

## Review article

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### ROLE OF HISTAMINE H<sub>3</sub> RECEPTORS IN THE REGULATION OF GASTRIC FUNCTIONS

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The role of central and peripheral histamine H<sub>3</sub> receptors in the regulation of gastric acid secretion and gastric mucosal integrity is reviewed. The activation of H<sub>3</sub> receptors by peripheral administration of the selective agonist (R)α-methylhistamine reduced acid secretion in cats, dogs, rats and rabbits, while increasing it in mice. The antisecretory effects were observed against indirect stimuli that act on vagal pathways or on enterochromaffin-like (ECL) cells, such as 2-deoxy-D-glucose, food or pentagastrin, but not against histamine or dimaprit. Inhibitory effects on acid production were observed in rats after central administration of histamine or of H<sub>3</sub> receptor agonists. In the conscious rat intragastric administration of (R)α-methylhistamine caused gastroprotective effects against the damage induced by absolute ethanol, HCl, aspirin and stress. The mechanism involved seems to be related to the increased mucus production, *via* nitric oxide-independent mechanisms. Gastroprotective effects against ethanol were also observed after central administration of histamine or its metabolite N<sup>ω</sup>-methylhistamine, suggesting that brain H<sub>3</sub> receptors participate in the histamine-mediated effects on gastric functions.

**Key words:** *Histamine H<sub>3</sub> receptors, gastric acid secretion, gastric mucosal integrity, Helicobacter pylori*

#### INTRODUCTION

Histamine is widely distributed in the gastrointestinal tract in various cell types, namely enterochromaffin-like (ECL) cells, mast cells, basophils and enteric nerves of the myenteric plexus, showing considerable variation among species (1–3). The amine is released in response to a variety of (patho)physiological stimuli and it is involved in the pathogenesis of gastroduodenal ulceration and gastric inflammation. Recently, evidence was provided that increased gastric histamine contributes to the inflammatory

changes and tissue damage associated with chronic *Helicobacter pylori* infection of the gastric mucosa (4, 5). Histamine modulates a variety of functions by interacting with specific receptors on target cells, namely H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub>, that belong to the G-protein-coupled receptor family (6). H<sub>1</sub> receptors are distributed in the brain, where they are involved in the control of arousal mechanism, attention and cognition and in peripheral tissues, mediating vascular and bronchial muscle responses to histamine during allergy (7). H<sub>2</sub> receptors, although widely distributed in body tissues, seem to have a central role only in the regulation of acid secretion, as confirmed by the widespread use of H<sub>2</sub> receptor blockers in the therapy of acid-related disorders (8, 9). Finally, H<sub>3</sub> receptors, originally described as presynaptic autoreceptors on brain histaminergic neurons controlling histamine synthesis and release (10), were subsequently characterized as heteroreceptors in non-histaminergic neurons either in central or peripheral nervous system. Moreover, they were found in paracrine and immune cells and, in some tissues, also in smooth muscle cells (11, 12). Over the last few years, the biochemical and functional characterization of H<sub>3</sub> receptors has been made possible by the discovery of highly selective and potent agonists and antagonists (13, 14).

Conversely from H<sub>1</sub> (15) or H<sub>2</sub> (16) receptors, H<sub>3</sub> receptors were cloned from different species only recently and after many unsuccessful attempts (17–20). The human and rat H<sub>3</sub> receptors exhibit a 97% homology in the transmembrane domains, but surprisingly they display a significant difference in the affinity for some H<sub>3</sub> ligands (e.g. thioperamide shows a tenfold preference for the rat receptor). Recent molecular studies have shown that a single form of the H<sub>3</sub> gene can give rise to multiple mRNA isoforms, named H<sub>3A</sub>, H<sub>3B</sub> and H<sub>3C</sub> in the rat (20) and H<sub>3L</sub> and H<sub>3S</sub> in the guinea pig (19), confirming the heterogeneity among H<sub>3</sub> receptors, that had long been suspected, based on agonist kinetics (21), radioligand binding characteristics (22, 23) and functional studies (24, 25). By contrast, the existence of variants in humans has not been definitely confirmed (26, 27). A constitutive activity of the human and, to a lesser extent, of the rat H<sub>3</sub> receptor has been reported in the brain, and it is responsible for the inhibition of neuronal histamine release (28).

Very recently, a novel subtype was added to the histamine receptor family, namely the H<sub>4</sub> receptor, that was cloned by several laboratories by screening the human genome databases (29–35). This new receptor subtype belongs to the G-protein-coupled receptors and was found to be highly expressed in the human bone marrow and, at moderate levels, also in the human colon. Due to the lack of selective agonists and antagonists (36), however, the functional role of H<sub>4</sub> receptors is still obscure.

The present review will outline the current knowledge on the role of histamine H<sub>3</sub> receptors in the stomach, with particular reference to the effects on gastric acid secretion and gastric mucosal integrity.

Table 1. Effect of H<sub>3</sub>-receptor activation on gastric acid production

| Species    | Technique                                   | Effect             | Reference |
|------------|---|--------------------|-----------|
| Mouse      | Whole stomach <i>in vitro</i>               | ↑                  | 39        |
| "          | Gastric glands <i>in vitro</i>              | 0                  | 40        |
| Rat        | Pylorus-ligated <i>in vivo</i>              | 0 (i.v.)           | 41        |
| "          | " "   | ↓ (i.c.v.)         | 42        |
| "          | Gastric fistula <i>in vivo</i>              | ↓ (i.c.v.)         | 43        |
| "          | Lumen-perfused stomach <i>in vivo</i>       | 0 (2DG, PGas, HA)  | 41        |
| "          |   | ↓ (E.S.)           | 44        |
| "          |   | 0 (Bet)            | 44        |
| "          | Vascularly-perfused stomach <i>in vitro</i> | 0                  | 45        |
| "          | Gastric fundus <i>in vitro</i>              | 0                  | 41        |
| Guinea pig | Gastric fundus <i>in vitro</i>              | 0                  | 12        |
| Rabbit     | Parietal cells <i>in vitro</i>              | 0                  | 46        |
| "          | Fundic glands <i>in vitro</i>               | ↓ (Bet)            | 47        |
| Cat        | Gastric fistula <i>in vivo</i>              | ↓ (2DG and BBS)    | 48        |
| "          | Gastric fistula and H.P.                    | ↓ (food and PGas)  | 49        |
| Dog        | Gastric fistula <i>in vivo</i>              | ↓ (2DG, PGas, BBS) | 50, 51    |
| Human      | Fundic membranes                            | no effect on AC    | 52        |
| "          | Gastric cell line HGT1                      | ↓ IP formation     | 53        |

2DG = 2-deoxy-D-glucose; BBS = bombesin; PGas = pentagastrin; HA = histamine; E.S. = electrical stimulation; Bet = bethanechol; BBS = bombesin; ↓ inhibition; ↑ stimulation; 0 = no effect; i.v. = intravenous; i.c.v. = intracerebroventricular; IP = inositol phosphate; AC = adenylyl cyclase; H.P. = Heidenhain pouch.

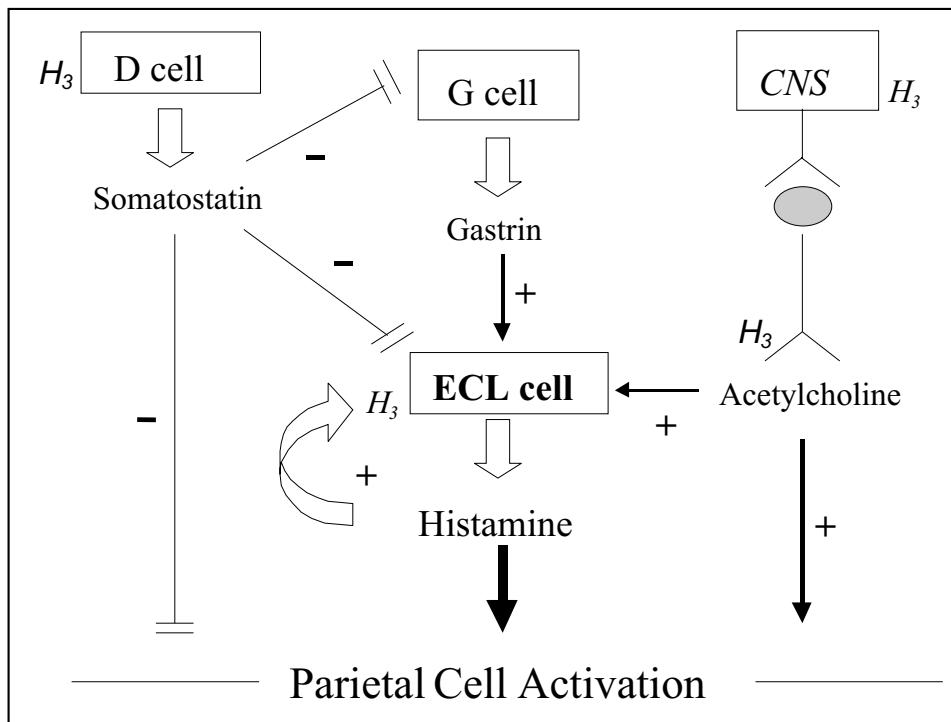
### Gastric acid secretion

The leading role of histamine in the control of gastric acid secretion was definitely assessed with the discovery of histamine H<sub>2</sub> receptors by Black and coworkers in 1972 (37). A bulk of experimental data clearly revealed that histamine is released from endogenous storing cells by several stimuli that include food intake, gastrin, vagus and the neuropeptides PACAP and VIP (38). Thus the ECL-parietal axis constitutes in various animal species and in humans the major stimulatory pathway for the acid secretion (38).

The discovery of histamine H<sub>3</sub> receptors led to a renewed interest in the physiologic role of histamine in gastric functions and several researchers re-examined histamine effects on the light of this new mechanism. In the gastrointestinal tract H<sub>3</sub> receptors are located in cholinergic and NANC neurones of the myenteric plexus, in endocrine and/or paracrine cells of the gastric mucosa and, at least in rabbits, also in parietal cells (12). In cats and dogs the activation of H<sub>3</sub> receptor leads to reduction of the acid response to different secretagogues (*Table 1*). The effect was evident against indirect stimuli, such as vagal stimulation or pentagastrin, but not against direct activation of H<sub>2</sub> receptors by dimaprit, indicating a location of H<sub>3</sub> receptors outside parietal cells (*Table 2*). In the dog, the inhibition of pentagastrin-induced acid secretion by the H<sub>3</sub> receptor agonist (R)α-methylhistamine was accompanied by a reduction of histamine levels in the gastrosplenic vein (62). In addition, a tonic autoinhibitory control of histamine release mediated by H<sub>3</sub> receptors is suggested by the threefold enhancement of histamine release observed with the H<sub>3</sub> antagonist thioperamide, when low doses of pentagastrin are used. This suggests that H<sub>3</sub> receptors are mainly located on histamine-producing cells, where they work as an endogenous inhibitory mechanism operated by histamine itself to control excess acid production. The inhibitory effect on ECL cell function was confirmed in rabbits (60, 61) and rats (45, 57—59); however, a location on parietal cells was

*Table 2.* Effect of H<sub>3</sub> receptor activation on gastric neuronal, endocrine and paracrine mediators

| Mediator      | Effect | Species | Reference |
|---------------|--------|---------|-----------|
| Acetylcholine | ↓      | Rat     | 54        |
| Gastrin       | 0      | Cat     | 55        |
|               | 0      | Dog     | 51        |
| Histamine     | ↑      | Mouse   | 39        |
|               | ↑      | Rat     | 56        |
|               | ↓      | Rat     | 45, 57—59 |
|               | ↓      | Rabbit  | 60, 61    |
|               | ↓      | Dog     | 62        |
|               | ↓      | Human   | 4, 64     |
| Somatostatin  | ↓      | Mouse   | 39        |
|               | ↓      | Rat     | 63—65     |
|               | ↓      | Dog     | 64        |
|               | ↓      | Human   | 4, 64     |



*Fig. 1.* Schematic model showing the effect of  $H_3$  receptors on the excitatory pathways that control acid secretion by the parietal cell. The model is based on experimental data obtained in the rat (see text for references). Besides the presence on enterochromaffin-like (ECL) cells, different groups indicated an additional presence of  $H_3$  receptors on fundic D cells and cholinergic neurons. Moreover, the possibility that  $H_3$  receptors regulate acid secretion by central mechanisms has also been raised. The inhibitory control does not involve directly parietal cell receptors.

suggested in rabbit, where a negative interaction of  $H_3$  receptors with muscarinic  $M_3$  receptors occurring through post-receptor mechanisms has been postulated (47). In rats, contrasting data were reported when acid secretion was considered (*Table 1*). Inhibitory effects after  $H_3$  receptor agonists were not observed in our lab in different acid secretion models (41) or in the vascularly-perfused isolated stomach (45). Subsequent studies, however, have suggested the existence of inhibitory  $H_3$  receptors on vagal terminals (54) and have shown inhibitory effects of (R) $\alpha$ -methylhistamine on vagally-induced acid secretion (44), indicating that  $H_3$  receptors have multiple cellular locations in the rat gastric mucosa (*Fig. 1*). However, according to recent experiments from our group, the very selective  $H_3$  receptor agonist immeppip (66) and the selective antagonists clobenpropit (66) and ciproxifan (67) did not induce any effect on either basal secretion or on the secretory response to pentagastrin (*Fig. 2*). A different contribution of  $H_3$  receptors located on fundic somatostatin D cells in the different experimental models, together with the use of non

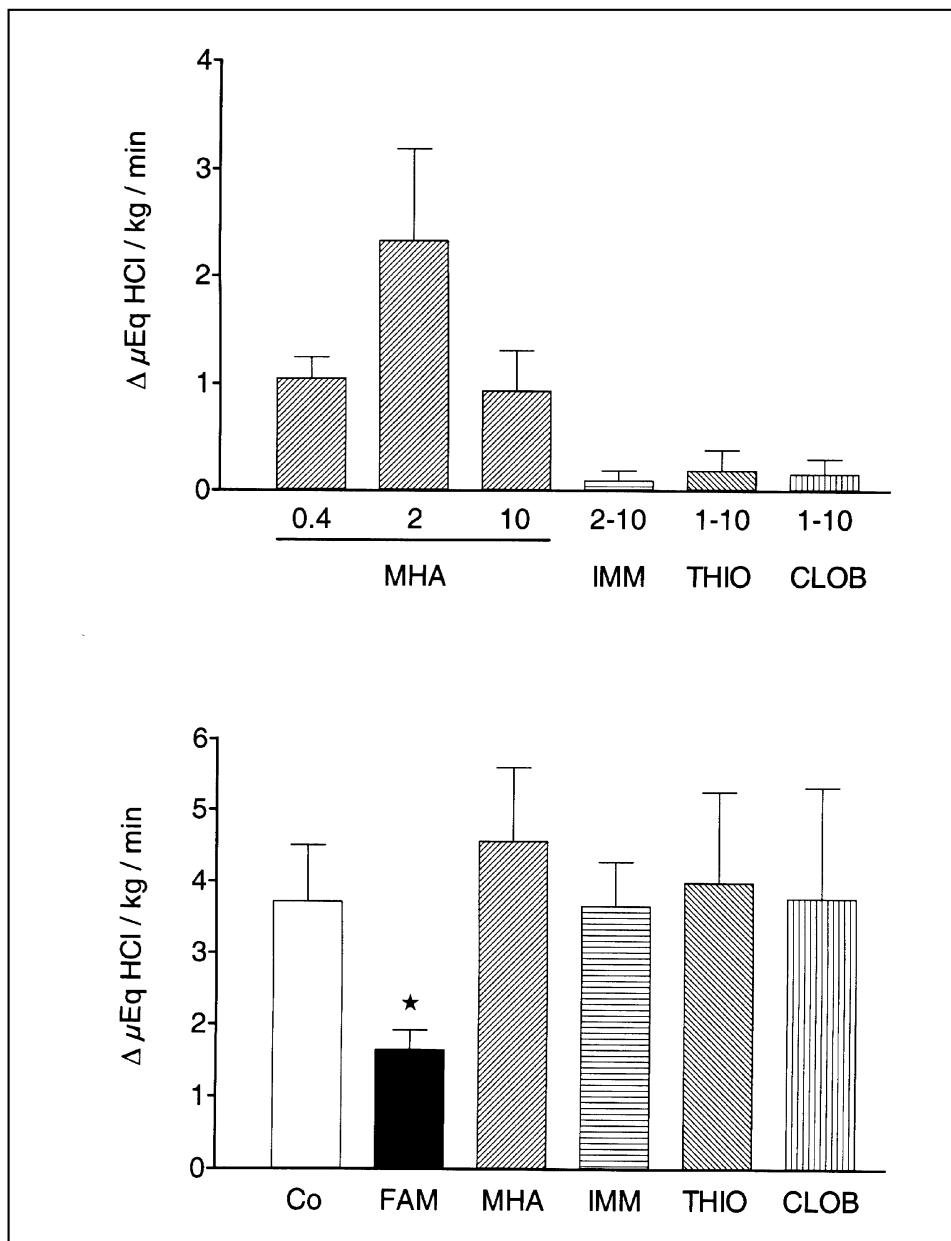


Fig. 2. Lumen-perfused stomach of the anaesthetized rat. *Upper panel*: Effect of different doses ( $\mu\text{mol}/\text{kg}/\text{h}$ ) of (R) $\alpha$ -methylhistamine (MHA), immeprine (IMM), thioperamide (THIO) and clobenpropit (CLOB) on basal acid secretion. *Lower panel*: Effect of famotidine (FAM, 1  $\mu\text{mol}/\text{kg}$  i.v.), (R) $\alpha$ -methylhistamine (MHA, 10), immeprine (IMM, 10), thioperamide (THIO, 1–10) and clobenpropit (CLOB, 1–10) on the acid secretion induced by pentagastrin (Co, 10 nmol/kg i.v.). H<sub>3</sub>-receptor ligands were administered ( $\mu\text{mol}/\text{kg}/\text{h}$ ) starting 30 min before pentagastrin. Mean values  $\pm$  SEM obtained from 6–8 data. \*P < 0.05 vs control (ANOVA and Newman-Keuls test).

selective compounds, might be responsible for the observed discrepancies. Indeed, (R) $\alpha$ -methylhistamine can activate H<sub>2</sub> receptors at high doses (12) and most H<sub>3</sub> ligands are also active at the newly discovered H<sub>4</sub> receptor (36), whose involvement in the acid secretion process is totally unexplored. A central site of action for histamine in the control of acid secretion has been suggested by some studies, that reported inhibitory effects on acid production after intracerebroventricular administration of histamine or of H<sub>3</sub> receptor agonists in the conscious rats (42, 43).

No functional studies concerning H<sub>3</sub> receptors and acid secretion are available at present in humans; however, the histamine H<sub>3</sub> receptor protein has been purified from the human gastric tumoral cell line HGT-1 and found to be negatively coupled with phosphatidylinositol turnover (53).

A role for H<sub>3</sub> receptors in the complex regulation of ECL cell proliferation was suggested by recent findings on isolated rat ECL cells: the selective H<sub>3</sub> receptor agonist imenit stimulated gastrin-induced ECL cell proliferation and the H<sub>3</sub> receptor antagonist thioperamide had the opposite effect (68). The recent finding (69) that rat antral G cells contain histamine suggests that histamine can influence gastric secretion also indirectly by regulating G cell function.

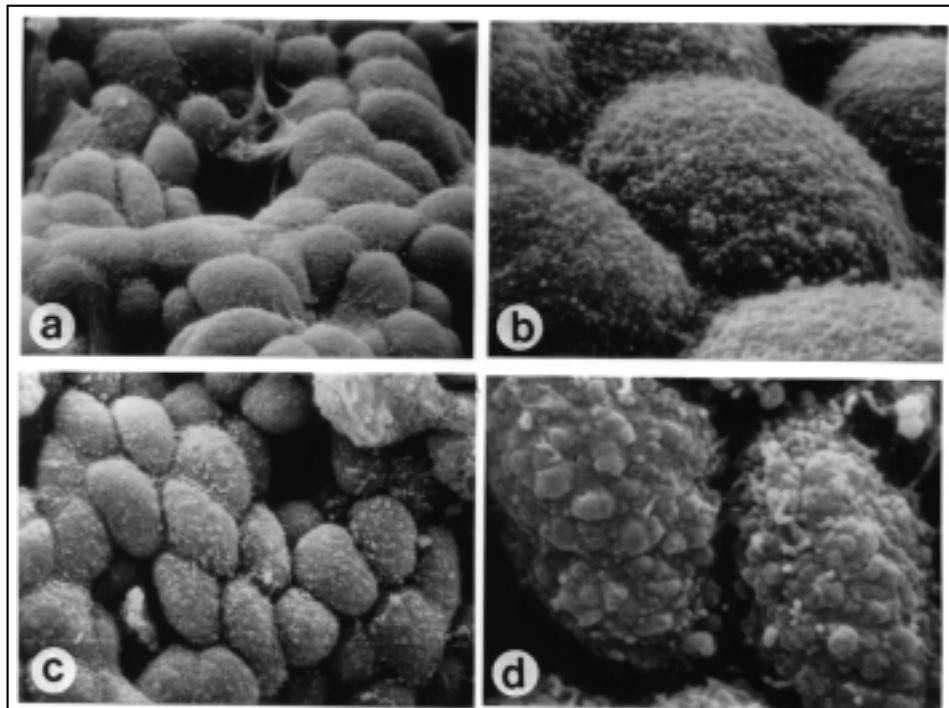
### *H<sub>3</sub> receptors and Helicobacter pylori*

The link between histamine and *Helicobacter pylori* (*Hp*) infection derived from recent observations, suggesting that stomachs from *Hp*-infected patients have enhanced activity of N-methyltransferase and, consequently, higher levels of N<sup>α</sup>-methylhistamine (4, 70). This unusual histamine metabolite is a potent H<sub>3</sub> receptor agonist (71, 72) and could be responsible for the reduced somatostatin secretion observed in *Hp*-positive patients (70) and, as a consequence, for the hypergastrinemia and acid hypersecretion.

N<sup>α</sup>-methylhistamine, however, could reduce acid secretion by reducing histamine synthesis and release through activation of H<sub>3</sub> receptors on ECL cells; this effect might counterbalance the acid secretagogue effect due to reduction of somatostatin release. Moreover, N<sup>α</sup>-methylhistamine is also a potent H<sub>2</sub> receptor agonist (73) and can increase acid secretion independently of H<sub>3</sub> receptor-mediated mechanisms. Taken together, these findings suggest that, despite the presence of a histamine-producing bacterium in the gastric mucosa (74), the involvement of H<sub>3</sub> receptors in the acid secretory effects of *Hp* needs further investigation.

### *Gastric mucosal defence*

Contrasting data have been reported on the role of histamine on gastric mucosal defense. Both ulcerogenic and gastroprotective effects have been re-



*Fig. 3.* Scanning electron micrographs of nonlesion areas from gastric fundus at 30 min after challenge with 0.6 N HCl in rats pretreated with saline (a,b) and with (R)- $\alpha$ -methylhistamine, 100 mg/kg intragastrically (c,d). In control rats superficial cells have a normal appearance (a, $\times$ 2,500; b, $\times$ 10,000). Following (R)- $\alpha$ -methylhistamine the apical membrane of surface mucous cells is rough, probably reflecting the increase in intracellular mucus content (c, $\times$ 2,500). Secreted mucus is closely apposed to the luminal surface (d, $\times$  10,000).

ported after administration of H<sub>1</sub> and H<sub>2</sub> receptor agonists (75—82). By contrast, there is ample evidence that the H<sub>3</sub> receptor agonist, (R)- $\alpha$ -methylhistamine, acts exclusively in a protective manner. (R)- $\alpha$ -methylhistamine proved to reduce the severity of acute gastric mucosal damage exerted by different noxious stimuli in the rat, such as cold-restraint stress, non-steroidal anti-inflammatory drugs, absolute ethanol and concentrated acid (83—88). Prevention of gastric lesions by (R)- $\alpha$ -methylhistamine has been causally linked to its ability to affect mucus-secreting cells, as confirmed by light, scanning and transmission electron microscopic techniques (85). Both surface mucous cells and mucous neck cells were increased in number and in volume after (R)- $\alpha$ -methylhistamine administration; the increase in intracellular and extracellular mucus content was paralleled by an increase in the secretory process (*Fig. 3*). These changes were particularly evident when the gastric mucosa was challenged with noxious stimuli. (R)- $\alpha$ -methylhistamine-induced gastroprotection does not seem to involve prostaglandins production or nitric oxide (NO)

generation, since the effect was scarcely affected by indomethacin (85) or by the NO-synthase inhibitor, L-NAME (89). L-NAME, while aggravating ethanol-induced gastric lesions, did not completely prevent the protective effect induced by (R) $\alpha$ -methylhistamine, suggesting that the underlying mechanism is largely independent of activation of NO system. A possible influence of (R) $\alpha$ -methylhistamine on other defence levels (microcirculation and mucosal immune system) cannot be excluded and warrants further investigation. In this connection, duodenal HCO<sub>3</sub><sup>-</sup> secretion was shown to be increased following (R) $\alpha$ -methylhistamine in the anaesthetized rat (12).

Recent studies have shown that i.c.v. administered histamine and N $^{\alpha}$  methylhistamine reduced the gastric lesions induced by ethanol; the protective effect was accompanied by increase in mucosal blood flow and plasma gastrin and was counteracted by clobenpropit (43). This could suggest the involvement of central mechanisms in the gastroprotection induced by H<sub>3</sub> receptor agonists.

The question as to whether histamine H<sub>3</sub> receptors mediate gastroprotection remains controversial. The protective effect of (R) $\alpha$ -methylhistamine was completely reversed by the selective H<sub>3</sub> receptor antagonists clobenpropit and ciproxifan (88). Furthermore, H<sub>3</sub> receptors have been demonstrated to be stereoselective, the (S)-configured isomer of  $\alpha$ -methylhistamine being 100 times less potent than the (R)-configured isomer (13); in keeping with this, the (S)-isomer was almost completely devoid of protective activity (87). On the other hand, recent experiments carried out with selective agonists, namely imetit, immepip and compound FUB 407 (90) indicate that only FUB 407 was effective against HCl-induced lesions with efficacy comparable to that of (R) $\alpha$ -methylhistamine, whereas immepip and imetit were ineffective (*Fig. 4*). These results are difficult to interpret; heterogeneity of histamine receptors or differences in the agonist kinetics or finally in the efficiency of receptor coupling in the different tissues could be responsible for the ineffectiveness of immepip and imetit. This, however, is the first evidence that H<sub>3</sub> receptors responsible for gastroprotection might be pharmacologically distinct from those found in the brain or in other peripheral tissues.

#### *Therapeutic potential of H<sub>3</sub> ligands*

Conversely from drugs acting on histamine H<sub>1</sub> and H<sub>2</sub> receptors, no H<sub>3</sub>-receptor ligand has been introduced so far into therapy, despite several highly potent ligands for H<sub>3</sub> receptors have been developed by various laboratories (66) and considerable progress in the understanding of the role of this receptor in tissue function. Experimental data may indicate future applications of H<sub>3</sub> receptor agonists in diseases characterized by excess acid secretion, or increased exposure of the gastric mucosa to noxious stimuli. The recent findings that histamine H<sub>3</sub>

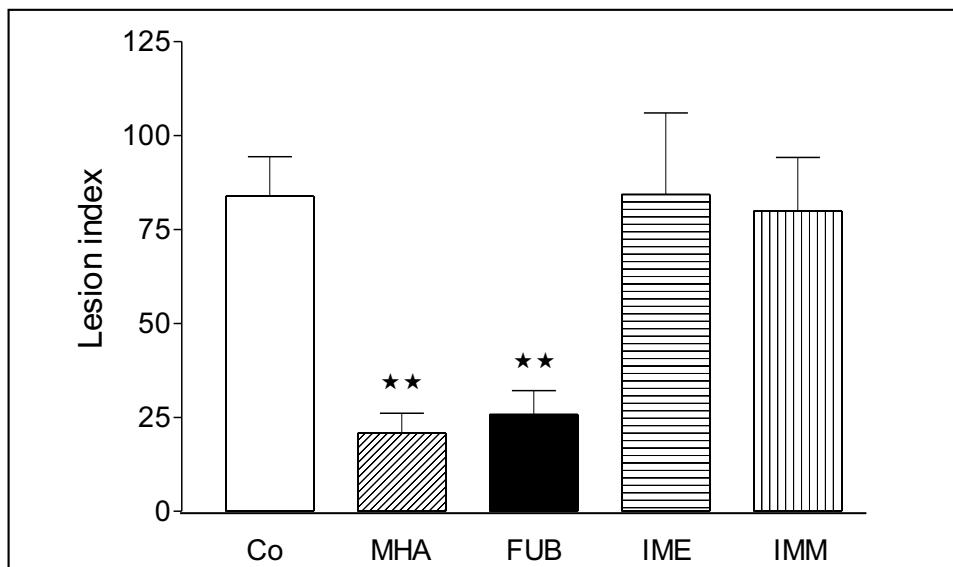


Fig. 4. Macroscopic lesion index, as assessed 30 min after 0.6 N HCl administration in rats pretreated with saline (Co) or with H<sub>3</sub>-receptor agonists at a dose equimolar to (R) $\alpha$ -methylhistamine, MHA, 100 mg/kg intragastrically (i.g.). FUB 407 = FUB, 92 mg/kg i.g.; Imetit = IME, 93 mg/kg i.g.; immepip = IMM, 92 mg/kg i.g. Each bar represents the mean  $\pm$  SEM from 5–6 rats per group. \*\* P < 0.01 compared with saline-pretreated group (ANOVA and Newman-Keuls test).

receptor agonists may have antinociceptive and antiinflammatory actions in different experimental models (91–93) may suggest novel therapeutic applications in inflammatory diseases. The mayor limit to the use of non-steroidal antiinflammatory drugs (NSAIDs), in fact, is due to their gastrolesive potential. H<sub>3</sub> receptor agonists, which combine antiinflammatory properties with antisecretory and gastroprotective effects, might represent the prototype of a novel class of gastrosparring NSAIDs, particularly of benefit in patients at risk of developing gastric lesions. This hypothesis is currently tested in ongoing clinical trials with the prodrug of (R) $\alpha$ -methylhistamine, BP 2.94 (94).

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