

STUDIES ON EXPERIMENTAL ITCH INDUCED BY KALLIKREIN AND BRADYKININ

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Abstract. Experimental itch, produced by i.c. injection of histamine, bradykinin and kallikrein, was compared in volunteer patients. The latent period until itch was perceived, the duration of the itch and the area of the flare response were measured. The effect of antihistamines on the itch and flare responses was studied. It was found that the latent period was not significantly shorter for bradykinin than for kallikrein, that itch induced by bradykinin but not by kallikrein was inhibited by the antihistamine levomepromazine, and that bradykinin induced a flare reaction which kallikrein did not. It was concluded that bradykinin is not the main final mediator of kallikrein-induced itch. Kallikrein is an itch-producing proteolytic enzyme which does not release histamine from dermal mast cells.

When itch is induced by intradermal injection of histamine or proteolytic enzymes there is a delay of about half a minute until itch is perceived, whereas virtually no delay is observed after mechanical stimulation of the cutaneous itch receptors (15). The reason for this latency is not known. One explanation could be that the delay period is accounted for by the time required for diffusion of the pruritic substance to the itch points (15), another that the injected substances are not pruritogenic in themselves but initiate chemical processes leading to formation or release of final mediators which act upon the itch receptors. When itch is produced by proteases it is conceivable that these itch mediators could be peptides formed by enzymic degradation of tissue proteins (3, 15).

There have been conflicting reports as to whether or not bradykinin can induce itch (4, 6). The present investigation was undertaken after we had confirmed that intracutaneous injection of both bradykinin and kallikrein produced itch. Bradykinin belongs to the group of vasoactive peptides (kinins) with pharmacological effects resembling the inflammatory reactions. Bradykinin is generated from its precursor,

bradykininogen, by a specific protease, kallikrein (14, 16). It may also be split off from its precursor by several other less specific proteases, e.g. trypsin and chymotrypsin, i.e. enzymes which, like kallikrein, are pruritogenic.

As most pharmacological effects of kallikrein are indirect and mediated by bradykinin (14, 16), and as both substances produce itch, the kallikrein-bradykinin system was considered to constitute a good experimental model to study whether a polypeptide (kinin) is the final mediator of protease-induced itch.

METHODS

Experimental itch was induced in human volunteers using the same technique as described in earlier papers (7, 8). Solutions of the itch-producing substances—about 0.02 ml—were injected intradermally on the lateral aspects of the upper arms. In most subjects histamine, bradykinin and kallikrein were compared on the same occasion. Because of limited supply, bradykinin was used in only one concentration and not given to all those who received kallikrein and histamine.

The area of the erythema was measured planimetrically 5 min after injection of histamine and bradykin (7). No measurable erythema was seen after kallikrein. The latent period between injection and itch perception was recorded, as well as the duration of the itch response.

The following itch-producing substances were used: kallikrein (Padutin®) generously supplied by Bayer AG, Leverkusen, BRD; synthetic bradykinin (BRS 640) kindly supplied by Sandoz AG, Basle, Switzerland, and histamine dihydrochloride. Bradykinin was used in the original concentration, 90 µg/ml; the other substances were dissolved and diluted in sterile saline immediately before use.

When antihistamines were studied, the pruritogenic substances were given both before and after administration of the drugs. The antihistamines, levomepromazine, 5 mg tablets (Nozinan, AB Leo, Hälsingborg, Sweden) and chlorcyclizine, 25 mg tablets (Di-Paralene, Abbott S.A., Brussels, Belgium) or placebo tablets were administered in a double-blind fashion during one day prior to the second itch test as described previously (7).

Table I. Itch duration and flare before and after administration of antihistamines

Exp.	series	Treatment	Histamine (10 µg/ml)				Kallikrein (20 IU/ml) Bradykinin (90 µg/ml)					
			Itch duration (sec)		Flare (mm ²)		Itch duration (sec)		Itch duration (sec)		Flare (mm ²)	
			Before	After	Before	After	Before	After	Before	After	Before	After
I		Levomepromazine (n=15)	108±18	20±7***	802±85	379±53***	131±17	126±19	63±8	15±10**	409±44	232±50*
		Placebo (n=10)	77±12	104±15	887±118	985±117	149±23	154±19	70±16	77±16	472±104	539±126
II		Chlorcyclizine (n=11)	105±24	10±5**	830±110	409±63***	140±20	131±22				
		Placebo (n=14)	71±17	77±17	852±112	874±93	172±44	163±44				

Mean values ± S.E.M.

** $p < 0.01$; *** $p < 0.001$.

RESULTS

All three injected substances elicited itch although some individuals stated that kallikrein itch had a more pricking character than the others and that the feeling of itch was weaker for bradykinin than for histamine.

Latency and duration of the itch response. If the final mediator of kallikrein itch was bradykinin, the latent period between injection and itch perception might be expected to be shorter for bradykinin than for kallikrein. In the present investigation it was found that after injection of bradykinin, 90 µg/ml, the delay was 23 sec and after injection of kallikrein,

5, 10 and 20 IU/ml, there was a latent period of 26, 18 and 13 sec respectively (Fig. 1, upper part). The delay after injection of histamine, 3 and 10 µg/ml, tended to be longer: 37 and 20 sec respectively.

Whereas the latency decreased with increasing concentration of the injected substances, the duration of the itch response increased (Fig. 1, lower part).

Effects of antihistamines on the itch and flare responses. In order to investigate whether histamine is involved in the kallikrein and bradykinin itch, experimental itch was elicited both before and after oral administration of antihistamines. It was found that kallikrein itch was not influenced by antihistamines, whereas both histamine and bradykinin itch were significantly reduced. The flare responses evoked by histamine and bradykinin also diminished. Kallikrein did not induce a flare even in the absence of antihistamines (Table I).

In Fig. 2 the itch duration and the flare area after levomepromazine are expressed as percentages of the responses obtained prior to administering the antihistamines. It is seen that the reactions induced by bradykinin and histamine are reduced to about the same extent, whereas kallikrein itch is not affected.

DISCUSSION

The present findings do not support the hypothesis that bradykinin is the final mediator of kallikrein itch. The observations that bradykinin itch, though not that of kallikrein, was inhibited by an antihistaminic drug, and that bradykinin evoked a flare reaction in the skin in contrast to kallikrein, both

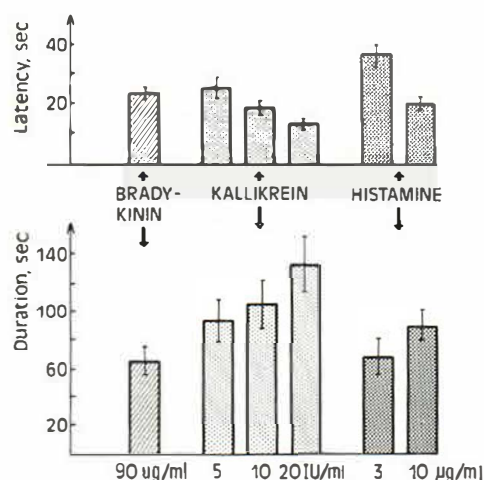


Fig. 1. Latency and duration of the itch response after i.c. injection of bradykinin (90 µg/ml), kallikrein (5, 10 and 20 IU/ml) and histamine (3 and 10 µg/ml).

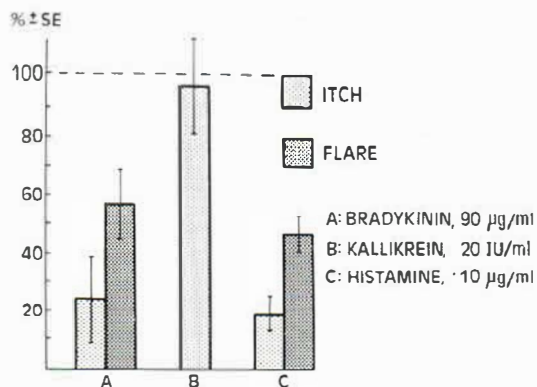


Fig. 2. Itch and flare responses after administration of levomepromazine (Nozinan), expressed as percentages of the values obtained before drug administration.

contradict such a hypothesis. From this it follows that the reason for the latent period remains to be explained. It cannot be due to a chemical reaction leading to formation of bradykinin, a conclusion which is supported by the fact that a latent period was observed not only after injection of kallikrein and histamine, but also after injection of bradykinin itself.

Previously it has been concluded that itch elicited by trypsin and chymotrypsin is, at least to some extent, mediated by histamine released from dermal mast cells, whereas papain produces itch without involving histamine (2, 7, 8). Kallikrein itch seems to resemble papain itch in many respects: no flare, itch of a pricking character, and no reduction of itch duration with antihistaminics. Thus, kallikrein like papain, is a protease which does not seem to release histamine from skin mast cells.

Bradykinin is one of the body's most potent pain-producing substances (1) and it might be expected that it should produce itch when injected close to the itch receptors at the dermo-epidermal junction. However, Cormia & Dougherty (4) reported that the itch-eliciting effect of bradykinin is weak, a finding which was confirmed in the present investigation. Greaves & Shuster (6) found no pruritogenic effect of intradermally injected bradykinin. One reason for this might be that they injected too large volumes (0.1 ml), since it is essential to inject volumes less than 0.05 ml to elicit experimental itch (15).

The antihistaminic drug levomepromazine reduced both the itch and the erythema induced by bradykinin. This inhibitory effect may be explained in two ways: (1) In addition to being an antihista-

mine, levomepromazine may be an antagonist of bradykinin. In favour of this alternative is the observation that some antihistamines also counteract the action of bradykinins (10, 12). (2) The responses observed after injection of bradykinin were due to histamine, i.e. bradykinin is a histamine liberator. This possibility is supported by the finding that bradykinin may release histamine in vitro (9, 13), and that mepyramine, which is considered to be a specific antihistamine, may reduce bradykinin-induced flare (11). At present this explanation seems to be the most probable one, i.e. the bradykinin itch is mediated by histamine.

To further illustrate the complicated interrelation between the pruritogenic factors it may be mentioned that injection of histamine or the histamine liberator compound 48/80 causes an increase in the kinin-forming activity in the lymph (5, 17). In these studies it was concluded that histamine, by increasing vascular permeability, allows an excess of fluid into the interstitial space, thus leading to activation of a kinin-forming enzyme, e.g. activation of prekallikrein to kallikrein.

From the above it is evident that bradykinin is not the main final mediator of protease-induced itch. Whether the pruritogenic substances directly stimulate the itch receptors or whether some other final itch mediators are formed is a question that still remains to be ascertained.

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