Comparative Study of the H₂-Receptor Antagonists Cimetidine, Ranitidine, Famotidine and Nizatidine on the Rabbit Stomach Fundus and Sigmoid Colon

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The H₂-receptor antagonists, cimetidine, ranitidine, famotidine and nizatidine, were tested for their effect on the isolated smooth muscle strips from the rabbit stomach fundus and sigmoid colon. These H₂-receptor antagonists were found to possess a concentration-dependent contractile effect on the above smooth muscle preparations and the order of potency was: ranitidine = nizatidine > famotidine > cimetidine. In addition, the smooth muscle preparations from the sigmoid colon were significantly more sensitive to the above compounds than the smooth muscle preparations from the stomach fundus.

Keywords — cimetidine; ranitidine; famotidine; nizatidine; stomach fundus; sigmoid colon; rabbit

Introduction

The H₂-receptor antagonists, cimetidine,¹⁾ ranitidine,²⁾ famotidine³⁾ and nizatidine⁴⁾ have been synthesized and shown to have a potency in reducing gastric acid production. It has also been reported that cimetidine,⁵⁾ ranitidine,⁶⁾ famotidine⁷⁾ and nizatidine^{8,9)} elicit a marked contractile effect on different parts of the intestinal tract from several animal species. The contractile effect of cimetidine,¹⁰⁾ ranitidine¹¹⁾ and nizatidine¹²⁾ on intestinal smooth muscle was attributed to their anticholinesterase activity. This cholinergic-like activity of the above H₂-receptor antagonists is a side effect and may modify the intestinal motility with diarrhoea or constipation, as it has been observed in a very small percentage of patients after their treatment with cimetidine¹³⁾ or ranitidine.¹⁴⁾

On the basis of the above findings we decided to investigate: a) The effect of cimetidine, ranitidine, famotidine and nizatidine on the smooth muscle of the rabbit stomach fundus and sigmoid colon, b) the activity of these compounds on the above smooth muscles in a comparative manner and c) the sensitivity of the stomach fundus and the sigmoid colon to the above compounds.

Materials and Methods

Preparations of the Rabbit Stomach Fundus and Sigmoid Colon — Rabbits of either sex (weighing approximately 2 kg) were killed by a severe blow on the neck and then exsanguinated. Segments from the stomach fundus and the sigmoid colon were taken and pinned on a wax block (mucosal side up) in a bath containing Krebs solution and the mucosa was removed. The composition (in mM) of Krebs solution was as follows: NaCl 118.00, NaHCO₃ 24.88, KH₂PO₄ 1.18, KCl 4.70, MgSO₄ 1.16, CaCl₂ 2.52 and glucose 11.10. The segments were cut into strips (20 mm length and 6 mm width). The strips from the stomach fundus were cut transversely to the greater curvature and the strips from the sigmoid colon were cut parallel to the oral-caudal axis. The smooth muscle preparations were suspended in organ baths (Hugo Sachs Elektronik G.K., Germany) containing 20 ml Krebs solution. The solution in the organ baths was maintained at a temperature of 37 °C and bubbled constantly with a mixture of 95% O₂–5% CO₂ gas. A resting tension of 500 mg was applied to the preparations which were allowed to be stabilized for a period of 30 min before any compound addition. During this period the or-

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gan bath fluid was replaced with fresh Krebs solution every 10 min. The contractions of the preparations were recorded on a physiograph recorder (desk model, type DMP-4A, NARCO Co., U.S.A.) *via* isotonic myograph transducers (NARCO Co., U.S.A.)

Drugs — The following compounds were used: acetylcholine chloride (E. Merck, Germany), cimetidine (Sigma Chemical Company, U.S.A.), ranitidine hydrochloride (Glaxo Operation, England), famotidine (M.S.D., Italia) and nizatidine (Eli Lilly, U.S.A.). The solutions of the above compounds were freshly prepared before each experiment, using Krebs solution as a solvent. All solutions were gently added to the organ bath fluid by microsyringe.

Concentration-Response Curves — After the 30 min stabilization period, the preparations were exposed to cumulatively increasing concentrations of acetylcholine (from 3.2×10^{-7} to 3.2×10^{-4} M), cimetidine, ranitidine, famotidine, nizatidine (from 10^{-5} to 3.2×10^{-3} M) in order to obtain full concentration-response curves. Each addition of the compound was made after the contractile response to the previous concentration had reached its maximal level.

Statistical Analysis of the Results — The responses obtained were expressed as a percentage of the maximum height attained in the control curve. Statistical evaluation of the data was performed using Student's *t*-test. The data were expressed as the mean $\pm$ S.E.M., and *p* values of < 0.05 were considered to be significant.

Results

Effect of Acetylcholine, Cimetidine, Ranitidine, Famotidine and Nizatidine on the Stomach Fundus Preparations

The addition of cimetidine, ranitidine, famotidine or nizatidine in the organ bath fluid exerted a concentration-dependent contractile effect on the smooth muscle preparations from the rabbit stomach fundus. The significant contractile effect started from a threshold concentration of 3.2×10^{-5} M for the above compounds. The average maximum responses (mean $\pm$ S.E.M.) caused by cimetidine, ranitidine, famotidine and nizatidine at the concentration of 3.2×10^{-3} M were 15.93 ± 2.06 , 86.46 ± 5.32 , 39.88 ± 7.41 and $85.26 \pm 7.67\%$ respectively of the average maximum response

Fig. 1. Cumulative Concentration-Response Curves for the Contractile Response to Acetylcholine (●), Ranitidine (○), Nizatidine (□), Famotidine (Δ) and Cimetidine (◇) of the Isolated Smooth Muscle Preparations from the Rabbit Stomach Fundus (a) and the Sigmoid Colon (b)

The ordinate is expressed as a percentage of the mean maximum response induced by acetylcholine (control). Each point represents the mean $\pm$ S.E.M. obtained from 21—22 preparations for acetylcholine and 5—8 preparations for each of the other compounds. No significant difference was noted between ranitidine and nizatidine. *a*) Shows significant difference between ranitidine and famotidine, *b*) between nizatidine and famotidine and *c*) between famotidine and cimetidine ($p < 0.05$).

of acetylcholine, produced at the concentration of 3.2×10^{-4} M. No significant difference was noted between the ranitidine and nizatidine contractile activity, while significant differences were noted among the ranitidine/nizatidine, famotidine and cimetidine contractile activity (Fig. 1a).

Effect of Acetylcholine, Cimetidine, Ranitidine, Famotidine and Nizatidine on the Sigmoid Colon Preparations

The addition of cimetidine, ranitidine, famotidine or nizatidine in the organ bath fluid exerted a concentration-dependent contractile effect on the smooth muscle preparations from the rabbit sigmoid colon. The significant contractile effect started from a threshold concentration of 10^{-5} M for ranitidine and of 3.2×10^{-5} M for the other compounds used. The average maximum responses (mean $\pm$ S.E.M.) caused by cimetidine, ranitidine, famotidine and nizatidine

Fig. 2. Cumulative Concentration-Response Curves for the Contractile Response to Ranitidine (a), Nizatidine (b), Famotidine (c) and Cimetidine (d) of the Isolated Smooth Muscle Preparations from the Rabbit Stomach Fundus (●) and the Sigmoid Colon (○)

The ordinate is expressed as a percentage of the maximum response induced by each of the above compounds on the smooth muscle preparations from the sigmoid colon. Each point represents the mean $\pm$ S.E.M. obtained from 5–8 preparations for each compound. a) Shows significant difference in sensitivity that was noted between the sigmoid colon and the stomach fundus preparations ($p < 0.05$).

at the concentration of 3.2×10^{-3} M were 38.12 ± 7.30 , 90.35 ± 9.83 , 53.13 ± 7.86 and $83.51 \pm 8.48\%$ respectively of the average maximum response of acetylcholine, produced at the concentration of 3.2×10^{-4} M. The significant differences among the above H_2 -receptor antagonists on the sigmoid colon were the same as on the stomach fundus preparations (Fig. 1b).

Sensitivity of the Stomach Fundus and Sigmoid Colon Preparations

The sigmoid colon preparations were significantly more sensitive than the stomach fundus preparations to the compounds used. The average maximum responses of the stomach fundus to cimetidine, ranitidine, famotidine and nizatidine compared to those of the sigmoid colon preparations were 28.69 ± 3.70 , 65.68 ± 4.84 , 51.55 ± 9.58 and $70.08 \pm 6.31\%$ respectively (Fig. 2a, 2b, 2c and 2d).

Discussion

The H_2 -receptor antagonists, cimetidine, ranitidine, famotidine and nizatidine, which are in clinical use, were found to exert a concentration-dependent stimulating effect on the rabbit stomach fundus and sigmoid colon smooth muscle. These results are in accordance with those which were reported for the above compounds in previous studies on different parts of the intestinal tract from several animal species.⁵⁻⁹ The mechanism of the cimetidine,¹⁰ ranitidine¹¹ and nizatidine¹² activity on the gastrointestinal tract was attributed to their anticholinesterase activity. The cholinergic-like activity of famotidine is unknown until now. Our data also showed that ranitidine has a potency similar to that of nizatidine, while ranitidine and nizatidine are more potent than famotidine, and famotidine is more potent than cimetidine on both the stomach fundus and the sigmoid colon smooth muscles. It should be reported here that, in a previous investigation of ours, it was shown that no significant difference was noted between the ranitidine and nizatidine activity on the rabbit taenia coli and the ascending colon smooth muscle.⁸ Concerning the sensitivity of the preparations, the sigmoid colon smooth muscle was significantly more sensitive than the stomach

fundus smooth muscle to the H_2 -receptor antagonists tested. It was reported by other investigators that there is a variety of the distribution of cholinesterase in different parts of the gastrointestinal tract.^{15,16} This variety could explain the difference in the sensitivity between the stomach fundus and the sigmoid colon to the above H_2 -receptor antagonists, which have an anticholinesterase activity. It is worth while mentioning here that, in our previous study, it was also shown that the rabbit taenia coli was more sensitive than the ascending colon to ranitidine and nizatidine.⁸

The conclusion which may be drawn is that the H_2 -receptor antagonists cimetidine, ranitidine, famotidine and nizatidine exert an excitatory effect on the rabbit stomach fundus and the sigmoid colon; the order of potency is ranitidine = nizatidine > famotidine > cimetidine and the sigmoid colon is more sensitive than the stomach fundus to the above compounds. This excitatory effect of these H_2 -receptor antagonists, which is a side effect, could modify the colon and the stomach motility and could lead to disorders which are expected to be of a greater potency in the colon than in the stomach.

References

- 1) R. W. Brimblecombe, W. A. Duncan, G. J. Durant, J. C. Emmett, C. R. Ganellin and M. E. Parsons: Cimetidine a non-thiourea H_2 -receptor antagonist, *J. Int. Med. Res.*, **3**, 86—92 (1975).
- 2) J. M. Daly, M. J. Humphray and R. Stables: Some *in vitro* and *in vivo* actions of the new histamine H_2 -receptor antagonist, ranitidine, *Br. J. Pharmacol.*, **72**, 49—54 (1981).
- 3) D. M. Campoli-Richards and S. P. Clissold: Famotidine, pharmacodynamic and pharmacokinetic properties and a preliminary review of its therapeutic use in peptic ulcer disease and Zollinger-Ellison Syndrome, *Drug*, **32**, 197—221 (1986).
- 4) J. T. Callaghan, R. F. Bergstrom, B. D. Obermeyer, E. P. King and W. W. Offen: Intravenous nizatidine kinetic and acid suppression, *Clin. Pharmacol. Ther.*, **37**, 162—165 (1985).
- 5) S. Vyas and S. C. Verma: The direct excitatory effects of cimetidine on the smooth muscle of guinea pig ileum, *Agents Actions*, **3**, 193—195 (1981).
- 6) G. Kounenis, M. Koutsoviti-Papadopoulou and V. Elezoglou: Cholinergic-like effect of the H_2 -receptor antagonist, ranitidine, on the rabbit small intestine,

- "Comparison Veterinary Pharmacology, Toxicology and Therapy," A. S. J. P. V. Miert, M. G. Bogoert and M. Debackere, MTP Press, Langaster, 1986, pp. 143—151.
- 7) G. Bertaccini, G. Coruzzi, E. Poli and M. Adami: Pharmacology of the novel H₂-antagonist, famotidine: *in vitro* studies, *Agents Actions*, **19**(3/4), 180—187 (1986).
 - 8) G. Kounenis, D. Voutsas, M. Koutsoviti-Papadopoulou and V. Elezoglou: Effect of the histamine H₂-receptor antagonists, nizatidine and ranitidine, on the smooth muscle of rabbit colon, *Hellenic. J. Gastroenterol.*, **1**, 129—135 (1988).
 - 9) G. Kounenis, M. Koutsoviti-Papadopoulou and V. Elezoglou: The excitatory effect of the new histamine H₂-receptor antagonist, nizatidine (LY 139037), on the guinea pig ileum, *J. Pharmacobio-Dyn.*, **10**, 669—672 (1987).
 - 10) G. Kounenis, D. Voutsas, M. Koutsoviti-Papadopoulou and V. Elezoglou: Inhibition of acetylcholinesterase by the histamine H₂-receptor antagonist, cimetidine; comparison with ranitidine, *Hellenic. J. Gastroenterol.*, **2**, 296—300 (1989).
 - 11) G. Kounenis, M. Koutsoviti-Papadopoulou and V. Elezoglou: The inhibition of acetylcholinesterase by ranitidine: A study on the guinea pig ileum, *J. Pharmacobio-Dyn.*, **9**, 941—945 (1986).
 - 12) G. Kounenis, D. Voutsas, M. Koutsoviti-Papadopoulou and V. Elezoglou: Inhibition of acetylcholinesterase by the H₂-receptor antagonist, nizatidine, *J. Pharmacobio-Dyn.*, **11**, 767—771 (1988).
 - 13) Package Insert: "Tagamet" Cimetidine hydrochloride. Smith, Kline and French Laboratories Ltd., 1983.
 - 14) G. Bertaccini and G. Coruzzi: H₂-receptor antagonists: side effect and adverse effects, *Ital. J. Gastroenterol.*, **16**, 119—125 (1984).
 - 15) B. Colas, J. P. Sine and R. Ferrand: Human intestinal mucosal cell cholinesterases (abstract), Proc. of 3rd International Meeting on Cholinesterases, La Grande Motte, France, 12—16 Mai, 1990, p. 90.
 - 16) J. P. Sine, R. Ferrand and B. Colas: Identification and characterization of cholinesterases in the intestine mucosal cells (abstract), Proc. of 3rd International Meeting on Cholinesterases, La Grande Motte, France, 12—16 Mai, 1990, p. 91.