. Sci. U.S.A.* 87:7346, 1990).
30. Yamamoto, J., Yatsunami, K., Ohmori, E., Sugimoto, Y., Fukui, T., Katayama, T., and Ichikawa, A. cDNA-derived amino acid sequence of L-histidine decarboxylase from mouse mastocytoma P-815 cells. *FEBS* 276:214-218, 1990.
31. Yamauchi, K., Sato, R., Tanno, Y., Ohkawara, Y., Maeyama, K., Watanabe, T., Satah, K., Yoshizawa, M., Shibahara, S. and Takishima, T. Nucleotide sequence of the cDNA encoding L-histidine decarboxylase derived from human basophilic leukemia cell line, KU-812-F. *Nucl. Acid Res.* 18:5891, 1990.
32. Zahnov, C.A., Hua-Fang, Y., McBride, W., and Joseph, D.R. Cloning of the cDNA encoding human histidine decarboxylase from an erythroleukemia cell line and mapping of the gene locus to chromosome 15. *DNA Sequence* 1:395-400, 1991.
33. Dimaline, R. and Sandvik, A.K. Histidine decarboxylase gene expression in rat fundus is regulated by gastrin. *FEBS Lett.* 281:20-22, 1991.
34. Mamune-Sato, R., Yamauchi, K., Tanno, Y., Ohkawara, Y., Ohtsu, H., Katayose, D., Maeyama, K., Watanabe, T., Shigeki, S., and Takishima, T. Functional analysis of alternatively spliced transcripts of the human histidine decarboxylase gene and its expression in human tissues and basophilic leukemia cells. *Eur. J. Biochem.* 209:533-539, 1992.
35. Watanabe, T., Kitamura, Y., Maeyama, K., Go, S., Yamatodani, A., and Wada, H. Absence of increase of histidine decarboxylase activity in mast cell-deficient W/W mouse embryos before parturition. *Proc. Natl. Acad. Sci. U.S.A.* 78:4209-4212, 1981.
36. Ohmori, E., Imanishi, N., Ohgoh, M., Fukui, T., and Ishikawa, A. Fluctuation of fetal rat hepatic histidine decarboxylase activity through the glucocorticoid-ACTH system. *Biochem. Pharmacol.* 41:844-847, 1991.
37. Imanishi, N., Nakayama, T., Asano, M., Yatsunami, K., and Ichikawa, A. Induction of histidine decarboxylase by dexamethasone in mastocytoma P-815 cells. *Biochim. Biophys. Acta.* 928:227-234, 1987.
38. Imanishi, N., Ohmori, E., Yatsunami, K., and Ichikawa, A. Effect of hydrocortisone on histidine decarboxylase activity in rat stomach. *Chem. Pharm. Bull. (Tokyo)* 36:4088-4094, 1988.
39. Kahlson, G. and Rosengren, E. Inhibition of histamine formation and some of its consequences. *J. Physiol.* 149:66P, 1959.
40. Kahlson, G. and Rosengren, E. Prevention of foetal development by enzyme inhibition. *Nature Lond.* 184:1238-1239, 1959.
41. Kahlson, G., and Rosengren, E. New approaches to physiology of histamine. *Physiol. Rev.* 48:155-196, 1968.
42. Ishikawa, E., Toki, A., Moriyama, T., Matsuoka, Y., Aikawa, T., and Suda, M. A study on the induction of histidine decarboxylase in tumor-bearing rat. *J. Biochem.* 68:347-358, 1970.
43. Watanabe, T., Taguchi, Y., Sasaki, K., Tsuyama, K., and Kitamura, Y. Increase in histidine decarboxylase activity in mouse skin after application of the tumor promoter tetradecanoylphorbol acetate. *Biochem. Biophys. Res. Commun.* 100:427-432, 1981.
44. Bartholeyns, J. and Bouclier, M. Involvement of histamine in growth of mouse and rat tumors: antitumoral properties of monofluoromethylhistidine, an enzyme-activated irreversible inhibitor of histidine decarboxylase. *Cancer Res.* 44:639-645, 1984.
45. Kahlson, G. and Rosengren, E. Histamine: entering physiology. *Experientia (Basel)* 28:993-1002, 1972.
46. Bouclier, M., Jung, M., and Gerhart, F. Histamine receptor blockade (H₂) versus inhibition of histamine synthesis in stress ulceration in rats. *Eur. J. Pharmacol.* 90:129-132, 1983.
47. Hosoda, S., Saito, T., Kumazawa, H., Watanabe, T., and Wada, H. Marked inhibition of histamine formation in transplantable histamine-producing gastric carcinoid of *Mastomys Natalensis* by (S)- α -fluoromethylhistidine and its potent antiulcer effect on tumor-bearing hosts. *Biochem. Pharmacol.* 34:4327-4329, 1985.
48. Brandes, L.J., Labella, F.S., Glavin, G.B., Paraskevas, F., Saxena, S.P., McNicol, A., and Gerrard, J.M. Histamine as an intracellular messenger. *Biochem. Pharmacol.* 40:1677-1681, 1990.

49. Fujimoto, K., Imamura, I., Granger, D.N., Wada, H., Sakata, T., and Tso, P. Histamine and histidine decarboxylase are correlated with mucosal repair in rat small intestine after ischemia-reperfusion. *J. Clin. Invest.* 1992; 89:126-133.
50. Sun, Y. and Li, Y. Induction of ornithine decarboxylase and histidine decarboxylase activities in rat colon mucosa after application of 12-o-tetradecanoylphorbol-13-acetate, sodium deoxycholate and indole. *Cancer Lett.* 39:77-84, 1988.
51. Sandvik, A.K., Dimaline, R., Marvik, R., Brenna, E., and Waldum, H.L. Gastrin regulates histidine decarboxylase activity and mRNA abundance in rat oxyntic mucosa. *Am. J. Physiol.* 267 (Gastrointest. Liver Physiol. 30): G254-G258, 1994.
52. Höcker, M., Zhang, Z., Fenstermacher, D.A., Tagerud, S., Chulak, M.B., Joseph, D., and Wang, T.C. The rat histidine decarboxylase promoter is regulated by gastrin through a protein kinase C pathway. *Am. J. Physiol.* 270 (Gastrointest. Liver Physiol. 33):G-619-G-633, 1996.
53. Maeyama, K., Taguchi, Y., Sasaki, M., Wada, H., Beaven, M.A., and Watanabe, T. Induction of histidine decarboxylase of rat basophilic leukemia (2H3) cells stimulated by higher oligomeric IgE or phorbol myristate acetate. *Biochem. Biophys. Res. Commun.* 151:1402-1407, 1988.
54. Mamune-Sato, R., Tanno, Y., Maeyama, K., Miura, Y., Takishima, T., Kishi, K., Fukuda, T., and Watanabe, T. Histidine decarboxylase in human basophilic leukemia (KU-812-F) cells. *Biochem. Pharm.* 40:1125-1129, 1990.
55. Kawai, H., Ohgoh, M., Emoto, S., Ohmori, E., Imanishi, N., Yatsunami, K., and Ichikawa, A. Synergistic effects of 12-O-tetradecanoylphorbol-13-acetate and dexamethasone on de novo synthesis of histidine decarboxylase in mouse mastocytoma P-815 cells. *Biochim. Biophys. Acta.* 1133:172-178, 1992.
56. Miyazaki, T., Ohgoh, M., Ohmori, E., Yamamoto, J., Emoto, S., Yatsunami, K., and Ichikawa, A. Synergistic effects of cyclic AMP and Ca²⁺ ionophore A23187 on de novo synthesis of histidine decarboxylase in mastocytoma P-815 cells. *Biochim. Biophys. Acta.* 1133:179-186, 1992.
57. Yatsunami, K., Nakagawa, S., Ohgo, M., Yamamoto, J., and Ichikawa, A. 12-O-tetradecanoylphorbol-13-acetate stimulates the transcription of the histidine decarboxylase gene. *Agents Actions* 38:C155-C157, 1993.
58. Young-Mee, L., Beinborn, M., McBride, E.W., Lu, M., Kolakowski, L.F., and Kopin, A.S. The human brain cholecystokinin-B/gastrin receptor. *J. Biol. Chem.* 268:8164-8169, 1993.
59. Ito, M., Matsui, T., Taniguchi, T., Tsukamoto, T., Murayama, T., Arima, N., Nakata, H., Chiba, T., and Chihara, K. Functional characterization of a human brain cholecystokinin-B receptor. *J. Biol. Chem.* 268:18300-18305, 1993.
60. Yassin, R.R., Clearfield, H.R., and Little, K.M. Gastrin's trophic effect in the colon: identification of a signalling pathway mediated by protein kinase C. *Peptides* 14:1119-1124, 1993.
61. Lundell, L. The role of cholinergic excitation in the formation of histamine within the rat gastric mucosa. *Acta. Physiol. Scand.* 97:145-149, 1976.
62. Dimaline, R., Evans, D., Dockray, G.J. Histidine decarboxylase (HDC) mRNA in rat stomach is rapidly increased by distention, and by a post-prandial cholinergic reflex. *Gastroenterology* 108: A963, 1995.