Histamine receptors in the gastric microcirculation¹

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SUMMARY The types and functions of histamine receptors in the submucosal arterioles of the corpus and antrum of the cat and rat stomach were studied using an *in vivo* microscopy technique. Change in arteriolar diameter in response to superfusion of histamine with and without antagonists was measured by an image-splitting technique. H₁ and H₂ histamine receptors subserving vasodilatation were demonstrated in both the antral and corpus submucosal arterioles of the cat and rat. However, the H₁ effect was predominant in the antrum (the H₂ antagonist inhibited histamine dilatation only in the presence of the H₁ antagonist), while H₁ and H₂ effects were approximately equal and independent in the corpus.

Histamine plays a major role in a number of physiological processes throughout the body, including the regulation of blood flow. It acts *via* two types of receptors; first, those blocked by mepyramine—for example, the receptors mediating bronchial constriction—termed H₁ (Ash and Schild, 1966), and, second, those not blocked by mepyramine—for example, the receptors mediating gastric secretion—termed H₂ (Black *et al.*, 1972). The recently developed H₂ antagonists (Black *et al.*, 1972) block the latter receptors. The two types of histamine receptor antagonists have been used to study the distribution and function of histamine receptors in different vascular beds. H₁ receptors subserving vasoconstriction and H₂ receptors subserving vasodilatation were found in the rabbit ear artery (Glover *et al.*, 1973; Parsons and Owens, 1973) while only H₂ receptors subserving vasodilatation were found in the human temporal artery (Glover *et al.*, 1973). Both H₁ and H₂ receptors subserving vasodilatation were observed in the general circulation—that is, depression of systemic arterial blood pressure—of the dog (Powell and Brody, 1973, 1976) and cat (Parsons and Owen, 1973), in the skeletal muscle of the dog (Powell and Brody, 1973, 1976) and in the intestinal circulation of the cat (Guth and Smith, 1978). When both receptors subserved vasodilatation, the H₁ receptor effects predominated—that is, an H₂ effect could not be demonstrated unless the H₁ receptor was blocked.

The purpose of the present investigation was to determine the types and function of histamine receptors in the submucosal arterioles of the corpus and antrum of the stomach of the cat and rat.

Methods

Cats weighing between 2 and 4 kg and male Sprague Dawley rats weighing between 150 and 200 g were used throughout this study. The animals were fasted but allowed free access to water for 24 hours before study. An *in vivo* microscopy technique was used to study gastric submucosal arteriolar responses (Guth and Rosenberg, 1972). The animals were anaesthetised with sodium pentobarbital, 35 mg/kg intraperitoneally, the abdomen opened, and the stomach exteriorised. For visualisation of the antrum a clad fibreglass rod was passed into the stomach through a small incision in the duodenum. For visualisation of the corpus, the rod was passed into the stomach through a small incision high in the fundus of the cat stomach or in the rumen of the rat stomach. The gastric wall was then transilluminated by passing light from a high intensity light source through this rod. Using a compound microscope with long working distance objectives, the microvascular bed was visualised. For optimum visualisation of the submucosal vascular network, it was necessary carefully to remove serosal and muscle layers from a small area of either the corpus or antrum thus exposing the submucosal plexi to direct viewing. The flow of blood through the submucosal arteriolar arcades to the mucosal arterioles and the capillary bed at the base of the mucosa and then back from the mucosa through collecting venules and into the submucosal

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venular network could clearly be seen. The area under study was continuously superfused with a Krebs solution at 38°C. Body temperature was monitored and maintained between 37 and 38°C by a heating pad. An image-splitting recording technique was used to monitor submucosal arteriolar diameter changes (Baez, 1966).

For study of histamine and the histamine antagonists, the compounds were added to the superfusing fluid so that the submucosal vascular network was continuously bathed with solutions of known molarity. The concentrations employed were: histamine 10^{-4} M, mepyramine, an H_1 antagonist, 10^{-5} M, and metiamide, an H_2 antagonist, 10^{-4} M. Previous studies revealed these were the optimally effective concentrations of mepyramine and metiamide (Guth and Smith, 1978). Higher concentrations of these agents by themselves inhibited noradrenaline vasoconstriction. While histamine produced readily measurable, marked dilatation of the rat corpus submucosal arterioles, the dilatation of cat and rat antral arterioles and cat corpus arterioles was relatively small. In order to amplify this effect so that the antagonists could be more readily studied, the antral submucosal bed in the cat and rat and the corpus bed in the cat were superfused with noradrenaline 10^{-5} – 10^{-7} M in order partially to constrict the arterioles. Subsequent superfusions with histamine with and without the antagonists contained noradrenaline. Six to 10 minutes were allowed between superfusion episodes to permit effects of the previously superfused agents to wear off. During these intervals superfusion with the Krebs solution alone was maintained. Repeat superfusions with histamine were performed at the end of each series of studies to be certain that the histamine response had not changed. To ascertain whether the H_1 and H_2 antagonist effects were specific for histamine, similar studies were performed with another vasodilator, papaverine 10^{-3} M.

The paired *t* test was used for statistical analysis of the results.

Results

CORPUS

Results of the microcirculatory studies in the corpus of seven cats are presented in Fig. 1. The size of the arterioles studied was similar in all cats, averaging $36.0 \pm 2.9 \mu\text{m}$ (mean $\pm$ SE, before noradrenaline constriction). Histamine caused dilatation in all animals, averaging $72.8 \pm 8.9\%$ above control diameter. Both the H_1 antagonist and the H_2 antagonist partially inhibited this response in all animals. The average diameters were 31.2 ± 8.1 and $44.2 \pm 7.3\%$ above control with the H_1 and H_2 antagonists

Fig. 1 Cat corpus: mean percent increase in submucosal arteriolar diameter in response to histamine and the histamine antagonists. Values are means $\pm$ SE of seven animals. In this and subsequent Figures: Δ Diameter (%): % above control diameter, H: histamine, H_1A = H_1 receptor antagonist, mepyramine, and H_2A = H_2 receptor antagonist, metiamide.

** significantly smaller than histamine-induced dilatation (H) at $P < 0.01$ (paired *t* test).

respectively. These were significantly less than the histamine dilated diameter ($P < 0.01$). When the antagonists were given together, there was complete inhibition of the histamine effect, the diameter being $5.6 \pm 7.4\%$ above control (not significantly different from 0). The effects of the antagonists appeared to be additive: 31.2% dilatation due to H_2 effect + 44.2% dilatation due to H_1 effect = 75.8% dilatation together (and histamine alone yielded $72.8 \pm 8.9\%$ dilatation).

Results of the microcirculatory studies in the corpus of seven rats are presented in Fig. 2. Vessels of similar size to those in the cat were studied, $32.9 \pm 2.6 \mu\text{m}$. Again dilatation occurred in all rats, the arteriolar diameter increasing on average to $36.1 \pm 5.1\%$ above control. Both H_1 and H_2 antagonists inhibited the histamine dilatation in all animals studied and this inhibition was statistically significant ($P < 0.01$). Interestingly, H_2 receptor antagonism alone nearly completely inhibited the response, the diameter being only $6.2 \pm 3.6\%$ above control. However, the H_1 antagonist alone also markedly inhibited the histamine effect, the average diameter being $10.8 \pm 3.2\%$ above control. The two antagonists together also completely inhibited the effect of histamine.

Fig. 2 Rat corpus: mean percent increase in submucosal arteriolar diameter in response to histamine and the histamine antagonists. Values are means $\pm$ SE of seven animals. ** significantly smaller than histamine-induced dilatation (H) at $P < 0.01$ (paired *t* test).

Fig. 3 Cat antrum: mean percent increase in submucosal arteriolar diameter in response to histamine and the histamine antagonists. Values are means $\pm$ SE of six animals. ** significantly smaller than histamine-induced dilatation at $P < 0.01$ (paired *t* test).

ANTRUM

Results of microcirculatory studies in six cats are presented in Fig. 3. The arterioles studied averaged $54.6 \pm 7.8 \mu\text{m}$ in diameter. Histamine produced arteriolar dilatation in all animals ($46.3 \pm 11.8\%$ above control). The H₁ antagonist partially but significantly ($P < 0.01$) inhibited this response in all animals, the dilatation now averaging only $17.3 \pm 10.6\%$ above control. The H₂ antagonist alone had no effect on the histamine response in any cat, but, when administered with the H₁ antagonist, there was complete inhibition of the histamine effect. The inhibition in the presence of both antagonists was

significantly greater than with the H₁ antagonist alone ($P < 0.05$, paired *t* test).

Results obtained in six rats are presented in Fig. 4. The arterioles studied averaged $32.7 \pm 2 \mu\text{m}$ in diameter. Histamine produced arteriolar dilatation in all six rats studied ($48.8 \pm 11.4\%$ above control). The H₁ antagonist significantly, and nearly completely, inhibited this response ($P < 0.05$), dilatation now averaging $12.7 \pm 10.1\%$ above control. The H₂ antagonist alone had no effect and, when administered with the H₁ antagonist, it did not significantly increase the inhibition obtained with the H₁ antagonist alone (the difference in diameter between H + H₁A and H + H₁A + H₂A was not statistically significant).

SPECIFICITY OF HISTAMINE ANTAGONISTS

The effect of the histamine antagonists on papaverine induced dilatation of corpus submucosal arterioles was studied in five cats. Papaverine produced a $45.2 \pm 7.5\%$ dilatation of the noradrenaline constructed arterioles. The H₁ and H₂ antagonists, separately or together, had no effect on the papaverine induced dilatation.

Discussion

The results of the present investigation indicate a distinct difference in the behaviour of histamine receptors on the submucosal arterioles of the antrum and corpus. In both regions the H₁ and H₂ receptors subserve vasodilatation. However, in the antrum the receptors behave in a fashion similar to that found in the small intestine submucosal arterioles (Guth and Smith, 1978). The H₁ receptor antagonist partially inhibited histamine dilatation and the effect of the H₂ antagonist could not be demonstrated unless the H₁ receptor was blocked. This was clear in the cat antrum (Fig. 3) but not in the rat antrum studies (Fig. 4) where, because of the nearly complete inhibition by the H₁ antagonist, an H₂ effect was not demonstrable. The predominance of H₁ receptors when both receptors subserve vasodilatation has also been found in skeletal muscle and the general circulation when the depressor effect of histamine was studied (Parsons and Owen, 1973; Powell and Brody, 1973; Powell and Brody, 1976). In the corpus, on the other hand, independent H₁ and H₂ receptor effects could be demonstrated (see Figs. 1 and 2). To date, similar independent H₁ and H₂ receptor effects have not been observed in any other vascular bed where both receptors subserved vasodilatation. In the cat studies, these effects were additive. In the rat studies, the inhibition by either antagonist was so marked that interpretation of the effect of the two antagonists administered together

Fig. 4 Rat antrum: mean percent increase in submucosal arteriolar diameter in response to histamine and the histamine antagonists. Values are means $\pm$ SE of six animals. *,** significantly smaller than histamine-induced (H) dilatation at $P < 0.05$ and 0.01 , respectively (paired t test).

was difficult. The more marked effect of the H_1 and H_2 antagonists alone seen in the rat study might be due to the smaller histamine effect seen in the rat without noradrenaline constriction, 36.1% dilatation, than in the cat with noradrenaline constriction, 72.8% dilatation.

The corpus and antrum perform different functions. The secretion of acid is a major function of the corpus. Histamine is an important stimulant of acid secretion. H_2 receptors on the parietal cell are responsible for this function of histamine. While an increase in mucosal blood flow will not stimulate acid secretion, an increase in flow is essential to meet the increased metabolic needs of the actively secreting parietal cell. Marked inhibition of mucosal blood flow will diminish acid secretion (Jacobson *et al.*, 1966). Therefore, a direct histamine effect on submucosal arterioles would not be unexpected. It is of interest that H_1 and H_2 receptors independently subserving vasodilatation were demonstrated on these arterioles. Since all stimuli of acid secretion increase mucosal blood flow (probably *via* vasoactive catabolites from the parietal cell), it is difficult to separate gastric secretory and primary blood flow effects using standard flow measurement techniques. For example, Jacobson and Chang (1969), found that both histamine and gastrin increased gastric mucosal blood flow, as determined by aminopyrine clearance, in the dog. However, the ratio of gastric mucosal blood flow to acid secretory rate was greater for histamine than for gastrin. This suggested, but did not prove, that the increased flow due to histamine represented both a direct pharmacological vasodilating effect and an indirect metabolic effect secondary to secretion. In previous studies we have shown that the gastric submucosal arterioles are the major resistance vessels regulating blood flow to the

gastric mucosa (Guth and Smith, 1975). When these constrict, mucosal flow decreases, when they dilate, flow increases. Thus the effect of histamine on H_2 receptors on the parietal cell to increase acid secretion would simultaneously call forth an increase in blood flow to these cells *via* the dilating effect of H_2 as well as H_1 receptors on the corpus submucosal arterioles.

The concentrations of mepyramine and metiamide employed in this study were chosen on the basis of maximum inhibition of histamine-induced vasodilatation without that concentration of the anti-histamine having any vasoactive effect by itself. The pA_2 for mepyramine is approximately 9 (Arunlakshana and Schild, 1959) and for metiamide approximately 6 (Black *et al.*, 1973). Therefore, relatively 100 times higher concentrations were used for mepyramine than for metiamide. Lower concentrations were not studied. Nevertheless the specificity of the agents in these concentrations for histamine receptor blockade was demonstrated by their failure to affect papaverine-induced vasodilatation.

The precise meaning of the interaction between H_1 and H_2 receptors in the antrum and other vascular beds in which the vasodilating effect is 'predominantly' an H_1 effect is not clear. It has been postulated (Powell and Brody, 1973) that this may be due to (1) a greater number of H_1 receptors, (2) greater affinity of histamine for H_1 receptors, or (3) greater smooth muscle action as a result of H_1 -receptor activation. Blockade of the dominant H_1 receptor is hypothesised to be necessary 'to allow the H_2 receptor to become more dominant and allow for a nearly pure H_2 -response'. Perhaps studies with the recently available pure H_1 and H_2 antagonists will shed some light on this question.

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References

- Arunlakshana, O., and Schild, H. O. (1959). Some quantitative uses of drug antagonists. *British Journal of Pharmacology*, **14**, 48-58.
- Ash, A. S. F., and Schild, H. O. (1966). Receptors mediating some actions of histamine. *British Journal of Pharmacology*, **27**, 247-439.
- Baez, S. J. (1966). Recording of microvascular dimensions with an image splitter television microscope. *Journal of Applied Physiology*, **21**, 299-301.
- Black, J. W., Duncan, W. A. M., Durant, C. J., Ganellin, C. R., and Parsons, M. E. (1972). Definition and antagonism of histamine thus 1m H₂-receptors. *Nature (London)*, **236**, 385-390.
- Black, J. W., Duncan, W. A. M., Emmett, J. C., Ganellin, C. R., Hesselbo, T., Parsons, M. E., and Wyllie, J. H. (1973). Metiamide—an orally active histamine H₂-receptor antagonist. *Agents and Actions*, **3**, 133-137.
- Glover, W. E., Carroll, P. R., and Latt, N. (1973). In *International Symposium on Histamine H₂-Receptor Antagonists*, pp. 169-174. Edited by C. J. Wood and M. A. Simkins. Smith, Kline and French Labs. Ltd: Welwyn Garden City.
- Guth, P. H., and Rosenberg, A. (1972). In vivo microscopy of the gastric microcirculation. *American Journal of Digestive Diseases*, **17**, 391-398.
- Guth, P. H., and Smith, E. (1975). Neural control of gastric mucosal blood flow in the rat. *Gastroenterology*, **69**, 935-940.
- Guth, P. H., and Smith, E. (1978). Histamine receptors in mesenteric circulation of the cat and rat. *American Journal of Physiology*, **234**, E370-E374.
- Jacobson, E. D., and Chang, A. C. K. (1969). Comparison of gastrin and histamine on gastric mucosal blood flow. *Proceedings of the Society for Experimental Biology and Medicine*, **130**, 484-486.
- Jacobson, E. D., Linford, R. H., and Grossman, M. I. (1966). Gastric secretion in relation to mucosal blood flow studied by a clearance technic. *Journal of Clinical Investigation*, **45**, 1-13.
- Parsons, M. E., and Owen, D. A. A. (1973). In *International Symposium on Histamine H₂-Receptor Antagonists*, pp. 127-135. Edited by C. J. Wood and M. A. Simkins. Smith, Kline and French Labs. Ltd: Welwyn Garden City.
- Powell, J. R., and Brody, M. J. (1973). In *International Symposium on Histamine H₂-Receptor Antagonists*, pp. 137-146. Edited by C. J. Wood and M. A. Simkins. Smith, Kline, and French Laboratories Ltd: Welwyn Garden City.
- Powell, J. R., and Brody, M. J. (1976). Identification and specific blockade of two receptors for histamine in the cardiovascular system. *Journal of Pharmacology and Experimental Therapy*, **196**, 1-14.