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CUTANEOUS REACTIONS TO KALLIKREIN, BRADYKININ AND HISTAMINE IN HEALTHY SUBJECTS AND IN PATIENTS WITH URTICARIA

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About 40 years ago Frey, Kraut and Werle found a blood pressure reducing principle in urine which seemed to originate from the pancreas. They named this substance kallikrein (Greek for pancreas). Later Werle (6) found that kallikrein was an enzyme which catalyzed the formation of an active serum substance called kallidin. Rocha e Silva (20) described in 1949 a peptide (bradykinin) with a similar pharmacological action which could be liberated from an alfa-2-globulin plasma fraction by snake venom and trypsin. Not until 10 years later did these two principles become connected and it was found that kallidin-9 and bradykinin were identical.

We now know that the kallikreins are a group of peptidases which liberate vasoactive peptides—called kinins—from an alfa-2-globulin. Kallikreins have been found in the pancreas, intestinal walls, salivary glands, saliva, sweat, cerebrospinal fluid, plasma and urine. They usually exist in an inactive state (kallikreinogens, prekallikreins) but are activated, for example, by the dilution of plasma. *In vivo* it is possible that extravascular leakage of plasma is an important factor for their activation. In the skin of man, the rabbit and the guinea pig, the kinins liberated by kallikrein give rise to increased capillary permeability. Since kinins produce both vasodilatation, edema, pain and leucocyte migration they have been assumed to be mediators of inflammatory reactions. Their formation, inhibition, and degradation is illustrated schematically in Figure 1.

There are now three chemically known kinins (plasma kinins): 1) Bradykinin (=Kinin-9); 2) Kallidin (=Kinin-10); 3) Methionyl-lysylbradykinin (=Kinin-11). Their pharmacological actions are almost

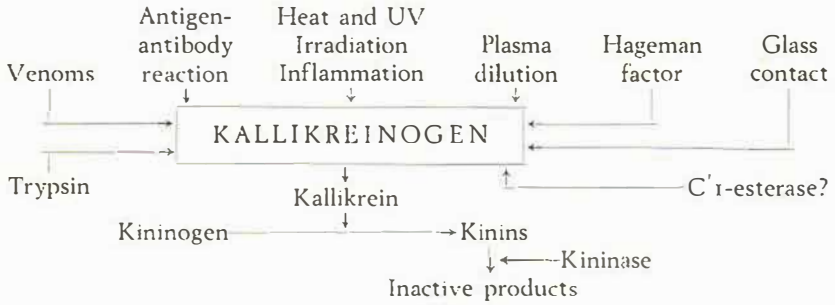

Fig. 1. Formation of kinins. Modified from Werle (27).

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the same. Bradykinin was synthesized in 1960 by Boisonnas *et al.* (2); this is the kinin that has been most studied and its effect is usually regarded as representative of all kinins (6 for ref.).

Further clinical interest in the kinins arose in 1962 when Landerman *et al.* (13) described a decreased amount of plasma inhibitor of kallikrein in patients with hereditary angioneurotic edema. Chronic urticaria is another disorder in which the kallikrein-kinin system might play an important role. That other factors than histamine may be involved is strengthened by the fact that these patients usually have little benefit of antihistamines. We have therefore studied the effect of intracutaneously injected histamine, bradykinin and kallikrein in normal subjects and in patients with various types of urticaria.

Material and Methods

The drugs used were: Histamine hydrochloride[1] 0.1 mg/ml. Synthetic bradykinin[2] 0.1 mg/ml. Kallikrein.[3] The dry powder containing kallikrein 40 U, thiomersalsodium 0.02 mg and sodium chloride 3.44 mg was dissolved in 1 ml. of saline. Lidocaine 1 %.[4]

Experimental procedure: Intradermal injections of 0.1 ml. of bradykinin, histamine and kallikrein were given on the volar side of the forearm. The area of the erythema and of the wheal with subsequent edematous infiltration was estimated by measuring the diameters and calculating that they were regular ellipses. All measurements were made by the authors, 20 minutes and 1, 2, 5, and 24 hours after injection. When repeated cutaneous tests were performed care was taken to avoid skin areas used previously. Freshly prepared solutions of kallikrein were used throughout. No patients had a known or suspected hypersensitivity to mercury.

Controls. Twenty-five apparently healthy men and women, mostly hospital personnel, aged 22–56 years, served as controls. Eleven of them were more than 30 years.

Chronic urticaria. Fourteen patients who had had frequent, almost daily urticarial eruptions for more than 4 months were being investigated on the wards in an attempt to determine the etiology of their urticaria. The patients are listed in Table 1 but the following comments should be added: Patient No. 1 had several attacks of cholecystitis 1962–64 and during the same period a recedivating urticaria which disappeared after a cholecystectomy in 1964. Since May 1967 there were again daily eruptions of urticaria. Patient No. 2 had a recidivating urticaria during 1961–62 and in 1967 she again had urticaria with slowly developing urticarial lesions which could remain unchanged for several days. Patient No. 3 had a giant type of urticaria which started after penicillin injections 18 years previously for a dental infection. During 4 pregnancies she was free from urticarial eruptions but within a few days after delivery there was an exacerbation. The eruptions became worse in the premenstrual period. Her last outbreak of urticaria was the most severe and started after an operation for varicose veins 4 months before the present investigation. In patient No. 4 the urticaria began the day after a cholecystectomy. She also had moderate rheumatoid arthritis, hemolytic anemia, L.E. cells and an increased sedimentation rate. Dental foci were found in patient No. 5. The urticaria and Quincke's edema of patient No. 6 started after a tonsillitis treated with sulphonamides. The condition was aggravated by mold-containing food such as cheese and beer. Patient No. 7 had had urticaria and Quincke's edema of unknown cause since 1963. Patient No. 8 had urticaria which started after a pneumonia treated with penicillin. He was also alcoholized. Patient No. 9 had urticaria of unknown

[1] Vitrum AB, Stockholm, Sweden.
[2] BRS, Bradykinin was kindly supplied by Sandoz, Stockholm, Sweden.
[3] Padutin®, Bayer AG, Leverkusen, Germany.
[4] Xylocain®, Astra AB, Södertälje, Sweden.

Table 1. *Reaction to intradermal injection of kallikrein in patients with different types of urticaria*

Pat. No.	Type of urticaria	Sex	Age	Area of edematous infiltration in mm² at various times after injection					Comments
				20′	1h	2h	5h	24h	
1	Chronic	f	58	90	226	734	1509	3449	
2	Chronic	f	25	113	314	392	1965	314	
3	Chronic	f	44	123	412	589	1981	4080	Penicillin-provoked
4	Chronic	f	50	99	225	419	847	1560	SLE w. hemolyt. anem.
5	Chronic	m	22	376	918	1810	1642	961	Dental foci
6	Chronic	f	48	685	1178	1700	4950	3140	Mold sensitive
7	Chronic	f	63	177	472	2160	7070	6280	Mold sensitive
8	Chronic	m	43	298	523	683	3454	4396	Penicillin-provoked?
9	Chronic	f	44	122	236	518	2591	6359	Shrimp sensitive
10	Chronic	f	49	236	825	904	1415	118	Aspirin sensitive, urin. inf.
11	Chronic	f	45	95	267	589	1031	1100	Aspirin sensitive
12	Chronic	m	36	79	200	707	942	0	Aspirin sensitive
13	Chronic	m	45	224	267	1100	2161	314	Aspirin sensitive
14	Chronic	f	44	542	942	1257	589	0	Aspirin sensitive
15	Acute	f	33	141	79	79	39	0	Taenia found
16	Acute	f	34	177	236	440	393	314	Shrimp sensitive
17	Acute	f	27	94	118	79	252	79	
18	Acute	f	58	141	314	550	707	314	
19	Acute	f	24	153	432	339	1532	314	Penicillin reaction
20	Acute	m	48	177	236	236	197	1730	Laxative reaction
21	Acute	f	67	204	707	361	1960	393	
22	Factitious	m	38	79	79	113	224	236	Taenia found
23	Factitious	f	34	79	362	314	314	314	
24	Factitious	f	23	95	79	961	1100	1730	
25	Factitious	f	25	118	39	39	0	706	
26	Factitious	f	32	94	283	425	314	0	
27	Factitious	m	22	86	79	153	42	20	
28	Cold	m	21	79	79	39	177	1570	
29	Cold	f	55	94	79	189	433	1375	
30	Heat	f	22	94	707	252	189	346	
31	Heat	f	25	138	113	267	345	79	

cause but in addition she had noticed a marked sensitivity to shrimps. Patient No. 10 had recidivating infections of the urinary tract since several years. Patients Nos 10 to 14 showed a marked increase in symptoms after provocation with 0.25 g. acetylsalicylic acid (aspirin). Their urticaria improved on a diet free from salicylates.

Thus a history of preceding infection was common, but our search for active infectious foci was negative in all patients except Nos 5 and 10. When developing the urticarial eruptions were tender to pressure in patients Nos 4 and 6. In patient No. 4 a bluish discoloration was often seen when the urticae had disappeared after 24–48 hours. Dermographism was not seen in any patients with chronic urticaria. Scratch or intracutaneous tests with molds were positive in patients Nos 6 and 7. Patients Nos 1, 2, 8, and 9 had delayed reactions to several of the antigens tested.

The level of C′1 esterase inhibitor in serum was estimated and found to be normal in patients Nos 1, 3, 4, 6, and 7.

Acute urticaria. The probable cause of

Table 2. *Reaction to intradermal injection of kallikrein*

	Number	Mean area of infiltration in mm² ± SE of the mean				
		20'	1h	2h	5h	24h
Controls 22–30 yrs	14	104 ± 11	119 ± 25	116 ± 25*	316 ± 90	766 ± 158*
Controls >30 yrs	11	108 ± 16	126 ± 13	199 ± 21	342 ± 59	359 ± 89
Chronic urticaria	14	233 ± 50*	500 ± 88***	992 ± 146***	2296 ± 479***	2291 ± 615**
Acute urticaria	7	155 ± 13	303 ± 81	298 ± 67	726 ± 278	449 ± 220
Factitious urticaria	6	92 ± 6	153 ± 55	334 ± 138	332 ± 163	501 ± 267

* $P<0.05$; ** $P<0.01$; *** $P<0.001$
P=The probability that the difference between controls over 30 years and others is caused by random factors.

Table 3. *Reaction to intradermal injection of histamine and bradykinin*

Diagnosis	Number of pat.	Mean area of wheal in mm² ± SE					
		Histamine			Bradykinin		
		20'	1h	2h	20'	1h	2h
Controls 16–30 yrs	10	217 ± 20	204 ± 24	0	94 ± 12	69 ± 13	0
Controls >30 yrs	10	180 ± 28	176 ± 29	0	103 ± 13	38 ± 12	0
Chronic urticaria	14	127 ± 14	111 ± 26	64 ± 24	100 ± 11	123 ± 17**	111 ± 26
Acute urticaria	7	285 ± 113	239 ± 64	87 ± 36	119 ± 18	160 ± 50*	67 ± 29
Factitious urticaria	6	143 ± 26	136 ± 39	38 ± 29	127 ± 29	52 ± 21	62 ± 42

* $P<0.05$; ** $P<0.01$ (see Table 2)

urticaria was in one patient infestation with Taenia, in two others drugs and in one a meal of shrimps. In three patients the cause was unknown. The patients are listed in Table 1.

Factitious urticaria. All these patients had a marked dermographism and their urticarial lesions were always referable to scratching or rubbing. Five of the six patients had a history of a long period of increased mental tension. In one of them Taenia was found.

Cold urticaria. Two patients who had had typical cold urticaria for 14 and 30 years were otherwise healthy. Tests with ice-cubes produced an urticarial reaction.

Heat urticaria. The patients showed small wheals typical for cholinergic urticaria. Patient No. 27 had no immediate dermographism to stroking but after 1 to 5 hours the stroked area showed a fragmented linear, red-cyanotic colored wheal without flare. She also had allergic rhinitis. Intracutaneous tests showed an immediate positive reaction to horse and pollens. After 5 hours these test areas were strongly red-cyanotic but with no wheals.

Results

Effects of histamine: The initial wheal and flare produced by intracutaneous injection of histamine varied widely both in the control group and in the patients. No significant differences were observed between controls and patients in this limited material.

In the control group the subjects less than 30 years old had a tendency to develop a larger mean flare area than the older subjects (mean ± SE 2282 ± 330 mm² and 1100 ± 420 mm², respectively, $P<0.05$). No differences were found with regard to wheal formation. Two hours after the injection no or only slight infiltration was present in the control group (Table 3).

In 8 of the 14 patients with chronic urticaria a wheal or infiltration was still pres-

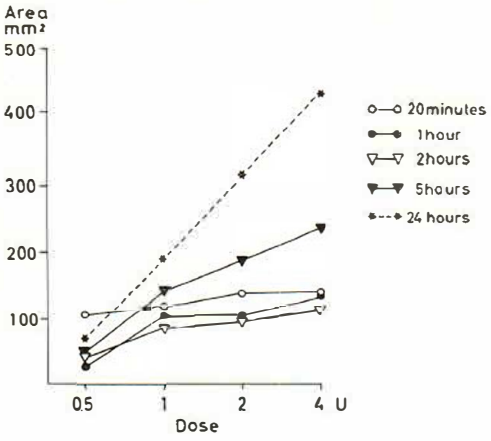

Fig. 2. Dose response curve for kallikrein measured 20 minutes to 24 hours after intradermal injection of 0.1 ml (5–40 U/ml). The points show the means from 7 subjects.

ent or reappeared 2 hours after injection of histamine. In 5 of these patients (Nos 1, 4, 7, 8 and 13) the delayed urticarial wheal had its maximum after 5–10 hours and was then slightly larger than at 20 minutes. The reactions were confirmed on repeated tests with negative saline controls. In 2 patients with acute urticaria and 1 with factitious urticaria the histamine reaction persisted for 2 hours and then disappeared. Otherwise patients with acute, factitious, cold and heat urticaria showed normal reactions to histamine.

Effects of bradykinin: In both controls and patients intradermal injection of bradykinin produced a wheal with a narrow reddish brim and a flare which had disappeared within one hour. The mean area of the flare in the younger control subjects was larger than for those over 30 years of age (mean ± SE 809 ± 130 and 275 ± 118 mm^2, respectively, $P < 0.01$). The axon-reflex was in 5 patients blocked by local injection of 1 percent lidocaine. No flare was seen in the anaesthetized skin in contrast to the typical flare in an untreated or saline treated area.

In patients with chronic urticaria the flare and initial wheal did not differ significantly from those in the controls but after one hour the wheal had increased in size (Table 3). At 2 hours, however, a reddish infiltration was palpable in 11 out of 14 patients. In 6 of them (Nos 1, 3, 4, 7, 8 and 13) the infiltration increased in size and reached a maximum at 5–6 hours. One patient (No. 4) had a maximal reaction at 6 hours with an erythematous tender infiltration which was visible for more than 24 hours. When it disappeared it left a bluish color of the same type as was seen in this patient after kallikrein. Another of the patients with chronic urticaria (No. 7) had on repeated tests an immediate reaction with a pale wheal which had almost disappeared at 1 hour, but at 2 hours there was an erythematous infiltrate which reached its maximal size at 5 hours.

In acute urticaria the wheal produced by bradykinin appeared to be increased at one hour (Table 3). Two patients with factitious and 4 with acute urticaria showed residual infiltration at 2 hours and one patient with severe generalized acute urticaria induced by penicillin had an erythematous reaction at 5 hours. One week later when she was free from urticaria the reaction was normal. Patients with acute, factitious, cold and heat urticaria otherwise had normal reactions.

Effects of kallikrein: In all control subjects intradermal injection of 1–4 U of kallikrein caused immediate pain persisting for 1–2 minutes but no itching. The wheal was surrounded by a flare which reached a maximum after 20 minutes. No significant differences were found between the size of the immediate flare reaction in subjects below 30 years of age and in the older subjects. After one hour the wheal had been replaced by a superficial, erythematous and tender but non-pruritic infiltration of the dermis which slowly increased after 2 hours. The infiltration attained its maximum after 5–24 hours and gradually disappeared within 48 hours. The same type of reaction was obtained with lower doses but they were less pronounced. As is evident from Figure 2 the slope of the dose response curve is steepest for the reactions at 24 hours. During the first hour after injection the size of the edema induced by kallikrein showed no correlation to the age of the subjects tested. At 2 hours, however,

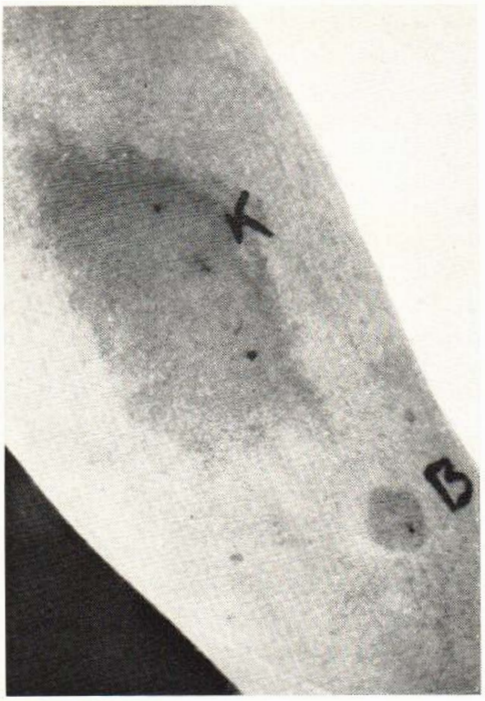

Fig. 3. Increased reaction to kallikrein and delayed reaction to bradykinin (3 hours after injection).

the mean area in control subjects below 30 years of age was smaller than in the older subjects ($P<0.05$). On the other hand, twenty-four hours after injection the mean area of the infiltrate in the younger subjects was larger than in those over 30 years of age ($P<0.05$) and an infiltration over 600 mm^2 was now more common among those below 30 years of age ($P<0.02$). The erythematous area was generally the same as the area of infiltration. Thus at 24 hours the control subjects less than 30 years of age had a larger erythematous area than the older controls ($P<0.01$).

When a second injection of kallikrein was given into the 24 hours old erythematous infiltration of 11 healthy subejcts the size of this area increased in 8 of them. The mean of the quotients—area after the second injection/the original area—was 1.9 one hour after the second injection and 2.0 twenty-four hours later. The infiltration then showed a bluish-green discoloration.

In patients with chronic urticaria the area—and especially the volume of the kallikrein induced edema—was much larger and was often seen as an elevation of the skin surface. It was palpable as a tender, fairly tense, deep infiltration. The overlying skin often had a reddish cyanotic hue which was not seen in normal subjects (Fig. 3). The areas of the edematous infiltrations are given in Tables 1 and 2. The increase of the edema compared to that in healthy subjects was most pronounced 1 to 5 hours after the injection (Table 2). It should be noted that the measures refer to the areas and not to the volume of the reactions. If the volume had been measurable the differences would have been even more striking. The strongest reactions were seen in patients with positive intradermal reactions to molds and the weakest in patients sensitive to salicylates.

As can be seen in Tables 1 and 2 the reaction to kallikrein was within normal limits in patients with acute, factitious, cold and heat urticaria.

Intradermal injections of serum and plasma

Blood was drawn from eleven of the patients with chronic urticaria and 1/10, 1/25, 1/100 saline dilutions of serum and citrated plasma were prepared and left for one hour in glass tubes. Intradermal tests were made with 0.1 ml. of the undiluted and diluted autologous serum and plasma.

One hour after the injection patient No. 7 developed increasingly tender erythematous infiltrates which reached a maximal size at about 24 hours. The strongest reaction was seen where undiluted plasma and serum had been injected. These infiltrates measured about 5000 mm^2. This patient had the same blood group as two patients with hereditary angioneurotic edema and she was also tested with their sera which lacked the inhibitor to C'1 esterase. She developed the same palm sized infiltrates to these sera as to autologous serum. A test with serum from a healthy subject gave a normal reaction and a test with the patient's serum in a healthy subject revealed no abnormal reaction. The reactions to autologous serum and plasma were un-

changed after i.v. infusion of 1000 ml. of fresh homologous plasma.

Patients Nos 1, 3, 4, 5, 6, 8, 9, 10, 11 and 31 showed normal reactions to tests with their own sera or plasma.

Discussion

Effects of histamine, bradykinin and kallikrein in healthy subjects

Histamine injected intradermally into human skin causes the triple response of a local vasodilatation, wheal and axon reflex flare which has been described in detail by Lewis (15). Bradykinin produces a wheal which in all subjects below 30 years of age was surrounded by an erythema with diffuse outer borders of the same type as seen in axon reflex mediated flare. When the flare disappeared a distinct red brim around the wheal could be seen. In older subjects the tendency to flare was less. The erythematous brim is believed to result from a direct effect on the vessels (8). Opinions differ as to if there is also a flare mediated by an axon reflex after bradykinin injection. Thus Schachter (22) found a wheal and flare similar to that of histamine after injection of 5 μg of synthetic bradykinin, whereas Greaves and Shuster (8), after injection of 10 μg, found an erythema which they did not consider to be mediated by an axon reflex since its spread was limited by a tight elastic band. On the other hand we found that in skin previously injected with lidocaine there was no erythema of the axon reflex type after bradykinin in contrast to the control skin where this was distinctly seen.

The effect of bradykinin and histamine in our subjects was maximal after 5 to 20 minutes and disappeared within 1–2 hours. These reactions are different from that seen after intradermally injected kallikrein. The initial wheal and erythema after kallikrein was followed by a usually red and always tender edematous infiltration which attained its maximum after 5–24 hours and then gradually disappeared. Injection of kallikrein also induced immediate pain which persisted for 1–2 minutes, and after 2 hours the area was tender to pressure and remained so for about 24 hours. Bradykinin is also known to cause a burning pain immediately after injection in some patients (8, 18). However, only a few of our control subjects complained of pain and it was certainly less than the pain produced by kallikrein. A low dose of kallikrein also induced immediate pain which might suggest that the pain is not so much due to a quantitative difference but rather to a qualitative one. It is possible that kallikrein in addition to bradykinin releases substances with more pain-producing activity.

There are few reports on the effects of kallikrein in normal human skin. Herxheimer and Schachter (9) injected in one subject a non-standardized preparation prepared from saliva. Mitchell and Krell (18) used the same preparation in nine subjects as we did but in lower concentrations (2 U per ml.). They found no flare and a wheal only seen for one hour. Cormia and Dougherthy (3) injected non-specified kallikrein. In contrast to our findings it produced a severe burning itching in 18 of 23 trials and only transient pain in 3 of 23 trials. They found small sharply demarcated wheals and less pronounced flares after kallikrein than after bradykinin.

When repeated doses of histamine are given intradermally into the same area the response is diminished (8). As pointed out by Schayer (23) this does not exclude a possible role of histamine in inflammation since the levels of histidine decarboxylase are increased after 5–6 hours in various types of inflammation. Such an 'induced' histamine differs in its activity from extrinsic histamine and its effects are not reduced by antihistamines. Reports of development of tachyphylaxis after bradykinin are divergent. Thus Fox *et al.* (7) found that tachyphylaxis developed fairly soon after bradykinin injection, while Greaves and Shuster (8) found no evidence of tachyphylaxis in respect to increased vascular permeability. Oyvin *et al.* (19) found, in animals, tachyphylaxis to histamine but no or only minor tachyphylaxis to bradykinin. Werle found that the blood pressure lowering effect of kallikrein decreased when repeated intravenous injections were

given at intervals of 10 minutes (28). He assumed that this was due to a lack of substrate for kallikrein. By waiting one hour before the injection was repeated, a full effect of kallikrein was obtained. When the same dose of kallikrein was injected into a 24 hours old lesion we found a marked enhancement of the pain, erythema and edema. The short duration of the derangement of the blood vessel permeability observed after bradykinin appears to depend upon a high activity of the kininase of the skin. This does not, however, rule out the participation of bradykinin as a mediator of sustained inflammatory reactions since we might have conditions with continuous formation of kinins by kallikrein. The prolonged effects of kallikrein further strengthen the importance of the kallikrein-kinin system as inflammatory mediators.

Our preparation contained about 4 U of kallikrein per mg. By injecting the same number of units of a 50 times more purified preparation we obtained a similar initial reaction but the effect disappeared sooner (11). These results suggest that the cruder preparation might contain some stabilizing factors for kallikrein or produce a more favorable milieu for its action such as, for example, a decrease of pH in the injected tissue. Thus a weakly acid tissue reaction is known to increase the formation of plasmakinins (31). When subcutaneously injected plasma was acidified to pH 6.3 about 10 times more kinins were formed. The increase was due both to a reduction of kininase and a reduction of kallikrein inhibitor (14).

Little is mentioned in the literature about variations of vascular reactions at different ages. Lamson (12) states that the senile atrophic skin shows the same reaction to histamine as ordinary skin. Schott (24) found a tendency to a smaller flare reaction with increasing age. He gives the followings means: 0–30 years 26.8 cm^2, 30–50 years 24.8 cm^2, 51–70 years 24.3 cm^2. It does not seem probable that the differences are significant. Roussy *et al.* (21) mention that the reactivity to histamine is poor in elderly people. This has been confirmed in the present study if by elderly people we mean those over 30 years. The flare produced by injection of bradykinin was also increased in younger people. It should be pointed out that it is apparently only the axon reflex mediated erythema which is increased. The erythema over the wheal which is caused by a direct effect on the vessels was not influenced by the age of the subjects. Whether or not a difference in the initial flare can influence the delayed erythema is uncertain. The increased reaction 2 hours after injection of kallikrein in older subjects possibly means that they produce kinins faster. In those less than 30 years the larger areas of both erythema and infiltrate 24 hours after injection could be explained by a delayed formation of kinins.

Vascular reactions in patients with urticaria

Histamine is thought to be the most important mediator in the production of urticaria. There are no reports that other agents such as kinins may be involved in the urticarial process. However, Winkelmann *et al.* (29) found that kinins are formed in dermographism produced by modest pressure in normal persons following the application of tetrahydrofurfuryl nicotinate ointment. This finding and the varying response of urticaria to antihistamines strengthen the possibility of some other mediators besides histamine.

In the present investigation we found strongly increased reactions to intradermally injected kallikrein in patients with chronic urticaria. The patients developed erythema localized over a tender large edematous infiltration which differed significantly from that in the controls. The differences were maximally pronounced 1–5 hours after the injections, but were still evident after 24 hours.

Another interesting finding in this study was a delayed reaction to histamine and bradykinin which was found in some patients with chronic urticaria. When the initial whealing had almost disappeared it was followed after 3–14 hours by a wheal or edematous infiltration which was often tender on pressure. This type of reaction to

histamine and bradykinin does not seem to have been described previously. The only report we have found in the literature is of an excessive reaction to histamine in a 56-year-old woman with mastocytosis leading to necrosis (25). A delayed type of histamine reaction might possibly be thought to be connected with delayed dermographic reactions which have been described in patients sensitive to molds (1). However, delayed dermographism was found in none of our patients with chronic urticaria, although two of them showed a positive immediate reaction to molds.

The cause of these delayed reactions to histamine and bradykinin are unknown, but it is possible that they are mediated by the kallikrein-kinin system. In favor of such a theory is the finding that the delayed reactions followed the same pattern as the reactions to kallikrein. The delayed reactions, like the increased reactions to kallikrein, were also eliminated by intravenous infusions of an identified kallikrein-inhibitor[5] (10). A possible mechanism for the delayed effects of histamine and bradykinin in chronic urticaria might be that they cause a leakage of plasma which activates kallikrein, to which these patients are very sensitive. Thus subcutaneous injection of plasma gives rise to extravascular formation of kinins (30). They can be formed either by activation of plasma kallikreinogen by an unknown tissue factor or by dilution of plasma (17). Histamine should in this way be able to initiate an inflammatory reaction by causing leakage of plasma. That histamine and histamine liberators actually increase the kinin forming activity was found by Edery and Lewis (5). The amount of kinins thus released is, however, normally probably too small to produce a sustained reaction after histamine injection. On the other hand in chronic urticaria, with highly increased sensitivity to kallikrein, it might be enough to give the typical delayed reaction. The probability that they lack an inhibitor to kallikrein will be discussed in the following.

Patients with hereditary angioneurotic edema are known to lack inhibitors to kallikrein and to C′ 1-esterase (4). C′ 1-esterase (C′1a) is an enzyme which is formed when the first component (C′) of the serum complement system interacts with an antigen-antibody complex. It has been suggested that C′ 1-esterase takes part in the formation of kallikrein (26). If this is correct it might be possible that also patients with increased sensitivity to kallikrein might lack C′1-esterase inhibitor. Patients with urticaria are, however, said to have normal or increased values of these inhibitors (4). Normal levels were also found in the five of our patients with the most severe form of urtciaria and the strongest reactions to kallikrein. This suggests that it is only the kallikrein inhibitor which is lacking, since the increased reaction to kallikrein could be inhibited by a kallikrein inhibitor (10).

Intradermal injections of autologous serum and serum from two patients with hereditary angioneurotic edema, of the same blood-group, induced in one of our patients a very strong reaction with a maximal size after 24 hours. Serum from a healthy subject gave no abnormal reaction and the patient's serum produced a normal reaction in a control subject. These findings suggest that she had an increased capacity to produce kinins or permeability increasing substances, which might be due to a lowered inhibitor level to some of the substances involved. She also reacted to serum of patients with hereditary angioneurotic edema who lacked the inhibitors to both C′ 1-esterase and kallikrein. It makes it probable that she lacked an inhibitor to kallikrein since her C′ 1-esterase inhibitor level was normal. The reason that the other patients with chronic urticaria did not react to autologous plasma might be that they had a small amount of inhibitor left, sufficient to inhibit the minute dose of kallikrein released by serum but not the higher doses of kallikrein injected. It should be noted here that the above patient is the one who showed the strongest reaction to injected kallikrein. Other explanations such

[5] Trasylol®, Bayer Ab, Leverkusen, Germany.

as lack of qualitatively different kallikrein inhibitors are also possible.

Intradermal tests with autologous sera have earlier been performed by Malmros (16) who tested over 900 patients with various internal disorders. He found immediate histamine-like reactions in 5.4 % of the patients, mainly in those in whom an allergic etiology was probable. The reaction usually disappeared within 1 hour and only very occasionally was a definite infiltration seen on the following day. Unfortunately it was not mentioned whether the tests were checked after 3–10 hours and the number of patients with urticaria was not given. In any case it seems that such a strong delayed reaction to autologous serum as was found in our patient must be rare.

SUMMARY

The effects of intradermally injected histamine, bradykinin and kallikrein were studied in normal subjects and in patients with various types of urticaria.

Intradermal injection of kallikrein caused in all subjects immediate pain persisting for 1–2 minutes. The wheal was usually surrounded by a flare which was maximal after 20 minutes. After one hour the wheal had been replaced by a superficial erythematous and tender infiltration which in normal subjects slowly increased after 2 hours and attained its maximum after 5–24 hours. The maximum of the reaction appeared earlier in older than in younger subjects. In patients with acute, factitious, cold and heat urticaria the reactions to kallikrein were within normal limits. However, a most striking effect of kallikrein was seen in patients with chronic urticaria. Here kallikrein induced a "giant" edema which was much more voluminous and tender than in normal subjects. The increase might possibly be due to a lack of inhibitor of kallikrein. The patient with the strongest reaction to kallikrein had a normal level of C'1-esterase inhibitor. She showed a palm-sized delayed infiltration 5–24 hours after injection of own serum and plasma and also of serum from patients with hereditary angioneurotic edema but not of normal sera. Ten other patients with chronic urticaria showed normal reactions to intradermal injections of autologous serum and plasma.

Histamine and bradykinin induced in normal subjects an immediate flare and a wheal and erythema which disappeared within 2 hours. The flare was larger in subjects less than 30 years of age than in older subjects. Among 14 patients with chronic urticaria the wheals after histamine and bradykinin injection persisted for more than 2 hours in 8 and 11 patients, respectively. A maximal wheal or infiltration was often seen after 5–10 hours. The theory is advanced that the delayed reaction is due to activation of the kallikrein-kinin system by the initial leakage of plasma in patients with a decreased level of kallikrein inhibitors. In patients with other types of urticaria these pronounced delayed reactions to histamine and bradykinin were not present.

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