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A COMPARISON OF THE *IN VIVO* EFFECTS OF KETOTIFEN, CLEMASTINE, CHLORPHENIRAMINE AND SODIUM CROMOGLYCATE ON HISTAMINE AND ALLERGEN INDUCED WEALS IN HUMAN SKIN

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1 The effect of ketotifen was compared with that of clemastine and chlorpheniramine, known antihistamines, and sodium cromoglycate, a drug considered to have mast cell 'stabilizing' properties on histamine and allergen wealing reactions in human skin, in random order, double-blind, placebo controlled studies.

2 Ketotifen was significantly more potent in the inhibition of both histamine ($P < 0.001$) and allergen ($P < 0.001$) skin wealing reactions than either clemastine or chlorpheniramine. Sodium cromoglycate had no significant effect on either histamine or allergen skin wealing reactions in any of the concentrations tested.

3 However ketotifen, like clemastine, had a significantly greater inhibitory effect on histamine than on allergen induced weals ($P < 0.001$) and both drugs were shown to act as competitive antagonists of histamine.

4 Ketotifen has been shown to be a potent anti-histamine but there is no evidence from these *in vivo* studies to suggest that it has any additional inhibitory activity on release of mediators from mast cells in human skin.

Introduction

Ketotifen is a benzocycloheptathiophene derivative, currently used in the treatment of asthma. Although studies have demonstrated its effectiveness in clinical practice in both adults and children (Lane, 1981; Kelly & Taylor, 1982) its mode of action remains uncertain. In a series of animal experiments, Martin & Romer (1978) showed that ketotifen had anti-histaminic properties in the guinea pig, cat, and in monkey skin. They also found that ketotifen and clemastine were equipotent at inhibiting histamine induced responses in rat skin but that only ketotifen inhibited passive cutaneous anaphylaxis (PCA). Both drugs inhibited the allergen-induced increase in airways resistance in the anaesthetised, sensitised rat, but ketotifen had a 90-fold greater potency than clemastine. Furthermore, ketotifen, at high concentrations, inhibited 48/80 induced histamine release from rat isolated peritoneal mast cells whereas clemastine tended to enhance release. They suggested, from this evidence, that ketotifen differed significantly from other antihistamines such as clemastine in its ability to inhibit mediator release. They also found evidence of phosphodiesterase inhibition but only insignificant anti-5-HT and anticholinergic activity in animal studies. Extrapolation of such results to humans should only be made with caution since species differences in drug effects do occur. Differences have also been shown in the effects of drugs on immediate hypersensitivity reactions in different tissues within the same species (Lichtenstein *et al.*, 1979).

The skin is an organ in which allergic reactions frequently occur, and has the advantage of allowing multiple drug comparisons to be performed simultaneously in the same subject. The purpose of this study was to evaluate the *in vivo* activity of ketotifen on allergen and histamine reactions in human skin by comparison with the effects of clemastine and chlorpheniramine, known anti-histamines, and sodium cromoglycate (SCG) a drug thought to have 'anti-allergic' properties.

Methods

Subjects

All the subjects studied were atopic. The criterion for

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atopy was the presence of one or more wealing responses of 2 mm diameter or greater on skin prick testing with 5 common allergens with no wealing response to the control solution. None of the subjects was taking any oral medication and informed consent was obtained from each.

Drugs and their administration

Drugs were obtained in solution at the following concentrations; sodium cromoglycate at 3.9×10^{-2} mol l^{-1}, clemastine at 2.2×10^{-3} mol l^{-1}, chlorpheniramine at 2.5×10^{-2} mol l^{-1} and ketotifen at 1.1×10^{-3} mol l^{-1}. All subsequent dilutions were made with 0.9% weight to volume (w/v) sodium chloride. All the drug solutions and the control solution (sodium chloride 0.9% w/v), were administered by intradermal injection of 0.05 ml using a 1 ml tuberculin syringe with a 25 gauge 16 mm sabre needle. All drug comparisons in an individual were performed concurrently, with the sites of drug administration distributed over the volar surface of the forearms.

Agonists and their administration

The following allergen solutions (w/v) were obtained from Bencard Ltd (Brentford, UK): *Dermatophagoides pteronyssinus* 1.2%, grass pollen 2.5%, cat fur 150%, dog hair 150% and horse hair 150%. All dilutions were made up on the day of administration. Histamine acid phosphate was prepared on the test days at concentrations of 256 mg ml^{-1}, 128 mg ml^{-1}, 64 mg ml^{-1}, 32 mg ml^{-1}, 16 mg ml^{-1}, 8 mg ml^{-1} and 4 mg ml^{-1}, in control solution.

Histamine and allergen solutions were administered over the site of previous drug administration using the modified skin prick test technique (Squires, 1950).

Measurement of skin prick test response

The weal area was measured by tracing its perimeter in biro and transferring the outline onto graphical paper using adhesive tape. The area was calculated and expressed in mm^2. Measurements were made at 10 min after prick testing with histamine and 12 min after prick testing with allergen solution in order to record the maximum weal response while still retaining good definition of the outline.

The effect of the time interval between drug administration and prick testing

Table 1 shows the drugs used in this study and their concentrations. Histamine was administered at 16 mg ml^{-1} to ten subjects and allergen in a two-fold dilution of the stock concentration to ten subjects. The time intervals between duplicate intradermal drug

Table 1 The drugs and their concentrations used in the four studies

Drugs	*Histamine (mol l^{-1})*	*Allergen (mol l^{-1})*
1. The effect of the time interval between drug administration and skin prick testing on the weal area		
Clemastine	1×10^{-6}	1×10^{-5}
Ketotifen	1×10^{-6}	1×10^{-5}
Chlorpheniramine	2.5×10^{-3}	2.5×10^{-3}
SCG	3.9×10^{-6}	3.9×10^{-4}
2. Drug dose-response relationships with histamine and allergen induced skin weals		
Clemastine	2×10^{-4}, 2×10^{-5}, 2×10^{-7}, 2×10^{-9}, 2×10^{-11}, 2×10^{-13}	
Ketotifen	1×10^{-4}, 1×10^{-5}, 1×10^{-7}, 1×10^{-9}, 1×10^{-11}, 2×10^{-13}	
Chlorpheniramine	2.5×10^{-3}, 2.5×10^{-4}, 2.5×10^{-6}, 2.5×10^{-8}, 2.5×10^{-10}, 2.5×10^{-12}	Drug concentrations as for histamine studies
SCG	3.9×10^{-3}, 3.9×10^{-4}, 3.9×10^{-6}, 3.9×10^{-8}, 3.9×10^{-12}- 3.9×10^{-14}, 3.9×10^{-16}	
3. Drug dose-response relationships with histamine and allergen induced weals of comparable area		
Clemastine	1×10^{-5}, 1×10^{-6}, 1×10^{-7}	
Ketotifen	1×10^{-5}, 1×10^{-6}, 1×10^{-7}	Drug concentrations as for histamine studies
Chlorpheniramine	1×10^{-3}, 1×10^{-4}, 1×10^{-5}	
SCG	1×10^{-3}, 1×10^{-4}, 1×10^{-5}	
4. Determination of pA_2 values		
Clemastine	0.5×10^{-6}, 1×10^{-6}, 0.5×10^{-5}	0.5×10^{-6}, 1×10^{-6}, 1×10^{-5}
Ketotifen	0.25×10^{-6}, 0.5×10^{-6}, 1×10^{-6}	1×10^{-6}, 0.5×10^{-6}, 1×10^{-5}

SCG sodium cromoglycate

administration and subsequent skin prick testing with either histamine or allergen in each subject were: 0, 10, 20, 30, 45 and 60 min. In addition skin prick testing was performed with a mixture of equal parts of drug and histamine or allergen: The concentrations of each in the mixture were equivalent to that in the previous studies in which the drug was administered intradermally and histamine or allergen by skin prick testing.

Drug dose response relationships with histamine and allergen induced weals

The drugs and their concentrations are shown in Table 1. All drugs and control solutions were administered in duplicate, double-blind and in random order to each subject. Skin prick tests were performed with histamine (16 mg ml^{-1}) in ten subjects and with allergen solutions in ten subjects 30 min after drug administration. The concentrations of the allergen solutions were those which gave a weal of between 15 to 30 mm^2 in each of the subjects.

Drug dose response relationships with histamine and allergen induced weals of comparable area

The drug concentrations are shown in Table 1. The concentrations of histamine and allergen solutions used for skin prick testing were adjusted to give weal areas of approximately 20 mm^2 in all ten subjects. All the drug solutions were administered in duplicate, double-blind and in random order to the right forearm 30 min prior to skin prick testing with histamine and similarly to the left forearm 30 min before skin prick testing with allergen.

Determination of the pA_2 values of ketotifen and clemastine for histamine and allergen

pA_2 values for histamine and for allergen were determined in two separate groups of eight subjects. Three concentrations of each drug were used in each patient (Table 1). These concentrations were chosen on the basis of the drug dose-response relationships previously established, to give partial inhibition of the weals. Drugs were administered in duplicate for each concentration.

A dose-response curve for histamine in the presence of saline was initially established in duplicate for the full range of histamine concentrations in all the subjects in the first group. Three concentrations of histamine were selected which had given wealing responses on the linear portion of the dose-response curve representing between 30% and 70% of the maximal response for that patient. pA_2 values for clemastine and ketotifen were determined from the shifts in the dose-response curve that resulted from drug administration. In order to demonstrate a shift in the histamine dose-response curve it was necessary to increase the histamine doses to obtain weal responses in the presence of drugs of similar area to those obtained in the presence of saline alone. In this study the necessary increments in the histamine concentration were determined from preliminary investigations, and 3 appropriate concentrations of histamine were skin prick tested in duplicate for each drug concentration. Skin testing was performed 30 min after drug administration.

The pA_2 values for clemastine and ketotifen for allergen were determined for each subject in the second group using the same methods as described for histamine. The dose-response curve for allergen in the presence of saline was obtained for each subject using serial two-fold dilutions of the appropriate stock allergen solution. It was not considered appropriate to increase the concentration of allergen that was skin prick tested above that of the standard solution, due to the risk of severe local and systemic reactions, but 3 concentrations of allergen were selected from the linear portion of the curve. Shifts of the allergen dose-response due to drug administration were obtained using the same methods as those employed in the histamine study.

Results

The effect of varying the time interval between drug administration and skin prick testing with histamine and allergen is illustrated in Figure 1. The results are illustrated for the ten subjects as the mean weal areas induced by histamine or allergen in the presence of each of the drugs expressed as a percentage of the weal areas induced by these agonists in the presence of the saline control. Maximal inhibition of both histamine and allergen weals by all the drugs studied occurred at 30 min. This time interval was used in all subsequent experiments.

The dose-response curves for the inhibition of histamine and allergen skin weals by each of the drugs are shown in Figure 2. The effect of different molar concentrations of chlorpheniramine, clemastine, ketotifen and sodium cromoglycate on histamine and allergen skin weals is expressed as a percentage of the weal obtained in the presence of the control solution (0.9% saline). The mean values ± s.e. mean are shown for the ten subjects in each group. Ketotifen and clemastine were the most effective drugs in the inhibition of histamine weals when compared to saline. Ketotifen at a concentration of 1×10^{-4} mol l^{-1} totally abolished the weal in eight out of ten subjects, and clemastine at a concentration of 2×10^{-4} mol l^{-1} abolished the weal in nine out of ten patients. Chlorpheniramine was only partially effective with a 20% inhibition of the weal response at a concentration of 1×10^{-3} mol l^{-1}. The administration

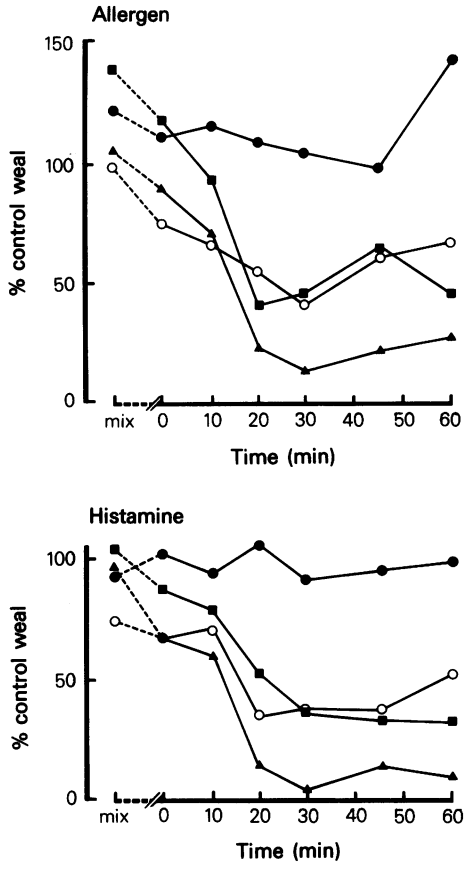

Figure 1 The influence of time on the inhibition of allergen and histamine induced weals in human skin by chlorpheniramine (○——○), sodium cromoglycate (●——●), clemastine (■——■) and ketotifen (▲——▲). The degree of inhibition is expressed as a percentage of the inhibition of the weals caused by the saline control.
Mix = The effect of the weal areas when the drugs were mixed with histamine or allergen prior to skin prick testing.

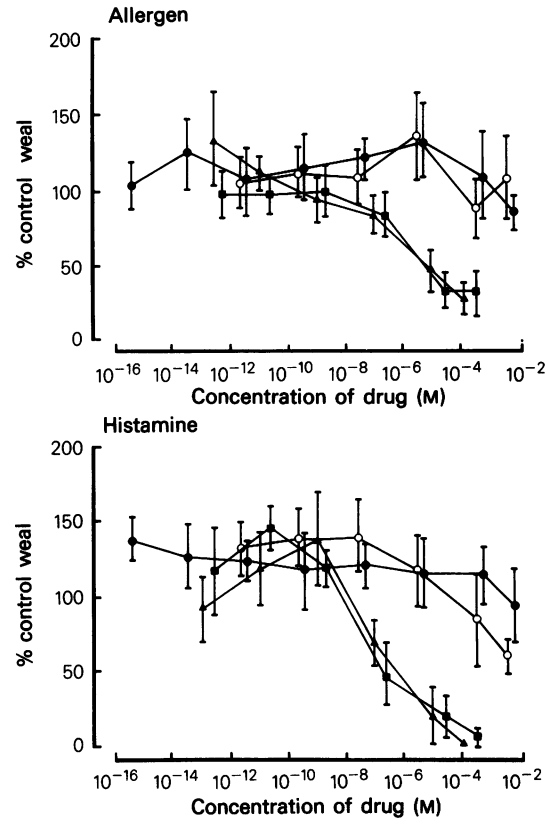

Figure 2a and b The effect of different molar concentrations of chlorpheniramine (○——○), sodium cromoglycate (●——●), clemastine (■——■) and ketotifen (▲——▲) on allergen and histamine skin weals expressed as a percentage of the inhibition of the weals caused by the saline controls. The results are shown as the means ± s.e. mean for the groups of 10 subjects.

of higher concentrations of chlorpheniramine resulted in skin irritation and could not, therefore, be studied. No concentration of SCG tested significantly inhibited histamine weals. A similar pattern was found for the inhibitory effect of the drugs on the allergen induced weals. However no drug totally inhibited the allergen induced weal in any subject and higher concentrations were required to produce the same degree of inhibition as that obtained with histamine weals. Again, no concentration of SCG tested had any significant inhibitory effect.

The drug dose-response curves for histamine and allergen induced weals of comparable area are illustrated in Figure 3. Mean values are shown for the group of ten subjects. The figure shows the effect of different molar concentrations of the drugs on the histamine and allergen skin weals in the same individuals expressed as a percentage of the weal obtained in the presence of the control solution (0.9% saline). The mean (± s.e. mean) of the histamine weal area for the group was 22.0 ± 1.88 mm^2 and for the allergen weal area was 19.8 ± 1.7 mm^2. The mean area together with the s.e. mean of the histamine and allergen weals in the presence of different concentrations of the drugs expressed as a percentage of the saline weal is shown in Table 2. Ketotifen was the most potent drug at inhibiting the histamine and allergen induced weals and was significantly better in both these respects than clemastine on analysis of variance ($P < 0.001$). Both

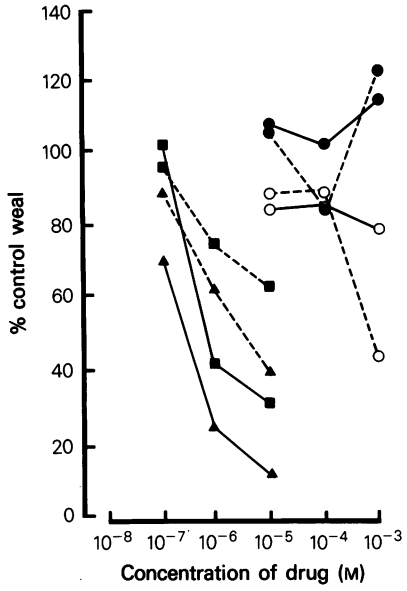

Figure 3 The effect of different molar concentrations of chlorpheniramine (○), sodium cromoglycate (●), clemastine (■) and ketotifen (▲) on histamine and allergen weals of similar area expressed as a percentage of the inhibition of the weals caused by the saline control. The results are shown as the means for the group of 10 subjects.
———Effect of drugs on histamine skin weals.
– – – –Effect of drugs on allergen skin weals.

ketotifen and clemastine had a significantly greater inhibitory effect on histamine induced weals than on allergen induced weals ($P < 0.001$). The IC_{50} values where recordable are also shown in Table 2.

Administration of chlorpheniramine had little inhibitory effect on the histamine induced skin weal and this effect could not be shown to be significantly different from saline. The lower concentrations of this drug (1×10^{-5} mol l^{-1} and 1×10^{-4} mol l^{-1}) had no significant inhibitory effect on allergen induced weals, but at the higher concentration (1×10^{-3} mol l^{-1}) there was a significant reduction in the skin response to allergen. Analysis of the results in each subject at this concentration of chlorpheniramine showed that total inhibition of allergen induced wealing occurred in four whereas in the other six subjects the percentage inhibition obtained was 27.6%, a degree of inhibition comparable to that obtained with the 2 lower concentrations of chlorpheniramine (1×10^{-4} mol l^{-1} and 1×10^{-5} mol l^{-1}). SCG showed no significant inhibition of either histamine or allergen weals.

A typical shift in the agonist dose-response curve resulting from drug administration is illustrated in Figure 4 for a subject in whom the effect of the 3

Table 2 Drug concentrations and their effects on histamine and allergen induced skin weals expressed as a percentage of the weal area obtained in the presence of the control solution (0.9% saline). Mean values together with the standard error of the mean are shown for the group of ten subjects. The IC_{50} for the drug effect on histamine and allergen induced weals is shown where recordable.

		Drug concentration (mol l^{-1})											
		1×10^{-3}		1×10^{-4}		1×10^{-5}		1×10^{-6}		1×10^{-7}			
Drug	*Agonist*	*% Control*	*s.e. mean*	*% Control*	*s.e. mean*	*% Control*	*s.e. mean*	*% Control*	*s.e. mean*	*% Control*	*s.e. mean*	*IC_{50} molar concentration*	
SCG	Histamine	116.9	10.8	102.7	12.0	107.8	8.9					—	
	Allergen	122.3	16.7	87.0	13.0	107.0	8.7					—	
Chlorpheniramine	Histamine	80.2	17.3	87.9	8.3	85.5	10.0					—	
	Allergen	44.2	12.5	92.1	28.0	90.8	7.5					—	
Clemastine	Histamine					31.7	12.7	41.6	7.4	102.3	7.9	9.2×10^{-7}	
	Allergen					62.2	22.0	74.0	18.0	97.0	6.7	—	
Ketotifen	Histamine					11.4	5.3	25.4	8.3	70.7	11.5	4.5×10^{-7}	
	Allergen					40.8	10.6	61.1	26.4	91.1	24.6	5.0×10^{-6}	

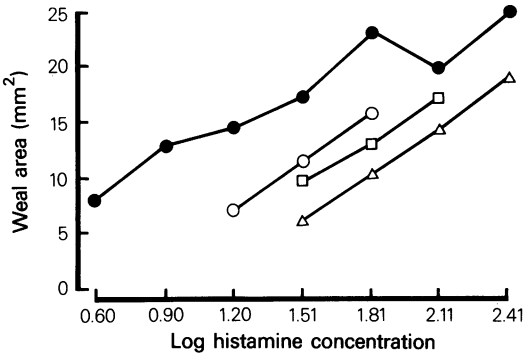

Figure 4 The effect on histamine dose-response curve in human skin of increasing doses of ketotifen.
●——●Histamine alone
○——○Histamine in the presence of ketotifen 0.25×10^{-6} mol l^{-1}
□——□Histamine in the presence of ketotifen 0.5×10^{-6} mol l^{-1}
△——△Histamine in the presence of ketotifen 1×10^{-6} mol l^{-1}

concentrations of ketotifen (c.f. Table 1) on the histamine dose-response curve was examined. Values for the pA_2 of the drug were determined from shifts in the agonist curve providing 2 criteria were satisfied: First that the shifts were approximately parallel; secondly that there was evidence of competitive antagonism, in that an increase in the agonist dose overcame the inhibitory effect of the drug. In all cases where these criteria were satisfied ratios were calculated taking a mean of between 3 and 5 readings distributed over the linear portion of the curve where parallel shifts had occurred in the presence of the drug. Schild plots were constructed for each patient using linear regression with $\log_{10}$ (dose ratio -1) as the ordinate variable and $\log_{10}$ (molar drug concentration) as the variable on the abscissa. The pA_2 value of the drug was obtained from the intercept of the line of regression on the abscissa. The mean pA_2 values and the mean slopes of the regression lines for the effect of the drugs on histamine and allergen induced skin weals for the group of eight subjects is shown in Table 3. Where agonist drugs are acting competitively theoretical considerations demand that the slope of the regression line is -1. In this study the slopes of all the lines approximated closely to -1 and did not differ significantly from this value. There are theoretical and statistical grounds (Tallarida *et al.*, 1979) for calculating the pA_2 values from plots where the slope is constrained to -1. These values are also shown in Table 3. Statistical comparisons between pA_2 values for the different drugs and agonists were performed using unpaired Student's *t*-tests as the values had a normal distribution. The pA_2 value of ketotifen for histamine was significantly different ($P < 0.001$) from its value for allergen and from the pA_2 value of clemastine for histamine. Other values were not significantly different from each other.

Discussion

Analysis of our results for drug dose-response relationships for histamine and allergen induced skin weals has shown that ketotifen significantly inhibits both the histamine and allergen induced skin weal, but is more effective against histamine than against allergen. Clemastine also markedly inhibits the histamine and allergen induced weals but is significantly less potent than ketotifen. Chlorpheniramine has only minor inhibitory activity and appears to be a relatively weak antagonist in comparison to ketotifen and clemastine. SCG had no significant effect on either the histamine or allergen induced weals. We have shown that maximal inhibition of the histamine and allergen induced weals by the different drugs occurred after a time interval of 30 min, although this inhibitory effect was prolonged beyond this time. The Schild plots showed that ketotifen and clemastine were acting as competitive antagonists to histamine and allergen in the skin and that ketotifen was significantly more potent at inhibiting histamine than allergen. The latter result suggests that other mediators as well as histamine are involved in the allergen induced skin weal, and that ketotifen does

Table 3 The pA_2 values of clemastine and ketotifen for histamine and allergen obtained from Schild plots when the slope of the plot is unconstrained and constrained to -1. The results are shown as the mean ± s.e. mean

			Schild plot		
			Unconstrained		*Slope constrained to* -1
Drug	*Agonist*	n	*Slope*	pA_2	pA_2
Ketotifen	Histamine	8	-0.94 ± 0.09	6.84 ± 0.65	6.79 ± 0.05
	Allergen	8	-0.81 ± 0.09	6.12 ± 0.65	5.94 ± 0.09
Clemastine	Histamine	8	-0.88 ± 0.17	6.13 ± 0.11	6.09 ± 0.08
	Allergen	8	-0.91 ± 0.17	6.14 ± 0.13	6.05 ± 0.14

not appear to have any anti-allergic activity in the skin additional to that explicable in terms of its antihistaminic properties.

It is well established that oral antihistamines have an inhibitory effect on both histamine and allergen induced skin weals (Cook *et al.*, 1973, Galant *et al.*, 1973). There are no previous studies to our knowledge in which antihistamines have been administered intradermally and their relative potencies against allergen and histamine induced skin weals compared by dose-response relationships. In our study a wide range of drug concentrations was initially used to establish drug dose-response relationships since the shape of the dose-response curve was unknown. Since the area of the histamine and allergen induced skin weals were not the same in this initial study, it was repeated after adjusting the concentration of allergen and histamine to give skin prick test weals of approximately 20 mm^2. The effect of 3 concentrations of each of the drugs was examined in each of the subjects. These dose-response studies showed that chlorpheniramine was a weak antihistamine. This is in agreement with previous studies (Hedges *et al.*, 1971) in which it has been shown to be less potent than clemastine. The inhibitory effect of chlorpheniramine on the allergen induced weal was also small, apart from an anomalous result obtained with the highest concentration of drug used in the dose-response study with histamine and allergen skin weals of equivalent size. At the highest concentration used, chlorpheniramine markedly reduced the mean area of the allergen induced weals for the group as a whole but at the lower concentrations it showed only minor inhibitory effects. A possible explanation for this may lie in the observation that antihistamines in high concentrations can cause release of mediators from rat and guinea pig mast cells and human basophils (Mota & de Silva, 1960; Lichtenstein & Gillespie, 1975; Moodley & Davies, 1982) and Church & Gradidge (1980) have shown that chlorpheniramine can cause histamine release from chopped human lung. It is possible that at high concentration chlorpheniramine might itself have caused mediator depletion from the mast cells at the challenge site so that subsequent allergen provocation could only result in a reduced response. Some support for this hypothesis comes from the finding that the low mean value for the group as a whole resulted from an absent response in four out of the ten patients: In the other six patients the inhibitory effect of chlorpheniramine was of a similar magnitude to that obtained at the two lower concentrations of the drug.

In this study clemastine appeared to be a potent antihistamine, capable of totally inhibiting histamine induced skin weals. It also had a marked inhibitory effect on allergen induced weals in the skin but did not abolish this response even at the highest concentration that could be administered intradermally without skin damage and at concentrations that totally inhibited histamine induced skin weals.

SCG was introduced as a drug which appeared to have a mode of action that was distinct from those of other established treatments for asthma (Cox, 1967). Goose & Blair (1969) demonstrated inhibition of allergic responses in the skin of the rat, and Sheard *et al.* (1967) showed that it inhibited mediator release from chopped human lung. It was thought that its mechanism of action was by stabilising mast cells, and support for this came from *in vitro* studies which demonstrated that it inhibited degranulation of rat peritoneal mast cells (Garland, 1974). It has been reported that SCG shows a bell shaped dose-response curve with regard to inhibition of mediator release from chopped human lung (Altounyan, 1979). We found no evidence of any inhibitory activity by SCG against either histamine or allergen induced skin weals over a wide concentration range. A similar lack of inhibitory effect of SCG on allergen challenge was found by Pearce *et al.* (1974) using sensitised human skin *in vitro*. In the only published study to examine its *in vivo* effect in human skin, Assem & Mongar (1970) found SCG to be ineffective in one patient in whom mixtures of SCG and allergen were skin prick tested and in three other patients in whom Prausnitz-Kustner reactions were performed, although they used a narrower range of drug concentrations than we employed. One explanation for these results might be that mast cells in the skin are inaccessible to SCG, perhaps due to its lipid insolubility, but this hypothesis appears unlikely in view of the fact that *in vitro* studies reveal similar results (Pearce *et al.*, 1974). Furthermore, SCG is capable of inhibiting the PCA reaction in rat skin indicating that mast cells in this situation are accessible to the drug (Goose & Blair, 1969). SCG has been shown to inhibit mediator release from chopped human lung, but it has no significant effect on sensitized human leucocytes (Assem & Mongar, 1970). It may be that mast cells in the skin differ in some critical way from those in the lung. Taylor & Sheldon (1977) have suggested that there may be an essential co-factor in lung tissue that enables SCG to affect mast cell stabilisation. It has also been argued (Stokes & Morley, 1981) that the effectiveness of SCG in the treatment of asthma could be unrelated to mast cell stabilization and be dependent upon other properties such as effects on irritant receptors. This would provide a further explanation for its lack of inhibitory effect on the allergen induced skin weal.

There has been a search for other drugs with 'anti-allergic' activity since the introduction of SCG in 1967, and ketotifen has been claimed to have similar mast cell stabilizing properties in addition to its antihistaminic actions. However, in this study the dose-response characteristics of ketotifen were totally

dissimilar to those of SCG but closely paralleled those of the antihistamine clemastine, although it was more potent than this drug against both histamine and allergen induced weals in human skin.

The time interval between drug administration and skin prick testing may have a critical effect on the response. SCG, when used in *in vitro* test models such as chopped human lung and sensitised rat peritoneal mast cells, exhibits the phenomenon of tachyphylaxis (Cairns, 1979). The inhibitory effect of SCG is maximal when administered at the same time as the allergen and declines when administered more than 5 min prior to challenge. This phenomenon is not seen when SCG is inhaled prior to allergen bronchial provocation tests in human subjects (Altounyan, 1979). The effect of the time interval between intradermal administration of antihistamines and skin prick testing has not been previously studied. We examined different intervals varying from 0 to 60 min and also looked at the effect of skin prick testing with a mixture of drug and agonist. Chlorpheniramine, clemastine and ketotifen exhibited maximal inhibition when given 30 min prior to skin prick testing with either histamine or allergen. In some instances, the inhibitory effect was maintained when the time interval was extended beyond 30 min but any such inhibition was not significantly different from that at 30 min. SCG was ineffective irrespective of the time interval between its administration and that of the agonist; it did not inhibit allergen or histamine skin weals even when administered concurrently.

The mechanism of action of ketotifen and clemastine on histamine and allergen induced skin weals was further examined by constructing Schild plots and obtaining pA_2 values (Schild, 1947). In situations where the antagonist drug is acting in a competitive fashion the pA_2 is related to the dissociation constant of the antagonist receptor interaction and is therefore useful in classifying drugs and receptors (Arunlakshana & Schild, 1959). If 2 agonists act on the same receptors they will be antagonised by the same competitive antagonist and give rise to the same value of pA_2 for the antagonist. The pA_2 value also provides a quantitative expression of the affinity of an antagonist for the receptor and is therefore a measure of its potency. Construction of a Schild plot provides a line whose intercept on the abscissa is the pA_2 value and whose theoretical slope is -1. Non-competitive antagonists will result in slopes that are markedly different from -1. The slopes for ketotifen and clemastine against both histamine and allergen were found to be close to -1 providing evidence that these drugs were acting as competitive antagonists in these situations.

In this study the pA_2 value for ketotifen when acting as an antagonist to histamine was significantly higher than the pA_2 value for clemastine suggesting that ketotifen is a more potent antihistamine at least in human skin. Ketotifen also has a significantly higher pA_2 value for histamine than for allergen. This implies that the mediators released by allergen challenge do not occupy exactly the same receptors as histamine. The allergen induced weal almost certainly results from the action of other mediators as well as histamine and ketotifen appears to be antagonising the histamine receptors only. This conclusion conflicts with our results for the pA_2 values for clemastine. Clemastine has identical pA_2 values for histamine and allergen, suggesting the involvement of perhaps only a single mediator—histamine. However, this is unlikely to be the case since our initial dose-response curves for clemastine showed that it has a significantly greater inhibitory activity against histamine than against allergen. The anomalous result is likely to be the pA_2 value for clemastine against allergen induced skin weals. We have found that clemastine causes significant release of histamine from sensitized human leucocytes at a concentration of 2.2×10^{-6} mol l^{-1} (Moodley & Davies, 1982). Ketotifen can also cause mediator release but a concentration of 2.6×10^{-5} mol l^{-1} is required to achieve the same effect as clemastine. It is possible that clemastine may be causing some degree of mediator depletion prior to allergen challenge, resulting in a reduced response and a high pA_2 value. Ketotifen may not show the effect at the doses used.

It has been suggested that there are both H_1 and H_2 receptors in human skin but the clinical relevance of the H_2 receptors remains controversial. Harvey & Schocket (1980) examined the effects of orally administered hydroxyzine and cimetidine, alone and in combination, on histamine induced cutaneous weal responses. Cimetidine, an H_2-receptor blocker, had no significant effects when given alone, but in combination with hydroxyzine, an H_1-receptor blocker, significantly increased the inhibitory activity from 75% to 84%: This effect is fairly minor and only limited conclusions can be drawn from the study as the authors did not examine drug dose-response relationships. Marks & Greaves (1977) using combined H_1- and H_2-receptor antihistamines produced a non-statistically significant reduction in the erythema resulting from histamine administration in human skin. In our studies both ketotifen and clemastine totally abolished the histamine induced skin weal and there is no evidence currently available to suggest that either drug has any H_2-receptor blocking activity (Kennedy, personal communication).

One of the questions we sought to answer in this study was whether ketotifen had any 'anti-allergic' action in human skin additional to its antihistaminic activity. If ketotifen were to significantly inhibit mediator release from mast cells in the skin, in addition to antagonising histamine at the receptor level, the drug would be a more effective inhibitor of allergen induced weals than of histamine weals of the

same size. We found the opposite to be true. This suggests that ketotifen does not possess any additional anti-allergic activity at least with regard to mast cells in human skin and when administered in a single dose within 30 min prior to allergen challenge.

In conclusion sodium cromoglycate proved to be ineffective against both histamine and allergen induced skin weals. Ketotifen and clemastine have been shown to be potent antihistamines in the skin but no additional 'anti-allergic' properties could be demonstrated for these drugs. Mast cells in the skin may differ however from those in the lung in the extent to which 'anti-allergic' drugs may inhibit mediator release.

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