Agents Actions, *Special Conference Issue* (1992)

0065-4299/92/02C428-03 $ 1.50+0.20/0

Effects of dimethindene maleate (Fenistil®) on histaminic, muscarinic and serotoninergic receptor systems

M. Sautel, R. Towart, M. Théraulaz-Lacroix and A. F. Weitsch

Innovation Department, Zyma Pharma SA, CH-1260 Nyon, Switzerland

Abstract

The effects of dimethindene maleate (DM) on histamine H_1, muscarinic and serotonin receptor systems were studied, using ligand binding studies (guinea-pig cerebral cortex membranes) and functional studies (guinea-pig ileum). DM was very potent at histamine H_1 receptors ($K_i = 1.5 \times 10^{-9}$ *M*, $pA_2 = 9.33$) using either method. DM showed a lower affinity for muscarinic receptors and a very low affinity for serotonin receptors. The only discrepancy found between receptor binding studies and functional tests concerned the activity of DM on muscarinic receptors where the K_i for binding studies using ^{3}H-pirenzepine was 6.4×10^{-8} *M*, but the pA_2 for the carbachol-stimulated ileum was 6.7. As the guinea-pig ileum possesses a rather low level of M_1 muscarinic receptors when compared to M_2 and M_3 muscarinic receptors, the difference observed indicates that DM is more potent at M_1 than other muscarinic receptors.

Introduction

Dimethindene maleate (DM) is a widely-used antihistamine which is effective in many allergic diseases such as pruritus, rhinitis, etc. [1], and is a potent antagonist of the histamine H_1 receptors. Many antihistamines also show some activity on other receptor systems such as serotoninergic and muscarinic receptors, which explains commonly observed side effects such as weight gain or dry mouth. In order to characterize the receptor profile of DM, we have investigated the effects of DM on histamine H_1, muscarinic and serotonin receptor systems using ligand binding assays on cerebral cortex membranes and functional pharmacology using stimulated guinea pig ileum.

Materials and methods

Male Hartley guinea-pigs, weighing 250–300 g and fed a standard diet, were used in this study. Displacement of ^{3}H-mepyramine [2], ^{3}H-pirenzepine [3], and ^{3}H-ketanserin [4] were performed on guinea-pig cerebral cortex membranes to determine the affinity of DM for H_1, M_1 and 5-HT_2 receptors, respectively. K_i values were calculated using the programme EBDA-Ligand (Biosoft, Cambridge).

The effects of DM on contractions of the isolated guinea-pig ileum induced by histamine and carbachol were assessed by standard techniques using an agonist exposure of 30 sec on a 3 min dose cycle. After 2 control histamine or carbachol dose-response curves (DRCs), DM was then added in concentrations ranging from 10^{-10} *M to* 10^{-5} *M*. The potency of DM in displacing histamine and carbachol DRCs was expressed as pA_2 values for competitive displacement and pD'_2 values for non-competitive displacement of the DRCs [5, 6].

For serotonin, there is a marked desensitization of receptors after the first serotonin DRC when using standard methods. To investigate the effects of DM,

the technique of Eglen et al. [7] was used and the ileum was allowed a 60 min recovery period after the first serotonin DRC. During this recovery time the bathing solution was replaced every 15 min. After that, only one single concentration of DM was applied to each ileal segment. A serotonin DRC without DM was run in parallel to correct for possible changes in the 5-HT receptor sensitivity and was taken as the control DRC.

Chemicals

Tritiated ligands were purchased from DuPont New England Nuclear (Boston, MA). All other chemicals were from Merck and Sigma. (±) Dimethindene maleate was supplied by Dr E. Moret, Zyma Pharma SA. In this study, dimethindene was diluted in the appropriate buffer in a concentration range of 10^{-10} to 10^{-4} *M*.

Results

Using ligand binding studies (see Table 1) in cerebral cortex membranes, DM showed approximately the same affinity ($K_i = 1.5 \times 10^{-9}$ *M*) for H_1 receptors as the reference compound mepyramine ($K_i = 9.7 \times 10^{-10}$ *M*).

DM showed a much lower affinity for M_1 ($K_i = 6.4 \times 10^{-8}$ *M*) and for 5-HT_2 ($K_i = 2.4 \times 10^{-6}$ *M*) receptors than for H_1 receptors (ca. 40 and 1500 times less, respectively).

Using functional studies (see Table 1), DM produced a dose-dependent displacement of the histamine DRCs [8]. At the lower concentrations DM acts competitively, showing a rightward shift of the histamine DRCs ($pA_2 = 9.33$), followed at higher concentrations by a decrease in the maxima ($pD'_2 = 8.01$) indicating a non-competitive antagonism at high DM concentrations.

Table 1
Calculated K_i (ligand binding studies), pA_2 and pD'_2 (guinea-pig ileum contractions, from [8]) values for DM on histamine, muscarinic and serotonin receptor systems.

Receptor system	K_i (*M*)	pA_2	pD'_2
Histamine	$1.5\,10^{-9}$	9.33	8.01
Muscarinic	$6.4\,10^{-8}$	6.7	<5
Serotonin	$2.4\,10^{-6}$	–	<6

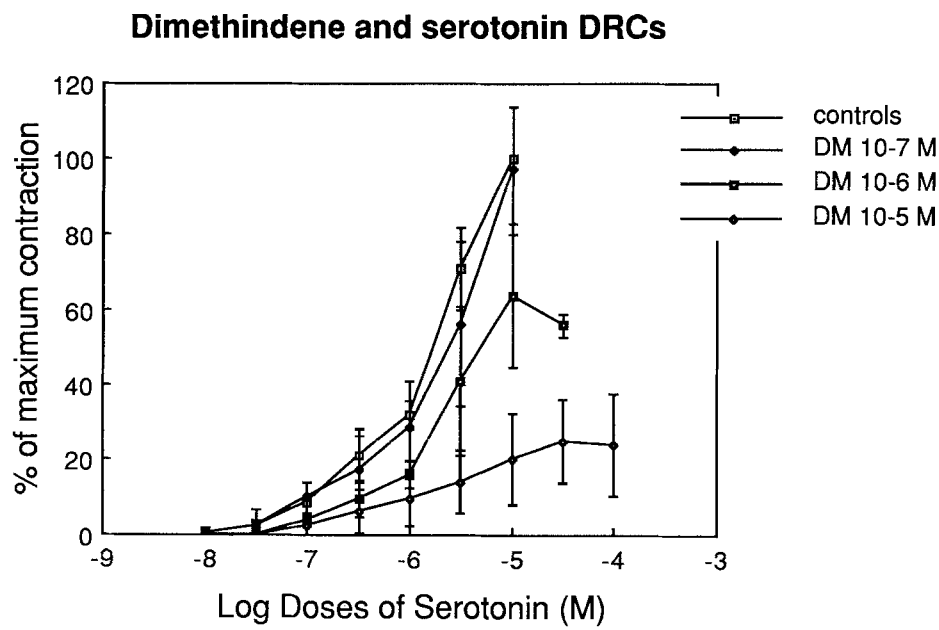

Figure 1
Serotonin dose-response curves (guinea-pig ileum) expressed as percent of the maximum response to serotonin after 3 different doses of DM. The serotonin dose-response (mean ± SD) curves were obtained as described in Methods.

Considering the carbachol-induced contraction of guinea-pig ileum, DM showed a classical competitive antagonism [8] characterized by a parallel rightward shift of the carbachol DRCs at concentrations up to 10^{-5} *M*, giving a pA_2 of 6.7, about 400 times less than the pA_2 calculated from the antagonism of the histamine DRC.

Concerning the effect of DM on the serotonin DRCs, the described methodology allowed us to study the effect of DM on serotonin DRCs, without any further desensitization of serotonin receptors induced by serotonin itself after a 60 min recovery period. Little displacement of the serotonin DRC was observed up to DM concentrations of 10^{-6} *M* and inhibition was only marked with 10^{-5} *M* (see Fig. 1).

Discussion

DM is a potent antihistaminic widely used against allergic diseases. In this study, we have shown that DM has a high affinity (about the same order as the reference compound mepyramine) for the histamine H_1 receptor. Previous studies have shown that DM does not affect histamine H_2 and histamine H_3 receptors [8]. The high affinity shown using ligand binding was reflected by the high affinity shown by DM in functional tests using displacement of histamine DRCs.

The K_i for binding to muscarinic M_1 receptors ($K_i = 6.4 \times 10^{-8}$ *M*) was surprising as the pA_2 against carbachol-induced contractions was 6.7 and a previous ligand binding study of DM on muscarinic receptors had yielded low potency, with a K_i of about 6×10^{-6} *M* (Williams 1986, Ciba-Geigy Summit, unpublished results). However Williams used ^{3}H-QNB, a non-specific muscarinic M_1/M_2 receptor ligand, in bovine striatum membranes, a brain region containing mostly M_2 receptors. Also about 70% of muscarinic receptors located on the guinea-pig ileum are M_3 receptors [9]. Thus, the rather high affinity of DM for muscarinic receptors measured here seems to be restricted to the M_1 receptors but not to the M_2 and M_3 receptors. Salivary secretion is associated with M_2 or M_3 receptors [10], which are little affected by DM. Finally, DM showed a very low affinity for serotonin $5HT_2$ receptors using either ligand binding or functional studies.

Acknowledgement

We are indebted to Valerie Dufour for her excellent technical assistance in developing the serotonin-induced DRC assay.

References

[1] D. Rehn, H. Geissler, O. Schuster, H. Lukas and G. Hennings, *Effect-kinetic characterization of dimethindene maleate following oral administration (Fenistil, Tropfen).* Fundam. Clin. Pharmacol. *4,* 673–683 (1990).

[2] V. T. Tran, R. S. L. Chang and S. H. Snyder, *Histamine H_1 receptors identified in mammalian brain membranes with ^{3}H-mepyramine.* Proc. Natl. Acad. Sci. USA *75,* 6290–6294 (1978).

[3] M. Watson, H. I. Yamamura and W. R. Roeske, *A unique regulatory profile and regional distribution of ^{3}H-Pirenzepine binding in the rat provide evidence for distinct M_1 and M_2 muscarinic receptor subtypes.* Life Sci. *32,* 3001–3011 (1983).

[4] J. E. Leysen, C. J. E. Niemegeers, J. M. Van Nueten and P. M. Laduron, *^{3}H-Ketanserin (R 41468) a selective ^{3}H-ligand for serotonin$_2$ receptor binding sites. Binding properties, brain distribution and functional role.* Mol. Pharmacol. *21,* 301–314 (1982).

[5] H. O. Schild, *pA, a new scale for the measurement of drug antagonism.* Br. J. Pharmacol. *2,* 189–206 (1947).

[6] J. M. Van Rossum, *Cumulative dose response curves II. Techniques for the making of dose-response curves in isolated organs and the evaluation of drug parameters.* Arch. Int. Pharmacodyn. *143,* 299–330 (1963).

[7] R. M. Eglen, S. R. Swank, L. K. M. Walsh and R. L. Whiting, *Characterization of 5-HT3 and "atypical" 5-HT receptors mediating guinea-pig ileal contractions in vivo.* Br. J. Pharmacol. *101,* 513–520 (1990).

[8] R. Towart, M. Sautel, E. Moret, E. Costa, M. Theraulaz and A. F. Weitsch, *Investigation of the antihistaminic action of Dimethindene maleate (Fenistil®) and its optical isomers.* In *New perspectives in histamine research* (Ed. H. Timmerman and H. Van der Goot) pp. 403–408, Birkhäuser, Basel 1991.

[9] R. L. Whiting, A. P. D. W. Ford, G. S. Baxter, G. C. Harris, N. A. Sharif and R. M. Eglen, *Pharmacological and biochemical analysis of muscarinic receptors in guinea-pig ileum in vitro.* Br. J. Pharmacol. *102,* 19P (1991).

[10] R. E. Delmondo, A. D. Michel and R. L. Whiting, *Affinity of muscarinic receptor antagonists for three putative muscarinic receptor binding sites.* Br. J. Pharmacol. *96,* 457–464 (1989).