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Ligands for histamine H_3 receptors modulate cell proliferation and migration in rat oxyntic mucosa

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1 (R)-α-methylhistamine, a selective agonist of histamine H_3 receptors, promotes mucus secretion and increases the number and volume of mucus-secreting cells. The hypothesis that the increased number of mucous cells could reside in an alteration of homeostasis in the gastric epithelium was investigated.

2 (R)-α-methylhistamine was administered to rats 1 h (10–100 mg kg^{-1} by intragastric and by intraperitoneal route) and 24 h (100 mg kg^{-1} by intragastric route) prior to killing. The (S)-isomer of α-methylhistamine (55.4 mg kg^{-1}), 100 times less potent than the (R)-isomer at H_3 receptors, and the H_3-receptor agonist FUB 407 (9.14–91.35 mg kg^{-1}) were intragrastically administered 1 h prior to killing. The H_1-receptor antagonist mepyramine (30 mg kg^{-1}), the H_2-receptor antagonist famotidine (3 mg kg^{-1}), and the H_3-receptor antagonists ciproxifan (3 mg kg^{-1}) and clobenpropit (30 mg kg^{-1}) were intragastrically administered 30 min before (R)-α-methylhistamine. Gastric tissue was processed for histology and immunohistochemistry.

3 Within 1 h, (R)-α-methylhistamine and FUB 407 dose-dependently increased the number of BrdU-positive cells and of apoptotic cells. (S)-α-methylhistamine failed to modify proliferation and apoptosis. The increase in proliferation by (R)-α-methylhistamine was reversed by ciproxifan and clobenpropit, but not by mepyramine and famotidine.

4 (R)-α-methylhistamine accelerated the differentiation towards pit cells and their outward migration 24 h after its administration. These effects were counteracted by ciproxifan. The apoptosis rate was unaffected at 24 h.

5 These findings reveal a primary role of histamine H_3-receptor ligands in modulating cell proliferation and migration in rat fundic mucosa.

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Keywords: (R)-α-methylhistamine; FUB 407; (S)-α-methylhistamine; clobenpropit; ciproxifan; H_3 receptor; gastric mucosa; gastric surface mucous cells; gastric cell proliferation

Abbreviations: BrdU, bromodeoxyuridine; PAS, periodic acid-Schiff; (R)α-MeHA, (R)-α-methylhistamine; SMCs, surface mucous cells; TGF-α, transforming growth factor α; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling.

Introduction

The gastric fundic mucosa is a self-renewing epithelium. The single-layered epithelium invaginates into blind tubular units, divided into four regions: pit, isthmus, neck, and base. The progenitor zone is located in the isthmus and it has a central position in the gastric unit. Isthmal cells turn over rapidly, with values of 33–40% per day, and by combined processes of migration and differentiation, give rise to the main cell lineages present in the oxyntic mucosa (Karam & Leblond, 1993a). Isthmal stem cells give rise to pre-pit cell precursors yielding pre-pit cells, to pre-neck cell precursors yielding pre-neck cells, and to pre-parietal cells, all located in the isthmus, and with the exception of pre-parietal cells, they are highly proliferative. Pre-pit cells and their precursors represent the majority of isthmal cells and generate pit cells, also referred to as surface mucous cells (SMCs), which migrate unidirectionally towards the lumen where they die (Karam & Leblond, 1993b).

We previously showed that the selective agonist of histamine H_3 receptors, (R)-α-methylhistamine ((R)α-MeHA) (Arrang *et al*., 1987), protects the gastric mucosa against acute damage from differently acting noxious agents in the rat (Morini *et al*., 1995; 1997b). Its protective effect is reversed by the highly selective H_3 receptor antagonists clobenpropit and ciproxifan (Ligneau *et al*., 1998; Morini *et al*., 2000). Ethanol-induced total damage was not reduced by the S isomer of α-methylhistamine ((S)α-MeHA (Morini *et al*., 1999), as expected on the basis of the high degree of stereoselectivity of H_3 receptors (Arrang *et al*., 1985). Prevention of damage was achieved with FUB 407, a reference compound for a novel class of histamine H_3-receptor (partial) agonists (Sasse *et al*., 1999; Morini *et al*., 2001). A relevant morphological change consequent on (R)α-MeHA administration was the increase in mucus secretion and in intracellular mucus content (Morini *et al*., 1997a). This latter effect could reflect an increase in the number and/or volume of mucus-secreting cells and, by inference, an alteration of homeostasis in the gastric mucosa. This hypothesis has been at the basis of the present study. This

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article examines how ligands for histamine H_3 receptors could influence proliferation, differentiation, and migration in the oxyntic mucosa of the adult rat.

Methods

Animals

Adult male Wistar rats (180–200 g, Harlan, Italy) were housed at 22°C on a 12 h light/dark cycle. Rats were deprived of food, but not of water, for 24 h before being sacrificed. All the experiments were approved by the Italian Animal Care and Use Committee.

Drugs

(R)α-MeHA dihydrogenmaleate, (S)α-MeHA dihydrochloride, FUB 407 (3-1*H*-imidazol-4-yl)propyl 3,3-dimethylbutyl ether) hydrogenmaleate and ciproxifan hydrogenmaleate were synthesized at the Free University of Berlin. Clobenpropit dihydrobromide was a kind gift from H. Timmerman, Vrije Universiteit, Amsterdam. The compounds were dissolved in saline. (R)α-MeHA at the dose of 100 mg kg^{-1} administered by intragastric route (i.g.), was shown to almost completely prevent gastric lesion formation and this effect was reversed by ciproxifan (3 mg kg^{-1} i.g.) and by clobenpropit (30 mg kg^{-1} i.g.) (Morini *et al*., 2000). FUB 407 was at least as effective as (R)α-MeHA in preventing damage, at the dose of 91.35 mg kg i.g., equimolar to (R)α-MeHA, 100 mg kg^{-1}. (S)α-MeHA was administered at the dose of 55.44 mg kg^{-1}, equimolar to (R)α-MeHA, 100 mg kg^{-1}. Famotidine was obtained from Sigma-Tau (Pomezia, Italy). The compound was dissolved in a small volume of 0.1N HCl adjusted to a pH of ~6 with 1N NaOH. Famotidine was tested at the dose of 3 mg kg^{-1} i.g., reported to reduce by 86% rat basal acid secretion following oral administration (Pendleton *et al*., 1985). Mepyramine was obtained from Sigma (St. Louis, MO, U.S.A.). The compound was dissolved in saline and tested at the dose of 30 mg kg^{-1} i.g., reported to significantly reduce by 60% ($P<0.05$) ethanol-induced gastric hyperemia in the rat following oral administration (Oates & Hakkinen, 1988).

Experimental design

The first set of experiments was carried out to examine the influence of (R)α-MeHA on cell proliferation at 60 min after its administration. The implication of histamine receptors was tested by treating rats with appropriate receptor agonists and antagonists. Groups of rats received either mepyramine (30 mg kg^{-1}), famotidine (3 mg kg^{-1}), ciproxifan (3 mg kg^{-1}), clobenpropit (30 mg kg^{-1}), or saline intragastrically in a 5 ml kg^{-1} volume. Thirty minutes later rats received either (S)α-MeHA (55.44 mg kg^{-1}), FUB 407 (9.14–91.35 mg kg^{-1}), saline intragastrically in a 5 ml kg^{-1} volume or (R)α-MeHA (10–100 mg kg^{-1}) intragastrically in a 5 ml kg^{-1} volume or intraperitoneally in a 1 ml kg^{-1} volume. The antagonists were administered 90 min and the agonists 60 min before death, respectively. All the animals received BrdU, 200 mg kg^{-1} intraperitoneally, 2 h before death. In the second set of experiments the influence of (R)α-MeHA on proliferation and migration at 24 h after its administration was examined. Groups of rats received either ciproxifan (3 mg kg^{-1}) or saline intragastrically in a 5 ml kg^{-1} volume. Thirty minutes later (R)α-MeHA (100 mg kg^{-1}) or saline were given intragastrically. (R)α-MeHA was administered 24 h before the animals were sacrificed. All the animals received BrdU, 50 mg kg^{-1} intraperitoneally, concurrently with (R)α-MeHA. The rats were killed by cervical dislocation and the stomachs were immediately removed and opened along the lesser curvature. A strip (5 × 10 mm) was excised from the glandular mucosa, 3–4 mm below and parallel to the limiting ridge, so that the greater curvature was approximately located in the middle of the strip. Three different tissue samples were taken from each strip, fixed in 10% neutral buffered formalin and embedded in paraffin. Serial sections, 4 μm thick, were cut, deparaffinized with xylene and rehydrated with graded concentrations of ethanol. Sections were stained either with H&E or with diastase-resistant periodic acid-Schiff (PAS). Adjacent sections were prepared for immunohistochemistry.

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 monoclonal 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). Apoptotic cells were identified using the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) assay kit (Dako) according to the manufacturer's suggested protocol.

Quantitative analysis

The image of the section was displayed on a colour monitor by means of a videocamera attached to the microscope (Nikon Optiphot) and quantitations were performed using a colour image analysis software system (LUCIA G, Nikon Laboratory Imaging, Japan). Measurements were performed in regions in which full-length glands were oriented perpendicular to the mucosal surface. The isthmus region was defined according to Karam & Leblond (1992). The number of positive cells was counted in fields being 100 μm in length with one field being considered in each section. At least 24 fields were examined for each rat. Mucosal thickness was measured from the epithelial surface to the muscularis mucosae in each field. For each rat the values from all the fields examined were averaged and these values used to calculate mean values for each experimental group.

Statistical analysis

Data shown are mean ± s.e.mean. Statistical analysis was performed using ANOVA followed by Newman-Keuls test; P values <0.05 were considered significant.

Results

Increase of cell proliferation by (R)α-MeHA

Examination of the rat oxyntic mucosa 2 h after the injection of BrdU in the control rats revealed the presence of S phase cells in the isthmal region in a number comparable to previously reported values in the adult rat (Lacy *et al*., 1996) (Table 1). Administration of (R)α-MeHA (10–100 mg kg^{-1}), *via* the intragastric or intraperitoneal routes 1 h before the stomach examination, resulted in a dose-dependent increase in the proliferative activity. With the highest dose tested, (R)α-MeHA approximately doubled the number of BrdU-positive cells with either route of administration (Table 1). Their position within the gland was not altered in comparison with the control group, in that positive cells were confined to the isthmal region with no positive cells outside the isthmus. To investigate the specificity of the effect of (R)α-MeHA, studies with the enantiomer (S)α-MeHA and with a further histamine H_3-receptor agonist, FUB 407, were conducted. Following (S)α-MeHA intragastric administration, the number of proliferating cells was 4.78 ± 0.32 ($n=5$), similar to that found in the saline-treated group. Intragastric administration of FUB 407 (9.14, 27.41, and 91.35 mg kg^{-1}) induced a dose-dependent increase in the number of proliferating cells, and following the dose of 91.35 mg kg^{-1} the number of positive cells was increased by 111% above that in the control group (Table 2). The specificity of the effect of (R)α-MeHA was further confirmed. The selective H_3-receptor antagonists, ciproxifan (3 mg kg^{-1}) and clobenpropit (30 mg kg^{-1}), intragastrically administered 90 min before stomach examination, did not affect the number of S phase cells as compared to the control group, while they fully counteracted the proliferative stimulus exerted by (R)α-MeHA (100 mg kg^{-1} i.g.) as shown in Table 3. Similarly ciproxifan (3 mg kg^{-1}) abolished the proliferative stimulus exerted by FUB 407 (91.35 mg kg^{-1}) (Table 2). By contrast the H_1-receptor antagonist, mepyramine (30 mg kg^{-1} i.g.), and the H_2-receptor antagonist, famotidine (3 mg kg^{-1} i.g.), did not significantly modify the number of BrdU-positive cells in comparison with the saline-treated group and failed to affect the increase in proliferation induced by (R)α-MeHA at 100 mg kg^{-1} i.g. (Table 3).

Migratory pathway of proliferating cells

Migration of the proliferating cells was followed in a second set of experiments by administering (R)α-MeHA (or saline) simultaneously with BrdU 24 h before stomach examination. In the control rats, the BrdU-positive cells formed a widened band corresponding to the isthmus and its vicinity. The proliferative zone was expanded, due to the longer time allowed for BrdU incorporation and to the early phases of the migratory process, but it was not altered in its location. Positive cells were absent in the upper as well as in the deepest portions of the gastric glands. In (R)α-MeHA-treated rats the number of positive cells was significantly higher, being increased by 121% (Table 4). The most relevant feature was the modification in the position of these cells, which appeared to be located predominantly towards the lumen. In

Table 1 Cell proliferation and apoptosis after 1 h (R)-α-methylhistamine treatment

		Route of administration						
		Intragastric			*Intraperitoneal*			
	Dose (mg kg^{-1})	n	*Mucosal thickness*	*BrdU-positive cells*	*Apoptotic cells*	n	*Mucosal thickness*	*BrdU-positive cells*	*Apoptotic cells*
Saline		7	605.16 ± 14.57	5.31 ± 0.65	0.29 ± 0.06	6	593.83 ± 8.65	4.81 ± 0.44	0.37 ± 0.08
	10	4	602.25 ± 9.04	5.15 ± 1.38	0.45 ± 0.08	4	584.00 ± 22.79	4.76 ± 0.88	0.61 ± 0.19
(R)-α-methylhistamine	30	6	612.50 ± 15.06	7.88 ± 1.65	0.94 ± 0.05	7	595.43 ± 19.62	6.67 ± 0.99	1.21 ± 0.61
	100	6	611.33 ± 14.93	9.62 ± 0.94**	2.39 ± 0.89*	5	625.50 ± 12.44	9.47 ± 1.48**	2.91 ± 0.86*

Cell proliferation and apoptosis were determined by BrdU and TUNEL immunohistochemistry. Values represent the mean ± s.e.mean of positive cells per field, each field being 100 μm in length. Mucosal thickness was measured in each field and mean values ± s.e.mean are reported. n = number of rats. Rats received saline, 5 ml kg^{-1} intragastrically, and 30 min later (R)-α-methylhistamine (10–100 mg kg^{-1} by intragastric or intraperitoneal route) or saline, 5 ml kg^{-1} intragastrically. Rats were killed 60 min after (R)-α-methylhistamine administration. BrdU was injected intraperitoneally 2 h before death. *$P<0.05$ and **$P<0.01$ compared with the corresponding saline-treated group.

Table 2 Cell proliferation and apoptosis after 1 h FUB 407 treatment

	Dose (mg kg^{-1})	n	*BrdU-positive cells*	*Apoptotic cells*
Saline		5	4.90 ± 0.77	0.33 ± 0.06
	9.14	4	5.02 ± 0.78	0.24 ± 0.03
FUB 407	27.41	4	8.17 ± 1.27	0.64 ± 0.18
	91.35	4	10.32 ± 1.73**	2.65 ± 0.85*
Ciproxifan/ FUB 407 (91.35 mg kg^{-1})		5	4.88 ± 1.19#	0.31 ± 0.04§

Cell proliferation and apoptosis were determined by BrdU and TUNEL immunohistochemistry. Values represent the mean ± s.e.mean of positive cells per field, each field being 100 μm in length. n = number of rats. Rats received ciproxifan, 3 mg kg^{-1}, or saline, 5 ml kg^{-1} intragastrically, and 30 min later FUB 407 (9.14–91.35 mg kg^{-1}), or saline, 5 mg kg^{-1} intragastrically was given. Rats were killed 60 min after FUB 407 administration. BrdU was injected intraperitoneally 2 h before death. *$P<0.05$ and **$P<0.01$ compared with the saline-treated group; §$P<0.05$ and #$P<0.01$ compared with the FUB 407 (91.35 mg kg^{-1})-treated group.

Table 3 Effect of histamine H_1-, H_2- and H_3-receptor antagonists on proliferation by 1 h (R)-α-methylhistamine treatment

		− (R)-α-methylhistamine			+ (R)-α-methylhistamine		
	Dose (mg kg^{-1})	n	*Mucosal thickness*	*BrdU-positive cells*	n	*Mucosal thickness*	*BrdU-positive cells*
Saline		7	605.16±14.57	5.31±0.65	6	611.33±14.93	9.62±0.94**
Mepyramine	30	4	600.00±37.91	5.46±2.01	4	621.05±19.63	11.69±0.79**
Famotidine	3	4	635.01±29.51	5.29±0.91	4	585.75±22.38	10.16±0.78**
Ciproxifan	3	4	609.03±16.74	5.42±1.29	4	621.11±23.71	5.36±0.65#
Clobenpropit	30	4	675.15±21.15	5.38±1.09	4	673.67±18.04	4.78±1.14#

Cell proliferation was determined by BrdU immunohistochemistry. Values represent the mean±s.e.mean of positive cells per field, each field being 100 μm in length. Mucosal thickness was measured in each field and mean values±s.e.mean are reported. n=number of rats. Rats received the histamine receptor antagonists or saline, 5 ml kg^{-1} intragastrically, and 30 min later (R)-α-methylhistamine, 100 mg kg^{-1}, or saline, 5 ml kg^{-1} intragastrically was given. Rats were killed 60 min after (R)-α-methylhistamine administration. BrdU was injected intraperitoneally 2 h before death. *$P<0.01$ compared with the corresponding group not receiving (R)-α-methylhistamine; #$P<0.01$ compared with the group receiving (R)-α-methylhistamine alone.

Table 4 Cell proliferation and apoptosis after 24 h (R)-α-methylhistamine treatment

	n	*Mucosal thickness*	*BrdU-positive cells*	*Apoptotic cells*
Saline	5	625.55±22.46	10.67±2.07	0.38±0.09
(R)-α-methylhistamine	5	597.63±28.30	23.57±5.28*	0.29±0.11
Ciproxifan	4	592.27±24.67	10.28±3.47	0.33±0.07
Ciproxifan/ (R)-α-methylhistamine	4	633.15±33.57	11.33±4.61	0.34±0.07

Cell proliferation and apoptosis were determined by BrdU and TUNEL immunohistochemistry. Values represent the mean±s.e.mean of positive cells per field, each field being 100 μm in length. Mucosal thickness was measured in each field and mean values±s.e.mean are reported. n=number of rats. Rats received ciproxifan, 3 mg kg^{-1}, or saline, 5 ml kg^{-1} intragastrically, and 30 min later (R)-α-methylhistamine, 100 mg kg^{-1}, or saline, 5 ml kg^{-1} intragastrically was given. Rats were killed 60 min after (R)-α-methylhistamine administration. BrdU was injected intraperitoneally 24 h before death. *$P<0.05$ compared with the saline-treated group.

order to quantify this effect, the pit region was arbitrarily divided into four segments: three segments of equal size named low, mid and high pit, and a fourth one facing the lumen or pit top (Karam & Leblond, 1993b). S phase cells in each segment were counted while, for convenience, positive cells in the isthmus and in the proximal regions were grouped into a single category, as shown in Figure 1. It was apparent that (R)α-MeHA promoted the preferential expansion of lineage of pit cells and strongly accelerated their migration rate. The hypothesis that the gastric epithelial cell hyperproliferation by (R)α-MeHA could interfere with the evaluation of its effect on migration was considered and fields having a similar number of labelled cells were compared. Even in this condition the difference in the distribution of BrdU-labelled cells between the control and (R)α-MeHA groups was evident (not shown). (R)α-MeHA did not apparently modify the mucin staining along the free luminal surface and the pit, compared with the control group, while the PAS staining in cells from the neck region was expanded (Figure 1). Ciproxifan alone did not alter the number and distribution of BrdU-labelled cells in comparison with the control group and it counteracted the proliferative stimulus exerted by (R)α-MeHA as well as the acceleration in the migration rate of pit cells consequent on (R)α-MeHA (Table 4 and Figure 1).

Apoptosis

The occurrence of apoptosis within the mucosal cells was detected by the TUNEL assay. In the control rats, there were only a few apoptotic cells, the majority being located in the pit region of the mucosa. The values are similar to published values for apoptosing cells in the adult rat (Li *et al*., 1999). Quantification revealed that 1 h after administration, both (R)α-MeHA and FUB 407 dose-dependently increased the number of apoptotic cells (Tables 1 and 2). Following (R)α-MeHA at 100 mg kg^{-1} or FUB 407 at 91.35 mg kg^{-1}, their number was significantly higher compared with the saline-treated group. This increase was accounted for almost exclusively by cells facing the lumen or situated in close proximity to it. Ciproxifan (3 mg kg^{-1}) alone was ineffective while it reversed the (R)α-MeHA- or FUB 407-induced effect (Tables 1 and 2). The number of apoptotic cells was not modified following (S)α-MeHA treatment. At 24 h after administration of (R)α-MeHA, apoptotic cells were comparable in number to those in control and in the other treatment groups (Table 4).

Thickness of the mucosa

The overall thickness of the mucosa was 605.41±11.26 μm (n=21) in the saline-treated group and it was not modified in any of the different treatment groups (see Tables). The pattern of glandular structure was not altered. There was no evidence of cell discontinuities at the level of superficial epithelium.

Discussion

The findings of this study clearly demonstrate that (R)α-MeHA promotes a dose-dependent increment in cell

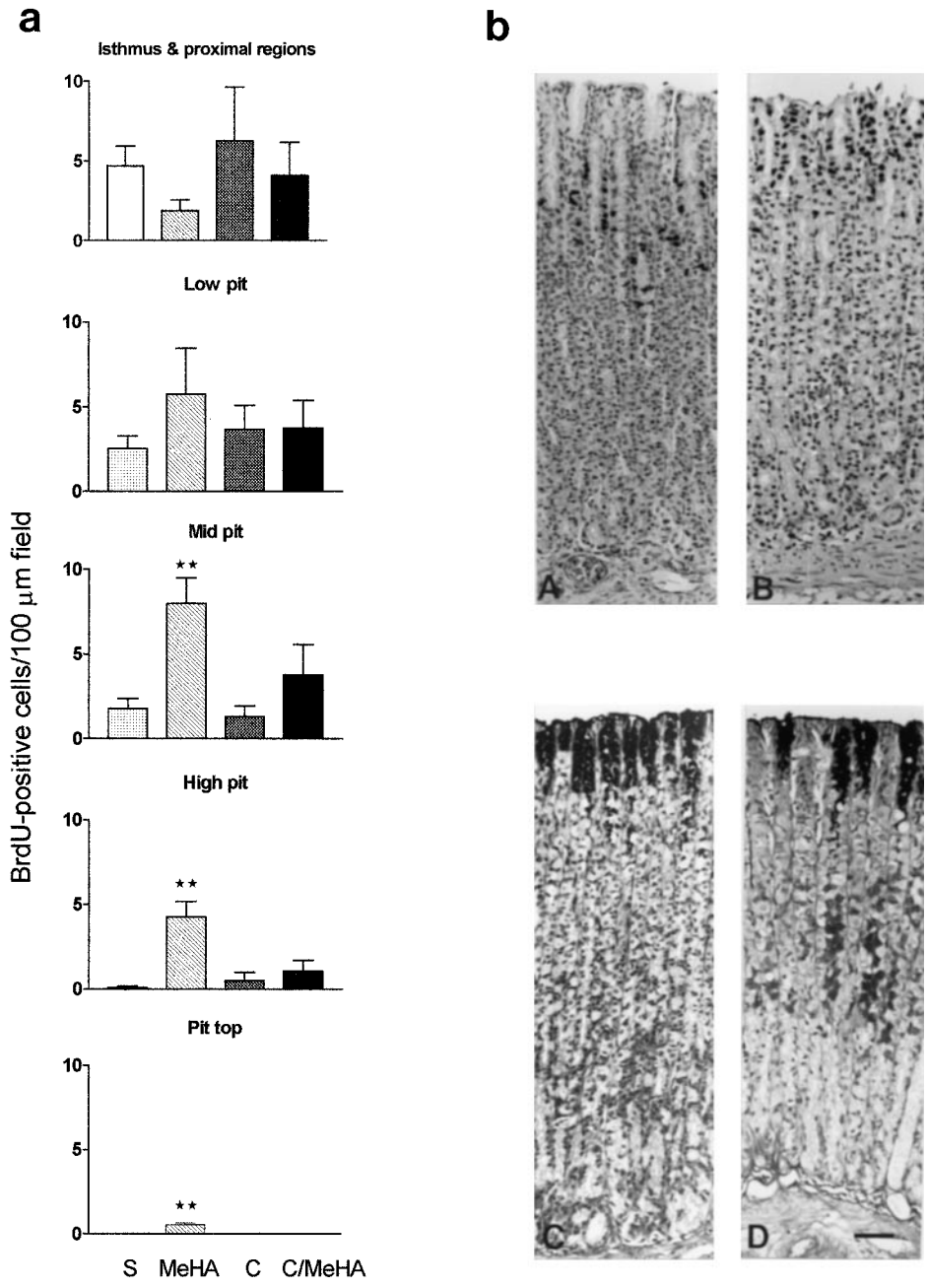

Figure 1 Migration of cells along the pit in rat oxyntic mucosa after 24 h (R)-α-methylhistamine treatment. BrdU was injected intraperitoneally concurrently with (R)-α-methylhistamine (MeHA), 100 mg kg^{-1}, or saline (S), 5 ml kg^{-1} intragastrically. Ciproxifan (C), 3 mg kg^{-1}, or saline, 5 ml kg^{-1} intragastrically, was administered 30 min before (R)α-methylhistamine or saline. (a) Quantitation of BrdU-positive cells. In each segment of the pit positive cells were counted. Mean ± s.e.mean, $n = 5$ rats. $^{**}P < 0.01$ compared with the saline-treated group. (b) Oxyntic mucosal sections from saline (A, C)- and (R)α-methylhistamine (B, D)-treated rats. BrdU incorporation was immunohistochemically identified (A, B). The accelerated outward migration of pit cells following (R)α-methylhistamine is evident. Adjacent sections were stained with diastase-resistant PAS to identify mucus secreting cells (C, D). Cells from the luminal surface and along the pit exhibit a comparable mucin staining in (R)α-MeHA- and saline-treated mucosa. The mucin staining in cells from the neck region is apparently increased in (R)α-MeHA-treated mucosa. Scale bar, 50 μm.

proliferation. Proliferation is increased in the same dose range and to a comparable extent by intragastric as well as by intraperitoneal route of administration, therefore suggesting the possibility that both local and systemic actions of (R)α-MeHA could be involved.

The stimulus exerted by (R)α-MeHA is not secondary to injury since the fundic mucosa was preserved in its integrity. In view of the restriction of BrdU-positive cells to the isthmus, isthmal cells appear to be the final target of (R)α-MeHA. It could be derived that (R)α-MeHA induces a large number of mitosable isthmal cells, well beyond those normally found, to enter the DNA-synthesizing phase of the cycle, almost without a latent period. The hyperproliferative response to (R)α-MeHA is achieved in a very short

time and it is unique in its rapidity, this feature not being shared by paracrine and endocrine factors active in stimulating epithelial cell proliferation in the oxyntic mucosa. Transforming growth factor α (TGFα), following a continuous infusion over 4 days, determined a 65% increase in PCNA immunoreactive cells relative to controls in the rat gastric mucosa (Turner *et al*., 2000). Gastrin, after a 3-h intravenous infusion in the rat (Casteleyn *et al*., 1977), increased the incorporation of tritiated thymidine in gastric mucosal extracts 8 h after starting the administration with a peak at the 16th h. A similar time course for the increase in cell proliferation by gastrin has been reported in the dog (Willems *et al*., 1972).

Proliferating cells are addressed by (R)α-MeHA towards the pit region, and (R)α-MeHA appears to be endowed with a powerful motogenic effect. Its dramatic effectiveness could be appreciated by considering that, under normal circumstances, pit cells take 4 days in the mouse (Karam & Leblond, 1993b) and 7 days in the rat (Yang *et al*., 1997) to migrate from the isthmus to the luminal surface, whereas under the influence of (R)α-MeHA the migratory process is achieved within 1 day. The degree of maturation of pit cells was not presently quantified. However, it is noteworthy that, while mucus production by pit cells was increased at 1 h following (R)α-MeHA treatment (Morini *et al*., 1997a), no apparent stimulation was observed at 24 h. The accelerated differentiation towards pit cells together with the increase in migration rate could impair the complete maturation of these cells and/or their responsiveness to stimuli. In contrast, the increase in mucus production by mucous neck cells was observed after 1 as well as after 24 h following (R)α-MeHA treatment, paralleled by the absence of any apparent influence on dynamics of these cells.

The incidence of apoptosis was increased 1 h but not 24 h after (R)α-MeHA. The increased apoptosing cells, found after 1 h, because of the location at the extreme top of the mucosa, are likely to be extruded to the gastric lumen within 24 h. Hence apoptosis would seem to be only transiently increased shortly after administration of (R)α-MeHA and, being induced in the oldest cells, its aim might be to expedite their loss and, in general, pit cell renewal.

The meaning of the events triggered by (R)α-MeHA remains speculative. Acute gastric injury increases mucosal proliferative activity. DNA synthesis peaks 16 h after a single dose of aspirin in the rat (Yeomans *et al*., 1973; Ohning & Guth, 1995), with a slight increase in labelling between 2 and 4 h (Yeomans *et al*., 1973). Following intragastric 2M NaCl, BrdU-positive cells increase steadily with time from 33% at 1 h after injury to 100% at 24 h (Fligiel *et al*., 1994). The relatively late occurrence of the increase in proliferation has been interpreted as evidence that proliferation and migration of SMCs are separate and independent defence mechanisms of the gastric mucosa (Silen & Ito, 1985; Allen *et al*., 1993). The capacity for active migration of SMCs would account for the process of restitution, responsible for the rapid repair of superficial mucosal damage (Lacy & Ito, 1984). Cellular proliferation would be required for the slower healing of deeply extending lesions. The great rapidity, by which (R)α-MeHA induces proliferation, combined with its protective activity, partly contradicts these statements. It is possible that proliferation, differentiation, and migration of SMCs are coordinated steps, essential in protecting the gastric mucosa, even though temporally distinct.

The present findings provide the pharmacological evidence that in the rat oxyntic mucosa, cell proliferation, differentiation, and migration are likely to be regulated through histamine H_3 receptors. Support for the specificity of the actions of (R)α-MeHA comes from finding that the H_3-receptor agonist FUB 407 is equivalent to (R)α-MeHA in its ability to stimulate cell proliferation, this effect being abolished by the H_3-receptor antagonist ciproxifan. The S isomer of α-methylhistamine was without effect. Both the H_3 receptor antagonists ciproxifan and clobenpropit inhibited the increase in proliferation by (R)α-MeHA. In contrast pretreatment with effective doses of the H_1 receptor antagonist mepyramine and the H_2 receptor antagonist famotidine did not modify the (R)α-MeHA-evoked hyperproliferation. Maintenance of gastric mucosal integrity by (R)α-MeHA was also characterized pharmacologically as mediated through H_3 receptors (Morini *et al*., 2000). H_3 receptors are proved to act both as autoreceptors, presynaptically located on histaminergic neurons regulating histamine synthesis and release (Arrang *et al*., 1983), and as heteroreceptors on non-histaminergic nerve fibers (Schlicker *et al*., 1994). The cDNA encoding for the histamine H_3 receptor in the rat has been recently cloned (Lovenberg *et al*., 2000). However detection of H_3 receptor mRNA expression in rat peripheral tissues, including the gastrointestinal tract, is controversial (Lovenberg *et al*., 2000; Héron *et al*., 2001). One possible explanation for the divergent results would be the existence of multiple isoforms of the rat H_3 receptor (Drutel *et al*., 2001; Morisset *et al*., 2001). Alternatively, as outlined by Lovenberg *et al*. (2000), the presynaptic nature of the receptor could make it difficult to correlate functional receptors with mRNA expression, mostly in the periphery.

Proliferation and migration triggered by histamine H_3 receptor agonists could be a direct mediated response or the result of complex interrelations with hormonal and/or paracrine mechanisms. Interestingly enough, apart from the effect on acid secretion (Morini *et al*., 2000; Guglietta *et al*., 1994), (R)α-MeHA shares a nearly identical spectrum of activity with TGFα in the gastric mucosa (Romano *et al*., 1992; Rutten *et al*., 1993; Turner *et al*., 2000). The close similarity highlighted here could represent a basis for evaluating whether (R)α-MeHA might act in an integrated way with growth factors (Taupin *et al*., 1999; Wong *et al*., 2000). In addition, studies, focusing on the control of gastric acid secretion with *in vitro* preparations (Prinz *et al*., 1993; Vuyyuru *et al*., 1997; Vuyyuru & Schubert, 1997), provided evidence that in rodents histamine release from enterochromaffin-like cells is not only stimulated by gastrin and inhibited by gastric somatostatin but also regulated by a feedback mechanism, with the H_3 receptor being the feedback receptor. Histamine in its turn, through the H_3 receptor, exerts an inhibitory influence on somatostatin secretion. A feedback loop, regulating acid production and sensitive to intraluminal pH, has been shown between gastric somatostatin and gastrin. This circuit could similarly operate in the control of cell proliferation and migration in the gastric mucosa. Both gastrin and somatostatin affect mucosal growth, with gastrin exerting a growth-positive and somatostatin a growth-negative action (Walsh, 1994).

There is considerable attention to the role of histamine in the regulation of cell proliferation. Because of the increase in histamine content and in histidine decarboxylase activity in rapidly growing tissues and in experimentally induced tumours, endogenous histamine is believed to have an important function in tissue growth (Bartholeyns & Fozard, 1985; Hill, 1990). Histamine H_2 receptors have been shown in many neoplastic tissues or cell lines (see Del Valle & Gantz, 1997 for review). High levels of both H_3 receptor and L-histidine decarboxylase mRNAs were recently observed in developing rat tissues (Héron *et al*., 2001). An intracellular receptor subtype has been also proposed to mediate the growth activity of histamine (Brandes *et al*., 1990). Furthermore, in different cell systems, activation of the H_2 receptor is coupled to proto-oncogene expression and cell proliferation (Shayo *et al*., 1997; Wang *et al*., 1997; 2000). Following the recent cloning of the histamine H_3 receptor, it became feasible to assess that, beside H_2 receptors, H_3 receptors can promote the activation of intracellular transduction pathways related to the stimulation of mitogenic activity. In CHO-K1 cells transfected with the full-length coding sequence of the rat H_3 receptor (Héron *et al*., 2001) or in COS-7 cells transfected with the rat H_3 receptor isoforms (Drutel *et al*., 2001), activation of H_3 receptors enhanced mitogen-activated protein kinase activity. However, in the stomach *in vivo* the putative function of endogenous histamine was not paralleled by the effects of exogenously administered histamine, in that either a decrease (Pishva *et al*., 1975) or no change (Willems *et al*., 1972) in cell proliferation are reported. Moreoever, the few studies evaluating the effects of selective ligands for histamine H_1 and H_2 receptors on gastric mucosal cell proliferation in rodents utilized prolonged treatments and have so far produced conflicting results (Waldum *et al*., 1991; Brenna *et al*., 1994; 1995; Modlin *et al*., 1996).

Present data are compelling in the light of an implication of endogenous histamine in cell cycle control and of a hypothetical role of ligands for histamine H_3 receptors in the development of neoplastic alterations in gastric mucosa.

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