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Epithelial cell proliferation is promoted by the histamine H_3 receptor agonist (R)-α-methylhistamine throughout the rat gastrointestinal tract

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Abstract

The temporal effect of (R)-α-methylhistamine on epithelial cell proliferation throughout the rat gastrointestinal tract was investigated. (R)-α-methylhistamine was administered at 100 mg/kg orally and the rats were sacrificed 1, 24, 48, 72 and 144 h later. All the animals received 5-bromo-2′-deoxyuridine, (BrdU), 200 mg/kg i.p., 2 h before sacrifice. Gastrointestinal tissue was processed for histology and immunohistochemistry. (R)-α-methylhistamine caused a progressive increase in mucosal thickness of gastric fundus, distal small intestine and distal colon. Statistically significant differences from control values were found between 48 and 72 h after (R)-α-methylhistamine. (R)-α-methylhistamine significantly increased the number of BrdU-positive cells in the gastric fundus and antrum, intermediate and distal small intestine and distal colon. Peak effects were observed between 1 and 24 h after (R)-α-methylhistamine administration. Proliferating cell number and mucosal thickness were comparable to those of control rats at 144 h. (R)-α-methylhistamine exerts a long lasting growth-promoting effect on the stomach, distal small intestine and distal colon. Present data support a role of histamine H_3 receptors in the normal regulation of cell cycle in epithelial tissue.

Keywords: (R)-α-methylhistamine; Histamine H_3 receptor; Gastrointestinal mucosa; Epithelial thickness and cell proliferation; (Rat)

1. Introduction

The epithelium of the gastrointestinal tract undergoes continuous rapid turnover. Self-renewal is committed to highly mitosable precursor cells. These cells are restricted to limited compartments and their position varies in the different regions of the gastrointestinal tract, probably depending on the migratory pathways of newly formed cells. In the gastric fundic and antral mucosa, where migration is bidirectional, progenitor cells are centrally positioned in a compartment known as the isthmus (Lee, 1985; Lee and Leblond, 1985; Karam and Leblond, 1993). Progenitor cells are localized to a few positions above the crypt base, just above the Paneth cells, in the small intestine (Cheng and Leblond, 1974; Potten and Loeffler, 1990), the mid-crypt in the ascending colon and the crypt base in the descending colon (Sato and Ahnen, 1992).

The progenitor zone is supposed to be the location of undifferentiated multipotent stem cells, from which all epithelial cell lineages originate. Terminal differentiation is achieved gradually and stem cells should produce undifferentiated and poorly differentiated transit cells, which still retain proliferative activity (Potten and Loeffler, 1990). The number of generations transit cells pass through before reaching full differentiation, varies within the lineages in the different regions of the gastrointestinal tract with a consequent variation in the total number of mitosable cells. In the adult mouse the size of the proliferative compartment is larger in the colon as compared to the small intestine (Cai et al., 1997; Bach et al., 2000).

An important role in the modulation of tissue growth has been attributed to endogenous histamine. Histamine content and histidine decarboxylase activity are increased in rapidly growing tissues and in experimentally induced tumours (Kahlson and Rosengren, 1968; Hill, 1990; Nissinen et al., 1995). On the other hand, depletion of histamine levels is associated with inhibition of tumor growth (Bartholeyns and Fozard, 1985; Hegyesi et al., 2001). Recent studies have shown

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that histamine H_3 receptor mRNA is highly expressed within the embryonic epithelia (Héron et al., 2001) and that (R)-α-methylhistamine and FUB 407, both agonists of histamine H_3 receptors, promote cell proliferation in rat oxyntic mucosa (Morini et al., 2002), suggesting a role of histamine H_3 receptors in mediating the mitogenic activity of histamine.

To further examine the role of histamine H_3 receptors, we analyzed the epithelial cell proliferation throughout the different regions of the rat gastrointestinal tract at different times, up to 144 h, after a single dose of (R)-α-methylhistamine.

2. Materials and methods

2.1. Drugs

(R)-α-methylhistamine dihydrogenmaleate was synthesized at the Institut für Pharmazie, Freie Universität Berlin. All other reagents were commercially available.

2.2. Animals and experimental protocol

Adult male Wistar rats (8 weeks old, 180–200 g; Harlan, Italy) were housed at 22 °C on a 12 h light/dark cycle. The rats were deprived of food, but not water, for 24 h before being sacrificed. All the experiments were approved by the Italian Animal Care and Use Committee. (R)-α-methylhistamine was dissolved in saline and administered at the dose of 100 mg/kg orally in a 5 ml/kg volume. The dose of (R)-α-methylhistamine was selected from a previous dose–response study, in which the compound was shown to produce a dose-dependent increase in proliferation in rat oxyntic mucosa over the range 10–100 mg/kg orally at 1 h after administration, with a highly significant increase at 100 mg/kg (Morini et al., 2002). Groups of 4–6 rats each were sacrificed at 1, 24, 48, 72 and 144 h after (R)-α-methylhistamine administration. The control rats were given saline, 5 ml/kg, orally. All animals received an intraperitoneal injection of the thymidine analog, 5-bromo-2′-deoxyuridine, (BrdU, 200 mg/kg, Sigma-Aldrich) 2 h before sacrifice. The rats were killed by cervical dislocation between 11 a.m. and 1 p.m.

2.3. Tissue preparation

On sacrifice, the abdomen was opened. The stomach and duodenum were isolated in whole by ligating and cutting the lower oesophageal end and the distal end of the duodenum. Formalin (10%, 1.5 ml) was injected into the gastric cavity, inflating the stomach. The stomach and duodenum were immersed in 10% formalin for 10 min and then washed after the removal of the nonglandular portion of the stomach. After overnight fixation in 10% formalin, 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. The remaining portion of the small intestine and colon were removed from the peritoneal cavity. The length of the small intestine and colon was expressed as 100% and 2-cm samples were taken corresponding to 20%, 50% and 90% of the small intestine length and to 80% of the colonic length. The excised segments were opened along the mesenteric border, rinsed, pinned flat and immersed in 10% formalin overnight. All tissue samples were embedded in paraffin. Serial sections, 4 μm thick, were stained either with hematoxylin–eosin or with diastase-resistant periodic acid-Schiff. Adjacent sections were prepared for immunohistochemistry.

2.4. Immunohistochemistry

Cells in the S phase of the cycle were identified by immunohistochemical detection of BrdU incorporation. Sections were placed in citrate buffer (10 mM, pH 6.0) and heated in a 650-W microwave three times for 5, 4, and 4 min each. Endogenous peroxidase activity was blocked with 3% H_2O_2/PBS for 8 min. The sections were then incubated with a mouse anti-BrdU antibody (1:20, Dako, Glostrup, Denmark) for 30 min at room temperature. An LSAB2 staining kit (Dako) was used as the secondary antibody reaction system. Colour was generated by incubation with 3,3-diaminobenzidine (Sigma-Aldrich).

2.5. Quantitative analysis

The image of the section was displayed on a colour monitor by means of a videocamera attached to the microscope and quantitations were performed using a colour image analysis software system (LUCIA G, Nikon Laboratory Imaging, Japan). Only the regions in which full-length glands, crypts or villi were oriented perpendicular to the luminal surface were considered for quantitative analysis. The number of immunoreactive cells was counted in at least 3 different sections, for a total of 50 glands or crypts per rat, from each region of the gastrointestinal tract at each time point. The results were expressed as the mean number of immunoreactive cells per unit. The height of the glands and villi and the depth of the crypts were measured from 15–20 glands, crypts or villi selected from three sections 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 total cell number was quantitated in a total of 15–20 glands, crypts or villi for each region of the gastrointestinal tract and the results expressed as the mean of the total number of cells per unit.

2.6. Statistical analysis

All values are expressed as mean ± S.E.M. for 4–6 rats per experimental group. ANOVA followed by the Newman–Keuls test was employed for the statistical analysis; P values < 0.05 were considered significant.

3. Results

3.1. Thickness of the gastrointestinal mucosa

In the control rats the thickness values of the gastric, duodenal, small intestinal and colonic mucosa were comparable

to those reported in literature (Tsujii et al., 1993; Fernández-Estívariz et al., 2003). Histological examination of the gastrointestinal sections at 1, 24, 48, 72 and 144 h after single p.o. administration of (R)-α-methylhistamine (100 mg/kg), revealed a progressive increase in mucosal thickness of gastric fundus, distal small intestine, consequent to the increase in villus height, and colon (Table 1). Statistically significant differences from control values were found only between 48 and 72 h after (R)-α-methylhistamine. To determine whether the increase in mucosal thickness was caused by an increased cell number, quantitation of total cell number was performed. Interestingly, increases in thickness were mirrored by increases in cell number compared with control values (Table 2). There was no evidence of other changes or damage in the gastrointestinal segments examined.

3.2. Proliferation of the gastrointestinal epithelial cells

Proliferation was quantitated by assessing BrdU incorporation at 1, 24, 48, 72 and 144 h after the administration of a single dose of (R)-α-methylhistamine, 100 mg/kg p.o. (R)-α-methylhistamine profoundly affected the rate of proliferation of the gastrointestinal mucosa.

In the fundic and antral regions of the stomach from control rats, the number of BrdU-positive cells was 1.12±0.13 and 1.43±0.15 per gland, respectively. A dramatic increase was observed in the fundus at 1 and 24 h after the administration of (R)-α-methylhistamine, when labeled cells were increased by 226% and 216% compared with control values. The number of cells with BrdU-incorporated nuclei declined at 48 h and subsequently increased at 72 h. The proliferation rate was comparable to that of the control animals at 144 h. At any time point the BrdU-labeled cells were confined to the isthmus, between the pit and neck regions (Fig. 1).

Table 1
Temporal effect of (R)-α-methylhistamine on gastrointestinal mucosal thickness

Tissue	Time (h)					
	Saline	1	24	48	72	144
Stomach						
Fundus	511±28	524±12	538±32	647±23[a]	559±18	539±15
Antrum	234±11	240±9	235±14	256±11	247±9	243±9
Duodenum						
Villus	468±11	536±9	454±37	471±24	500±63	442±11
Crypt	150±6	199±4	164±17	155±1	182±20	161±11
Distal ileum						
Villus	240±10	269±23	283±28	307±11	320±21[a]	254±18
Crypt	131±3	128±7	148±18	141±4	151±1	126±5
Colon	230±10	259±19	284±23	292±14[a]	302±11[a]	217±16

(R)-α-methylhistamine was administered at 100 mg/kg orally and rats were sacrificed at 1, 24, 48, 72 and 144 h later. Control rats received saline.
Data are expressed in μm as mean±S.E.M. for $n=4–6$ rats per group.
[a] $P<0.05$ compared with saline-treated group (ANOVA and Newman–Keuls test).

Table 2
Temporal effect of (R)-α-methylhistamine on total number of cells in gastrointestinal epithelium

Tissue	Time (h)		
	Saline	48	72
Stomach			
Fundus	77.3±3.5	104.4±3.8[a]	74.3±3.5
Distal ileum			
Villus	94.7±3.5	110.5±8.8	136.4±4.5[a]
Crypt	44.5±3.5	43.4±2.8	45.7±4.0
Colon	43.2±2.0	63.8±3.7[a]	65.4±2.06[a]

(R)-α-methylhistamine was administered at 100 mg/kg orally and rats were sacrificed at 48 and 72 h later. Control rats received saline. Data are expressed as mean of the total number of cells per gland, crypt or villus±S.E.M. for $n=4–6$ rats per group.
[a] $P<0.01$ compared with saline-treated group (ANOVA and Newman–Keuls test).

A significant increase in the rate of proliferation of the antrum was induced by (R)-α-methylhistamine at 1 and 24 h after administration, with a 97% and 103% increase, respectively over control values. The number of positive cells appears

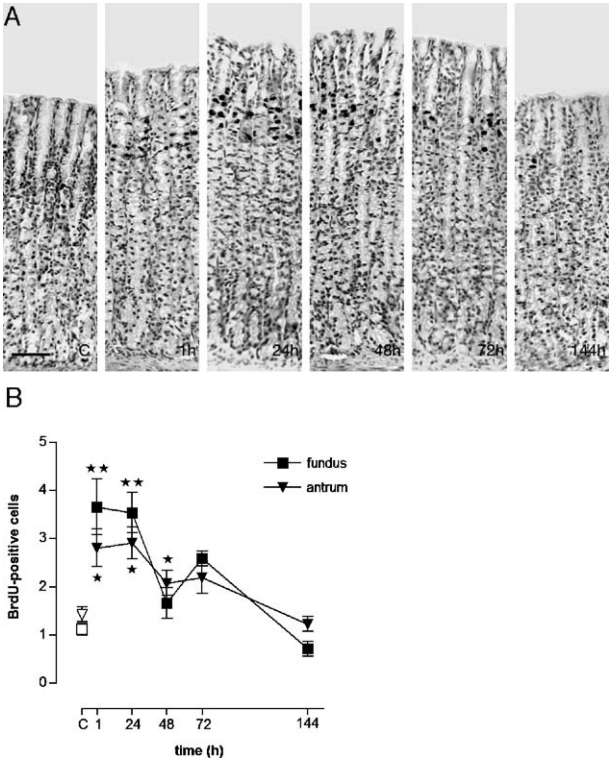

Fig. 1. Temporal effect of (R)-α-methylhistamine on epithelial cell proliferation in the rat stomach. (R)-α-methylhistamine was administered at 100 mg/kg orally and the rats sacrificed at 1, 24, 48, 72 and 144 h later. Control rats received saline. All the animals received BrdU 2 h before sacrifice. A: Representative fundic mucosal sections from a control rat (C) and from rats sacrificed at the indicated times after the single administration of (R)-α-methylhistamine. Scale bar = 70 μm. B: Quantitation of BrdU-positive cells in gastric fundic and antral glands. Mean±S.E.M. of positive cells per gland; $n=4–6$ rats per group. *$P<0.05$ and **$P<0.01$ compared with saline-treated group (C).

to be increased at 48 and 72 h, although this difference did not reach statistical significance. Values observed at 144 h were comparable to those of control rats (Fig. 1). (R)-α-methylhistamine did not alter the position of proliferating cells, as compared with control rats.

Examination of the small intestine mucosa from untreated rats revealed a proximal-distal gradient of BrdU-positive cell number. The highest number of positive cells, 18.64±0.53 per crypt, was observed in the duodenum and their number progressively decreased, values being 14.78±1.06, 12.22±0.81 and 10.95±0.46, respectively in proximal, intermediate and distal portions of small intestine. (R)-α-methylhistamine did not modify the proliferation rate in the duodenum and in the proximal small intestine, while a significant increase was induced in the intermediate and distal small intestine. Peak effects were observed between 1 and 24 h after administration, and the positive cells increased by 32% and 36% in the intermediate and by 28% and by 49% in the distal portion, respectively. At 48 h and at longer time periods no statistical difference was observed by comparing values from untreated and treated rats, despite an increase being present at 72 h (Fig. 2). Positive cells in both control and (R)-α-methylhistamine-treated rats were found to be located in the crypts with the highest occurrence near to the base.

Colonic mucosa revealed to be extremely sensitive to the effect of (R)-α-methylhistamine. Positive cells were infrequent in untreated animals, the mean number being 0.61±0.14 per crypt, and they were polarized towards the base of the crypts. Examination of the colonic mucosa at 1 and 24 h after (R)-α-methylhistamine administration revealed an increase of 459% and 387%, respectively. The increased proliferation was associated with a broadening of the proliferative zone. Values were higher but not statistically different compared with controls at 48 and 72 h; they returned to control values at 144 h (Fig. 3).

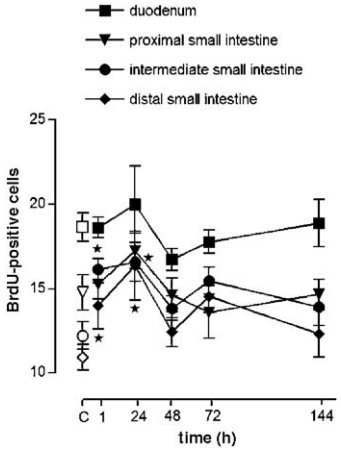

Fig. 2. Temporal effect of (R)-α-methylhistamine on epithelial cell proliferation in the rat small intestine. (R)-α-methylhistamine was administered at 100 mg/kg orally and the rats sacrificed at 1, 24, 48, 72 and 144 h later. Control rats received saline. All the animals received BrdU 2 h before sacrifice. Crypt proliferation was determined by BrdU immunohistochemistry and expressed as mean number±S.E.M. of positive cells per crypt. $n=4–6$ rats per group. *$P<0.05$ compared with saline-treated group (C).

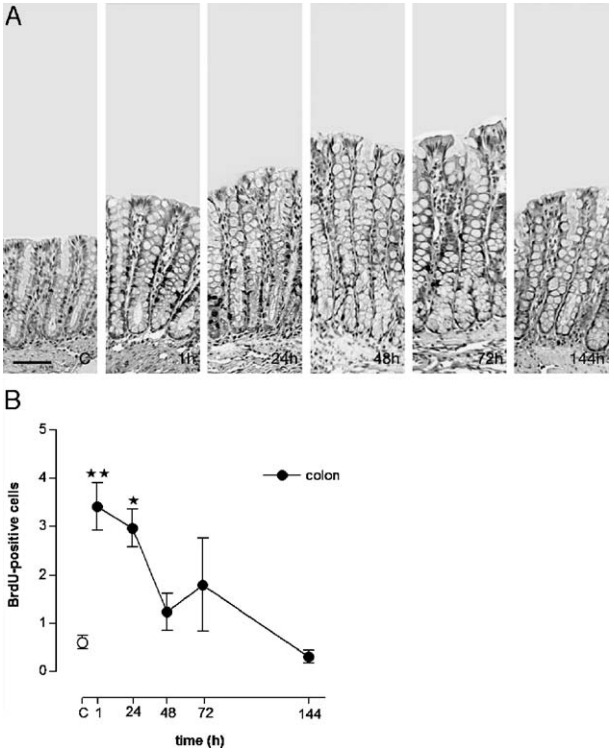

Fig. 3. Temporal effect of (R)-α-methylhistamine on epithelial cell proliferation in the rat distal colon. (R)-α-methylhistamine was administered at 100 mg/kg orally and the rats sacrificed at 1, 24, 48, 72 and 144 h later. Control rats received saline. All the animals received BrdU 2 h before sacrifice. A: Representative colonic mucosal sections from a control rat (C) and from rats sacrificed at the indicated times after the single administration of (R)-α-methylhistamine. Scale bar=70 μm. B: Quantitation of BrdU-positive cells in colonic mucosa. Mean ±S.E.M. of positive cells per crypt; $n=4–6$ rats per group. *$P<0.05$ and **$P<0.01$ compared with saline-treated group (C).

4. Discussion

Present findings provide evidence that acute administration of (R)-α-methylhistamine promotes proliferation of epithelial cells throughout the gastrointestinal tract. The most striking features of the action of (R)-α-methylhistamine were its rapid onset, with maximal response being achieved within 1 h after (R)-α-methylhistamine administration, and the selective influence on cells located in the proliferative compartment. BrdU-positive cells were observed almost exclusively in areas corresponding to the proliferative compartments of the gastrointestinal epithelium, which could be partly expanded but not modified in their location.

The gastric fundus and colon appeared to be highly sensitive to the mitogenic effect of (R)-α-methylhistamine, while the small intestine exhibited a modest response, with a gradual increase in responsiveness from the duodenum, not significantly affected, to the intermediate and distal ileal segments, which exhibited a significant increase in proliferating cell BrdU labeling. The different responsiveness could be attributed to the different cell proliferation kinetics of the epithelium in the

various regions of the gastrointestinal tract. In rats, the fundic and colonic epithelium are reported to have a lower percentage of dividing cells per gland and crypt, respectively, and a less rapid rate of proliferation, as compared with the small intestine (Messier and Leblond, 1960; Lipkin, 1985; Butler et al., 1992; Berlanga-Acosta et al., 2001; Mandir et al., 2005). In the small intestine, the entry rate of cells into mitosis is higher in the duodenum than in the jejunum (Wille et al., 2004) and the percentage of mitotic cells or ^{3}H-thymidine labeled cells is higher in the duodenum and jejunum than in the distal portion of the small intestine (Cheng and Leblond, 1974). A further difference lies in the number of generations of proliferating transit cells, which in rodents are reported to be six in the small intestinal crypts and eight in the colonic ones (Cai et al., 1997; Bach et al., 2000). The colonic epithelium appears to have a higher number of cell generations in the transit compartment, which could ensure a larger proliferative potential. (R)-α-methylhistamine causes an almost immediate entry of mitosable cells in the S-phase of the cycle. The regions, such as proximal portions of the small intestine, in which the proliferation rate is elevated, might be unable to further increase proliferation within a very short time. Alternatively the differential sensitivity could be explained by a region-specificity of the mitogenic activity of (R)-α-methylhistamine, potentially attributable to a region-specific distribution of the receptor for (R)-α-methylhistamine or of endogenous factors mediating its effect.

(R)-α-methylhistamine is a short-lived compound. When orally administered at 30 mg/kg in rats, the plasma level reached a plateau from 30 min to 2 h, followed by a progressive decrease, and the decrease half-life was calculated to be 64 min (Garbarg et al., 1989). As a consequence at 24 h and at longer times thereafter, it is no longer detectable in plasma. Furthermore the compound is methylated by the enzyme histamine-*N*-methyltransferase and the product is devoid of any agonist activity at H_3 receptors (Rouleau et al., 1997). The cycle duration of epithelial cells varies in the different segments of the gastrointestinal tract. It is reported to be 13.9 h in the mouse antrum (Lee and Leblond, 1985), 13 to 16 and 10 to 14 h in the mouse and rat small intestine (Cheng and Leblond, 1974) and 18 to 32 h in the mouse and rat colon (Potten et al., 1974; Tutton and Barkla, 1976; Kellett et al., 1992). Therefore cells found to be labeled 1 h after the single administration of (R)-α-methylhistamine, have duplicated by mitosis within 24 h. Duplication can be presumed to be complete in the stomach and small intestine and near to complete in the colon, because of the longer duration of the cell cycle in this segment. When the 2 h-BrdU incorporation is evaluated 24 h after the single administration of (R)-α-methylhistamine, labeled cells can be considered newly formed cells. Their number is equal or slightly higher than that observed at 1 h in the stomach and proximal and distal portions of the small intestine and slightly lower in the colon, that is, they are present in a number well above that found in control animals, indicating that newly formed cells retain a high proliferative activity. If we consider that at 24 h after administration, plasmatic levels of (R)-α-methylhistamine have fallen below detectable levels, it is evident that at this time hyperproliferation is achieved in absence of the compound. The present finding could be explained by considering the type of cells influenced by (R)-α-methylhistamine. Their location and proliferative ability indicate that they are undifferentiated or poorly differentiated cells, destined to divide repeatedly before migration/differentiation and extrusion into the lumen, even in absence of external stimuli. Furthermore as hyperproliferation declines, as observed 48 h after (R)-α-methylhistamine administration, the mucosal thickness of the fundus and colon increased. This increase was primarily attributable to the increase in cell number. It is interesting to note that the fundus and colon, where the increase in proliferation was more pronounced, also exhibited an expansion of cell number and a concurrent increase in mucosal thickness. However, on the whole, the increase in thickness was short-lasting. This feature suggests that newly formed cells are rapidly extruded with a consequent impairment of a complete maturation. A condition comparable to that of control rats, in terms of the number of proliferating cells and mucosal thickness was observed at 144 h after (R)-α-methylhistamine. In conclusion it could be hypothesized that (R)-α-methylhistamine causes an initial massive entry of mitosable cells in the S-phase of the cell cycle and activates a process comparable to a wave, which continues through proliferation, differentiation and extrusion irrespective of the initial stimulus, and gradually returns to normal conditions.

(R)-α-methylhistamine is a selective agonist of histamine H_3 receptors. It was found to have only a weak affinity at the H_1 and H_2 receptor (Arrang et al., 1987a) and is 208 times more potent at the H_3 compared to H_4 receptor (Jablonowski et al., 2003). In rat fundic mucosa, with the use of a selective H_3-receptor agonist and selective H_1-, H_2- and H_3-receptor antagonists, pharmacological evidence has been provided that the proliferative activity of (R)-α-methylhistamine is mediated by H_3 receptors (Morini et al., 2002). H_3-receptor activation inhibits histamine release (Arrang et al., 1983) and synthesis (Arrang et al., 1987b) by a negative feedback mechanism. Histamine deficiency was achieved in mice by deletion of L-histidine decarboxylase (HDC) (Hunyady et al., 2003; Nakamura et al., 2004), the only enzyme able to produce histamine in vivo. In HDC-KO mice, stomach weight and thickness of fundic mucosa increased progressively from 1 to 9 months after birth, a condition which was caused by an increase in the number of parietal and enterochromaffin-like cells. It is therefore apparent that a decrease of histamine content, achieved by histamine H_3-receptor activation or, more completely, by HDC deletion, determines a trophic effect. Increased thickness of fundic mucosa, ascribed to an increased number of parietal and enterochromaffin-like cells, was also evident in histamine H_2 receptor-deficient mice (Kobayashi et al., 2000; Fukushima et al., 2003). Taken together these findings suggest that the regulatory role exerted by histamine in cell proliferation and differentiation involves different histamine receptors, likely to mediate opposite effects. Furthermore it is worth noting that both HDC-KO and H_2-KO mice were hypergastrinemic. The amidated form of the peptide hormone gastrin is an acid secretagogue and a trophic factor to the oxyntic mucosa; the less processed forms of the peptide have growth factor properties in

the colon while a possible proliferative effect of members of the gastrin family on the small intestine remains uncertain (Wang et al., 1996; Dockray, 1999; Koh et al., 1999; Koh, 2002). Thus, it appears that the sensitivity of the gastrointestinal segments to the trophic effect of gastrin parallels the sensitivity to (R)-α-methylhistamine, as observed in the present study. In the stomach, gastrin promotes histamine release and activation of HDC; in its turn histamine, acting via H_3 receptors, is reported to inhibit not only its own release from ECL cells but also the release of somatostatin, with a consequent increase of locally released gastrin and histamine (Vuyyuru and Schubert, 1997). The gastrin–histamine axis, shown to operate at a gastric level in the control of gastric acid secretion, could be relevant in the regulation of proliferation and differentiation along the gastrointestinal tract.

In conclusion we further define the proliferative activity of (R)-α-methylhistamine, by showing that this compound exerts a long lasting influence on cell proliferation in the stomach, distal small intestine and distal colon. The present data provide support for a role of histamine H_3 receptors in normal cell cycle regulation in epithelial tissue.

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