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Histamine H_3-receptor antagonists inhibit gastroprotection by (R)-α-methylhistamine in the rat

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1 (R)-α-methylhistamine, a selective agonist of histamine H_3 receptors, is capable of protecting the gastric mucosa against differently acting damaging agents. The objective of the present study was to determine whether H_3 receptors mediate its protective action in the rat.

2 Gastric mucosal lesions were induced intragastrically (i.g.) by 0.6 N HCl, 1 ml rat^{-1}. (R)-α-methylhistamine, 100 mg kg^{-1} i.g., substantially reduced the severity of macroscopically and histologically assessed damage caused by concentrated acid. Prior treatment with highly selective H_3-receptor antagonists, ciproxifan (0.3, 1 and 3 mg kg^{-1} i.g.) and clobenpropit (3, 10 and 30 mg kg^{-1} i.g.), dose-dependently inhibited the protection exerted by (R)-α-methylhistamine up to a complete reversal. When given alone at high doses, both antagonists tended to worsen the HCl-induced histologic damage.

3 During basal conditions, (R)-α-methylhistamine, 100 mg kg^{-1} i.g., caused a significant increase in titratable acidity of the gastric juice. Prior treatment with ciproxifan (3 mg kg^{-1} i.g.) and clobenpropit (30 mg kg^{-1} i.g.) did not alter the secretory response to (R)-α-methylhistamine. Clobenpropit alone, but not ciproxifan, increased the volume of gastric juice, and both compounds alone had no effect on titratable acid.

4 Present findings support evidence that H_3 receptors are actively involved in the maintenance of gastric mucosal integrity, with no apparent role in the regulation of basal gastric acid secretion.
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Abbreviations: (R)α-MeHA, (R)-α-methylhistamine

Introduction

(R)-α-methylhistamine [(R)α-MeHa], a selective agonist of histamine H_3 receptors, exerts protective effects in various experimental models of gastric injury (Morini *et al*., 1995; 1997). Based on the high degree of stereoselectivity of H_3 receptors (Arrang *et al*., 1985), the failure of the S-isomer of α-methylhistamine to reduce total gastric lesions by absolute ethanol favours the hypothesis that H_3 receptors mediate gastroprotection by (R)α-MeHA-stimulation (Morini *et al*., 1999). The present study was carried out to further validate this hypothesis. To circumvent the interaction of H_3-receptor antagonists with absolute ethanol, responsible for central nervous system effects, gastric mucosal lesions were induced by 0.6 N HCl. Thus, the ability of two selective antagonists of H_3 receptors, ciproxifan and clobenpropit (Ligneau *et al*., 1998; van der Goot *et al*., 1992), to counteract protection provided by (R)α-MeHA against 0.6 N HCl-induced gastric lesions in rat, has been evaluated. The effects of the agonist and the antagonists of H_3 receptors on basal gastric acid secretion, which could influence the responsiveness of the mucosa to damage, have also been investigated.

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Methods

Animals

Male Wistar strain rats (Harlan, Italy), weighing 180–200 g, deprived of food, but not of water, for 24 h before the experiments, were used.

0.6 N HCl-induced gastric mucosal lesions

Macroscopy Rats were randomized to receive intragastrically either a single dose of ciproxifan (0.3, 1 and 3 mg kg^{-1}) or of clobenpropit (3, 10 and 30 mg kg^{-1}) or saline (0.9% NaCl) in a 5 ml kg^{-1} volume. Thirty minutes later animals received intragastrically either a single dose of (R)α-MeHA (10, 30 and 100 mg kg^{-1}) or saline in a 5 ml kg^{-1} volume. After an additional 30 min period 0.6 N HCl, 1 ml rat^{-1}, was given intragastrically. Rats were killed by cervical dislocation 30 min after HCl administration.

The stomachs were immediately removed, opened along the lesser curvature, rinsed, and layed on a flat surface. Each individual haemorrhagic lesion was measured along its greatest length (1 mm: rating of 1; 1–2 mm: rating of 2; >2 mm: rating according their greatest length). The overall total was designated 'lesion index'. Each treatment subgroup consisted of 6–8 animals.

Microscopy The experimental protocol described above was followed. Rats were randomized to receive intragastrically either a single dose of ciproxifan (0.3, 1 and 3 mg kg^{-1}) or of

clobenpropit (3, 10 and 30 mg kg^{-1}) or saline in a 5 ml kg^{-1} volume. Thirty minutes later animals received intragastrically either (R)α-MeHA (100 mg kg^{-1}) or saline in a 5 ml kg^{-1} volume. After an additional 30 min period either 0.6 N HCl or saline, 1 ml rat^{-1}, was given intragastrically. Rats were killed by cervical dislocation 30 min after the last administration. Five to six rats were employed for each treatment subgroup. A strip ($\approx 5 \times 10$ 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% formaldehyde and embedded. Serial sections, 5 μm thick, in which the gastric pits and glands were oriented perpendicular to the surface of the mucosa, were cut from each block. The sections were stained with eosin-haematoxylin, PAS, alcian-PAS. For the morphometric analysis of gastric lesions, 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 M, Nikon Laboratory Imaging). The damage was scored in three grades on the basis of its depth: grade I = damage to luminal surface mucous cells only. Cells detached from the basal lamina in clusters. Exfoliation resulted in a frequent though small discontinuities in the surface epithelium; grade II = damage to luminal surface and gastric pit mucous cells. Cell exfoliation was present; grade III = damage extending into the glandular region. For each stomach the total length of mucosa examined, the total length of damaged mucosa and the length of mucosa with each grade of damage were measured.

Basal gastric acid secretion

Rats were randomized to receive intragastrically either ciproxifan (3 mg kg^{-1}) or clobenpropit (30 mg kg^{-1}) or saline in a 5 ml kg^{-1} volume. Thirty minutes later animals received intragastrically either (R)α-MeHA (100 mg kg^{-1}) or saline in a 5 ml kg^{-1} volume. Rats were killed by cervical dislocation 60 min after the last administration. Five rats were employed for each treatment subgroup. The oesophagogastric junction and the pylorus were clamped, the stomach removed, and an incision in the forestomach was made. The gastric contents were collected gravimetrically and centrifuged. The volume was measured, and the amount of titratable acidity was determined by titration to pH 7 with 0.01 N NaOH, using an automatic titrator (Radiometer, Copenhagen, Denmark).

Materials

(R)α-MeHA dihydrogenmaleate and ciproxifan hydrogenmaleate were synthesized by H. Stark and W. Schunack. Clobenpropit dihydrobromide was a kind gift from Prof H. Timmerman. The compounds were dissolved in saline. All other reagents were from Sigma (St. Louis, MO, U.S.A.).

Statistical analysis

Data are expressed as means ± s.e.mean. Comparisons between data were made by ANOVA followed by Newman-Keuls test or by Student's *t*-test for unpaired data. *P* values of less than 0.05 were taken as significant.

Results

0.6 N HCl-induced gastric mucosal lesions

Macroscopy Intragastrically administered 0.6 N HCl determined the formation of red to black linear streaks in the glandular portion of the stomach (lesion index 89.30 ± 11.07, $n = 8$). Mucosal damage caused by concentrated acid was substantially reduced by (R)α-MeHA at the dose of 100 mg kg^{-1} (lesion index 15.18 ± 4.49, $n = 7$; $P < 0.01$ *vs* saline/saline/HCl group). The effect of lower doses of (R)α-MeHA did not reach significance.

Ciproxifan and clobenpropit alone did not significantly modify the damaging effect of concentrated acid. Both H_3-receptor antagonists dose-dependently inhibited protection exerted by (R)α-MeHA, 100 mg kg^{-1}, and an almost complete reversal was obtained with ciproxifan at 1 mg kg^{-1} (lesion index 63.45 ± 14.21, $n = 7$) and clobenpropit at 10 mg kg^{-1} (lesion index 61.99 ± 9.48, $n = 7$). Lesion index values were not different from those in the saline/saline/HCl group and significantly increased ($P < 0.05$) when compared with those in the saline/(R)α-MeHA 100 mg kg^{-1}/HCl group.

Microscopy Results are presented in Table 1 and Figure 1. Luminal exposure to 0.6 N HCl caused damage involving 85% of the total mucosal length evaluated. (R)α-MeHA reduced the

Table 1 Histologically assessed gastric mucosal damage produced by 0.6 N HCl. Effect of histamine H_3-receptor antagonists on the protection by (R)-α-methylhistamine

			−(R)-α-Methylhistamine			*+(R)-α-Methylhistamine*	
Antagonist	*Dose* (mg kg^{-1})	n	*Total length measured*	*Total length of damaged mucosa*	n	*Total length measured*	*Total length of damaged mucosa*
Saline		6	32.80 ± 1.51	27.85 ± 1.94	6	32.20 ± 1.25	10.82 ± 2.72*,†
Ciproxifan	0.3	5	36.99 ± 2.05	32.69 ± 2.52	5	34.42 ± 1.04	18.75 ± 2.41*,†,#
	1	5	35.74 ± 2.92	33.51 ± 1.73	5	36.98 ± 1.59	22.20 ± 1.65*,†,#
	3	5	35.43 ± 1.76	32.89 ± 1.94	5	37.10 ± 2.35	26.52 ± 2.04**
Clobenpropit	3	5	34.64 ± 1.15	30.10 ± 3.51	6	34.57 ± 1.77	12.17 ± 2.56*,†
	10	5	34.18 ± 1.87	30.65 ± 1.12	6	35.99 ± 2.23	22.84 ± 3.77*,#
	30	5	37.44 ± 0.35	32.00 ± 1.33	5	35.44 ± 1.82	30.82 ± 4.22**

Values are mm and are expressed as means ± s.e.mean. n = number of rats. Rats received ciproxifan, clobenpropit, 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. After an additional 30 min period 0.6 N HCl, 1 ml rat^{-1}, was given intragastrically. Rats were killed 30 min after 0.6 N HCl administration. *$P < 0.01$ compared with the total length measured in the same treatment group. †$P < 0.01$ compared with the corresponding value in the corresponding group not given (R)-α-methylhistamine; #$P < 0.05$ and **$P < 0.01$ compared with the corresponding value in saline/(R)-α-methylhistamine/HCl group (ANOVA and Newman-Keuls test).

total extent of HCl-induced damage to 33%. The two H_3-receptor antagonists alone did not affect the integrity of the gastric mucosa in the absence of HCl exposure (data not shown), while they tended to increase the total damage by HCl, though these trends did not reach statistical significance. Prior treatment with both compounds caused a progressive decrease in the protective activity of (R)α-MeHA paralleled by the increase in the dose of the antagonist. At the highest dose of either ciproxifan or clobenpropit there was no significant difference between the length of total damage in the group given saline and that in the group given (R)α-MeHA before the exposure to HCl. When examining the distribution of grades of damage (Figure 1), it is evident that H_3-receptor antagonists alone determined a dose-dependent trend towards the deepening of HCl-induced injury. This was reflected by a larger and significant percentage of grade II damage with respect to that of the corresponding group not given the antagonist, while grade I damage was correspondingly lowered.

Damage to the glandular region was not altered. The complete reversal of (R)α-MeHA protection obtained with the highest dose of both antagonists could be ascribed almost exclusively to a greater grade I damage for ciproxifan and to a greater damage of all grades for clobenpropit.

Basal gastric acid secretion

Results are presented in Table 2. (R)α-MeHA caused a small increase in the volume of gastric juice with a concomitant marked and significant increase in the amount of titratable acidity. The stimulation of acid secretion by (R)α-MeHA was unaffected by prior treatment with either ciproxifan or clobenpropit. Both H_3-receptor antagonists alone had no significant effect on titratable acidity, whereas clobenpropit, but not ciproxifan, significantly increased the volume of the juice present in the stomach.

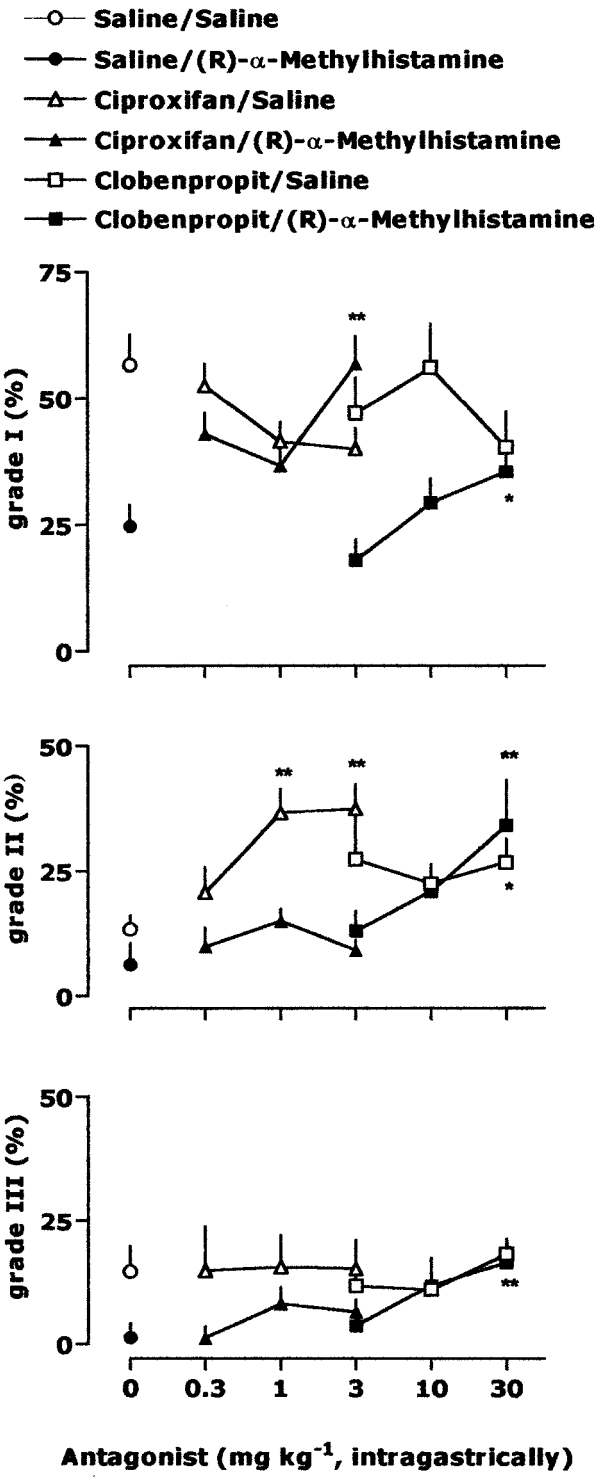

Figure 1 Effect of histamine H_3-receptor antagonists, ciproxifan and clobenpropit, on the effect of (R)-α-methylhistamine on the different grades of histologic damage. Length values of the gastric mucosa exhibiting damage of different grades are expressed as a percentage of the total length of mucosa measured reported in Table 1. Values are means ± s.e.mean from 5–6 rats. $*P<0.05$ and $**P<0.01$ compared with the corresponding group not given the antagonist (ANOVA and Newman-Keuls test).

Discussion

The present study indicates the (R)α-MeHA effectively preserves the integrity of rat gastric mucosa against damage by 0.6 N HCl, as assessed using both macroscopic and histologic evaluation.

In the present model of gastric damage ciproxifan and clobenpropit, shown to be pure and selective antagonists of histamine H_3 receptors *in vitro* and *in vivo*, displayed similar effects. When administered alone at high doses, they slightly worsened the HCl-induced histologic damage, this effect not being significant when total damage is considered and significant when the increase in depth of damage is considered. Most importantly they dose-dependently inhibited the protective action of (R)α-MeHA on both macroscopic and histologic gastric mucosal damage induced by concentrated acid, up to the abolishment of protection of (R)α-MeHA.

The mechanisms responsible for the protective action of (R)α-MeHA and for the increased exacerbation of damage caused by the two H_3-receptor antagonists appear to be unrelated to their effects on basal gastric acid secretion. Protection by (R)α-MeHA is not attributable to a reduction in gastric acidity, since acid production was stimulated. Furthermore, ciproxifan did not affect basal secretion, and clobenpropit increased the amount, but not the acidity, of gastric fluid, indicating a dissociation between their effects on acid secretion and their influence on the susceptibility of the mucosa to lesion formation. There is some debate as to whether the stimulation of acid secretion evoked by (R)α-MeHA, when peripherally administered in rodents, is attributable to H_3-receptor activation (Vuyyuru & Schubert, 1997; Coruzzi & Bertaccini, 1998). However, the present failure of both antagonists to influence (R)α-MeHA-stimulated acid secretion at doses causing a complete inhibition of (R)α-

Table 2 Basal gastric acid secretion. Effect of histamine H_3 receptor antagonists in rats treated with or without (R)-α-methylhistamine

			−(R)-α-*Methylhistamine*			+(*R*)-α-*Methylhistamine*	
	Dose		*Volume*	*Titratable acidity*		*Volume*	*Titratable acidity*
Antagonist	(mg kg^{-1})	n	(μl)	(μEq)	n	(μl)	(μEq)
Saline		7	100±39	3.10±1.28	6	275±76	13.23±4.36†
Ciproxifan	3	6	125±62	2.38±1.07	6	213±61	10.30±2.33†
Clobenpropit	30	6	378±76*	5.50±0.28	6	235±52	12.98±2.71†

Values are expressed as means±s.e.mean. n=number of rats. Rats received ciproxifan, clobenpropit, 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 the last administration. *$P<0.05$ compared with the corresponding value in the group not given the antagonist (ANOVA and Newman-Keuls test); $^{\dagger}P<0.05$ compared with the corresponding value in the corresponding group not given (R)-α-methylhistamine (Student's *t*-test).

MeHA-induced protection appears to rule out the involvement of H_3-receptors in the acid secretory response to (R)α-MeHA.

Caution is required when comparing the presently effective dose range of the two H_3-receptor antagonists with doses reported to be effective *in vivo* after peripheral administration. The two compounds have been investigated almost exclusively for their H_3-receptor antagonist activity in the central nervous system (Ligneau *et al*., 1998; Barnes *et al*., 1993; Yokoyama *et al*., 1994; Fischer & van der Goot, 1998), their potency depending on their ability to cross the blood-brain barrier. While data on brain penetration by ciproxifan are not yet available, penetration by clobenpropit is incomplete (Mochizuki *et al*., 1996). Only a limited number of studies concerns *in vivo* peripheral effects of ciproxifan and clobenpropit (Mazenot *et al*., 1999; Coruzzi *et al*., 1996; Godlewski *et al*., 1997), and in these studies the compounds were administered intravenously. Beside the difference in animal species, ciproxifan being studied in the dog, bioavailability should be considered for comparison, and data on oral bioavailability of ciproxifan (62%) are reported only (Ligneau *et al*., 1998). Moreover, ciproxifan and clobenpropit are likely to differ considerably in their pharmacokinetic properties, considering that both compounds display a similar *in vitro* potency at the H_3 receptor (Ligneau *et al*., 1998), whereas *in vivo* potency of ciproxifan is at least 10–20 fold higher than that of clobenpropit. In any case, in agreement with *in vivo* studies in rodents, ciproxifan is more potent than clobenpropit, and both are active after oral administration.

In summary, the inhibition of the gastroprotection by (R)α-MeHA exerted by ciproxifan and clobenpropit and their high selectivity for histamine H_3 receptors validate the hypothesis that the ability of (R)α-MeHA to counteract mucosal damage is mediated through histamine H_3 receptors. Here we present the first evidence that the activation of histamine H_3 receptors is of relevance for maintaining the gastric mucosal integrity which implies the complex role of histamine in regulating the functioning of the gastric mucosa. The precise mechanism through which H_3 receptors mediate protective effects and the location of H_3 receptors remain to be elucidated.

References

ARRANG, J.-M., SCHWARTZ, J.-C. & SCHUNACK, W. (1985). Stereoselectivity of the histamine H_3-presynaptic autoreceptor. *Eur. J. Pharmacol.,* **117,** 109–114.

BARNES, J.C., BROWN, J.D., CLARKE, N.P., CLAPHAM, J., EVANS, D.J. & O'SHAUGHNESSY, C.T. (1993). Pharmacological activity of VUF 9153, an isothiourea histamine H_3 receptor antagonist. *Eur. J. Pharmacol.,* **250,** 147–152.

CORUZZI, G. & BERTACCINI, G. (1998). Histamine H_3 receptors and gastric acid secretion. *Gastroenterology,* **115,** 245–246.

CORUZZI, G., GAMBARELLI, E. & BERTACCINI, G. (1996). Stimulatory effect of (R)α-methylhistamine on duodenal HCO_3^- secretion in anaesthetized rats. *Ital. J. Gastroenterol.,* **28,** 520–522.

FISCHER, W. & VAN DER GOOT, H. (1998). Effect of clobenpropit, a centrally acting histamine H_3-receptor antagonist, on electroshock- and pentylenetetrazol-induced seizures in mice. *J. Neural Transm.,* **105,** 587–599.

GODLEWSKI, G., MALINOWSKA, B., BUCZKO, W. & SCHLICKER, E. (1997). Inhibitory H_3 receptors on sympathetic nerves of the pithed rat: activation by endogenous histamine and operation in spontaneously hypertensive rats. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **355,** 261–266.

LIGNEAU, X., LIN, J.-S., VANNI-MERCIER, G., JOUVET, M., MUIR, J.L., GANELLIN, C.R., STARK, H., ELZ, S., SCHUNACK, W. & SCHWARTZ, J.-C. (1998). Neurochemical and behavioral effects of ciproxifan, a potent histamine H_3-receptor antagonist. *J. Pharmacol. Exp. Ther.,* **287,** 658–666.

MAZENOT, C., RIBOUT, C., DURAND, A., JOULIN, Y., DEMENGE, P. & GODIN-RIBOUT, D. (1999). *In vivo* demonstration of H_3-histaminergic inhibition of cardiac sympathetic stimulation by R-α-methyl-histamine and its prodrug BP 2.94 in the dog. *Br. J. Pharmacol.,* **126,** 264–268.

MOCHIZUKI, T., JANSEN, F.P., LEURS, R., WINDHORST, A.D., YAMATODANI, A., MAEYAMA, K. & TIMMERMAN, H. (1996). Brain penetration of the histamine H_3 receptor antagonists thioperamide and clobenpropit in rat and mouse, determined with ex vivo [^{125}I]iodophenpropit binding. *Brain Res.,* **743,** 178–183.

MORINI, G., GRANDI, D. & BERTACCINI, G. (1995). (R)-alpha-methylhistamine inhibits ethanol-induced gastric lesions in the rat: involvement of histamine H_3 receptors? *Digestion,* **56,** 145–152.

MORINI, G., GRANDI, D., BERTACCINI, G., LESCHKE, C. & SCHUNACK, W. (1999). Stereoselective inhibition of ethanol-induced gastric lesions in the rat by the H_3-receptor agonist (R)-α-methylhistamine and its (S)-configured isomer. *Pharmacology,* **59,** 192–200.

MORINI, G., GRANDI, D., KRAUSE, M. & SCHUNACK, W. (1997). Gastric mucosal injury by nonsteroidal anti-inflammatory drugs is reduced by (R)-α-methylhistamine and its pro-drugs in the rat. *Inflamm. Res.,* **46,** S101–S102.

VAN DER GOOT, H., SCHEPERS, M.J.P., STERK, G.J. & TIMMERMAN, H. (1992). Isothiourea analogues of histamine as potent agonists or antagonists of the histamine H_3-receptor. *Eur. J. Med. Chem.,* **27,** 511–517.

VUYYURU, L. & SCHUBERT, M.L. (1997). Histamine, acting via H_3 receptors, inhibits somatostatin and stimulates acid secretion in isolated mouse stomach. *Gastroenterology,* **113,** 1545–1552.

YOKOYAMA, H., ONODERA, K., MAEYAMA, K., SAKURAI, E., IINUMA, K., LEURS, R., TIMMERMAN, H. & WATANABE, T. (1994). Clobenpropit (VUF-9153), a new histamine H_3 receptor antagonist, inhibits electrically induced convulsions in mice. *Eur. J. Pharmacol.,* **260,** 23–28.

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