REVISTA ESPAÑOLA DE FISIOLOGIA, 35, 261-264. 1979

Modification by Histamine H_2-Receptor Blockade of Acid Secretion Stimulated by Histamine, Pentagastrin and Methacholine in the Isolated Whole Mouse Stomach

E. Martí-Bonmatí, S. F. Aliño, E. Morcillo and J. Esplugues

Departamento de Farmacología
Facultad de Medicina
Valencia (Spain)

(Received on October 27, 1978)

E. MARTI-BONMATI, S. F. ALIÑO, E. MORCILLO and J. ESPLUGUES. *Modification by Histamine H_2-Receptor Blockade of Acid Secretion Stimulated by Histamine, Pentagastrin and Methacholine in the Isolated Whole Mouse Stomach.* Rev. esp. Fisiol., 35, 261-264. 1979.

The direct influences of the blockade of the gastric histamine H_2-receptors on the secretory actions induced by histamine, pentagastrin and methacholine, have been studied on the isolated perfused whole mouse stomach.

According to the results cimetidine did not modify the spontaneous basal acid secretion. The interactions of cimetidine with the secretagogues were of a competitive nature with histamine and non-competitive with pentagastrin, while no modification of methacholine stimulated acid secretion.

Since the discovery by BLACK *et al.* (3), of a new group of drugs — the histamine H_2-receptor antagonists — a great leap forward to the elucidation of basic mechanisms involved in the regulation of gastric acid secretion has been made.

For research on direct action of drugs on gastric secretion, isolated gastric mucosa or whole stomach preparations are necessary in order to minimize indirect influences which may occur in whole animals.

The pioneering work with the isolated whole stomach preparation was made by DAVENPORT and CHAVRÉ (7). Recently, several papers have been published on the methods for making this kind of preparation (5, 14), and on the dose-response relationship obtained with different secretagogues (2, 4-6, 13, 14).

Studies on the effect of metiamide on gastric acid secretion from the isolated rat (4, 5) or mouse (14) stomach have been recently published.

The present report describes a quantitative study of the inhibitory effect of cimetidine — a histamine H_2-receptor antagonist — on the acid secretion induced

by histamine, pentagastrin and methacholine using a continuously perfused isolated whole mouse stomach.

Materials and Methods

The preparation used in this study is very similar to that employed by WAN (14). Briefly, isolated whole stomachs from fed immature mice were suspended in organ baths containing Krebs-Henseleit solution at 37° C and perfused at a rate of 1 ml/min^{-1} with unbuffered Krebs-Henseleit solution. The perfused from the stomach lumen was passed over a microcombined electrode clamped 20 cm higher than the stomach. Changes in pH of the effluent were continuously recorded. The secretory response to a single concentration of an agonist was studied in the absence or presence of cimetidine (10^{-4}M). Agonists were added 40 min after the addition of cimetidine. Only one response per stomach was considered. Responses were calculated by measuring the amount of acid secreted at peak response minus preceding basal level. H^+ secretory rate was then expressed as nmol min^{-1} (mean ± S.E.M.). Statistical calculations were made by analysis of variance.

Results

Basal acid secretion. Values for basal acid secretion were 28.6 ± 2.3 nmol/min^{-1} and 31.2 ± 3.3 nmol/min^{-1} for control and cimetidine-incubated stomach, respectively. These data indicate that blockade of histamine H_2-receptors does not modify the *in vitro* spontaneous acid secretion.

Histamine. Responses to histamine (10^{-5}M or 10^{-4}M) alone or after incubation with cimetidine (10^{-4}M) are shown in figure 1A. Cimetidine produced a significant ($p < 0.05$) parallel rightward

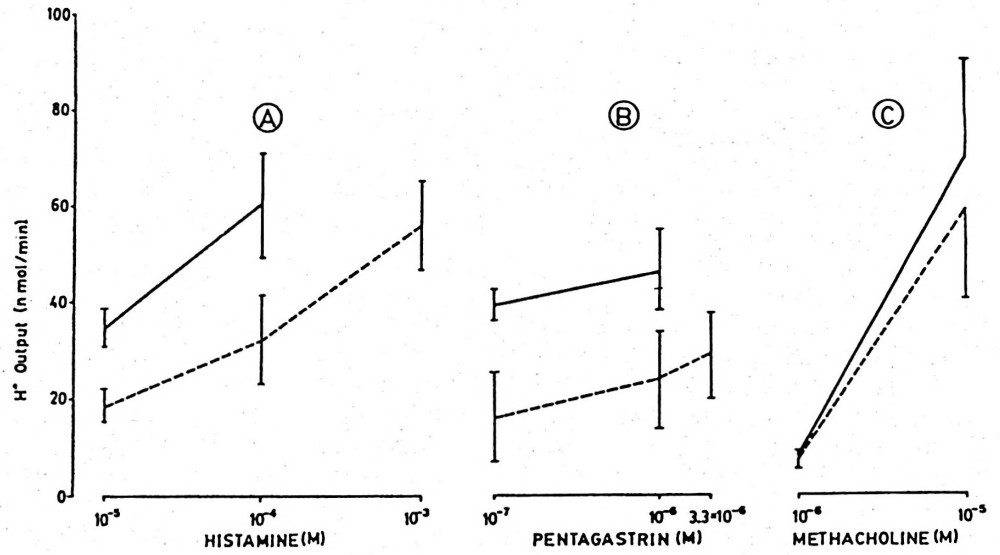

Fig. 1. *Dose-response curves to histamine (A), pentagastrin (B) and methacholine (C) in the absence (continuous lines) and presence (dotted lines) of cimetidine (10^{-4} M).* Each point represents the mean ± S.E.M. from a minimum of six preparations. Analyses of variance showed that cimetidine causes a significant displacement of the control dose-response curve obtained with histamine and pentagastrin. Metacholine dose-response curve was not significantly modified by cimetidine.

shift of the two-point dose-response curve to histamine. The inhibitory effect of cimetidine was surmounted by a concentration of histamine of 10^{-3}M. These results suggest that cimetidine inhibited histamine stimulation through a competitive type of antagonism.

Pentagastrin. Responses to pentagastrin (10^{-7}M or 10^{-6}M) alone or after incubation with cimetidine (10^{-4}) are shown in figure 1B. Cimetidine produced a significant ($p < 0.05$) rightward shift of the two-point dose-response curve to pentagastrin. The inhibitory effect of cimetidine was not surmounted by increasing pentagastrin concentration to 3.3×10^{-6}M. These results suggest a non-competitive type of antagonism of pentagastrin-induced acid secretion.

Methacholine. Results from the two-point dose-response curve to methacholine (10^{-6}M to 10^{-5}M) in the absence or presence of cimetidine 10^{-4}M are presented in figure 1C.

Cimetidine did not significantly ($p < 0.05$) displaced the two-point dose-response curve to methacholine.

Discussion

The present findings along with results reported by others (2, 13, 14) confirm that the isolated whole mouse stomach is a suitable preparation for quantitative studies on gastric secretion since it gives reproducible and dose-related responses to histamine, pentagastrin, acetylcholine and also to other physiological stimuli.

Cimetidine produced a parallel displacement to the right of the two-point dose-response curve to histamine without significantly affecting the slope or maximum effect, (figure 1A) therefore suggesting that the inhibition of histamine-stimulated acid secretion by cimetidine is of a competitive nature. Similar findings have been obtained with cimetidine in isolated guinea pig gastric mucosa (11) and with other histamine H_2-receptor antagonists (5, 14) in the isolated whole rat and mouse stomachs.

The inhibition of pentagastrin stimulated gastric acid secretion by cimetidine seems to be of a non-competitive nature (figure 1B). This kind of antagonism, also reported for metiamide in isolated whole rat stomach (4), may be explained by the three receptor hypothesis (8, 12) which assumes the existence of separate receptors for histamine, gastrin and acetylcholine on the surface of the parietal cell.

Thus, blockade of one receptor alters the responsiveness of one or both of the others. Interference of cimetidine with the histamine mediation of pentagastrin action (9, 10, 15) should also be taken into account although recent data have shown that larger concentrations of cimetidine are required to interact at this intermediate step (1).

The failure of cimetidine to inhibit gastric acid secretion induced by methacholine (figure 1C) is consistent with results obtained by other groups of workers for metiamide in *in vitro studies* (4, 14). The later observation supports the view that the *in vitro* cholinergic excitation of gastric acid secretion is unlikely mediated by the release of either gastrin or histamine. There seems to exist a direct muscarinic pathway leading to gastric acid secretion.

In conclusion, the present data are consistent with a model in which the parietal cell has specific receptors for histamine, gastrin and acetylcholine and in which H_2-receptor antagonists are specific in their action against histamine, modify the responses to gastrin by a still unknown mechanism and do not interfere with the cholinergic pathway to gastric secretion.

Resumen

Las influencias directas del bloqueo de los receptores histaminérgicos-H_2 gástricos sobre

la acción secretagoga de histamina, pentagastrina y metacolina han sido estudiadas en estómago aislado y perfundido de ratón. Los resultados indican que la cimetidina no modifica la secreción ácida basal. La interacción de la cimetidina con los secretagogos utilizados es de tipo competitivo con la histamina y no competitivo con la pentagastrina. No se observa ninguna modificación con la metacolina.

References

1. ALBINUS, M., FEMPPEL, J., MASLINSKI, S. and SEWING, K. FR.: In «Hormonal Receptors in Digestive Tract Physiology» (Bonfils, S., Fromageot, P., Rosselin, G. eds.). North-Holland Publishing Company, Amsterdam, 1977, pp. 409-411.
2. ANGUS, J. A. and BLACK, J. W.: *Proc. 7th Inter. Congress Pharmacol.*, Abs. 853, p. 338, 1978.
3. BLACK, J. W., DUNCAN, W. A. M., DURANT, G. J., GANELLIN, C. R. and PARSONS, M. E.: *Nature* (London), **236**, 385-390, 1972.
4. BUNCE, K. T., PARSONS, M. E. and ROLLINGS, N. A.: *Br. J. Pharmac.*, **58**, 149-156, 1976.
5. BUNCE, K. T. and PARSONS, M. E.: *J. Physiol.* (London), **258**, 453-465, 1976.
6. BUNCE, K. T., MARSH, G. and PARSONS, M. E.: *Br. J. Pharmac.*, **61**, 279-284, 1977.
7. DAVENPORT, H. W. and CHAVRÉ, V.: *Gastroenterology*, **15**, 467-480, 1950.
8. GROSSMAN, M. I. and KONTUREK, S. J.: In «Int. Symp. on Histamine H_2-receptor antagonists» (Wood, C. J. & Simkins, M. A. eds.). Deltakos, London, 1973, pp. 297-303.
9. KAHLSON, G., ROSENGREN, E., SVANH, D. and THUNBERG, R.: *J. Physiol.* (Lond.), **174**, 400-416, 1964.
10. MAIN, I. H. M. and PEARCE, J. B.: *Br. J. Pharmacol.*, **61**, 461 P, 1977.
11. SJÖSTRAND, S. E., RYBERG, B. and OLBE, L.: *Naunyn. Schmiedeberg's Arch. Pharmacol.*, **296**, 139-142, 1977.
12. SOLL, A. H.: *J. Clin. Invest.*, **61**, 370-380, 1978.
13. WAN, B. Y. C.: *Br. J. Pharmac.*, **56**, 357-358, 1975.
14. WAN, B. Y. C.: *J. Physiol.* (Lond.), **266**, 327-346, 1977.
15. WEIDLE, E. and SEWING, K. FR.: *J. Pharm. Pharmac.*, **25**, 234-238, 1973.