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Selectivity of 4-methylhistamine at H_1- and H_2-receptors in the guinea-pig isolated ileum

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1 The selectivity of 4-methylhistamine (4-MH) as an agonist at histamine H_1- and H_2-receptors has been evaluated in the guinea-pig isolated ileum.

2 The EC_{50} values of 4-MH on H_1- and H_2-receptors that mediate contractile responses were determined. The EC_{50} at H_1-receptors was estimated after selective blockade of H_2-receptors by tiotidine and the EC_{50} at H_2-receptors estimated after selective blockade of H_1-receptors by mepyramine.

3 The $-\log EC_{50}$ values at H_1- and at H_2-receptors were 4.57 and 5.23, respectively.

4 The dissociation constants for the interaction of 4-MH with H_1- and H_2-receptors were determined.

5 The $-\log K_D$ values at H_1- and H_2-receptors were 3.55 and 4.27, respectively.

6 These results suggest that 4-MH is only about 5 times as potent at H_2- as it is at H_1-receptors in the guinea-pig ileum and that 4-MH should be used with caution to discriminate between H_1- and H_2-receptors.

Introduction

4-Methylhistamine (4-MH) is frequently used as a selective H_2-agonist. It has been noted (Hough, Weinstein & Green, 1980) that some investigators impart a selectivity to 4-MH that is not warranted by the original observations of Black, Duncan, Durant, Ganellin & Parsons (1972). In addition to its action at H_2-sites, 4-MH is also a full agonist at H_1-receptors (Black *et al.*, 1972, this paper). The selectivity of 4-MH for H_2-relative to H_1-receptors has only been defined in terms of its potency relative to that of histamine for actions on ileal H_1- and atrial H_2-receptors.

Because EC_{50} values for agonist action at one receptor can vary among tissues (independent of agonist affinity), the present study was undertaken to re-evaluate the selectivity of 4-MH on H_1- and H_2-receptors in one tissue, the guinea-pig isolated ileum. The presence of H_1- and H_2-receptors in the guinea-pig ileum has been established (Ash & Schild, 1966; Barker & Jones-Ebersole, 1982). H_1-receptors are located predominantly on smooth muscle cells (Chang, Tran & Snyder., 1979) and H_2-receptors are present on some myenteric plexus interneurones (Barker & Jones-Ebersole, 1982). Activation of either receptor results in a contractile response. Further, in the isolated ileum, either receptor can be selectively antagonized and the response mediated by the other receptor studied in relative isolation.

These studies were designed to determine the selectivity of 4-MH for ileal H_1- and H_2-receptors and to evaluate the utility of a two receptor-one response model in describing our results. We show that 4-MH has a selectivity for ileal H_2-receptors, but the selectivity is much less than suggested by the initial observations of Black *et al.* (1972). A preliminary report of part of this study has been given (Barker & Hough, 1982).

Methods

Male and female Hartley albino guinea-pigs, 400–700 g, were used. Segments, 3 cm, were taken from the ileum between 15 to 30 cm from the ileo-caecal junction and suspended in a 5 ml jacketed organ bath for recording isometric contractions as previously described (Barker & Jones-Ebersole, 1982). Concentration-response curves were gener-

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ated by the sequential variation of agonist concentration; the exposure time was 30 s and the interval between doses was 4 min. Initially, duplicate concentration-response curves were generated in the absence of antagonists. In addition, the responses to 5 μM histamine and 200 μM dimaprit were obtained. These concentrations of histamine and dimaprit are about 35 times their respective EC_{50} values on the ileum (Barker, 1981; Barker & Jones-Ebersole, 1982). The responses produced by these concentrations of histamine and dimaprit were taken as the maximal response obtainable at H_1- and H_2-receptors respectively. Subsequently, a second set of concentration-response curves was generated after 1 h incubation with either 0.5 μM mepyramine or 15 μM tiotidine. During the equilibration period with antagonists, the bath solution was changed at 15 min intervals. The concentrations of mepyramine and tiotidine used are 1000 times their K_B values for antagonism at H_1- and H_2-receptors, respectively (Arunlakshana & Schild, 1959; Yellin, Buck, Gilman, Jones & Wardleworth, 1979; Barker, 1981). At these concentrations, neither drug exhibits any measurable antagonism of the other histamine receptor in the guinea-pig isolated ileum (Barker, 1981; Barker & Jones-Ebersole, 1982). The use of highly selective antagonists permits one to evaluate the agonist activity of 4-MH independently at either H_1- or H_2-receptors.

Concentration-response data generated in the presence of an antagonist were evaluated for a fit to the equation:

$$E = (Em)/(1 + (EC50/A)^{nH}) \quad (1)$$

where E is the measured response, Em the maximal response, A the concentration of agonist, and nH the Hill coefficient. Iterative curve fitting was done using the programme, 'Regression of an arbitrary function in one variable' (Heilborn, 1981) on an Apple II Plus computer.

Concentration-response data generated for 4-MH in the absence of any antagonists were fitted to the equation for two receptors mediating a common response by one agonist (Ariens, Van Rossum & Simonis, 1956):

$$E = E1 + E2 - E1 \times E2/Emax \quad (2)$$

where E is the observed effect, Emax the maximal tissue response, and E1,2 each have the form of equation (1). Rearranging and substitution gives the form used for fitting:

$$E/Emax = 1\text{-}[(Emax1/(1 + (K1/A)^{nH1}))\text{-}1] \times [(Emax2/(1 + (K2/A)^{nH2}))\text{-}1] \quad (3)$$

where K1 and K2 are the EC_{50} values for H_1- and H_2-receptors, respectively, and all other terms are as defined above. A two stage fit to this equation to determine the variables nH1, nH2, K1, and K2 was performed with the PROPHET computer system's Public Procedure FITFUN (Baig & Reid-Miller, 1980). The criteria for a good fit were a significant correlation coefficient and a random distribution of residuals, the difference between fitted values and observed values. The fitting was performed as follows: Emax1 was always 1 since H_1-receptors curves for this tissue always gave the same maximum response as did curves composed of both receptors (Figure 1). Emax2 was determined experimentally with dimaprit in the absence of either antagonist and expressed relative to Emax1 (e.g. Emax2 = 0.3 in Figure 1). Initial estimates of K1 and K2 were made by inspection of the data, and nH1 and nH2 were fitted, with the only constraint that they be positive. These fitted values were then used as the initial estimates for a second fit of all four variables, with the second constraint that the EC_{50} values be between 0 and 1 M. Initially, attempts were made to fit all six parameters in equation 3 simultaneously. While such fits converged and gave significant correlation coefficients, the fitted values did not agree with the experimentally observed values. In a second attempt to carry out a simultaneous fit, the values of Emax and nH were fixed to those obtained from the analyses of the individual components. Again, the fits converged and gave significant correlation coefficients, but the residuals showed a systematic variation. For these reasons, the two stage fit described above was used to estimate the four parameters, Emax1, Emax2, nH1, and nH2. This approach satisfied both criteria for a good fit..

The apparent dissociation constants (K_D) of 4-MH for H_1- and H_2-receptors were determined by the method of Furchgott (1966). Irreversible inactivation of a fraction of the H_1-receptor population was achieved by exposure of the ileum to dibenamine HCl, 1 μM, for 30 min. Partial irreversible inactivation of H_2-receptors was achieved by treatment of the ileum with L-643,441(3-N-[3-[3-(1-piperidinomethyl) phenoxy] propyl] amino-4-amino-1, 2, 5-thiadiazole-1-oxide), 50 nM, for 15 min. L-643,441 is a selective irreversibly acting H_2-antagonist (Pendelton, Torchiana, Hanson & Clineschmidt, 1982). When the K_D for H_1-receptors was determined, 0.2 μM tetrodotoxin was present to mask any actions of 4-MH on H_2-receptors Barker & Jones-Ebersole, 1982). Similarly when the K_D for H_2-receptors was determined, mepyramine (0.5 μM) was present to preclude actions at H_1-receptors. Agonist dissociation constants were obtained from a regression of equation (4)

$$[A']/[A] = ((1-q)/qK_D)[A'] + 1/q \quad (4)$$

where [A] and [A'] are the concentrations of agonist giving equal responses before and after partial inactivation of receptors, respectively; q is the fraction of active receptors remaining after treatment with the irreversible antagonist; and K_D is the apparent dissociation constant. Equation 4 is an algebraic rearrangement of the original equation of Furchgott (1966). Duplicate dose-response curves were generated by the sequential method before and after inactivation of a fraction of the active receptors.

The results are presented as mean and 95% confidence limits (tables) or ± s.e. mean (figures and text).

Drugs

All chemicals used were of reagent grade. L-643,441 was kindly provided by Dr R.G. Pendleton, Merck Sharp & Dohme Research Laboratories, West Point, PA. 4-MH and dimaprit were kindly provided by Dr C.R. Ganellin, Smith Kline and French Research, LTD, Welwyn Garden City. Tiotidine was kindly provided by Dr D. McCurdy, Stuart Pharmaceuticals Division of ICI Americas, Willmington, DE. Mepyramine was obtained from Pfaltz and Baer, Stamford, CT. Tetrodotoxin and 5-hydroxytryptamine (serotonin) were purchased from Sigma Chemicals, St Louis, MO, and dibenamine from ICN Pharmaceuticals, Plainview, NY. Substance P was obtained from Bachem Inc., Torrance, Ca. Histamine dihydrochloride and carbachol chloride were bought from Aldrich Chemicals, Milwaukee, WI. Carbachol was converted to the perchlorate salt and twice recrystallized from methanol before use.

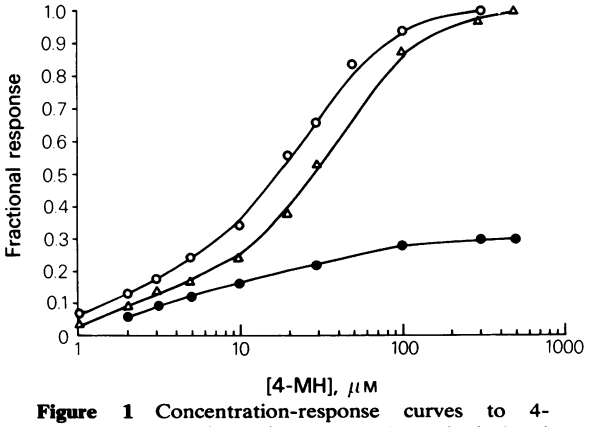

Figure 1 Concentration-response curves to 4-methylhistamine (4-MH) in the guinea-pig isolated ileum generated in the absence (○) or presence of either 0.5 μM mepyramine (●) or 15 μM tiotidine (△). Control curves were generated on adjacent segments of ileum taken from the same animal. One segment was subsequently treated with mepyramine and the other with tiotidine. Responses are expressed relative to the maximum histamine response. Data are from a single experiment.

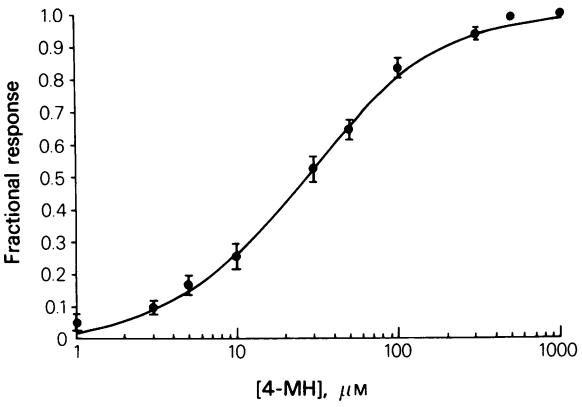

Figure 2 Mean concentration-effect curve to 4-methylhistamine (4-MH) at H_1-receptors in the guinea-pig isolated ileum as determined in the presence of 15 μM tiotidine ($n = 5$) or 0.2 μM tetrodotoxin ($n = 5$). Responses are expressed relative to the maximum histamine response. Data points and bars are mean and s.e.mean for 10 observations. Line is that predicted from equation 1 using the mean values of EC_{50} and nH (Table 1).

Results

Concentration-response parameters of 4-methylhistamine determined by the analyses of the individual components

Figure 1 shows the effect of selective blockade of either H_1- or H_2-receptors on the response to 4-MH. Concentration-response curves for the actions of 4-MH on H_1- and H_2-receptors are shown in Figures 2 and 3. The results of curve fitting analyses to determine EC_{50} values and nH for the actions of 4-MH at H_1- and H_2-receptors based on data obtained under conditions of selective blockade of either site are shown in Table 1. The EC_{50} value for 4-MH at H_1-receptors determined in the presence of tiotidine, 23.2 ± 6.8 μM ($n = 5$) was not significantly different from that determined in the presence of tetrodotoxin, 30.4 ± 4.0 μM ($n = 5$, $P > 0.05$, Student's t test). Data from both sets of experiments are presented as a single mean concentration-response curve in Figure 2.

The maximal response obtainable for the actions of

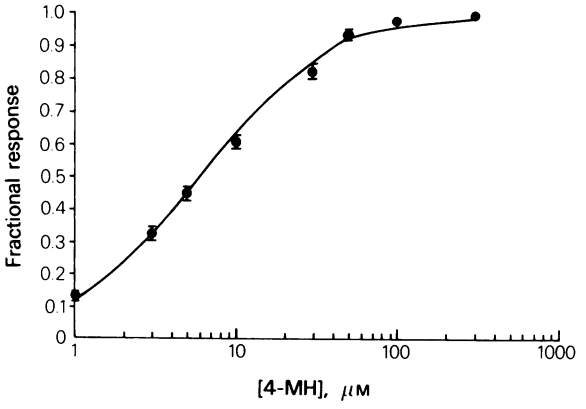

Figure 3 Mean concentration-effect curve to 4-methylhistamine (4-MH) at H_2-receptors in the guinea-pig isolated ileum as determined in the presence of 0.5 μM mepyramine. Responses are expressed relative to the maximum dimaprit response. Data points and bars are mean with s.e.mean for 17 observations. Line is that predicted from equation 1 using the mean values of EC_{50} and nH (Table 1).

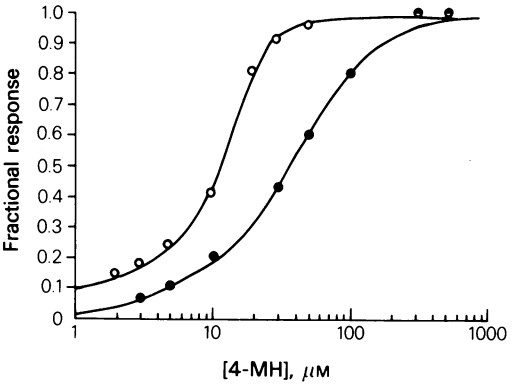

Figure 4 Representative results of curve fitting experiments. Data points are experimentally observed responses. Lines are lines of best fit obtained from iterative nonlinear regression analysis for fits to equation 3 (see methods).

4-MH at H_2-receptors was about 30% of that due to actions at H_1-receptors. The same was true for the actions of dimaprit on ileal H_2-receptors whether determined in the presence or in the absence of 0.5 μM mepyramine (data not shown). Relative to histamine, 4-MH was a full agonist at H_1-receptors.

Concentration-response parameters of 4-MH estimated by computer analyses of dose-response curves generated to 4-MH in the absence of histamine receptor antagonists

In 7 out of 10 experiments, the results were described quite well by a fit to equation (1). Although such analyses gave statistically good fits, they were inadequate because such a model does not reflect the physical reality of the system as revealed by the experiments described above. The results of iterative non-linear regression analyses of data obtained in the absence of either tiotidine or mepyramine for fits to equation (3) are shown in Table 2. The data from ten experiments were analysed. In each case, the concentration-response curve predicted by equation 3 showed excellent agreement with the experimentally observed results. Examples of fits to two individual experiments are shown in Figure 4. The two-stage fitting procedure produced curves that agreed well with the observed results and satisfied the criteria of a significant correlation coefficient and random distribution of residuals. The estimates of the EC_{50} values for agonism by 4-MH at H_1- and H_2-receptors obtained by this method agreed well with those obtained from an analyses of the individual components. However, the average of estimates of the slope parameter, nH, for agonism at H_1-receptors obtained from fits of data obtained in the absence of antagonists was double that obtained from fits of data generated in the presence of tiotidine. No such disparity was observed between the two methods for estimating the slope parameter for actions of 4-MH at H_2-receptors.

Table 1 Dose-response parameters for the independent interaction of 4-methylhistamine with ileal histamine receptors

			Receptor	
	n	H_1	*n*	H_2
$-\log ED_{50}$	10	4.57 (4.45-4.74)	17	5.23 (5.18-5.29)
E_{max}	10	1.0	10	0.30 (0.16-0.44)
n_H	10	1.06 (1.0-1.12)	17	1.10 (1.03-1.17)
$-\log K_D$	5	3.55 (3.34-3.99)	8	4.27 (4.15-4.44)
e	5	10 (8-12)	8	10 (7-13)

Values are mean and 95% confidence limits; *n* = number of animals from which tissue was obtained; E_{max} is maximal response relative to tissue maximal histamine response; K_D is the dissociation constant; and e is efficacy as defined by Stephenson (1956), $e = (K_D + EC_{50})/EC_{50}$.

Table 2 Dose-response parameters obtained from fits to the equation, $E = E_1 + E_2 - E_1E_2/E_{max}$

	Receptor	
	H_1	H_2
$-\log ED_{50}$	4.72 (4.70-4.73)	5.49 (5.36-5.68)
n_H	2.1 (1.4-2.8)	1.1 (0.9-1.3)

Data are from 10 experiments. Values are mean and 95% confidence limits.

Dissociation constants of 4-methylhistamines for H_1- and H_2-receptors

The results of experiments in which the apparent dissociation constant of 4-MH for H_1- and H_2-receptors were determined are shown in Table 1. Figures 5 and 6 show the results of the inactivation of a fraction of either H_1- or H_2-receptors, respectively, on the response to 4-MH at these sites.

In separate experiments, the selectivity of L-643,441 was evaluated. Treatment of ilea with L-643,441, 50 nM for 15 min, did not affect dose-response curves generated to carbachol, 5-HT or substance P and did not alter the response of the ileum to electrical field stimulation. In each case, three experiments were performed.

Discussion

Previous estimates of the selectivity of 4-MH were based on a comparison of its activity relative to that of histamine on ileal H_1- and atrial H_2-receptors (Black *et al.*, 1972; Durant, Ganellin & Parsons., 1975). The ratios of EC_{50} values, i.e. histamine/4-MH, were 0.0023 and 0.41 respectively. At first approximation, this suggests that 4-MH is about 200 times more potent at H_2- than at H_1-receptors. However, the value of 200 is not a ratio of the EC_{50} values for 4-MH actions at H_1- and H_2-receptors. Instead it is that ratio multiplied by the ratio of EC_{50} values, H_2/H_1, for histamine. Since histamine is about 10 times more potent at H_1- than H_2-receptors, it is likely that the potency of 4-MH at H_2-receptors exceeds that at H_1-receptors by no more than a factor of 20.

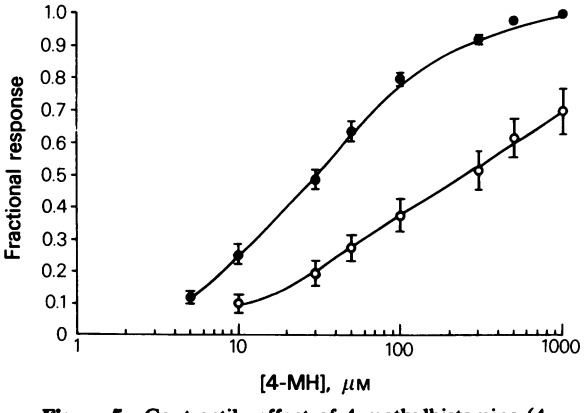

Figure 5 Contractile effect of 4-methylhistamine (4-MH) on the ileum at H_1-receptors before (●) and after (○) a 30 min incubation with 1 μM dibenamine. Responses are relative to the maximum histamine response. Data points and bars are mean with s.e.mean for 5 experiments.

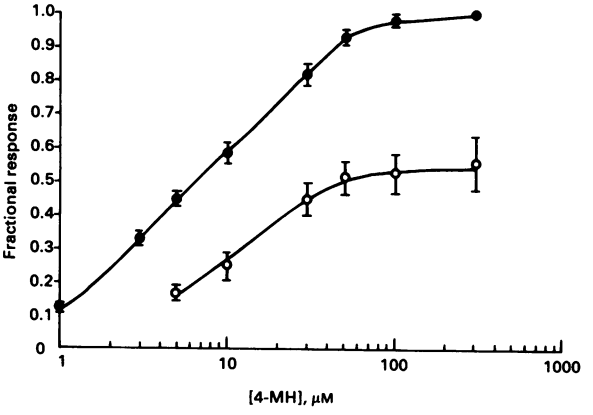

Figure 6 Contractile effect of 4-methylhistamine (4-MH) on the ileum at H_2-receptors before (●) and after (○) a 15 min incubation with 50 nM L-643,441. Responses are relative to the maximum dimaprit response. Data points and bars are mean with s.e.mean for 9 experiments.

The results of the present study show that 4-MH is only about 5 times more selective for H_2- than H_1-receptors in the guinea-pig ileum. These results were obtained from experimental determinations of the EC_{50} values for the independent interaction of 4-MH with H_1- and H_2-receptors as well as from computer analyses of dose-response curves generated in the absence of either mepyramine or tiotidine.

The results of curve fitting experiments differ from those of the analyses of the individual components in one major respect which was in the estimation of the slope parameter, nH, for the H_1- component. The results of the curve fitting analyses gave a value of 2.1 and those from the analyses of the H_1-component in isolation gave a value of 1.1. The basis for this difference is not clear. In the presence of tiotidine at 1000 times its K_B value, 4-MH at the concentration range used would exert negligible actions at H_2-receptors. Under these conditions, equation (3) reduces to equation (1). Thus, one would expect that the two analyses would yield similar estimates for nH values as was the case for EC_{50} values. The lack of agreement on estimations of the slope parameter for

agonism at H_1-receptors may indicate that the two-receptor model is not valid. Alternatively, the model may be appropriate and the results have revealed an interaction between the two receptors and their effector systems that could not be predicted by an investigation of the individual receptors in isolation.

Because the maximal obtainable response due to activation of H_2-receptors is less than that for H_1-receptors, there may be some objection to determining the relative potency of 4-MH at these sites by a ratio of EC_{50} values. For this reason, the apparent dissociation constants for the interaction of 4-MH with ileal histamine receptors were determined. The results of these experiments also showed that the selectivity of 4-MH for H_2-receptors is only about 5 times that for H_1-receptors.

The theoretical basis for the pharmacological determination of agonist dissociation constants has been discussed in detail (Furchgott, 1966; Furchgott & Bursztyn, 1967). Of particular importance to the present study is the requirement that the irreversibly acting antagonist alters the sensitivity of the effector to the agonist only by reducing the concentration of active receptors for the agonist and not by actions at other sites in the effector chain (Furchgott, 1966). The utility of dibenamine for the pharmacological estimation of apparent agonist K_D values at ileal H_1-receptors has been established (Furchgott, 1966). Because the contractile respose by the ileum to H_2-agonists is mediated by the sequential release of 5-HT, substance P and acetylcholine with an involvement of a product(s) of the arachadonic acid cascade (Barker & Jones-Ebersole, 1982), it was necessary to establish that L-643,441 did not alter the response to these agents. At the concentration and time of exposure used in these studies, L-643,441 produced no measurable effects at receptors mediating responses to 5-HT, substance P or acetylcholine. In addition, L-643,441 did not alter the response of the ileum to electrical field stimulation; showing that it did not impair the response of the ileum to H_2-agonists by a mechanism similar to that of cyclo-oxygenase inhibitors (Barker & Jones-Ebersole, 1982). These results validate the use of L-643,441 in the present studies.

The results of the present study show that 4-MH is selective for H_2-receptors, but that the selectivity is too low for the utilization of 4-MH alone in the characterization of responses mediated by H_2-receptors. The selectivity ratio for 4-MH agonism at H_1- and H_2-receptors in other tissues will depend on the relative concentration of the two receptor populations. In tissues where the relative concentrations of H_1- and H_2-receptors are not known, 4-MH should be used with caution to characterize functions mediated by H_2-receptors.

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References

ARIENS, E.J., VAN ROSSUM, J.M. & SIMONIS, A.M. (1956). A theoretical basis of molecular pharmacology. *Arzneim. Forsch.*, **6**, 737–746.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug agonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

ASH, A.S.F. & SCHILD, H.O. (1966). Receptors mediating some actions of histamine. *Br. J. Pharmac. Chemother.*, **27**, 427–439.

BAIG, H. & REID-MILLER, M. (1980). *PROPHET Statistics–A User's Guide to Statistical Analysis on the PROPHET System.* ed. Kush, T., Bethesda: NIH Publication No. 80-2169.

BARKER, L.A. (1981). Histamine H1-and muscarinic receptor antagonist activity of cimetidine and tiotidine in the guinea pig isolated ileum. *Agents and Actions*, **11**, 699–705.

BARKER, L.A. & HOUGH, L.B. (1982). Relative histamine H1- and H2-agonist activity of 4-methylhistamine (4-MH) on the guinea pig ileum. *Pharmacologist*, **24**, 123.

BARKER, L.A. & JONES-EBERSOLE, B. (1982). Histamine H2-receptors on guinea-pig ileum myentric plexus neurons mediate the release of contractile agents. *J. Pharmac. exp. Ther.*, **221**, 69–75.

BLACK, J.W., DUNCAN, W.A.M., DURANT, G.J., GANELLIN, C.R. & PARSONS, M.E. (1972). Definition and antagonism of histamine H2-receptors. *Nature, Lond.*, **236**, 485–490.

CHANG, R.S.L., TRAN, V.T. & SYNDER, S.H. (1979). Characteristics of histamine H1-receptors in peripheral tissues labelled with [^{3}H]mepyramine. *J. Pharmac. exp. Ther.*, **209**, 437–442.

DURANT, G.J., GANELLIN, C.R. & PARSONS, M.E. (1975). Chemical differentiation of histamine H1- and H2-receptor agonists. *J. med. Chem.*, **18**, 905–909.

FURCHGOTT, R.F. (1966). The use of beta-haloalkylamines in the determination of dissociation constants of receptor-agonist complexes. In *Advances in Drug Research*, Vol. 3. ed. Harper, N.J. & Simmonds, A.B. pp. 21–55. London: Academic Press.

FURCHGOTT, R.F. & BURSZTYN, P. (1967). Comparison of dissociation constants and of relative efficies of selected agonists acting on parasympathetic receptors. *Ann. N.Y. Acad. Sci.*, **144,** 882–899.

HEILBRON, J. (1981). *Science and Engineering Programs.* Apple II Edition. Berkley: Osborne/McGraw Hill.

HOUGH, L.B., WEINSTEIN, H. & GREEN, J.P. (1980). One agonist and two receptors mediating the same effect: histamine receptors linked to adenylate cyclase in the brain. In *Receptors for Neurotransmitters and Peptide Hormones.* ed. Pepeu, G. & Kuhar, M. pp. 183–192. New York: Raven Press.

PENDELTON, R.G., TORCHIANA, M.L., C.A. & CLINES-CHMIDT, B.V. (1982). 3-N-[3-[3-(1-piperidinomethyl) phenoxy]propyl]amino-4-amino-1,2,5-thiadiazole-1-oxide (L-643,441) as a competitive, irreversible histamine H2-receptor antagonist. *Fedn. Proc.*, **41,** 1555.

STEPHENSON, R.P. (1956). A modification of receptor theory. *Br. J. Pharmac. Chemother.*, **11,** 379–392.

YELLIN, T.O., BUCK, S.H., GILMAN, D.J., JONES, D.J. & WARDLEWORTH, J.M. (1979). ICI 125,211: A new gastric antisecretory agent acting on histamine H2-receptors. *Life Sci.*, **25,** 2001–2009.

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