DUALIST OR PSEUDO-DUALIST INTERACTIONS OF Mepyramine, Diphenhydramine and Eprozinol with Histamine at H1-RECEPTORS

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Accepted February 14, 1977

Abstract—The types of interaction of mepyramine (M), diphenhydramine (D) and eprozinol (E), with histamine H1-receptors of guinea pig ileal and tracheal smooth muscle, were comparatively studied in vitro. According to the concentrations used, all three substances showed an apparent dualist mechanism of action on both preparations when histamine (dihydrochloride) was used as the agonist. The competitive component of this mechanism (at low concentrations) was characterized by the following pA2 values: 9.01 (M), 7.80 (D) and 5.64 (E) with the ileum; 8.06 (M), 7.00 (D) and 6.02 (E) with the trachea. The so-called non-specific component (at high concentrations) was of comparable intensity in the two organs. The pD'2 values were 5.54-5.66 (M), 4.65-4.38 (D) and 3.82-3.55 (E) with ileal and tracheal muscle, respectively. At low concentrations the equi-active dose-ratio for M/D/E (1/16/2300 on the ileum) became 1/12/110 when the trachea was used as the effector. This is surprising since histaminergic receptors of the two preparations are of the same H1-type. It is suggested that only diphenhydramine and eprozinol are really dualistic, and that the non-specific mechanism of activity differs for each drug with that of eprozinol being effective on tracheal muscle.

The first known antihistamines—which acted only on H1-receptors—have been succeeded by others which are either specific for H2-receptors or, in contrast, are much more polyvalent. The latter have less affinity for histaminergic receptors than their predecessors, and in particular show pronounced interference with various fundamental membrane phenomena. Mepyramine remains one of the reference products for the “classical” or anti-H1 receptor antihistamines. However, the specificity when using certain biological receptors is questionable. Edvinsson and Owman (1) have recently shown that mepyramine reduces the slope and maximum amplitude of histamine dose-response curves. Diphenhydramine, another reference product of the same class, is known to antagonize acetylcholine (2, 3) as well as histamine. In addition, it competitively inhibits transmembrane Ca2+ influx (Ohmura and Matsumoto, 4; Rubin, 5). Under certain conditions, it has a protective action on H2-receptors which prevents blockade by irreversible antagonists (Watanabe and Goto, 6). Certain aspects of the molecular pharmacology of mepyramine and diphenhydramine were therefore deemed worthy of further investigation. Our studies were extended to include eprozinol, a disubstituted piperazine, which, as the chemical structure shows, is related to cinnarizine.
The main difference is the replacement of the benzhydryl fraction of the cinnarizine molecule by the 2-phenyl-2-methoxy-ethylamine of eprozinol. Cinnarizine, the most characteristic compound of the disubstituted piperazine group, has been the object of numerous recent studies (7, 8, 9, 10, 11, 12, 13). Preliminary pharmacological studies with eprozinol suggest that it possesses a particular anti-histamine tropism toward bronchial smooth muscle. In contrast, its inhibitory action on histamine with regard to other organs remains weak (Duchene-Marullaz et al., 14). In view of these findings, the present *in vitro* studies were carried out in an attempt to determine the types of histamine interaction with mepyramine, diphenhydramine and eprozinol when guinea pig intestinal and tracheal smooth muscle served as the experimental tissues. The classical parameters which characterized the affinities of substances for receptor sites (pA<sub>2</sub>) and the intensities of non-specific antagonism (pD<sub>2</sub>) were calculated and compared.

**MATERIALS AND METHODS**

The terminal ileum was removed from both male and female guinea pigs (300±50 g) sacrificed by pithing after an 18 hr fast. 2-5 mm sections were set up in Van Rossum (15) organ baths (containing oxygenated Ringers at 33.5°C). The trachea was removed from male or female guinea pigs (600±100 g) sacrificed under the same conditions. Spiral 4 mm wide strips were suspended in an organ bath containing oxygenated Krebs solution at 37°C. Contractions were recorded on Helec "C 10" smooth muscle transducer connected to a variable amplification coefficient recorder (Sefram Servotrace). Classical techniques based on a study of variations in biological response (muscular contraction here) as a function of agonist concentration (histamine) and the method of cumulative doses, without intermediate rinsing of the preparation were used (15). With each preparation, two consecutive similar control dose-response curves were required before exposure to an antagonist. Two dose-ratios were measured with the trachea, and four with the ileum. After each addition of the antagonist, control dose-response curves were recorded until two identical dose-response control curves were obtained. The order of experiments with the different concentrations of antagonists was previously randomized. An initial approximation of the type of interaction was gained by a qualitative study of the series of recorded dose-response curves. When the curves between the control and test dose-response curves were in good parallel (without lowering of the height of the latter) a strictly competitive antagonism or a competitive dualism was assumed; this was confirmed by testing the weighted regression straight line of log (x-1) on log (agonist concentration) after Arunlakshana and Schild (16). In the two model cases of strictly competitive antagonism and competitive dualism, the regression of log. (x-1) on log. [B], concentration of antagonist, is linear with slope = 1. In general, the ratio x is calculated by using agonist concentrations giving 0.5 maximal responses on each curve. In this study, however, log. (x-1) was directly read from Van
Rossini tables (15) after measuring the distance between the 50% effect points in control curves and those made in the presence of the antagonist. A comparison of the slopes of these regression lines was carried out using the Student t-test to determine differences between observed and theoretical slopes (17). The degree of parallel shift or lowering of the curves induced by preliminary addition of different antagonist concentrations to the bath, was used to calculate parameters (pA2 and pD'2). The final antagonist concentrations and the number of tests carried out in each case, are shown on the mean dose-response curves in Figs. 1 and 2.

RESULTS

Fig. 1 shows the series of dose-response curves for histamine with guinea pig ileum, obtained in the presence of different concentrations of the three antagonists. These three graphs show a certain similarity. After a shift to the right (when curves remain parallel to the control curve and are only slightly lowered), there is a sudden change in slope, accompanied by a clear lowering of the maximum amplitude. Identical observations may be applied to Fig. 2 in which dose-response curves were obtained with guinea pig tracheal muscle. In both cases, the interaction between histamine and mepyramine, diphenhydramine or eprozinol may be considered either as (1) the manifestation of a competitive antagonism with the appearance of a non-competitive antagonism above a certain concentration

Fig. 1. Contractant effect of histamine on isolated guinea pig ileum, in the presence of different concentrations of mepyramine, diphenhydramine or eprozinol. [A], histamine concentration (M); [B], antagonist concentration (M)—mepyramine, diphenhydramine or eprozinol. EAB/E_{max. A}: Effect of a certain concentration of histamine in the presence of a fixed concentration of antagonist (EAB), expressed as a fraction of the maximal effect with histamine alone (E_{max. A}). n, number of tests carried out in each experimental situation. N.B.: For clarity, not all dose-response curves are reported on this graph.
FIG. 2. Contractant effect of histamine on isolated guinea pig trachea, in the presence of different concentrations of mepyramine, diphenhydramine or eprozinol. [A], histamine concentration (M); [B], antagonist concentration (M)-mepyramine, diphenhydramine or eprozinol. $E_{AB}/E_{max}$: Effect of a certain concentration of histamine in the presence of a fixed concentration of antagonist ($E_{AB}$), expressed as a fraction of the maximal effect with histamine alone ($E_{max}$). $n$, number of tests carried out in each experimental situation. N.B.: For clarity, not all dose-response curves are reported on this graph.

(II), the superposition of non-competitive antagonism and competitive antagonism from the lowest effective concentrations onwards, (III) a functional antagonism (18), (IV) an irreversible antagonism with receptor reserve, (V) a non-competitive antagonism with a reserve in the receptors (19), or (VI) a potent competitive antagonism with a low dissociation constant, acting as an irreversible competitive antagonism (20, 21). A study of the slopes of the regression lines of log. (x−1) on log. [B] traced on Figs. 3 and 4 shown in Tables 1 and 2, allows for clarification.

It appears that in all cases the slopes of the regression lines are not significantly different from 1. Thus, in the previously described experimental conditions, the antagonism between histamine and mepyramine, diphenhydramine or eprozinol is really competitive, at least for the range of antagonist concentrations outlined in Tables 1 and 2. It is possible, however, that a non-competitive mechanism completes the strictly competitive component. The different parameters calculated are summarized in Table 3.

From this table, remembering that the $pA_2$ are expressed on a logarithmic scale, the molar concentrations of mepyramine, diphenhydramine and eprozinol which cause the same competitive antagonism of histamine, may be calculated. Thus, with the guinea pig...
Fig. 3. Histamine/antagonist interactions. Regression straight lines for log \((x-1)\) on log \([B]\) with guinea pig isolated ileum. \(x = \frac{A}{a}\), ratio of agonist concentrations necessary to obtain the same fraction of the maximal contractant effect, in the presence (A) or absence (a) of antagonist; \([B]\), concentration (M) of antagonist (mepyramine, diphenhydramine or eprozinol). • M, Mepyramine \((y = 0.951x - 8.576)\); ■ D, Diphenhydramine \((y = 1.014x + 7.918)\); *E, Eprozinol \((y = 1.002x + 5.650)\).

Fig. 4. Histamine/antagonist interactions. Regression straight lines for log \((x-1)\) on log \([B]\) with guinea pig tracheal muscle. \(x = \frac{A}{a}\), ratio of agonist concentrations necessary to obtain the same fraction of the maximal contractant effect, in the presence (A) or absence (a) of antagonist; \([B]\), concentration (M) of antagonist (mepyramine, diphenhydramine, or eprozinol). • M, Mepyramine \((y = 1.023x + 8.243)\); ■ D, Diphenhydramine \((y = 0.993x + 6.954)\); *E, Eprozinol \((y = 1.043x + 6.283)\).
### Table 1. Histamine-antihistamine interaction on smooth muscle of guinea pig ileum

| Antagonist    | Concentration intervals* | Correlation coefficient | Ordinate at Origin | Estimation | Slope Standard error | t test | Statistical conclusion** |
|---------------|---------------------------|-------------------------|---------------------|------------|----------------------|--------|--------------------------|
| Mepyramine    | $1 \times 10^{-8}$ M, $1 \times 10^{-7}$ M | 0.977                   | 8.58                | 0.95       | 0.0076               | -1.772 | p > 0.05                 |
| Diphenhydramine | $1 \times 10^{-8}$ M, $1 \times 10^{-7}$ M | 0.949                   | 7.92                | 1.01       | 0.008                | -0.378 | p > 0.05                 |
| Eprozinol     | $1 \times 10^{-6}$ M, $1 \times 10^{-5}$ M | 0.999                   | 5.65                | 1.00       | 0.0004               | -1.642 | p > 0.05                 |

Characteristics of regression lines of log. (x-1) on log. (antagonist concentration).
* The regression lines were only studied for antagonist concentration intervals which led to a parallel shift of dose response curves, without lowering of their maximum amplitude. ** Result of study of significance of difference between calculated and theoretical slopes.

### Table 2. Histamine-antihistamine interaction on smooth muscle of guinea pig trachea

| Antagonist    | Concentration intervals* | Correlation coefficient | Ordinate at Origin | Estimation | Slope Standard error | t test | Statistical conclusion** |
|---------------|---------------------------|-------------------------|---------------------|------------|----------------------|--------|--------------------------|
| Mepyramine    | $1 \times 10^{-8}$ M, $3 \times 10^{-7}$ M | 0.927                   | 8.24                | 1.02       | 0.012                | -0.544 | p > 0.05                 |
| Diphenhydramine | $3 \times 10^{-7}$ M, $1 \times 10^{-6}$ M | 0.943                   | 6.95                | 0.99       | 0.016                | -0.104 | p > 0.05                 |
| Eprozinol     | $3 \times 10^{-7}$ M, $3 \times 10^{-6}$ M | 0.973                   | 6.28                | 1.04       | 0.0072               | -2.143 | p > 0.05                 |

Characteristics of regression lines of log. (x-1) on log. (antagonist concentration).
* The regression lines were only studied for antagonist concentration intervals which led to a parallel shift of dose response curves, without lowering of their maximum amplitude. ** Result of study of significance of differences between calculated and theoretical slopes.
TABLE 3. Mean parameters (§) characterizing dualist interactions of mepyramine, diphenhydramine and eprozinol, with histamine, at H₁ receptors

| Antagonist | Guinea pig ileum | Guinea pig trachea |
|------------|------------------|-------------------|
|            | pA₂             | pD₂              | pA₂             | pD₂              |
| Mepyramine | 9.01±0.252      | 5.54±0.072       | 8.06±0.031*     | 5.66±0.094       |
|            | (13)            | (8)              | (12)            | (9)              |
| Diphenhydramine | 7.80±0.031     | 4.65±0.158       | 7.00±0.184*     | 4.38±0.138       |
|            | (24)            | (7)              | (14)            | (4)              |
| Eprozinol  | 5.64±0.0046     | 3.82±0.054       | 6.02±0.006*     | 3.55±0.125       |
|            | (14)            | (20)             | (8)             | (15)             |

* The mean pA₂ values for the same antagonist, calculated for the two experimental series, are significantly different (p < 0.05). § Each mean is followed by the standard error and (in brackets) the number of experimental results.

ileum, a certain concentration of mepyramine would be as effective in competitively antagonizing the effect of histamine as concentrations of diphenhydramine and eprozinol respectively 16 and 2300 times higher. On the trachea, the ratios of equiactive concentrations are considerably narrowed. In effect, in this case, a molar concentration of mepyramine has the same activity as diphenhydramine and eprozinol at concentrations only 12 and 110 times higher. Finally it is noteworthy that in the two experimental series, the non-competitive component of the anti-histamine action of the three compounds occurs at absolutely parallel levels of activity. The scale of equiactive concentrations (pD₂) goes from 1/8/52 (ileum) to 1/19/129 (trachea), which is not a statistically significant variation. Moreover, eprozinol produced a change in basal tone of the tracheal muscle. This relaxant action was constant and significant for the highest concentration used (1×10⁻³ M). Such a change was not observed either with mepyramine or diphenhydramine on the two isolated preparations, or with eprozinol on the ileum.

DISCUSSION

The apparent dualist nature of the activity shown by the three anti-histamines, was clear in both experimental situations. Similar results for diphenhydramine have previously been shown by Ohmura and Matsumoto (4). They found the muscle relaxant activity independant of the action at the membrane receptor level. Such a clear activity for mepyramine has not previously been demonstrated, although Edvinsson and Owman (1) recorded a non-competitive type histamine/mepyramine interaction using cat intra-cranial artery. This dualist activity indicates that even at the lowest doses of each antagonist, the competitive anti-histamine action may be completed by a non-competitive or non-specific action. The affinity of the reference products for their membrane receptor sites, calculated at the beginning of this study, may be compared with previously published values. The pA₂ values found for mepyramine (9.01±0.25) and diphenhydramine (7.80±0.03) are of the same order as those found in the literature, when the guinea pig ileum is used as the effector. For
mepyramine, quoted pA₂ values are 9.2 to 9.3 (15, 23, 24) and for diphenhydramine 7.5 to 8.15 (2, 15, 25, 26, 27). In contrast, our studies with guinea pig tracheal muscle gave pA₂ values which were lower by nearly 1.0 than those reported by Arunlakshana and Schild (16). The closing up of the ratios of equiactives doses for competitive anti-histamine activity in the two organ systems is also remarkable. This was 1/16/2300 (mepyramine/diphenhydramine/eprozinol) for ileal smooth muscle and 1/12/110 for tracheal muscle. In agreement with Duchene-Marullaz et al. (14) we consider that this substantial lessening of the potentiality ratios (with tracheal muscle) is not only the result of increased anti-histamine activity for eprozinol at this level. Although pA₂ values for eprozinol are significantly different in the two organ models (5.64 ± 0.004 and 6.02 ± 0.006), it appears that the narrowing of the ratios of equiactives doses is also due to a lower affinity of mepyramine and diphenhydramine for tracheal H₁ receptors. In fact, the pA₂ of mepyramine falls from 9.01 ± 0.252 (ileum) to 8.06 ± 0.031 (trachea) while that of diphenhydramine goes from 7.80 ± 0.031 to 7.00 ± 0.184. The mean values are significantly different for the two effector organs.

To explain these observations, it is possible to imagine two similar but slightly different forms of H₁ receptor in the two organ systems. The eprozinol molecule may have physico-chemical properties allowing it to adapt equally well to both receptor forms. In contrast, mepyramine and diphenhydramine may be better adapted structurally to intestinal smooth muscle receptors than to tracheal receptors. This hypothesis of different receptor forms depending on the organ is worthy of consideration. Frankhuysen and Bonta (22) have also suggested similar differences between tryptaminergic D receptors in different organs. Bouhuys et al. (28) have shown that the spasmogenic action of histamine at the bronchial level was modulated by the interference of several other biological mediators. As cyclic AMP plays the role of "second messenger", the antagonism of a mediator other than histamine may modify the bronchospasmogenic effect of histamine. In the present study, a non-strictly competitive antagonism was observed. However, the mechanisms implied in these "non-competitive" activities were not demonstrated in preliminary experiments. Thus, if the non-specific antagonist mechanisms of mepyramine, diphenhydramine and eprozinol are quite different, it is possible that, according to the effector, a given mechanism may predominate. In this case, a drug acting by this mechanism could be a more active non-specific antagonist and the narrowing of the equiactive dose-ratios with the trachea may be explained. It should be noted that only eprozinol exerted a proper significant relaxant effect and only on the tracheal muscle. A conflicting argument must be discussed: in fact, dose-ratios are based on pA₂ values, which are calculated with experimental results using the lower concentrations of each drug. However, for these low doses, only competitive interaction between histamine and anti-histamines was noticed. On the other hand, Ariëns (19) showed that, in the case of a dualist drug acting on an effector with a receptor reserve, the non-competitive facet of the antagonist also induces a shift in the log dose-response curves (until the reserve in the stimulus is exhausted) followed then by a depression of the curves. This theoretical model appears to be in good agreement with experimental results obtained during this work. The possibility of a superposition of non-competitive and
competitive antagonism from the lowest effective concentrations onwards cannot however, be excluded.

The dualist antagonism seen with eprozinol is similar to mechanisms evoked for flunarizine and cinnarizine, which are chemically related to eprozinol. Van Nueten and Janssen (11) showed that with guinea pig ileum low doses of flunarizine and cinnarizine caused shifting of histamine dose-response curves to the right. At higher doses these compounds reduced maximum contractions. In the case of mepyramine where a lowering of the dose-response curves is induced (20), dissociation kinetics have to be considered. Paton (20) estimated the dissociation half-life of the antagonist receptor complex, in guinea pig ileum, to be about 10 min for mepyramine. Thus, this potent reversible competitive antagonist would be acting as an irreversible competitive antagonist (a non-equilibrium antagonist) during such a test. In other words, the so-called non-competitive component of the apparent dualist antagonism observed in Figs. 1 and 2 for mepyramine, might actually only be the consequence of its low dissociation constant. If such is the case then this drug would really be a strictly competitive anti-histamine. Finally, Ohmura and Matsumoto (4) have shown that anti-spasmodic action induced by high concentrations of diphenhydramine is not related to a particular type of biological receptor. In this case, diphenhydramine would inhibit transmembrane movements of Ca ions. The non-specific component above-mentioned could proceed from such a mechanism. In conclusion, it appears that lowering of histamine dose-response curves, provoked by mepyramine, diphenhydramine or eprozinol pretreatment, is induced by different mechanisms. From the literature, it seems that only diphenhydramine and eprozinol are characterized by a dualist anti-histaminergic antagonism and such would explain the narrowing of relative activities when tracheal muscle is used.

Acknowledgements: Gratitude is due to Mr. Christopher Houldsworth for translation and kind advice and to Ms. A.M. Menrier and B. Bordes for technical assistance.

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