Biochemical Pharmacology, Vol. 24, pp. 1215–1219 Pergamon Press, 1975. Printed in Great Britain

OCCURRENCE OF H_2-RECEPTORS FOR HISTAMINE IN THE GUINEA-PIG INTESTINE*

IVONE BAREICHA† and M. ROCHA E SILVA

Department of Pharmacology, Faculty of Medicine, USP, Ribeirão Preto, São Paulo, Brazil

(*Received* 12 *November* 1973; *accepted* 20 *December* 1974)

Abstract—Burimamide, which specifically blocks H_2-receptors for histamine, potentiated the stimulating effect of histamine upon the guinea-pig ileum at a concentration of 10^{-6} M. This was interpreted as due to the relaxing effect induced by H_2-receptors present in the preparation. Similar results were obtained with pieces of the guinea-pig duodenum. On the basis of Clark's equation, a theoretical treatment permitted the calculation of the proportion of H_2/H_1 receptors in several preparations of the ileum and duodenum of the guinea-pig; an average value of 15·7% was found for the ileum and 12·4% for the duodenum. This small percentage of H_2/H_1 receptors may account for the fact that only the smallest concentrations of histamine appeared to be potentiated by burimamide and that this effect tended to disappear as the concentrations of histamine approached those giving maximal contraction.

The stimulating effects of histamine on the isolated guinea-pig ileum have been attributed mainly to the interaction with H_1-receptors, since anti-histaminics such as diphenhydramine and mepyramine, well known anti-H_1-receptors, entirely block such effects [1–3]. The recent introduction of a specific anti-H_2 agent, burimamide [4] provided an important tool to analyse the interference of a concomitant relaxing effect of histamine acting through H_2-receptors. In the present paper this possibility was studied starting from the observation that burimamide potentiates the contracting effects of histamine, especially at low concentrations. A theoretical calculation of the proportion of H_2/H_1 receptors was made possible by application of Clark's equation to the data, in the absence and presence of the anti-H_2 agent burimamide.

MATERIALS AND METHODS

Smooth muscle experiments. The experiments performed upon the isolated strips of guinea-pig intestine (ileum and duodenum) were done in the usual way, in a 10-ml chamber with Tyrode solution as the perfusion fluid, and the contractions registered on a smoked drum, with an isotonic lever [3]. The dose–response curves were obtained by adding in triplicate or quadruplicate increasing doses of histamine, in the absence (control) and presence of 10^{-6} M burimamide. Atropine at a concentration of 10^{-7} M was always present in the perfusing fluid.

Method of calculation. The average responses to histamine were plotted by taking the reciprocals of the doses added and of the responses obtained (double reciprocal plot). In most experiments, to test the potentiating effect of burimamide, the range of doses of histamine was extended to very small concentrations, since we have observed that the responses to the small doses of histamine were usually those most affected by burimamide. Therefore, in the present experiments, the concentrations of histamine varied from $1{\cdot}7 \times 10^{-6}$ M to 5×10^{-8} M.

In previous experiments [3] in which the parameters of the line were calculated to derive the slopes (K_n) only the higher concentrations of histamine were used and found to fall along the straight line of the double reciprocal plot. In the present experiments the use of very small concentrations of histamine in the presence of burimamide gave responses falling accurately upon the line obtained with the larger concentrations of histamine. Therefore, the present experiments show that with the exclusion of H_2-receptors the reciprocals of the responses accurately follow the line of responses (double reciprocal plot) to lower and higher concentrations of histamine.

RESULTS

Potentiation of the effects of histamine by burimamide. As shown in Figs. 1 and 2, the potentiating effect of burimamide on the contractions produced by histamine is more clearly seen with the smaller concentrations of histamine. As the doses of histamine increase, there is a tendency of the responses to coincide with those produced in the presence of burimamide. In Fig. 2, the lower straight line is that obtained in the presence of burimamide, and the upper points are those obtained in the absence of the anti-H_2 agent. In Fig. 1 the columns represent the average effects ($\bar{y} \pm$ S.E.) to each concentration of histamine in the absence (open rectangles) and presence of burimamide (hatched rectangles) in six different preparations of the guinea-pig ileum. It is clear that the potentiating effect of burimamide is far stronger for the smaller concentrations of histamine, so that the responses tend to equalize when the concentrations of the agonist increase.

Proportion of H_2-receptors in relation to the total number of H_1-receptors for histamine in the guinea-pig ileum. The enhanced potentiation by burimamide of

* Supported in part by Conselho Nacional de Pesquisas and Fundação de Amparo à Pesquisa do Estado de São Paulo.

† From the School of Pharmacy and Odontology, Ribeirão Preto, São Paulo, Brazil.

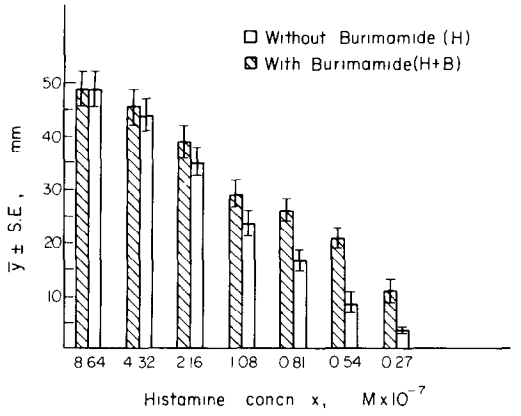

Fig. 1. Mean responses ($\bar{y}$) of six pieces of guinea-pig ileum to various molar concentrations of histamine (x). Open columns, mean responses in the absence of burimamide; hatched columns, means of responses in the presence of 10^{-6} M burimamide. The vertical bars indicate the S.E.M. Note that the potentiating effect is more marked for the smaller concentrations of histamine.

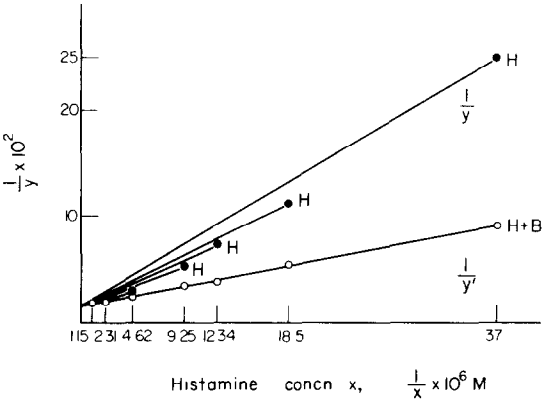

Fig. 3. Double reciprocal plots of Exp. VIII, on a single piece of the guinea-pig ileum. Ordinates, reciprocals of the responses ($\times 10^2$); abscissae, reciprocals of the histamine concentrations ($\times 10^6$ M). Lower continuous line (H + B): reciprocal of the responses in the presence of 10^{-6} M burimamide. Upper points: responses in the absence of burimamide. The upper lines converging to the same intercept are drawn to show that the lower line (with B) is a limit to which tend the lines drawn from the individual points.

the response to the lower concentrations of histamine indicates that the ratio of H_2/H_1 receptors is rather small in that smooth muscle preparation. In order to evaluate such a ratio, a method of calculation was introduced based on Clark's approach to describe the interaction of an agonist with its own receptors [5]. To analyse quantatively the phenomenon described above, we have considered each point representing the responses to histamine in the absence of burimamide as a separate experiment giving a separate slope in the double reciprocal plot in such a way that its position in the graph, would indicate the magnitude of the interference of the H_2-receptors in the responses to histamine. As shown in Fig. 3, each point could give a separate line converging on the same intercept with the basic line obtained in the presence of burimamide. As seen in the figure, such lines drawn from each experimental point tend to coincide with the basic line obtained in the presence of burimamide, as the concentration of histamine increases. From many similar experiments it became obvious that the slope of the line in the presence of burimamide is a limit to which converge the slopes of the lines drawn from each experimental point obtained in the absence of the anti-H_2 agent. Again it can be seen that the slope is maximal for the responses obtained with the smallest concentrations of histamine. Figure 4 describes a similar experiment with a piece of guinea-pig duodenum, and the final calculation of the percentage c is given in Table 2 for two experiments with such a smooth muscle preparation.

In order to calculate an index α, representing the proportion of H_2-receptors in relation to the H_1-receptors present in the ileum of the guinea-pig, the following theoretical interpretation was given. If one assumes that interaction with H_2-receptors reduces the stimulating effect of histamine, the assumption can be made that in the absence of burimamide to

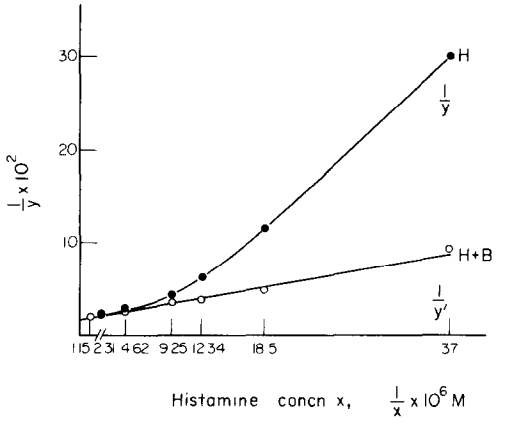

Fig. 2. Double reciprocal plots of the data from Fig. 1 in the absence (upper line) and in the presence of 10^{-6} M burimamide (lower line). Ordinates, reciprocal of the responses ($\times 10^2$); abscissae, reciprocal of the concentrations of histamine ($\times 10^6$ M).

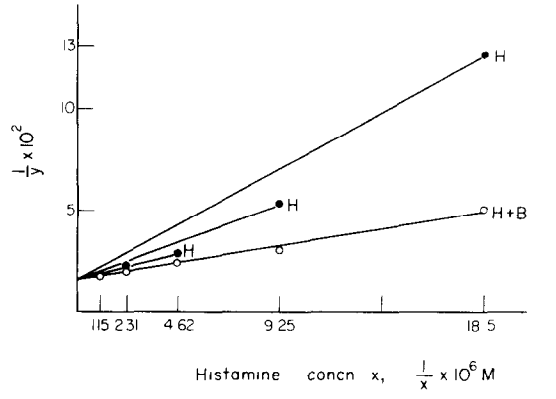

Fig. 4. Double reciprocal plots of Exp. I, on a single piece of the guinea-pig duodenum. Ordinates, reciprocals of the responses ($\times 10^2$); abscissae, reciprocals of the histamine concentrations. Other connotations, as in Fig. 3.

produce the same effect, as in the presence of burimamide, a certain proportion of αy of H_1-receptors has to be acted upon in addition to the number of receptors y, that according to Clark's equation would measure the number of 'occupied receptors'. In other words, to produce a certain measured effect y', the agonist must interact with a number $y + \alpha y$ of receptors, where α is the proportion of H_2-receptors in relation to the number of H_1-receptors (y) actually occupied. In such a case, $1 - y - \alpha y$ represents the number of unoccupied receptors, and Clark's equation can be set as

$$y + \alpha y = K_c(1 - y - \alpha y)x \qquad (1)$$

Where x is the histamine concentration applied and K_c is Clark's constant. Replacing K_c by its reciprocal K_n, equation (1) becomes,

$$\frac{K_n}{x} = \frac{1}{y(1 + \alpha)} - 1 \qquad (2)$$

In the limiting case where $\alpha = 0$, i.e. in the absence of H_2-receptors or in presence of burimamide, and substituting y' for y, the response in the presence of burimamide is expressed by

$$\frac{K_n}{x} = \frac{1}{y'} - 1 \qquad (3)$$

Solving for $1/y$ and $1/y'$, we have the pair of equations:

$$\frac{1}{y} = (1 + \alpha)\left(\frac{K_n}{x} + 1\right) \qquad (4)$$

and

$$\frac{1}{y'} = \frac{K_n}{x} + 1 \qquad (5)$$

for the points in the absence of burimamide (4) and in the presence of burimamide (5). From equations (4) and (5), we deduce the simple relationship

$$\alpha = \frac{y'}{y} - 1 \qquad (6)$$

giving the proportion (referred to a maximum of 1·00) of H_2-receptors in relation to the number of H_1-receptors present. The values of α can be deduced directly from the double reciprocal plots, for each dose of histamine, using the relation

$$\alpha = \frac{y' - y}{y}$$

and the percentage c of H_2-receptors referred to the maximum (100%) can be calculated as

$$c = 100\alpha y$$

or

$$c = 100(y' - y)$$

The steps for the calculation of α and c are indicated in Table 1 for a typical experiment, and the values obtained for different experiments are indicated in Table 2.

It is seen from Table 1, that the values of α tend to decrease with the size of the response (y), indicating that for smaller concentrations of histamine the proportion of H_2-receptors participating in the reaction is much larger than that for the higher concentrations of histamine, a fact that has been already stressed in the first section of this paper.

Table 1. Calculation of α and c for the guinea-pig ileum in Exp. VIII

Histamine concn $X \times 10^{-7}$ (M)	Responses Without B y%	With B y'%	$\alpha = (y'/y^{-1})$	$c^* = \alpha y = y' - y$
0·27	0·081	0·21	1·59	0·13
0·54	0·18	0·34	0·89	0·15
0·81	0·28	0·50	0·78	0·21
1·08	0·39	0·54	0·38	0·15
2·16	0·67	0·79	0·18	0·11
4·32	0·90	0·96	—	—
8·64	1·00	1·00	—	—

* Average: 0·15 (S.E. = ±0·017).

Table 2. Values of the proportions and percentages of H_2-receptors over H_1 in different smooth muscle preparations

Exp. No.	Nature of the preparation	αy*	Per cent of H_2-receptors over H_1† $c = 100\alpha y$
I	Guinea-pig duodenum	0·123	12·3
II	Guinea-pig duodenum	0·126	12·6
III	Guinea-pig ileum	0·076	7·6
IV	Guinea-pig ileum	0·136	13·6
V	Guinea-pig ileum	0·303	30·3
VI	Guinea-pig ileum	0·126	12·6
VII	Guinea-pig ileum	0·128	12·8
VIII	Guinea-pig ileum	0·150	15·0
IX	Guinea-pig ileum	0·249	24·9
X	Guinea-pig ileum	0·150	15·0
XI	Guinea-pig ileum	0·170	17·0

Averages, guinea-pig ileum: *0·157; †15·7 S.E. = ±2·27.

However, the values of c that indicate the apparent proportion of H_2-receptors in relation to H_1 in the preparation tend to be constant (7·6–30·3%) with an average of 15·7% for the ileum of the guinea-pig. This rather moderate percentage of H_2 in relation to H_1-receptors explains why the line in the double reciprocal plot tends to bend upwards for the smaller concentrations of histamine, as shown in Fig. 2.

Potentiation of the histamine effects by inhibition of histamine metabolizing enzymes. Part of the potentiation produced by burimamide, however, might be due to inhibition of enzymes that could destroy histamine. One of these enzymes, histamine methyl transferase, is inhibited by concentrations of burimamide of the order of 10^{-5} M [8]. In our experiments, concentrations of burimamide of the order of 10^{-6} M were sufficient to fully potentiate the effect of the smaller doses of histamine. Burimamide may interfere with histaminase activity at concentrations of the order of those utilized in this paper [9]. Since aminoguanidine is a common inhibitor of histaminase [10], we have tested the effect of 10^{-6} M aminoguanidine, as shown in Fig. 5; in parallel experiments aminoguanidine was tested in combination with burimamide, and the effects compared with burimamide added alone. As shown in Fig. 5, aminoguanidine only partially rectified the line of the double reciprocal plot, in contrast with the addition of burimamide alone which fully rectified the plot.

If we apply the calculation described above to the experiments in which histamine (H) was applied alone, and histamine + aminoguanidine (H + A) and finally histamine + aminoguanidine + burimamide

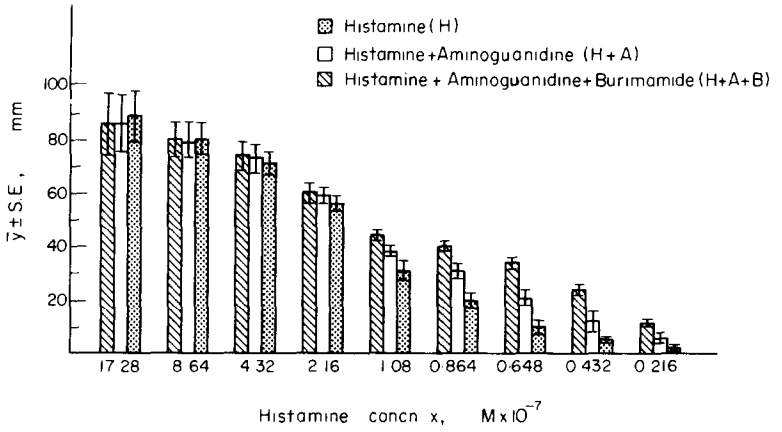

Fig. 5. Averages of five experiments in which the responses ± standard error ($\bar{y} \pm SE$) are indicated on the ordinates; abscissae, the concentrations of histamine applied (10^{-7} M). Note that A alone was unable to fully potentiate the effect of the smaller doses of histamine, as done by B alone.

(H + A + B) as shown in Fig. 6, the alleged percentage of H_2 receptors would fall from a value of 16·20 ± 2·27 (comparing H with H + A) to 8·82 ± 1·70 (comparing H + A with H + A + B). This result would still be compatible with the idea of the presence of H_2 receptors in the guinea-pig ileum.

DISCUSSION

The introduction of burimamide, a specific agent capable of blocking H_2-receptors in several biological structures such as the guinea-pig atrium, rat uterus and the dog gastric mucosa [4], contributed to the analysis of histamine receptors.

As far as the ileum of the guinea-pig is concerned, the overwhelming presence of H_1-receptors precluded any clear-cut demonstration of the occurrence of receptors such as H_2 that might have a relaxing effect. In the presence of the classic synthetic antihistaminics such as diphenhydramine or mepyramine, the stimulating effect of histamine due to interaction with H_1-receptors is entirely blocked, but since the tonus of the usual preparations of the ileum of the guinea-pig is already low, any residual relaxing effect in the presence of anti-histaminics is difficult to observe. Along that line, it is interesting to note the observation by Ambache *et al.* [6] that burimamide is able to block the partial inhibitory effect of histamine on the contractions of the guinea-pig ileum subjected to rhythmic electrical stimulation in the presence of atropine and mepyramine.

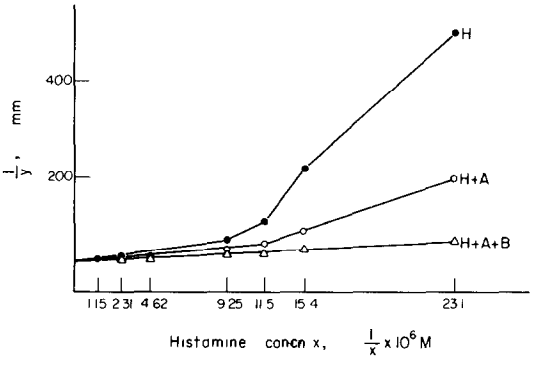

Fig. 6. Double reciprocal plots showing the partial effect of aminoguanidine (H + A) and the full effect when burimamide + aminoguanidine (H + A + B) were added. Note that burimamide alone was enough to correct the bending of the line for the smallest concentrations of histamine, as shown in Figs. 1–4.

It was, however, a common observation to everyone using the double reciprocal plot, that the line which is straight when high concentrations of histamine are used, tends to bend upwards when very small concentrations of histamine are used. This was generally thought to result from friction of the needle on the smoked drum, or to destruction of the smallest concentrations of histamine by histaminase, or to some cholinergic effect that should however be blocked by atropine. The use of atropine and of an inhibitor of histaminase, such as aminoguanidine, did not prevent the bending of the line.

The fact that burimamide, when added at a suitable concentration, fully corrects the bending observed with the smallest concentrations of histamine was a strong indication that this effect might depend to a large extent upon the participation of a moderate proportion of H_2-receptors inducing a relaxation that would reduce the stimulating effect of histamine acting upon H_1-receptors. As the effect only appeared for the smaller concentrations of histamine, it would indicate that the proportion of H_2/H_1 receptors is too small to affect the larger doses of histamine.

The results presented above show that Clark's equation in its classical presentation, and its inversion (Lineweaver–Burke transformation) covers a wide range of histamine concentrations provided the interference of H_2-receptors is blocked by burimamide. The correction introduced by this procedure affects predominantly the contractions induced by the smallest concentrations of histamine (below 10^{-7} M) and becomes less and less important as the concentrations of histamine approach those producing the maximum response. This has been shown by the experimental treatment presented above to be due to the small percentages of H_2-receptors in relation to the much larger number of H_1-receptors present in the guinea-pig intestine.

The present findings have the following consequences: (a) the calculations presented before of pK_n and y_m for histamine on the guinea-pig ileum based on the inverted equation (double reciprocal plots) are still valid; (b) there was no significant correction to be made on the parameter of affinity pK_n calculated as the slope of the line near the maximal effect, nor on the maximal effect (y_m) obtained as the intercept of the line with the ordinates ($1/y$), since only the higher doses of histamine were used to calculate such parameters by the double reciprocal plot [3, 7].

Acknowledgement—We are indebted to Prof. J. W. Black, of the Research Institute, Smith Kline and French Laboratories Ltd., Welwyn Garden City, Herts., England, for a generous supply of the anti-H_2 agent burimamide (*N*-methyl-*N'*-(4-4(5)-imidazolyl) butyl) thiourea.

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