urine (Timsina & Hewick 1992)) may have a considerable influence. The degradation will lower the detectable concentration of DSFab in the urine, which will decrease the calculated clearance and hence the filtration fraction. However, whatever proportion of renally delivered DSFab is filtered by the glomerulus, it seems that they impair glomerular function and may interfere with their own elimination.

In our study, normal renal function was affected and the possibility exists that where impairment is already present, the adverse effect may be more marked. This is relevant in view of the finding in a recent postmarketing surveillance survey (Smith 1991) that three-quarters of patients receiving DSFab had some degree of renal dysfunction and 32% of these patients had severe renal failure. It is therefore important that renal function is thoroughly investigated after the administration of clinically-used doses of DSFab. These investigations may include the measurement of inulin clearance for the more accurate determination of GFR and the inclusion of suitable control animals injected with normal saline (vehicle in which DSFab are normally dissolved).

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Effects of selective histamine receptor antagonists on skin responses to intradermal bradykinin in healthy volunteers

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Abstract—The effects of chlorpheniramine and cimetidine on the cutaneous responses to intradermal injections of bradykinin were investigated in a randomized, double-blind, placebo-controlled, cross-over study. Chlorpheniramine significantly attenuated the increase in cutaneous blood flow and erythema induced by bradykinin but not the weal response. Cimetidine was without influence on these parameters and the effects of the combined therapy of chlorpheniramine and cimetidine were not significantly different from those due to chlorpheniramine alone. These results suggest that the cutaneous vasodilator effect of bradykinin is in part due to histamine release acting on histamine H_1-receptors,

Intradermal injection of bradykinin produces erythema and wealing in human skin (Greaves & Shuster 1967). The erythema reflects an increase in local blood flow in keeping with the vasodilator effect of bradykinin and the weal formation indicates an increase in vascular permeability. These actions of bradykinin may result from a direct action on receptors in the cutaneous microvasculature or may be mediated via the secondary release of vasoactive chemical substances.

Histamine is a potential candidate mediator of these effects of bradykinin. Intradermal injection of histamine elicits the 'triple response' (Lewis 1927). This consists of an initial local erythema due to histamine-directed vasodilatation, a circumferential erythema due to vasodilatation mediated via an axon reflex, and finally a central oedematous weal due to a histamine-directed increase in permeability, and represents a combined histamine H_1- and H_2-receptor response (Marks & Greaves 1977; Greaves et al 1977; Robertson & Greaves 1978). Bradykinin and histamine are released simultaneously when there is tissue damage (Rocha e Silva & Rosenthal 1961), and any tissue damage produced by bradykinin may trigger a release of histamine and vice versa. Bradykinin stimulates histamine release when it is injected into granuloma pouches in rats. This effect and the inflammatory reaction to bradykinin were considerably reduced in histamine-depleted skin following pretreatment of the animals with the histamine liberator, compound 48/80, suggesting that the effects of bradykinin are in part mediated by histamine release (Stern et al 1962). Antihistamines reduce the exudation produced by injections of bradykinin in the rat hindpaw (Maling et al 1974) and rabbit skin (Marceau et al 1981) indicating possible participation of endogenous histamine in the increased vascular permeability evoked by bradykinin. Bradykinin has also been reported to liberate histamine from rat peritoneal mast cells in-vitro (Johnson & Erdös 1973; Ishizaka et al 1985), but similar findings were not observed with human skin mast cells or basophils (Lawrence et al 1989). However, Zachariae et al (1969) previously demonstrated that the mean histamine content of the human skin is reduced after local injections of bradykinin into the subcutaneous tissue, support-

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ing the idea that bradykinin is a histamine liberator in man. The cutaneous effects of intradermal injections of other peptides such as substance P (Barnes et al 1986), calcitonin gene-related peptide and neurokinin A (Fuller et al 1987) have been associated with histamine release.

This study explored the possibility that the actions of bradykinin in the human skin may, in part, be due to release of histamine by examining the effects of selective histamine H_1- and H_2-receptor antagonists, chlorpheniramine and cimetidine, respectively, on the increase in local cutaneous blood flow measured by the non-invasive technique of laser Doppler flowmetry (LDF) (Stern 1975; Holloway & Watkins 1977), erythema and weal reactions induced by intradermal injections of bradykinin in healthy volunteers.

Materials and methods

Eight volunteers (4 males, 4 females), aged 21 to 33 years (mean 25 years), gave informed consent to take part in this randomized, double-blind, placebo-controlled, cross-over study which had approval from the City and Hackney District Ethical Committee, London. They were all healthy and had no history of any dermatological abnormalities i.e. eczema, angioedema or dermatographism. They were on no medication for at least one week before the start of the study and only the study medications were allowed during the trial. They fasted from 2200 h preceding each study day and did not smoke or consume alcohol or caffeine-containing beverages for 24 h before and during each trial day.

The volunteers attended on 4 separate mornings at least 1 week apart. On each study day, they took two study capsules with 100 mL water: placebo + placebo, chlorpheniramine 4 mg + placebo, placebo + cimetidine 200 mg, or chlorpheniramine 4 mg + cimetidine 200 mg, the order of the treatment being randomized. Two hours later, solutions of 0·1 mL of 0, 1, 2·5, 5 and 10 μg of bradykinin in 0·9% NaCl (saline) were injected intradermally at 3 min intervals into each forearm. These solutions were coded to maintain blindness and to reduce measurement bias and they were assigned randomly to marked sites on the volar surface of the forearms. Each injection was made with a 1 mL Sable syringe and a 25 g × 5/8 sterile needle, and the injection sites were at least 6 cm apart in order to avoid interactions between injections at adjacent sites. The skin responses were assessed at 15 min after each injection by 3 methods: LDF output, area of erythema and weal volume (Li Kam Wa et al 1990). LDF output, expressed in arbitrary units (a.u.), was recorded for a 15 s period at 4 separate sites at the edge of the induced weal and a mean LDF value was obtained from these. The outlines of the erythema and weal reactions were traced onto transparent acetate sheets once the blood flow recordings were completed and the areas measured from these tracings by digitalized computer planimetry (Hewlett-Packard). The skin thickness was measured with modified skin callipers and the weal thickness was determined by subtracting pre- from post-injection values and dividing by 2 as described by Cook & Shuster (1980). Weal volume was calculated by multiplying weal thickness by area.

The investigations were carried out at approximately the same time of the morning under identical conditions in the same room with the ambient temperature maintained at 23–25°C and relative humidity at 30–40%. The subjects were lightly clad and seated with their forearms supported at an angle of 30° to the horizontal so that the hands were at heart level. They rested in the quiet clinical laboratory for a minimum of 20 min before the tests were performed.

The LDF output, erythema area and weal volume data were analysed by multiple linear regression analysis with treatment and subjects as discrete independent variables, employing the dummy variable technique, and bradykinin dose as a continuous independent variable. Two-way analysis of variance was used to identify any significant difference between treatment in the cutaneous responses to intradermal injection of saline alone which acted as control.

Results

Compared with placebo, pretreatment with chlorpheniramine significantly reduced the increase in LDF output (regression coefficient, $B = -0{\cdot}14$ a.u., $F(1,116) = 6$, $P < 0{\cdot}025$) and erythema formation ($B = -107$ mm^2, $F(1,116) = 6{\cdot}92$, $P < 0{\cdot}01$) induced by intradermal bradykinin (Figs 1, 2). The reduction in local cutaneous blood flow measured by these two variables was seen mainly with the 10 μg dose of bradykinin where the mean reduction in LDF output and erythema area were 34 and 36%, respectively. Chlorpheniramine did not alter the bradykinin-induced weal response (Fig. 3).

Cimetidine had no effect on the cutaneous effects of bradykinin, and the effects of the combined treatment of chlorpheniramine and cimetidine on the bradykinin-induced skin responses were not significantly different from those produced by chlorpheniramine alone (Figs 1–3).

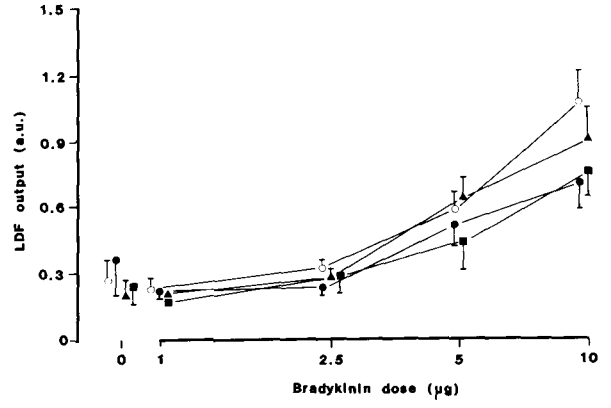

FIG. 1. LDF output recorded at 15 min after intradermal injections of 0, 1, 2·5, 5 and 10 μg bradykinin in saline following pretreatment with placebo + placebo (○), chlorpheniramine + placebo (●), placebo + cimetidine (▲) and chlorpheniramine + cimetidine (■). Values are shown as mean and s.e.m. (n = 8).

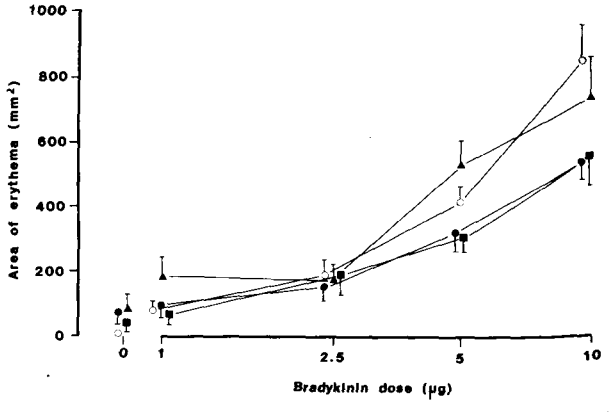

FIG. 2. Erythema induced by intradermal injections of 0, 1, 2·5, 5 and 10 μg bradykinin in saline following pretreatment with placebo + placebo (○), chlorpheniramine + placebo (●), placebo + cimetidine (▲) and chlorpheniramine + cimetidine (■). Values are shown as mean and s.e.m. (n = 8).

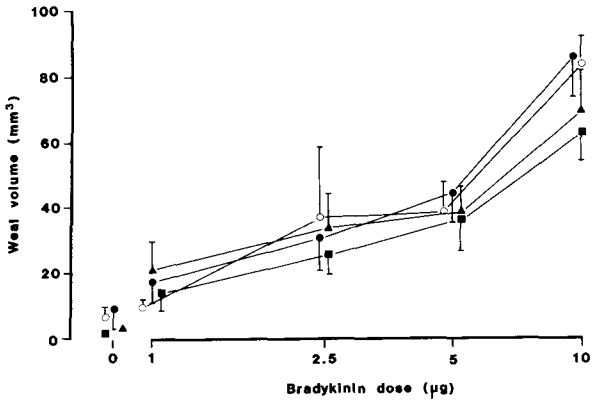

FIG. 3. Weal induced by intradermal injections of 0, 1, 2·5, 5 and 10 μg bradykinin in saline following pretreatment with placebo + placebo (○), chlorpheniramine + placebo (●), placebo + cimetidine (▲) and chlorpheniramine + cimetidine (■). Values are shown as mean and s.e.m. (n = 8).

The skin responses following intradermal injection of the vehicle, i.e. saline only, measured by the above parameters, were not significantly different on the 4 study days.

Discussion

Chlorpheniramine, a histamine H_1-receptor antagonist, significantly diminished the increases in local cutaneous blood flow measured by LDF and the erythema induced by intradermal injections of bradykinin, but not the weal response. The histamine H_2-receptor antagonist cimetidine had no effect on these actions of bradykinin and the effects of the combined therapy of chlorpheniramine and cimetidine were not significantly different from those due to chlorpheniramine alone.

The attenuation of bradykinin-induced increases in cutaneous blood flow and erythema formation by a histamine H_1-receptor antagonist, but not an H_2-receptor antagonist, in this study provides evidence that the vasodilator effects of bradykinin in the human skin are in part mediated by histamine release acting on H_1-receptors. These results appear to contradict those of Crossman & Fuller (1988). The latter chose to study the effect of H_1-receptor blockade on the erythema produced by 1 μg dose of bradykinin only and measured the erythema response at 5 min. There is, however, little erythema formation induced by that dose of bradykinin as they observed and the technical problems of accurate measurements of differences in sizes of small erythema reactions make it difficult to demonstrate any difference between the responses. In the present study, the reduction in erythema area or blood flow was more evident at the higher doses of bradykinin and there was approximately 35% reduction in the vasodilator response induced by the 10 μg dose of bradykinin. Measurement of the vasodilator response at 5 min after injection also includes the change in blood flow secondary to the trauma of needle injection, so that it may be more appropriate to measure the vasodilator response later (Holloway 1980; Bisgaard & Kristensen 1984; Li Kam Wa et al 1989).

Chlorpheniramine did not modify bradykinin-induced weal in contrast to its effects on local cutaneous blood flow and erythema formation. Cimetidine, either alone or in combination with chlorpheniramine, also exerted no effect on the weal response. These findings suggest that histamine does not contribute to the vascular permeability enhancing effect of bradykinin. It is, however, possible that the histamine release by bradykinin was not extensive enough to induce a detectable oedema and this may explain why chlorpheniramine affected only the erythema and blood flow increases but not the weal induced by bradykinin. It is also evident that vasodilation and weal formation are two separate and distinct entities, although they may interact (Williams & Peck 1977). The anatomical substratum for exudation or weal formation produced by bradykinin and other agents is the contraction of the endothelial cells and the concomitant generation of gaps between them, whereas relaxation of arteriolar smooth muscle is responsible for vasodilation, and the vasodilation and exudation-promoting activities are dissociable (Gabbiani et al 1970; Wilhelm 1973). Chlorpheniramine may therefore affect one component of the bradykinin-induced skin reactions but not another. Similarly, it has been demonstrated that the H_1-receptor antagonist terfenadine reduced the flare but not the weal induced by substance P (Barnes et al 1986).

The failure to demonstrate an effect on bradykinin-induced weal or a greater suppression of the vasodilator effect of bradykinin in the present study may also mean that the antihistamines were not present at concentrations sufficient to substantially block endogenously released histamine. The time selected for measurement of the cutaneous responses to bradykinin was on the basis of earlier pharmacokinetic studies as the time when plasma chlorpheniramine (Huang et al 1982; Rumore 1984) and cimetidine (Grahnén et al 1979; Mihaly et al 1984) concentrations were near peak levels, and the timing and doses of the drugs administered were similar to those used by Robertson & Greaves (1978) who demonstrated an effect of these drugs on the erythematous reactions induced by histamine H_1- and H_2-receptor agonists. Interestingly, they also failed to demonstrate an effect on weal formation and it is possible that the doses of the drugs used in their, and also our, study were too low. The lack of effect of H_1-receptor blockade on bradykinin-induced weal contrasts with the observations of Marceau et al (1981) who demonstrated that the histamine H_1-receptor antagonist mepyramine, conjointly injected with bradykinin, reduced exudation produced by bradykinin in the rabbit skin. The divergence in results may indicate that local application of antihistamines is more effective than systemic administration in antagonizing the effects of an intradermally injected agent or it may be due to species difference in bradykinin-induced effects. Our results, however, are in agreement with other studies in the human skin showing a lack of effect of H_1-receptor blockade on wealing caused by bradykinin (Greaves & Shuster 1967; Crossman & Fuller 1988). The absence of any effect of histamine suppression upon bradykinin-induced weal implies that mediators other than histamine are involved or that the vascular permeability property of bradykinin is due to a direct effect of bradykinin on vascular receptors, probably bradykinin B_2-receptors (Stewart 1979; Marceau et al 1981).

In conclusion, we have shown that the histamine H_1-receptor antagonist chlorpheniramine but not the H_2-receptor blocker cimetidine at the dosages used, significantly reduced the cutaneous vasodilator effect of intradermally injected bradykinin. Chlorpheniramine and cimetidine, administered alone or in combination, were without effects on bradykinin-induced weal formation. These results may be taken as an indication that bradykinin may owe some of its cutaneous vasodilator effect to endogenous histamine release acting on histamine H_1-receptors.

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