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# Immunolocalization of histamine $H_3$ receptors on endocrine cells in the rat gastrointestinal tract

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**Summary.** The histamine $H_3$ receptor ($H_3R$) has been identified in the gastrointestinal tract of the rat by immunohistochemistry, using the first validated anti-$H_3$ receptor antibody. Immunoreactivity to $H_3R$ was exclusively localized to the endocrine cells scattered in the gastrointestinal mucosa, with positive cells being prominently abundant in the gastric fundus, while they were rarely found in the other regions. In the fundus, positive cells were distributed in the lower half of the mucosa and their number significantly decreased after a 24 h-fasting period. Double-labeling studies were undertaken to identify the $H_3R$-immunoreactive cell types in the fundic and antral mucosa. The $H_3R$-immunoreactive cells were positive for chromogranin A. In the fundus, approximately 90% of cells positive to $H_3R$ were also positive to the histamine-forming enzyme, histidine decarboxylase. None of the cells expressing $H_3R$ displayed immunoreactivity for gastrin, somatostatin or ghrelin. Location, the influence of food deprivation and colocalization with histidine decarboxylase indicate that $H_3R$ positive cells correspond to the enterochromaffin-like cells (ECL).

**Key words:** Histamine $H_3$ receptor, Gastric fundus, Histidine decarboxylase, Enterochromaffin-like cells, Food deprivation

## Introduction

The histamine $H_3$ receptor ($H_3R$) is a presynaptic receptor which regulates the release of histamine and of various neurotransmitters (Arrang et al., 1983; Schlicker et al., 1994). There is a general agreement that the histamine $H_3$ receptor is abundantly expressed in the central nervous system of different animal species (Lovenberg et al., 1999; Tardivel-Lacombe et al., 2000), while its presence in the periphery remains controversial. $H_3R$ mRNA expression, analyzed by in situ hybridization and Northern blotting, is reported to be either undetectable in peripheral tissues of humans and rats (Lovenberg et al., 1999, 2000; Hemedah et al., 2001) or alternatively to be restricted to liver and epithelia, including the mucosa of the gastrointestinal tract (Heron et al., 2001) or to brown adipose tissue (Karlstedt et al., 2003), in developing and adult rats. In distinct studies, based on Reverse Transcription-Polymerase Chain Reaction (RT-PCR), the $H_3R$ was found to be highly expressed in rat epithelia (Heron et al., 2001), absent in mouse peripheral organ tissues (Chen et al., 2003) while in human peripheral tissues, expression of $H_3R$ mRNA was reported to be either restricted to the small intestine, prostate and testis (Lovenberg et al., 1999) or to the stomach and lymphocytes (Wellendorph et al., 2002) or to be detectable at low levels in five of the 66 samples considered (Sander et al., 2006). Immunostaining of human intestinal tissues with a commercial antibody failed to reveal the presence of $H_3R$ (Sander et al., 2006). The ganglia of autonomic and enteric nervous systems are reported to be negative for $H_3R$ mRNA expression (Karlstedt et al., 2003; Sander et al., 2006).

Conversely, pharmacological studies suggest the presence of $H_3Rs$. Indeed, histamine and selective agonists and antagonists of histamine $H_3$ receptors have been demonstrated to exert multiple effects on peripheral organs, particularly in the gastrointestinal tract they influence secretion, motility, protection of the gastric mucosa and epithelial cell proliferation (Coruzzi et al., 1999; Morini et al., 2002).

In the present study, optimised immuno-histochemical protocols with a validated selective anti-$H_3R$ antibody were used to investigate the possible existence and location of histamine $H_3R$ within the rat gastrointestinal tract, and thereby to elucidate further the

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relevance of this receptor in mediating the effects of histamine at the gastrointestinal level. The influence of fasting was also investigated.

## Materials and methods

### *Animals*

Twelve male Wistar strain rats (8 weeks of age, 180-200 g, Harlan, Italy) were housed at 22°C on a 12 h light/dark cycle. Six rats had free access to food and water. Another six rats were deprived of food, but not of water, for 24 h before the sacrifice. The animals were sacrificed by cervical dislocation. The experiments were approved by the Italian Animal Care and Use Committee.

### *Tissue preparation*

On sacrifice, the abdominal cavity was opened. The stomachs were ligated at the oesophagus and duodenum, inflated by injecting the fixative (10% formalin, 1.5 ml), immersed in the fixative for 10 min, and then washed after the removal of the nonglandular portion of the stomach. After overnight fixation in 10% formalin, 2-3 tissue samples were obtained from the fundus, by excising the mucosa 3-4 mm below and parallel to the limiting ridge, with the greater curvature approximately located in the middle of the strip, from the antrum, with the lesser curvature approximately located in the middle, and from the duodenum. Small intestine and colon were removed from the peritoneal cavity, 2-cm tissue samples were taken from distal small intestine and distal colon, opened along the mesenteric border, pinned flat and immersed in 10% formalin overnight. All tissue samples were embedded in paraffin and sectioned at 4 µm. Serial sections were stained with hematoxylin-eosin or prepared for immunohistochemistry.

### *Generation of $H_3R$ antibody*

An anti-$H_3R$ antibody was generated in rabbits to an oligopeptide corresponding to a sequence within the third intracellular loop ($H_3$ 349-358) of the human $H_3R$, as described in Chazot et al. (2001). This sequence is largely identical in both human, rat and mouse $H_3R$ orthologs. Serum was collected and purified using $H_3$ (349-358) peptide-affinity chromatography with a mean yield of 244 µg antibody/ml original serum. Specificity of the antibody has been previously confirmed using immunoblotting +/- antigen peptide, and immunohistochemical techniques with $H_3$ (+/+) and $H_3$ (-/-) knockout mice (Cannon et al., 2007).

### *Immunohistochemistry*

Serial sections were depararaffinized, rehydrated, immersed in citrate buffer (10 mM citric acid; pH 6) and heated for 30 min at 98°C and then washed with distilled water. Thereafter sections were incubated for 10 min in 3% (vol/vol) $H_2O_2$/PBS to inhibit endogenous peroxidase activity, and washed in PBS. The preparations were incubated with normal goat serum, to minimize nonspecific binding of the primary antibody. They were then incubated with the primary $H_3R$ antibody (concentration 298 µg/ml) diluted to 1:220 for 60 min at room temperature, optimal dilution being determined in pilot experiments. For comparison two antibodies for $H_3R$ were obtained commercially from Chemicon, International Inc., Temecula, CA, USA. The Chemicon AB5660P (final dilution 1:200) was targeted against an 18 amino acid sequence in the C-terminus region of rat $H_3R$. The Chemicon AB9189 (final dilution 1:200) was targeted against a synthetic peptide from the third cytoplasmic domain of human $H_3R$. In addition, serial sections from gastric fundus and antrum were incubated for 60 min at room temperature with the following primary antibodies: mouse monoclonal anti-chromogranin A (CgA) (Neomarkers, Fremont, CA, USA; final dilution 1:250), rabbit polyclonal anti-histidine decarboxylase (HDC) (Eurodiagnostica, Malmo, Sweden; final dilution 1:500), rabbit polyclonal anti-somatostatin (Zymed Laboratories Inc., San Francisco, CA, USA; final dilution 1:100), rabbit polyclonal anti-gastrin (Chemicon International Inc., Temecula, CA, USA; final dilution 1:500), rabbit polyclonal anti-ghrelin (Phoenix Pharmaceuticals Inc., Belmont, CA, USA; final dilution 1:2,000). Following washing in PBS, secondary antibodies were supplied with the LSAB2 staining kit (DakoCytomation, Glostrup, Denmark) and used according to the manufacturer's suggested protocol. Immunoreactivity was visualized with freshly prepared 3,3'-diaminobenzidine (DAB) (Sigma, St. Louis, MO, USA). The specimens were counterstained with hematoxylin, dehydrated and mounted. To determine the level of non-specific staining the preparations were incubated without the primary $H_3R$ antibody and/or with the primary antibody blocked by overnight preincubation with an excess of the peptide, against which the antibody was raised (Cannon et al., 2007).

### *Double-labeling nonfluorescent immunohistochemistry*

Double labeling was used to identify the cell types expressing $H_3R$ in the fundic and antral regions of the stomach. The procedure described by Lan et al. (1995) was followed. Sections were stained for CgA, HDC, somatostatin, gastrin or ghrelin and binding sites were visualized with DAB, as previously described. Sections were then microwaved twice for 5 min and incubated with normal goat serum for 10 min. Thereafter the tissues were incubated with anti-$H_3R$ antibody (1:220) for 60 min at room temperature. After washing in PBS, sections were reacted with biotinylated link antibody (DakoCytomation), followed by incubation with alkaline phosphatase–conjugated streptavidin (DakoCytomation). Immunoreactivity signal was developed using Dako Fast

Red Substrate System (DakoCytomation). The specimens were counterstained with hematoxylin. By double staining, cells immunoreactive to CgA, HDC, somatostatin, gastrin or ghrelin stained brown and cells immunoreactive to $H_3R$ stained red.

### *Double-labeling immunofluorescence of $H_3R$ and HDC*

Double immunofluorescence was used to confirm the colocalization of $H_3R$ and HDC on ECL cells. Since both primary antibodies are made in rabbit, the procedure described by Rice et al. (1997) was followed. Serial sections of the fundic region of the stomach were prepared as described above. Sections were incubated with anti-$H_3R$ antibody (1:220) for 60 min at 4°C. The tissues were then incubated with biotinylated link antibody (DakoCytomation) for 30 min at room temperature, followed by incubation with FITC conjugated streptavidin diluted 1:200 for 1 h at room temperature (Southern Biotech, Birmingham, AL, USA). Sections were then microvawed twice for 5 min and incubated with normal goat serum for 10 min. Subsequently, the sections were incubated with the second primary antibody directed against HDC diluted 1:500 for 60 min at 4°C. The tissues were then incubated with biotinylated link antibody for 30 min at room temperature, followed by incubation with TRITC conjugated streptavidin diluted 1:200 for 1 h at room temperature (Southern Biotech, Birmingham, AL, USA). Each step was separated by a PBS wash. Alternate sections on other slides were prepared by reversing the order of the primary antibodies. All sections were counterstained with DAPI (Biogenex, San Ramon, CA, USA). The slides were examined using the Olympus BX60 microscope and images were captured by the Olympus DP10 digital camera.

### *Quantitative analysis*

Quantitations were performed using a colour image analysis software system (LUCIA G, Nikon Laboratory Imaging, Japan). Only regions in which full length glands, crypts or villi were oriented perpendicular to the luminal surface were considered for quantitative analysis. Counts for cells positively immunoreactive were determined in at least 3 different sections, for a total of 60 glands, crypts or villi per rat, for each region of the gastrointestinal tract. For each rat the values from each region were averaged and these values used to calculate the mean values for each group. The results were expressed as the mean number of immunoreactive cells per unit.

### *Statistical analysis*

All values were expressed as means ± SEM. The significance of differences between the means was determined by Wilcoxon rank sum test. P values <0.05 were considered statistically significant.

## Results

### *$H_3$-R immunoreactivity*

Immunoreactivity to the $H_3R$ was present in all regions of the rat gastrointestinal tract examined, but with profound variations in density.

$H_3R$ immunoreactivity ($H_3R$-IR) was predominantly localized in the gastric fundus. In this region, studies were performed first with the antibody developed by Chazot et al. (2001). $H_3R$-IR was intense. Positive cells were distributed in the lower half of the mucosa, mostly at the bottom, and were virtually absent in the superficial portion (Fig. 1). The number and morphology of positive cells was influenced by food deprivation. In freely fed rats, the number of positive cells per gland was 5.08±0.25 and cells were large, often exhibiting long processes (Table 1; Fig. 1A,B). Compared with freely fed rats, a 24 h-fasting period significantly decreased the number of positive cells per gland to 3.09±0.31 (Table 1). Moreover positive cells appeared to be smaller (Fig. 1C,D). Location, morphology and the influence of food deprivation suggest that $H_3R$ positive cells correspond to enterochromaffin-like (ECL) cells. Studies were then repeated with the two commercially available $H_3R$ antibodies. Labeling pattern was comparable to that obtained with the antibody from Chazot, though the labelling intensity was much lower. In the subsequent and in double-labeling studies only the Chazot antibody was used.

$H_3R$ positive cells were also found in the antral mucosa, where they were mostly located in the basal portion of the glands (Fig. 2). Their density was extremely low, mean number of positive cells per gland

**Table 1.** Numbers of immunoreactive (IR) cells in the fundic mucosa of freely fed and fasted rats.

| | $H_3R$-IR cells (no/gland) | Chromogranin A-IR cells (no/gland) | HDC-IR cells (no/gland) | Somatostatin-IR cells (no/gland) | Ghrelin-IR cells (no/gland) |
|---|---|---|---|---|---|
| Freely fed rats | 5.08±0.25 | 4.85±0.37 | 4.10±0.24 | 0.85±0.10 | 0.84±0.09 |
| 24 h-Fasted rats | 3.09±0.31[a] | 4.94±0.28 | 2.38±0.16[a] | 0.93±0.06 | 1.56±0.07[a] |

Data are expressed as mean of the total number of immunoreactive cells per gland ± SEM; n=6 rats per group. a P<0.05 vs freely fed rats (Wilcoxon rank sum test).

being 0.48±0.06 (Table 2).

$H_3$R-IR cells were scattered in the epithelium of both villi and crypts from duodenum and distal ileum and of crypts from distal colon. They were very rare with less than one positive cell per unit routinely observed. Positive cells had the typical morphology of intestinal endocrine cells (Fig. 3). In regions other than the gastric fundus, the frequency of $H_3$R positive cells was not influenced by food deprivation. In all the regions examined, immunoreactivity for the $H_3$R was lost when the primary $H_3$R antibody was omitted or when the antibody was preadsorbed with an excess of the peptide, confirming the specificity of the immunostaining to the $H_3$R protein.

Throughout the gastrointestinal regions examined, no $H_3$R-IR was found in the submucosa, mast cells, muscle layer, neuronal cells, nerve fibers and vessels.

### *Double-labeling studies*

Double-labeling nonfluorescent immunohistochemistry was undertaken in order to identify the $H_3$R-IR cell types, focusing on the fundic and antral mucosa. CgA was used as a marker of endocrine cells. In gastric fundus of freely fed rats, CgA-IR cells were comparable in number to $H_3$R-IR cells and they were also similarly located in the basal region of the mucosa. Most of the CgA-IR cells were positive to $H_3$R (Fig. 4A). The

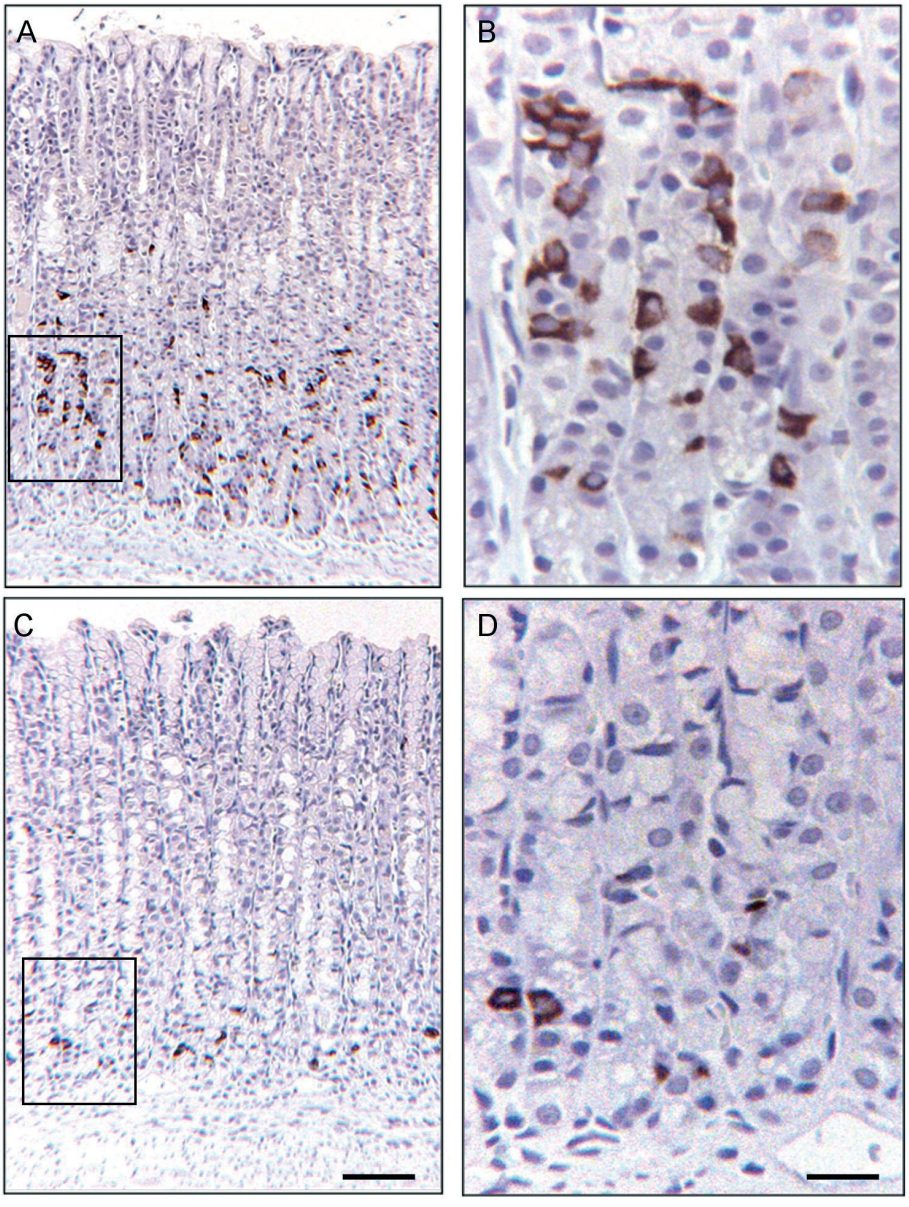


**Fig. 1.** Immunolocalization of histamine $H_3$ receptor in the gastric fundus from freely fed **(A, B)** and 24 h-fasted rats **(C, D)**. **B and D** represent higher magnification of the rectangle shown in **A and C**. Immunoreactive cells are scattered in the basal region of the mucosa. Immunoreactivity is intense and present in the cytoplasm, while nuclei are not stained. Cells are more large and numerous in freely fed as compared with fasted rats. Scale bars: A, C, 75 µm; B, D, 16 µm.

density of CgA-IR cells was not influenced by food deprivation, and their number was similar in fed and fasted rats (Table 1). Immunostaining for HDC was used to demonstrate ECL cells in the fundic mucosa. In fed rats, the number of HDC-IR cells was slightly lower than that of $H_3$R-IR cells (Table 1). Positive cells were similar in location and shape to $H_3$R-IR cells. Approximately 90% of cells positive to $H_3$R were also immunoreactive to HDC (Fig. 4C). Food deprivation caused a 42% decrease in number of HDC-IR cells and a reduction in cell size, as similarly observed for $H_3$R-IR cells (Table 1). Somatostatin-IR cells were present in small number and not affected by food intake. Ghrelin-IR cells, low in number in fed rats, significantly increased in fasted animals. Double immunostaining for somatostatin/$H_3$R and for ghrelin/$H_3$R revealed that cells immunoreactive to somatostatin (Fig. 4E) as well as to ghrelin (Fig. 4G) were $H_3$R negative

In the gastric antrum CgA-IR cells were present in higher number (5-fold) than $H_3$R-IR cells and their number was not influenced by food intake (Table 2). In double labeling studies approximately all the cells immunoreactive to $H_3$R were also positive to CgA (Fig. 4B). In the gastric antrum, cells positive to gastrin, somatostatin and ghrelin were identified. Ghrelin-IR cells increased while, as expected, gastrin-IR cells decreased in number in the fasted state (Table 2). Double immunostaining revealed that cells positive to gastrin, somatostatin and ghrelin were $H_3$R-IR negative (Fig. 4D,F,H).

Colocalization of $H_3$R and HDC in the fundic mucosa was substantiated by double immunofluorescence staining. Consistent with nonfluorescent staining, the results shown in Fig. 5 provide a good indication that $H_3$R-positive cells coincided with HDC-positive cells.

## Discussion

Present findings provide clear evidence that $H_3$R-IR is present throughout the rat gastrointestinal tract. $H_3$R-IR appears to be exclusively localized to the endocrine cells scattered in the gastrointestinal mucosa, with $H_3$R-positive cells being prominently abundant in the gastric fundus, whereas they were rarely found in the other regions.

Expression and localization of $H_3$R in the

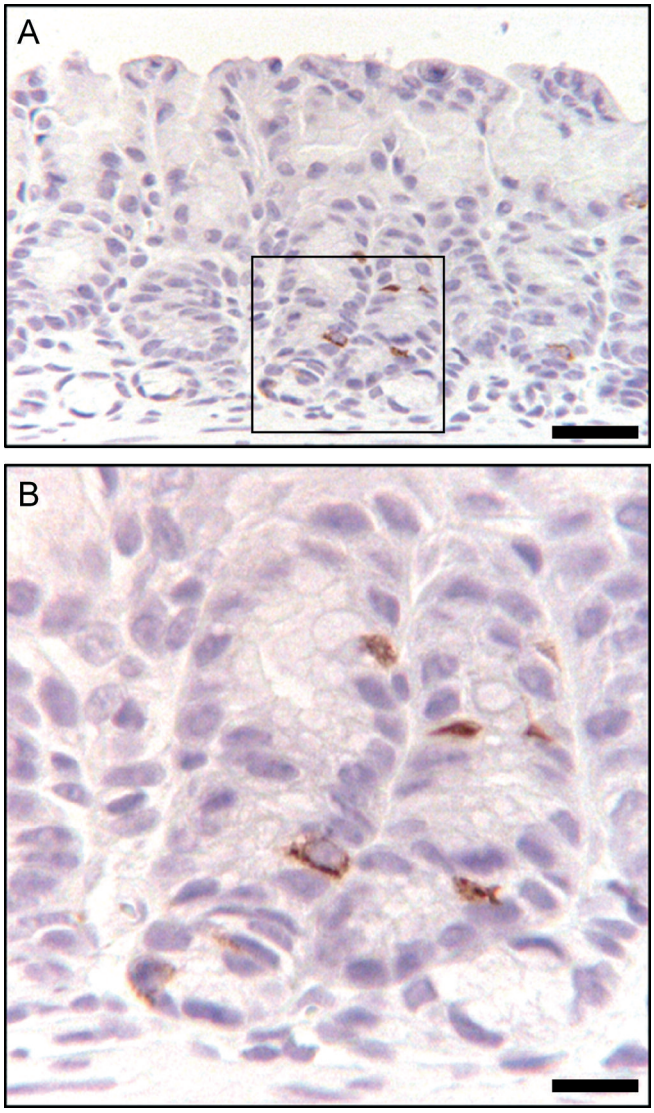


**Fig. 2. A.** Immunolocalization of histamine $H_3$ receptor in the gastric antrum from a freely fed rat. **B** represents the higher magnification of the rectangle shown in **A.** Immunoreactive cells are few and mostly located in the basal region of the mucosa. Nuclei are not stained. Scale bars: A, 60 µm; B, 12 µm.

**Table 2.** Numbers of immunoreactive (IR) cells in the antral mucosa of freely fed and fasted rats.

| | $H_3$R-IR cells (no/gland) | Chromogranin A-IR cells (no/gland) | Gastrin-IR (no/gland) | Somatostatin-IR cells (no/gland) | Ghrelin-IR cells (no/gland) |
|---|---|---|---|---|---|
| Freely fed rats | 0.48±0.06 | 2.57±0.07 | 1.71±0.15 | 0.54±0.07 | 0.21±0.01 |
| 24 h-Fasted rats | 0.42±0.04 | 2.33±0.26 | 0.76±0.06[a] | 0.58±0.07 | 0.41±0.03[a] |

Data are expressed as mean of the total number of immunoreactive cells per gland ± SEM; n = 6 rats per group. a P< 0.05 vs freely fed rats (Wilcoxon rank sum test).

gastrointestinal tract as well as in other peripheral tissues has not yet been established. Most studies investigating the presence of peripheral $H_3R$ both in humans and in rodents analyzed the localization of receptor mRNA. Lovenberg et al. (1999) attributed the absence of a clear peripheral expression expression of $H_3R$ mRNA to the presynaptic nature of the receptor, and to the consequent difficulty to correlate functional receptors with mRNA expression or to the possible existence of receptor subtypes. Alternatively the failure of Northern blot analysis or in situ hybridization to detect $H_3R$ has been attributed to the low density of the receptor in the periphery (Hemedah et al., 2001). However the use of RT-PCR amplification has not provided consistent findings, if we consider that the receptor is reported to be highly expressed in rat stomach and small intestine (Heron et al., 2001), absent in mouse (Chen et al., 2003) while in humans it is reported to be expressed in small intestine or in stomach only or to be absent (Lovenberg et al., 1999; Wellendorph et al., 2002; Sander et al., 2006). This present study has taken an alternative approach to detect the $H_3R$, based on our previously validated anti-$H_3R$ antibody, and an optimised immunohistochemical protocol (Chazot et al., 2001). Our anti-$H_3R$ antibody detects all the major isoforms present in the rat (Shenton and Chazot, unpublished

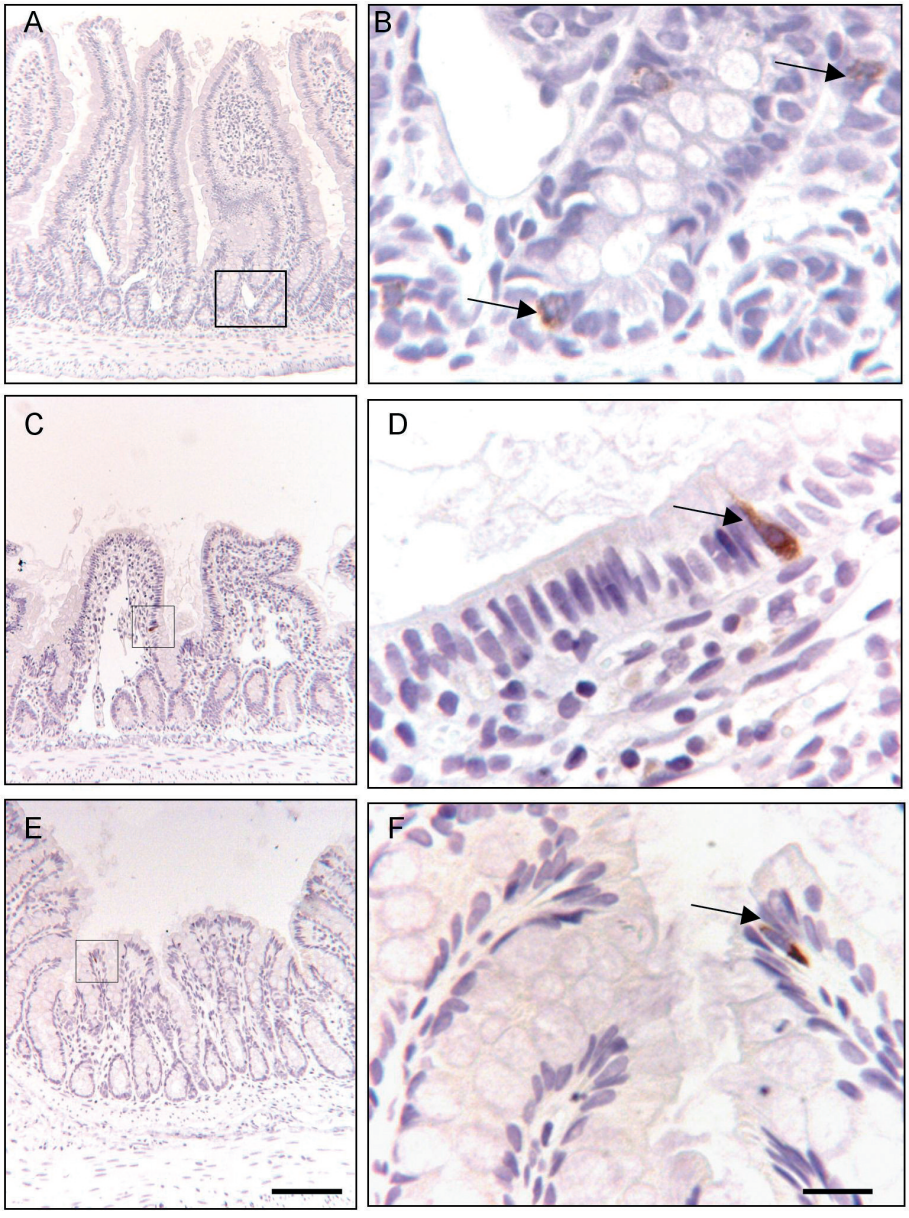


**Fig. 3.** Immunolocalization of histamine $H_3$ receptor in the duodenum **(A, B)**, distal ileum **(C, D)** and distal colon **(E, F)** from freely fed rats. **B, D and F** represents the higher magnification of the rectangle shown respectively in **A, C and E.** Immunoreactive cells are rare and located in the epithelium of both villi **(D)** and crypts **(B)** from duodenum and distal ileum and of crypts **(F)** from distal colon. Scale bars: A, C, E, 110 µm; B, D, F, 16 µm.

data). Further studies with isoform-specific $H_3R$ antibodies, which we are currently developing, will shine further light on the significance of receptor splicing in the gastrointestinal tract (Bakker et al., 2006). Our anti-$H_3R$ antibody showed strong immunoreactivity on the endocrine cells of the gastrointestinal mucosa. The commercially available antibodies, used in the present study, though less sensitive, exhibited a similar pattern of labelling in the gastric fundus. Sander et al. (2006) recently examined $H_3R$ expression in human small and large intestine, but not in stomach, by immunostaining with commercial $H_3R$ antibodies (Acris Antibodies GmbH, Hiddenhausen, Germany). They found that antibodies did not stain any cellular structures and only non-specific staining was detected in and around the walls of blood vessels and in the myenteric plexus, leading them to conclude that the receptor is absent. The sensitivity of the immunoprobes used could be at the basis of discrepancy between present and literature findings. However, a further possibility is that

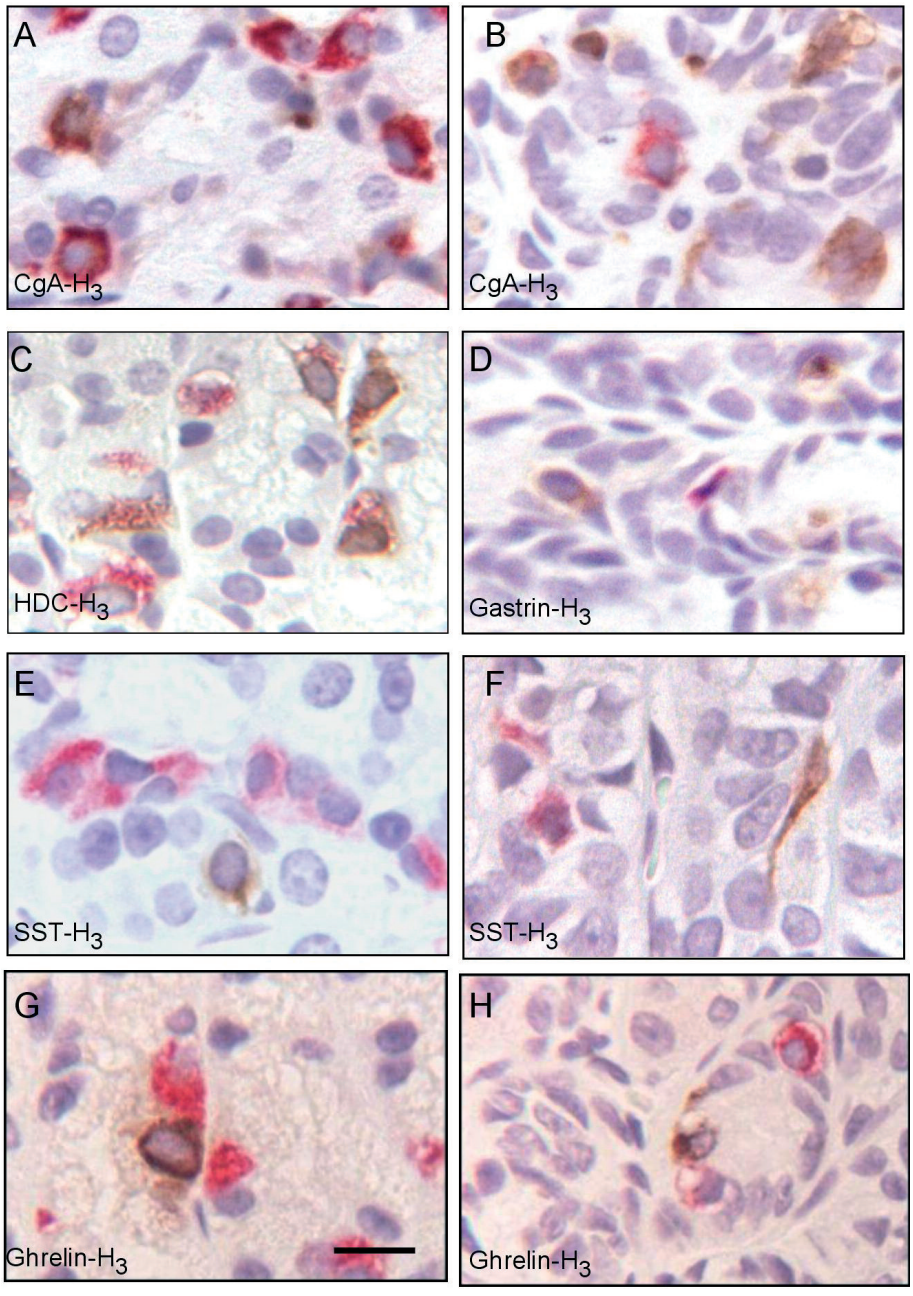


**Fig. 4.** Double-label nonfluorescent immunostaining of the fundic (**A, C, E, G**) and antral (**B, D, F, H**) mucosa from freely fed rats. Cells immunoreactive to chromogranin A (CgA), histidine decarboxylase (HDC), gastrin, somatostatin (SST) and ghrelin stained brown and cells immunoreactive to $H_3$ receptor stained red. Most of the $H_3$ receptor-immunoreactive cells are immunoreactive to CgA in the fundus **(A)** and antrum **(B)** and to HDC in the fundus **(C)**. Cells positive for gastrin **(D)**, somatostatin **(E, F)** and ghrelin **(G, H)** were negative for $H_3$ receptor. Scale bar: 12 µm.

the paucity of signal simply reflects the fact that endocrine cells occur in a very small number throughout the gastrointestinal tract. In mice endocrine cells represent about 7% of all epithelial cells in the stomach fundus, 3% in the pyloric antrum, 0.5% in the small intestine and 0.4% in the colon (Karam, 1999). Moreover if their number were estimated for the gastrointestinal tissue throughout its entire thickness, the relative proportion of endocrine cells would further decrease. In the present study, the $H_3R$ has been identified exclusively in the endocrine cells and the frequency of cells expressing the $H_3R$ closely mirrors the progressive decrease of endocrine cell number from the gastric fundus to the colon. Accordingly the inability to clearly localize $H_3R$ in human small and large intestine (Sander et al., 2006) could be attributable to the minor population of endocrine cells in these regions. On the whole the relative infrequency of endocrine cells, besides differences in the antisera or in species utilised, could be at the basis of the failure to clearly detect the expression of receptor.

In the present study, $H_3R$-positive cells in the oxyntic mucosa have been identified as ECL cells. It is well known that ECL cells contain the histamine-forming enzyme, HDC, synthesize and release histamine, which then activates $H_2$ receptors on parietal cells, stimulating acid secretion (Lindström et al., 2001). ECL cell function is mainly under control of two hormones, namely gastrin and somatostatin. Gastrin, present in antral gastrin (G)-cells, activates $CCK_2$ receptors of ECL cells, stimulating histamine synthesis and release (Lindström et al., 2001; Chen et al., 2006). Somatostatin, present in fundic and antral D cells, activates $sst_2$ receptors of ECL as well as of G and parietal cells, exerting an inhibitory action (Chen et al., 2006). Functional studies in isolated rat ECL cells (Prinz et al., 1993) and in isolated mouse (Vuyyuru and Schubert, 1997) and rat (Bado et al., 1994) stomach provided evidence that the $H_3R$ exerts an inhibitory influence on histamine and somatostatin secretion, leading to suggestion that the $H_3R$ could be present both in ECL and D cells (Vuyyuru and Schubert, 1997). However, contrary to these reports, histamine and histamine $H_3$-receptor ligands were also shown to be without effect on secretion of both histamine and pancreastatin from isolated rat ECL cells (Lindstrom et al.,1997). In the present study, we have demonstrated, for the first time, the presence of the $H_3R$ in ECL cells only and not in G and D cells. In line with their role, $H_3Rs$, localized on ECL cells, could be responsible for the inhibition of histamine synthesis and release by a negative feedback mechanism. The inability to identify $H_3R$ on G, D and A-like cells strongly suggests the lack of a direct influence of histamine, through the activation of $H_3R$, on the release of gastrin, somatostatin or ghrelin. As a consequence of their localization, activation of $H_3R$ by selective agonists, by inhibiting histamine synthesis and release, could be expected to profoundly affect gastric acid secretion. However a major influence is doubtful, since $H_3R$ selective agonists are reported to be ineffective, as well as to stimulate or inhibit gastric acid secretion in the rat (Sandvik et al., 1989; Coruzzi et al., 1992; Ballabeni et al., 2002). The selective $H_3R$ agonists exert antiulcer activity (Morini et al., 1995) and promote

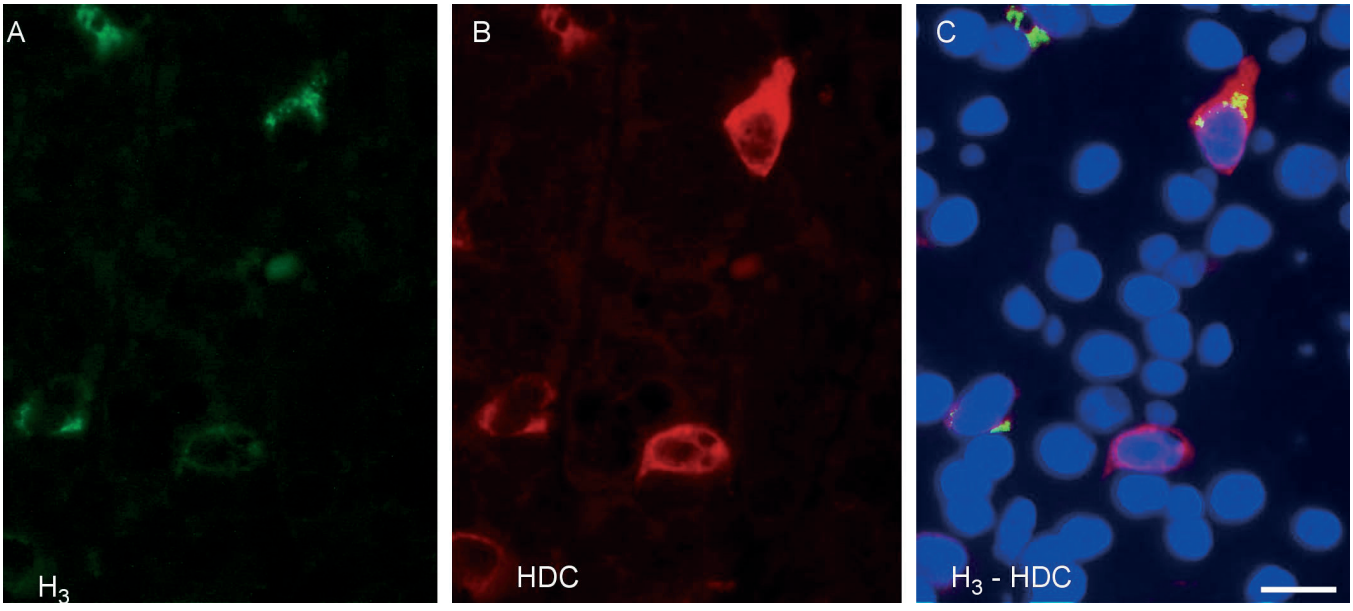


**Fig. 5.** Double-label immunofluorescent staining of fundic mucosa from a freely fed rat. $H_3$ receptor (green) colocalizes with histidine decarboxylase (HDC, red). Sections were weakly counterstained with DAPI (blue). Scale bar: 8 μm.

epithelial cell proliferation (Morini et al., 2002). Mitogenic effect is promoted throughout the gastrointestinal epithelium, and peaked in the gastric fundus and colon (Grandi et al., 2006) and consequently the responsiveness of gastrointestinal regions to $H_3R$ agonists does not appear to parallel the regional distribution of $H_3R$. This requires further investigation.

$H_3R$ and HDC immunostaining decreased rapidly and to a comparable extent during fasting. Fasting causes a decrease in density of antral G cells, as also presently observed, and in circulating gastrin, while histamine immunostaining appears to be largely unaffected (Schwarting et al., 1986; Ohning et al., 1998). The decrease in HDC activity, observed during fasting, has been attributed to the decrease in circulating gastrin (Chen et al., 1998) and to the increased L-histidine substrate depletion (Lambrecht et al., 2007). Present findings suggest a strong correlation between enzymatic activity and the $H_3R$, even though the role of $H_3R$ in the regulatory pathway, highlighted by food deprivation, remains unclear.

The minimal expression of $H_3R$ observed in antrum, small and large intestine suggests a minor role of the $H_3R$ in these regions.

In conclusion, we report the first detailed description of the anatomical framework for understanding the role of the histamine $H_3$ receptor in the rodent gastrointestinal tract. Furthermore, we show, for the first time, that the $H_3$ receptor expression in the gastric fundic ECL cells is highly regulated by feeding behaviour. The location of $H_3R$ is potentially interesting and suggests a role in the regulation of the activity of ECL cells.

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