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INHIBITION OF HISTAMINE RELEASE FROM HUMAN LUNG *in vitro* BY ANTIHISTAMINES AND RELATED DRUGS

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1 A series of cationic, lipophilic histamine H_1-receptor antagonists, neuroleptics, antidepressants and monoamine oxidase inhibitors were tested for their effects on anti-IgE-induced histamine release from human lung fragments *in vitro*.

2 They had a biphasic effect: at low concentrations a dose-related inhibition of histamine release was observed whereas, at higher concentrations, the drugs liberated histamine even in the absence of antigen.

3 Mepyramine, a much less lipophilic drug than the others tested, was only weakly active on mast cells at pharmacological concentrations.

4 The potency of the drugs as release inhibitors was not related quantitatively to their histamine liberating potency.

5 There was no correlation between activity on mast cells and histamine H_1-receptor antagonism.

6 Mast cell stabilization may play a part in the activity of these drugs as anti-allergic agents.

Introduction

Antihistamines and related structures have been known for some years both to inhibit anaphylactic histamine release and to act as histamine liberators (Arunlakshana, 1953; Mota & Dias da Silva, 1960). However, the potency of these drugs in producing these effects does not appear to be correlated with their histamine H_1-receptor blocking activity (Lichtenstein & Gillespie, 1975; Guschin, Deryugin & Kaminka, 1978). More recently, two new histamine H_1-receptor blocking drugs, oxatomide and ketotifen, which may be useful in the treatment of hay fever and asthma, have been found to inhibit anaphylactic histamine release from animal tissues (De Beule, Vannieuwenhuyse, Callier, Verstraete, Degreef, Gregoire, Robience, Stevens & Liebert, 1977; Craps, Greenwood & Radielovic, 1978; Martin & Roemer, 1978; Borgers, De Brabander, Van Reempts, Awouters & Janssen, 1978; De Clerk, Van Gorp, Vanparijs, Borgers & Awouters, 1978).

In this paper the effects are examined of a series of classical antihistamines, tricyclic antidepressants, neuroleptics and monoamine oxidase inhibitors, all compounds with histamine H_1-receptor blocking and local anaesthetic activity, on inhibition of histamine release and drug-induced histamine liberation from human lung fragments *in vitro*. A comparison of these activities with histamine H_1-receptor blocking activity is also made.

Methods

Human lung obtained from lobectomy specimens was chopped finely with scissors, divided into 200 mg replicates and sensitized with 0.2 ml serum from an allergic donor, in a total of 2 ml Tyrode solution for 18 h at room temperature followed by 1 h at 37°C. Samples were then washed, resuspended in oxygenated Tyrode solution, brought to 37°C and challenged with anti-IgE (1/1000 dilution) in a final volume of 2.0 ml. After 15 min incubation, the supernatant was removed and the tissue frozen then thawed to release the remaining histamine. Histamine in the two solutions was assayed spectrofluorimetrically (Evans, Lewis & Thomson, 1973) and that released by anti-IgE expressed as a percentage of the original total histamine content of the lung sample. Samples incubated in the absence of anti-IgE were used to correct for spontaneous histamine release. Drugs under investigation were added 30 s before challenge with anti-IgE.

Because anti-IgE-induced histamine release by lungs from different donors varied markedly (14 to 34% of total lung histamine) histamine release in the

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absence of drug was designated as 100% for each experiment. All drug effects were calculated in terms of this figure.

Drugs

Mepyramine maleate, promethazine hydrochloride, chlorpromazine hydrochloride and trimeprazine tartrate were kindly donated by May and Baker, diphenhydramine hydrochloride by Parke Davis, chlorpheniramine maleate by Allen and Hanbury's, cyclizine hyrochloride by Burroughs Wellcome, amitriptyline hydrochloride and phenelzine sulphate by William R. Warner, cinnarizine and oxatomide by Janssen Pharmaceuticals and ketotifen hydrogen fumarate by Sandoz. All drugs were dissolved or suspended in Tyrode solution immediately prior to use. The anti-IgE used was anti-human IgE raised in the goat (Miles-Yeda).

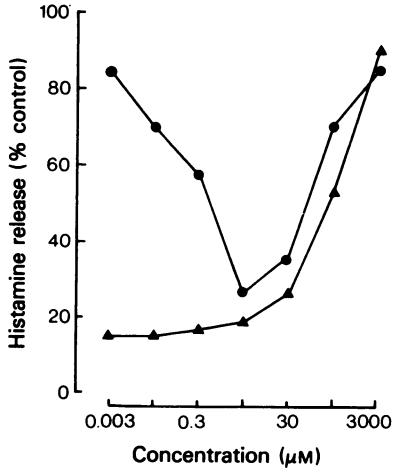

Figure 1 Effect of promethazine on histamine release from human lung. Each point is the mean of results obtained in three experiments. (●) Promethazine in the presence of anti-IgE; (▲) promethazine alone.

Results

All twelve drugs tested affected histamine release from passively sensitized human lung fragments *in vitro* in a qualitatively similar fashion (e.g. promethazine in Figure 1). At low concentrations they caused a linear dose-related inhibition of anti-IgE-induced histamine release. From this an IC_{50} value (the concentration of drug which reduced anti-IgE-induced histamine release by 50%) was calculated for each drug (Table 1). At higher concentrations, usually above 0.1 to 1.0 μM, the drugs caused a dose-related release of histamine both in the presence and absence of anti-IgE. The maximum amount of drug-induced histamine release was, in the majority of experiments, very close to that induced by anti-IgE, i.e. 14 to 34% of total lung histamine and in no cases significantly exceeded immunological release. For comparison of histamine releasing potencies of drugs, an RC_{50} (the concentration of drug which released an amount of histamine equivalent to 50% of that released by anti-IgE) was calculated for each drug (Table 1).

The least effective compound tested, mepyramine, inhibited histamine release induced by anti-IgE only in concentrations above 1 μM (Figure 2). Its IC_{50} was 850 μM. Mepyramine was also a very weak releaser of histamine, significant release only being attained at exceedingly high concentrations, around 0.3 M.

Table 1 IC_{50} and RC_{50} values of antihistamines and related drugs in human lung

Compound	*IC_{50} (μM)*	*RC_{50} (μM)*
Oxatomide	0.0045 (0.0028–0.0072)	21.32 (12.69–35.86)
Amitriptyline	0.0049 (0.0016–0.014)	4.24 (3.06–5.58)
Trimeprazine	0.019 (0.012–0.030)	1.03 (0.22–4.93)
Chlorpheniramine	0.036 (0.017–0.077)	384.80 (127.32–1163.06)
Ketotifen	0.052 (0.028–0.096)	24.87 (8.96–69.05)
Chlorpromazine	0.12 (0.040–0.39)	1.10 (0.53–2.25)
Promethazine	0.34 (0.22–0.53)	109.95 (53.68–225.23)
Phenelzine	0.55 (0.19–1.55)	51.48 (31.16–71.32)
Diphenhydramine	1.46 (0.57–3.72)	73.60 (41.72–129.8)
Cinnarizine	1.87 (0.30–11.30)	38.40 (26.82–55.96)
Cyclizine	5.42 (0.14–20.44)	10.81 (5.67–20.59)
Mepyramine	855.0 (657.1–1191.8)	>100,000

IC_{50}: concentration of drug calculated to reduce anti-IgE-induced histamine release by 50% (with standard error limits). RC_{50}: concentration of drug calculated to release an amount of histamine equivalent to that released by anti-IgE (with standard error limits). Each value is derived from results obtained in 3 to 6 experiments.

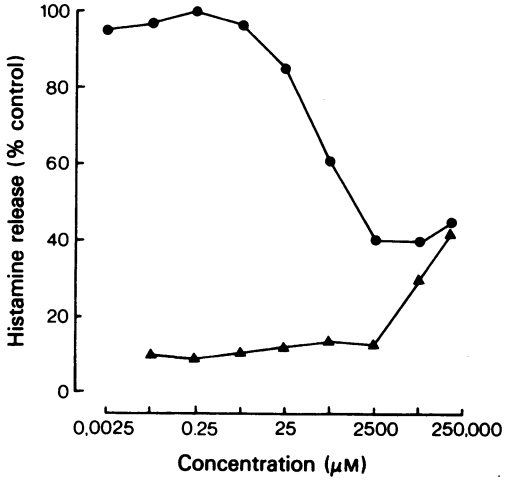

Figure 2 Effects of mepyramine on histamine release from human lung. Each point is the mean of results obtained in five experiments. (●) Mepyramine in the presence of anti-IgE; (▲) mepyramine alone.

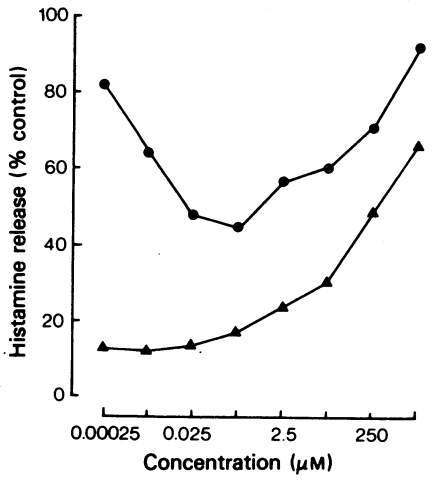

Figure 3 Effects of chlorpheniramine on histamine release from human lung. Each point is the mean of results obtained in five experiments. (●) Chlorpheniramine in the presence of anti-IgE; (▲) chlorpheniramine alone.

Chlorpheniramine differed from the general pattern of activity in that drug-induced histamine release in the presence and absence of anti-IgE was significantly different (Figure 3). This suggests that immunological histamine release was never completely suppressed by the drug. Consequently, apparent drug-induced release in the presence of anti-IgE is the sum of true drug-induced release plus residual immunological release.

Table 1 shows that there is no correlation ($r = 0.03$) between the ability of a drug to inhibit anti-IgE-induced histamine release (IC_{50}) and to release histamine (RC_{50}). The most potent inhibitors were oxatomide and amitriptyline with IC_{50}s of 4.5 and 4.9 nM respectively. The most potent releasers were trimeprazine and chlorpromazine with RC_{50}s of 1.03 and 1.10 μM respectively.

Furthermore, there is no correlation between

Table 2 Comparison of histamine H_1-receptor blocking activity with inhibition of anti-IgE-induced histamine release and drug-induced release

	H_1-receptor blocking activity (pA_2)	*Inhibition of anti-IgE induced histamine release ($-\log IC_{50}$)*	*Drug-induced histamine release ($-\log RC_{50}$)*
Oxatomide	8.43	8.35	4.67
Amitriptyline	8.29*	8.31	5.37
Trimeprazine	8.11*	7.72	5.99
Chlorpheniramine	8.83	7.44	3.41
Ketotifen	9.49	7.28	4.60
Chlorpromazine	8.14	6.92	5.96
Promethazine	8.84	6.47	3.96
Phenelzine	—	6.26	4.29
Diphenhydramine	7.59	5.84	4.13
Cinnarizine	7.43	5.73	4.42
Cyclizine	7.62	5.27	4.79
Mepyramine	8.97	3.07	—

pA_2 values (Arunlakshana & Schild, 1959), calculated from displacement of histamine dose-response lines on guinea-pig ileum, were supplied by Dr J.M. Van Nueten. * $-\log IC_{50}$, calculated from antagonism of a single maximal dose of histamine (Schild, 1957).

histamine H_1-receptor blocking potency of this series of drugs, as assessed from pA_2 values on guinea-pig ileum, and either their capacity to inhibit histamine release ($r = 0.01$) or their histamine releasing potency ($r = 0.06$) (Table 2).

Discussion

The results show that this series of antihistamines and structurally related compounds are potent inhibitors of anti-IgE-induced histamine release from passively sensitized human lung fragments *in vitro*. The most active compounds, oxatomide and amitriptyline (IC_{50}s 4.5 and 4.9 nM respectively) are, in this system, of comparable potency to the β-adrenoceptor stimulants, salbutamol and isoprenaline (IC_{50}s 1.6 and 32 nM respectively; Church, unpublished data) and some 20,000 times more potent than sodium cromoglycate (IC_{50} 92.5 μM; Church & Gradidge, 1980). However, unlike β-adrenoceptor stimulants and sodium cromoglycate, the concentration-response curves for the antihistamines were biphasic, an inflexion occurring at concentrations which inhibited histamine release by 50 to 65%. At higher concentrations, drug-induced histamine release occurred.

The concentrations of drugs required to inhibit immunological histamine release were not related quantitatively to the concentrations required for drug-induced release, e.g. the ratio between IC_{50} and RC_{50} for oxatomide was 1:4700 whereas that for chlorpromazine was 1:9. This suggests that different structure-activity relationships may apply for inhibition of histamine release and drug-induced release. If this is so, it may be possible to design a drug of this type which inhibits release but does not liberate histamine at higher concentrations.

The finding that neither inhibition of histamine release nor histamine liberating potency was correlated with histamine H_1-receptor blocking potency indicates that the effects of these drugs on lung mast cells are not mediated through histamine H_1-receptors. Similar conclusions have been reached from effects of antihistamines, tricyclic antidepressants, neuroleptics and local anaesthetics on drug-induced histamine release and on inhibition of degranulation of rat mast cells (Frisk-Holmberg & Van der Kleijn, 1972; Kazimierczak, Peret & Maslinski, 1976; Guschin *et al.*, 1978; Johnson & Miller, 1979). Frisk-Holmberg & Van der Kleijn (1972) suggest that the mast cell histamine liberating activity of tricyclic compounds is governed primarily by their lipophilicity. Our observations that highly lipophilic tricyclic compounds are amongst the most potent tested and that mepyramine, a much less lipophilic compound (Rooney, Gore & Lee, 1979), was the weakest compound, supports this hypothesis.

The biphasic effects of these drugs may be explained by the observations of Seeman (1972) that cationic local anaesthetic drugs, a class which includes all the drugs tested here, readily adsorb to biomembranes and competitively displace calcium, whereas at higher concentrations they dissolve in membranes which they expand and eventually disrupt. The former effect may reduce calcium entry into mast cells and hence inhibit degranulation (Foreman & Mongar, 1972). Membrane disruption at higher concentrations has been suggested as the mechanism of drug-induced release (Frisk-Holmberg, 1971).

The mast cell stabilizing activity of the more potent drugs tested is in the same concentration range as their histamine H_1-receptor blocking activity and may therefore contribute towards their clinical anti-allergic activity. This may be the case in hay fever, where histamine is a major mediator and where H_1-receptor antagonists provide a very effective treatment. In asthma, where the role of histamine as a mediator is less clear, antihistamines are generally considered to be of little value in treatment (Lancet, 1955). However some beneficial effects have been reported: (1) Amitriptyline and ketotifen have been shown to be of some benefit in chronic asthma (Sugihara, Ishiara & Noguchi, 1965; Meares, Mills, Horvath, Atkinson, Pun & Rand, 1971; Göbel, 1978; Craps *et al.*, 1978). (2) In antigen and exercise provocation tests, the bronchospasm following challenge may be reduced by antihistamines (Booij-Noord, Orie, Berg & de Vries, 1970; Girard & Cuevas, 1977; Zielinski & Chodosowska, 1977; Eiser, Guz, Mills & Snashall, 1979). (3) Antihistamines cause bronchodilatation in asymptomatic chronic asthmatics (Popa, 1977; Nogrady, Hartley, Handslip & Hurst, 1978). It is tempting to speculate that prevention of mast cell degranulation contributes to the beneficial effects of antihistamines in asthmatics.

Bronchial irritation and spasm occur following inhalation of aerosols containing high concentrations (about 3%) of antihistamines but not with aerosols containing less than 1% antihistamine (Charlier & Philippot, 1949; Herxheimer, 1952). This irritation may be due to drug-induced histamine release. After systemic administration, drug-induced histamine release is unlikely to occur because the required tissue concentrations may not be reached.

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