

Respiratory pathophysiologic responses

Skin responses to bradykinin, kallidin, and [desArg⁹]-bradykinin in nonatopic and atopic volunteers

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Background: Kinins are potent vasoactive oligopeptides that may act as mediators in a variety of inflammatory diseases of the skin by interacting with specific receptors designated B₁ and B₂. In this study we have investigated the structure-activity relationship of intradermally injected bradykinin, kallidin (lysine-bradykinin), and [desArg⁹]-bradykinin in atopic (n = 8) and nonatopic (n = 8) subjects.

Methods: On two separate occasions, each separated by a week, subjects randomly underwent intradermal challenge with incremental doses (0.5, 5, and 50 nmol) of either the B₁-agonist [desArg⁹]-bradykinin, the B₂-agonists bradykinin or kallidin, or vehicle placebo. In a separate randomized double-blind study we have also examined the effect of an orally administered antihistamine, terfenadine, on kinin-induced wheal and flare responses and their repeatability in a group of nine volunteers. The skin responses were monitored objectively by measurement of wheal and flare areas.

Results: Both bradykinin and kallidin induced a dose-dependent increase in wheal and flare areas in all subjects studied. Although the effects of the two lowest doses (0.5 and 5 nmol) of [desArg⁹]-bradykinin on skin responses were indistinguishable from those of placebo, this kinin at the highest dose administered (50 nmol) caused a significant increase in wheal and flare areas in all subjects studied. No difference could be identified in the skin responses to kinins between atopic and nonatopic subjects. In addition kinin-induced cutaneous responses were not altered by pretreatment with terfenadine.

Conclusions: These in vivo structure activity studies suggest that in human beings the skin responses to kinins may be compatible with the stimulation of B₂ receptors, which is unrelated to histamine release from cutaneous mast cells. (*J ALLERGY CLIN IMMUNOL* 1993;92:683-9.)

Key words: Skin, wheal, flare, bradykinin, kallidin, [desArg⁹]-bradykinin, terfenadine

Wheal and flare formation in the skin in response to trauma or allergic reaction follows both antidromic nerve stimulation, as part of an axon reflex, and local inflammatory mediator production, including production of kinins.¹⁻³ Moreover, the activation of a kinin-forming pathway seems to be responsible for the acute episodes of swelling that characterize patients with hereditary angioedema and associated C1 inactivator deficiency.⁴ The mammalian kinins, bradykinin, kallidin

Abbreviations used

[desArg⁹]-Bk: [des-Arginine⁹]-bradykinin
CV: Coefficient of variation

(lysine-bradykinin), and methionine-lysine-bradykinin are potent vasoactive oligopeptides formed as cleavage products from the action of kallikrein-like enzymes on high and low molecular weight kininogens.²

Although direct evidence of kinin formation in human skin in vivo is still lacking, recent studies have demonstrated that large quantities of bradykinin and kallidin are recovered in the lavage fluid of mucosal surfaces of the upper^{5, 6} and lower airways^{7, 8} in human beings in vivo during both the immediate and late responses to allergen challenge and in tears in response to conjunctival

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provocation with allergen.⁹ When injected intradermally, bradykinin elicits a marked wheal and flare response,^{10, 11} which is not altered by either cyclooxygenase inhibition or antihistamine pretreatment.¹¹ The mechanism(s) for these effects are incompletely understood. As with other autacoids, kinins produce their effects by interacting with specific cell surface receptors. To date, two major kinin receptor subtypes have been described, B₁ and B₂, on the basis of studies of different agonist potencies in different tissue preparations.² In cat ileal strips¹² and in isolated canine tracheal preparations,¹³ both bradykinin and kallidin, via an action on B₂ receptors, induce contraction, whereas the B₁ agonist [desArg⁹]-bradykinin ([desArg⁹]-Bk) is without effect. In rabbit aorta the situation is reversed: [desArg⁹]-Bk induces contraction,² whereas bradykinin and kallidin are inactive. In addition to their actions on vasculature and smooth muscle, it is well documented that kinins may also induce histamine release from rodent mast cells and that this effect is independent of bradykinin receptor stimulation.¹⁴⁻¹⁶

Although it is known that cultured human skin fibroblasts express high numbers of B₂ receptors,¹⁷ the receptor subtype responsible for the cutaneous response to intradermal kinin injection in human beings has not been defined. This has implications for the development of a human model for the specific receptor antagonism of kinins on cutaneous surfaces. Furthermore, the vascular actions of kallidin and [desArg⁹]-Bk within the human skin in vivo are unexplored. Thus the aim of the present study was to investigate the receptor specificity and the comparative potencies of bradykinin, kallidin, and [desArg⁹]-Bk in the skin, measuring changes in wheal and flare areas. In addition, to determine whether a disease-specific difference exists, these studies have been undertaken in both atopic and non-atopic volunteers. As part of these studies, the effect of a potent and selective H₁-receptor antagonist, terfenadine, on kinin-induced wheal and flare areas was also examined in a separate randomized double-blind study.

METHODS

Subjects

Eight nonatopic (five men) and eight atopic (six men) volunteers participated in the study. They were comparably matched for age, with respective mean ($\pm$ SEM) ages of 21.3 ± 0.7 years and 25.9 ± 1.9 years. None of our volunteers had a history of skin disease, atopic dermatitis, chronic urticaria, or angioneurotic edema. The atopic subjects were not receiving any form

of medication at the time of the study, and none had ever received specific immunotherapy. Atopy was defined as positive (>3 mm wheal diameter) skin prick test results to one or more of the common aeroallergens: mixed grass pollens, *Dermatophagoides pteronyssinus*, *D. farinae*, cat fur, feathers, mixed tree pollens (Bencard, Brentford, U.K.). Skin prick tests were carried out according to the guidelines set by the Scandinavian Society of Allergology.¹⁸ The other eight volunteers were healthy control subjects who had no history of allergies and negative skin prick test responses. All of the subjects gave written informed consent, and the study was approved by the Southampton Hospitals and University Joint Ethical Sub-Committee.

Drug administration

Bradykinin, kallidin, and [desArg⁹]-Bk (Nova Biochem Ltd., Nottingham, U.K.) were prepared in 5% ethanol in 0.9% sodium chloride under sterile conditions by the pharmacy department of the hospital to produce increasing doses of 0.5, 5, and 50 nmol (in 50 μ l). The purity of the synthetic kinin used was confirmed by high-performance liquid chromatography. The control solution was 5% ethanol in normal saline solution. For local intradermal challenge, 50 μ l of bradykinin, kallidin, [desArg⁹]-Bk (0.5, 5, and 50 nmol), and the control solution were injected into the volar surface of each subject's forearm with a 27-gauge needle and Lo-dose 0.5 ml syringe (Becton Dickinson, Paramus, N.J.).

Study design

The study consisted of two phases. In phase 1, subjects attended the laboratory on two separate occasions at the same time of day; they were randomly allocated to receive intradermal injections of bradykinin, kallidin, [desArg⁹]-Bk, or vehicle diluent (control solution) in a double-blind fashion. At each visit, separated by at least 7 days, coded solutions were injected into the skin of the volar aspect of each subject's forearm; sites for injection were carefully randomized to minimize bias. Each set of three increasing doses of the solution to be tested was injected in each subject's forearm. Thus on study day 1 subjects received a total of six injections (three increasing doses for each arm) and six additional injections on study day 2. Flare responses were recorded 5 minutes after injection, and wheal areas were measured at 15 minutes. The wheal and flare areas were recorded by an independent observer who was blinded to the protocol. The wheal-and-flare perimeters were first outlined directly on the skin surface and then transferred onto transparent sheets with a felt-tip pen. Areas of the tracings were measured by microcomputer-assisted planimetry and expressed as square centimeters.¹⁹ All of the subjects were asked to report on the presence or absence of pain during each intradermal challenge.

In phase 2, nine subjects attended the laboratory on two additional occasions at least 7 days apart. On each

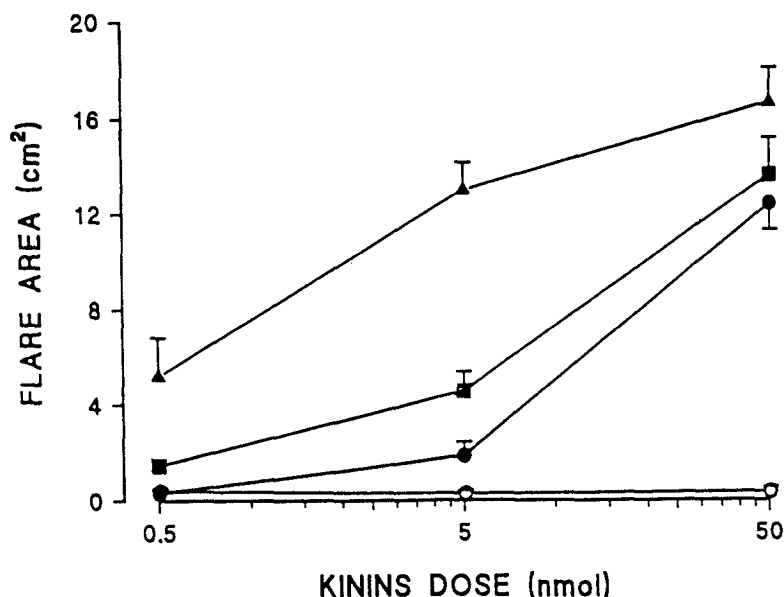


FIG. 1. The flare response after intradermal injections of placebo (open circles) and increasing doses of kallidin (filled triangles), bradykinin (filled squares), and [desArg⁹]-Bk (filled circles). Each point represents the mean $\pm$ SEM response in 16 subjects.

occasion subjects received intradermal injections of a single dose (5 nmol in 50 μ l) of bradykinin, kallidin, [desArg⁹]-Bk, or vehicle diluent (control solution) 3 hours after oral dosing with terfenadine (180 mg) or matched placebo administered in double-blind fashion and random order. Wheal and flare responses were recorded in a manner similar to that described for phase 1.

Data analyses

For all subjects, mean ($\pm$ SEM) values of wheal and flare areas were calculated for each dose of mediator injected and a p value of less than 0.05 was considered significant. Dose-response curves were constructed by plotting the maximum wheal or flare area observed against the dose of mediator injected on a logarithmic scale.

For the nine subjects who took part in both phases of the study, the repeatability of the challenge procedure with intradermally injected test solutions was calculated for each duplicate series by working out the individual coefficient of variation (CV) ($CV = \text{standard deviation/arithmetic mean} \times 100$) between repeated observations obtained on the open (phase 1) and placebo (phase 2) study days for each subject and by averaging the individual CVs of the subjects studied. Low values of the CV represent a high repeatability and vice versa.

In all experiments the significance of differences in the relative cutaneous vascular efficacy of the three kinins tested was evaluated with two-way analysis of variance followed by linear contrasts by Sheffé's method. Skin responses obtained in both atopic and nonatopic subjects were compared by means of Student's t test for unpaired data. The comparisons between skin wheal and flare areas after premedication

with placebo and terfenadine were carried out by Student's t test for paired data.

RESULTS

Phase 1

In both the nonatopic and atopic subjects all three kinins caused dose-dependent increases in wheal and flare areas when compared with the vehicle placebo, which produced little or no response. Because there were no differences between the nonatopic and atopic responses, the data were combined for further analysis.

For flare, kallidin was significantly more active than either bradykinin ($p < 0.01$) or [desArg⁹]-Bk ($p < 0.01$); the mean $\pm$ SEM flare areas were respectively: 5.19 ± 1.63 , 1.46 ± 0.27 , and 0.30 ± 0.13 cm² at 0.5 nmol and 12.97 ± 1.17 , 4.54 ± 0.83 , and 1.84 ± 0.57 cm² at 5 nmol (Fig. 1). At the highest dose administered (50 nmol) any difference between the kinins in terms of flare was lost; the mean flare areas were 16.56 ± 1.42 , 13.55 ± 1.56 , and 12.36 ± 1.11 cm² for kallidin, bradykinin, and [desArg⁹]-Bk, respectively (Fig. 1). The difference in the three kinins in eliciting flare responses was most marked at the 5 nmol dose, with a potency order of kallidin $\gg$ bradykinin $>$ [desArg⁹]-Bk = placebo and with kallidin being approximately 4.1 times more potent than bradykinin and 12.9 times more potent than [desArg⁹]-Bk on a molar basis.

For wheal, kallidin and bradykinin were significantly more active than [desArg⁹]-Bk or placebo

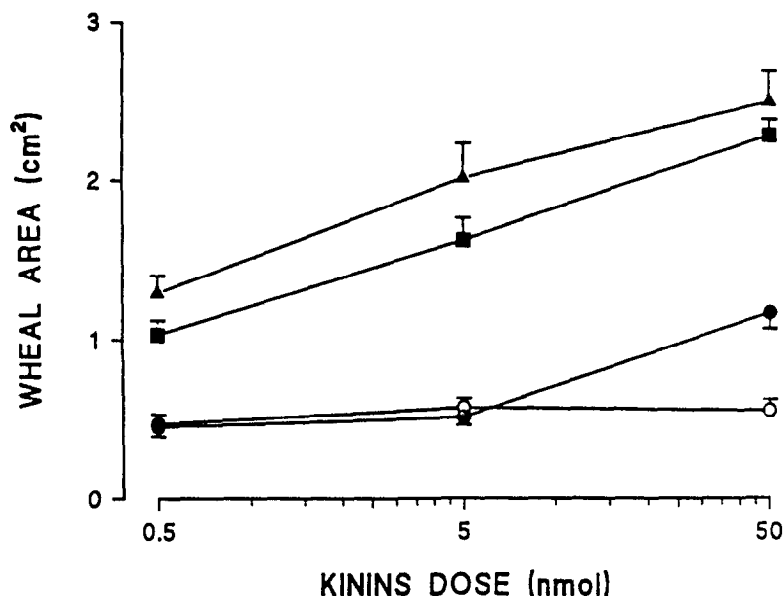


FIG. 2. The wheal response after intradermal injections of placebo (open circles) and increasing doses of kallidin (filled triangles), bradykinin (filled squares), and [desArg⁹]-Bk (filled circles). Each point represents the mean $\pm$ SEM response in 16 subjects.

at all doses used ($p < 0.01$). The mean $\pm$ SEM wheal areas for kallidin, bradykinin, and [desArg⁹]-Bk were respectively: 1.29 ± 0.11 , 1.03 ± 0.09 , and 0.45 ± 0.06 cm² at 0.5 nmol, 2.01 ± 0.22 , 1.62 ± 0.14 , and 0.51 ± 0.05 cm² at 5 nmol and 2.48 ± 0.19 , 2.27 ± 0.10 , and 1.16 ± 0.10 cm² at 50 nmol (Fig. 2). The difference in the three kinins in inducing wheal areas was most marked at the 5 nmol dose, with the similar potency order of kallidin = bradykinin $\gg$ [desArg⁹]-Bk = placebo seen for flare responses and with kallidin being approximately 1.6 times more potent than bradykinin and 6.8 times more potent than [desArg⁹]-Bk on a molar basis.

After intradermal injections with vehicle diluent solution and [desArg⁹]-Bk, no consistent pain occurred in any of the 16 subjects studied, whereas bradykinin and kallidin caused a marked pain sensation, especially at the highest dose administered (50 nmol). No difference existed between atopic and nonatopic individuals regarding the subjective sensation of pain. In addition, flushing was reported by four subjects after administration of the highest doses of bradykinin and kallidin but not [desArg⁹]-Bk.

Phase 2

The skin challenge procedure was found to be repeatable with CVs of 26%, 15%, and 33% for flare areas induced by bradykinin, kallidin, and [desArg⁹]-Bk (5 nmol in 50 μ l), respectively. In-

tradermal injections with bradykinin, kallidin, and [desArg⁹]-Bk also produced repeatable wheal responses; CVs were 14%, 11%, and 10%, respectively.

When compared with placebo, terfenadine 180 mg administered orally 3 hours before intradermal challenge with kinins failed to alter both wheal and flare responses. On the placebo study day, the bradykinin-, kallidin-, and [desArg⁹]-Bk-induced wheal areas were respectively 1.51 ± 0.08 , 1.41 ± 0.10 , and 0.52 ± 0.04 cm², which were not significantly different from their respective values of 1.49 ± 0.16 , 1.39 ± 0.08 , and 0.45 ± 0.06 cm² obtained on the terfenadine study day. After treatment with placebo the mean flare areas of $6.50 (\pm 1.03)$, $11.03 (\pm 2.28)$, and $3.76 (\pm 0.63)$ cm² with bradykinin, kallidin, and [desArg⁹]-Bk, respectively, were not significantly different from those obtained after terfenadine; their respective values were $4.51 (\pm 0.76)$, $7.34 (\pm 1.03)$, and $4.72 (\pm 0.90)$ cm².

DISCUSSION

The present study demonstrates that both bradykinin and kallidin induce a dose-related increase in wheal and flare areas when injected intradermally. In contrast, the kinin metabolite, [desArg⁹]-Bk at doses of 0.5 and 5 nmol is indistinguishable from vehicle placebo in its effects on both wheal and flare responses. At doses of 0.5 and 5 nmol kallidin and bradykinin were consis-

tently more potent than [desArg⁹]-Bk in inducing wheal and flare responses. These findings indicate the presence of kinin B₂ receptors but not B₁ receptors within human skin. Comparison between atopic and nonatopic individuals reveals no disease-related change in either receptor subtype or end organ sensitivity to these intradermally injected kinins. We have also shown that premedication with the selective H₁-receptor antagonist, terfenadine, failed to alter wheal and flare responses to bradykinin, kallidin, and [desArg⁹]-Bk, suggesting that the action of kinins within the skin is not mediated by histamine release.

The doses of kinins for intradermal challenge were chosen on the basis of previous pilot studies carried out in our laboratory and represent submaximal doses for wheal response induced with the kinins tested. The dose of orally administered terfenadine used in the present study and the timing of administration before intradermal challenge have been shown to completely abolish the histamine-mediated vascular responses in human skin,^{20, 21} thereby indicating its ability to antagonize the H₁ cutaneous effects of histamine *in vivo*.

The value of the observations on pharmacologically induced skin response depends on the reproducibility of this response. In the present study we have found that kinin-induced wheal and flare responses are highly repeatable with the technique used, because the calculated CVs ranged from 10% to 14%, which is in accordance with previously reported reproducibility limits ranging from 10% to 30% when the mean wheal area was used for analysis.²²⁻²⁴

Critical to the discussion of the present data is also the possibility that there might be a difference in the cutaneous response to injected kinins in the presence of ethanol. However, in a controlled trial of eight volunteers we could not elicit any significant difference in the skin responses induced by the injection of 5 nmol bradykinin prepared in 5% ethanol in normal saline solution and in normal saline solution alone (R. Polosa, unpublished observations).

The comparative dose-response investigation of bradykinin, kallidin, and [desArg⁹]-Bk in the same subjects has allowed a crude evaluation of the receptor specificity of the dermal responses to these kinins and an assessment of their relative potencies. Although previous studies have reported the skin effects of bradykinin in inducing wheal and flare areas^{10, 11} and the pain responses of bradykinin and [desArg⁹]-Bk in the human blister base model,²⁵ to our knowledge this is the

first study to show a definite skin response to intradermally injected kallidin and [desArg⁹]-Bk. However, because [desArg⁹]-Bk, a product of the catabolism of bradykinin, is active at the highest dose administered, a non-specific effect cannot be excluded at this dosage.

The demonstration that at lower doses both bradykinin and kallidin but not [desArg⁹]-Bk elicit marked wheal and flare responses, suggests that these skin responses are mediated through B₂ receptor stimulation. These findings are consistent with the results of ligand binding studies in the nose with iodine 125-labeled bradykinin, which identify bradykinin receptors at the level of the walls of small muscular arteries, arterioles, capillaries, small venules, venous sinusoids, and possibly sensorimotor fibers²⁶ and indicate that these vascular and neural bradykinin receptors are of the B₂ subtype. *In vitro* the effects of bradykinin and kallidin on B₂ receptor-mediated events are similar,^{12, 13} and attempts to differentiate their actions by pharmacologic manipulation have been unsuccessful.²⁷ Similarly in human beings *in vivo* the effects of bradykinin and kallidin in the upper²⁸ and lower²⁹ airways are thought to be mediated via the activation of surface receptors of the B₂ type and to be directly comparable on a molar basis. In the present study at the dosage of 5 nmol, kallidin was approximately four times more potent than bradykinin in inducing flare response on a molar basis, and this was significant. We do not know the reason for this, but perhaps the presence of lysine in position 1 confers an ability to kallidin to degranulate skin mast cells similar to that observed for basic secretagogues such as poly-lys peptides *in vitro*.³⁰ However, the lack of inhibition of kallidin-induced cutaneous responses by terfenadine and the similar magnitude of wheal areas elicited by intradermal injections with bradykinin and kallidin argues against this possibility. In interpreting the findings of the present study, consideration must be given to the metabolism of kallidin, because a significant amount of this peptide may be converted to bradykinin by cleavage of the N-terminal lysine residue by aminopeptidase.² Although very little is known about the enzymatic cleavage of exogenously administered kinins *in vivo*, it is unlikely that the cutaneous responses of kallidin are mediated via prior conversion to bradykinin, because if this were the case, it should not be possible for kallidin to be more potent than bradykinin. Ascribing receptor subtypes to *in vivo* responses solely on the basis of agonist potencies alone is

fraught with difficulties, and ideally it is also desirable to use selective receptor antagonists. Selective antagonists for B₁ and B₂ receptors have been described,³¹ but they are not yet available for study in human beings.

The mechanisms by which B₂ receptor stimulation produces skin responses are incompletely defined. Several possible mechanisms have been suggested from the results of rodent studies including mast cell degranulation,¹⁴⁻¹⁶ sensory neural stimulation,³² and stimulation of the synthesis and release of prostaglandins.^{33, 34} Although the ability of kinins to induce mediator release from rodent mast cell is well documented,¹⁴⁻¹⁶ we failed to demonstrate any significant inhibitory effect of terfenadine on kinin-induced skin responses, thus suggesting that in human beings the wheal and flare responses elicited by kinins do not require the release of histamine. This finding is in keeping with the observation of a recent double-blind study of six subjects that the cutaneous responses to intradermal challenge with bradykinin was uninfluenced by prior treatment with antihistamines.¹¹ Moreover, in the same study cyclooxygenase inhibitors did not attenuate bradykinin-induced wheal and flare responses. Our failure to show a significant inhibitory effect of orally administered terfenadine on the cutaneous responses to kinins is at variance with what has been reported in rodent mast cells and highlights important interspecies differences. Indeed, in human skin mast cells kinins exhibited very limited histamine release, even at high doses.^{16, 30} These findings are consistent with the lack of efficacy reported by several investigators in treating patients who had acute attacks of hereditary angioedema with antihistamines.^{35, 36} Another possibility is that kinins may stimulate sensory nerve endings, thus activating an axon reflex, which in turn is responsible for the local release of vasoactive peptides such as substance P, neurokinin A, and calcitonin gene-related peptide.³² In support of this a recent study by Crimi et al.³⁷ has demonstrated that pretreatment with topical capsaicin, an irritant that is known to induce neuropeptide depletion from sensory nerve endings, greatly reduces both wheal and flare responses induced by intradermally injected bradykinin in human beings.

In conclusion, we have demonstrated that bradykinin and kallidin are potent agonists in inducing wheal and flare responses in human skin and provided evidence that these responses are likely to be mediated through stimulation of a B₂

receptor subtype. However, only when suitable antagonists become available will it be possible to establish the importance of these mediators in the cutaneous inflammatory response. This study also enhances the observation that mast cell release of histamine does not contribute to cutaneous responses induced by kinins in human beings.

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