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EFFECTS OF ANTIHISTAMINES, ACETYLSALICYLIC ACID AND PREDNISONE ON CUTANEOUS REACTIONS TO KALLIKREIN AND PROSTAGLANDIN E_1

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Abstract. The effects of antihistamines, acetylsalicylic acid and prednisone on the reactions to intradermally injected kallikrein, prostaglandin E_1, bradykinin and histamine were studied in patients with various minor skin disorders and in patients with chronic urticaria.

Systemic treatment with mepyramine reduced the size of the histamine-induced weal and both mepyramine and cyproheptadine strongly inhibited the axon-reflex mediated flare. None of them significantly lessened the weal produced by bradykinin. Nor did acetylsalicylic acid or prednisone change the response to histamine or bradykinin.

The antihistamines slightly reduced the reactions to kallikrein in patients with a normal kallikrein sensitivity. A more pronounced inhibition of the kallikrein reactions was seen in patients with chronic urticaria who initially showed strong reactions to kallikrein. An inhibition of the kallikrein reaction was also seen after treatment with prednisone and, to some extent, after acetylsalicylic acid.

A slight, but inconsistent, decrease of the erythematous reaction to prostaglandin E_1 was observed during treatment with antihistamines and prednisone while acetylsalicylic acid did not influence the reaction.

The vascular reactivity in human skin to intracutaneously injected kallikrein and prostaglandins E has recently been studied (9, 10). Abnormally increased reactions to both of these substances were found in patients with chronic urticaria. These findings actualized the need of further knowledge about the possibilities of altering the effects of kallikrein and prostaglandins E. It was considered logical to begin with a study of the effects of antihistamines and corticosteroids, as they are the drugs mainly used for symptomatic treatment of chronic urticaria. Since it is possible that the kallikrein-kinin system and the prostaglandins are involved as mediators of inflammatory reactions, the influence of an antiphlogistic, acetyl-salicylic acid was also studied.

MATERIAL AND METHODS

Patients. The study was carried out on seventy-one patients (thirty men and forty-one women). Their ages varied between twenty and sixty-three years. Sixty of them were older than thirty years. Fifteen patients had chronic urticaria and the remainder had various minor dermatoses, such as localized eczematous dermatitis (thirty-four patients), rosacea (five patients) and varicose leg ulcer (four patients). None of the patients had atopic dermatitis. The patients had no treatment with any drugs other than those administered for this study.

Substances used for intradermal tests. 1) Kallikrein (Padutin®, Bayer AG, Leverkusen, Germany). The dry powder containing kallikrein 40 U, thiomersal sodium 0.02 mg and sodium chloride 3.44 mg was dissolved in one or two milliliters of saline, giving a concentration of 40 or 20 U/ml. 2) Prostaglandin E_1, (PGE_1) (kindly supplied by Professor Sune Bergström, Stockholm, Sweden). A stock solution containing 50 μg/ml was diluted in saline to concentrations of 5μg and 1μg/ml. 3) Histamine hydrochloride, 0.1 mg/ml. 4) Synthetic bradykinin (BRS, Bradykinin, kindly supplied by Sandoz, Stockholm, Sweden), 0.1 mg/ml.

Procedure. On the first day the patients were given a primary intradermal test on the volar sides of the forearms with kallikrein, 4 U and/or 2 U; PGE_1, 0.5 and/or 0.1 μg; bradykinin, 0.01 mg; histamine, 0,01 mg; all in a volume of 0.1 ml. Often only 2 U of kallikrein were used in patients with chronic urticaria because of their pronounced sensitivity to kallikrein. On the second and third day the patients were given one of the following tablets: mepyramine (Anthisan®, Pharma Rodia, Birkeröd, Denmark), 100 mg three times a day; cyproheptadine (Periactin®, Merck, Sharp & Dohme, Int., New York, USA), 4 mg three times a day; acetylsalicylic acid, 1 g five times a day; prednisone, 50 mg before breakfast. On the third day, two hours after the patients had taken their morning dose of the drug, injections were given at corresponding sites on the other arm. A single dose of 50 mg prednisone or 1 g of acetylsalicylic acid was given to another series of patients.

The test reactions were measured and estimated as previously described in detail (9, 10).

Table I. *Influence of antihistamines, acetylsalicylic acid and prednisone on cutaneous reaction to intradermally injected kallikrein*

Drugs	No. of patients	Mean values ± S.E. of the mean of individual ratios for size of kallikrein reaction after/before treatment			
		0.3 h	1 h	2 h	5 h
		Kallikrein 4 U			
Mepyramine	16	0.91±0.15	0.74±0.14	0.75±0.14	0.64±0.11[c]
Cyproheptadine	11	1.18±0.28	0.84±0.15	0.74±0.16	0.52±0.17[b]
Acetylsalicylic acid	11	1.19±0.07	0.88±0.15	0.78±0.13	0.86±0.13
Prednisone (repeated doses)	11	1.21±0.17	0.82±0.16	0.81±0.14	0.56±0.10[d]
Prednisone (single dose)	15	1.27±0.16	1.30±0.19	0.95±0.18	0.76±0.13
		Kallikrein 2 U			
Mepyramine	17	0.64±0.07[d]	0.75±0.10[a]	0.68±0.12[b]	0.60±0.12[c]
Cyproheptadine	11	0.80±0.13	0.66±0.14[a]	0.70±0.21	0.54±0.10[d]
Acetylsalicylic acid	11	1.30±0.19	0.82±0.06[b]	0.64±0.11[c]	0.49±0.08[d]
Prednisone (repeated doses)	15	1.05±0.14	0.86±0.13	0.86±0.12	0.55±0.10[d]

p = the probability that the differences between the ratios and 1.00 are caused by random factors.
[a] $p<0.05$. [b] $p<0.02$. [c] $p<0.01$. [d] $p<0.001$.

Reproducibility of intradermal reactions. Intradermal tests were carried out for determination of the reproducibility of the test reaction in twenty-five patients with chronic urticaria and with various other skin disorders.

Kallikrein (4 U) and PGE_1 (0.5 μg) were injected at symmetrical sites on the left and right arms either at the same time or on two consecutive days. The mean ratio at 5 hours of the reaction in the left to that in the right arm for kallikrein tests performed at the same time was 1.01±0.07 and 1.06±0.14 for tests performed on two consecutive days. The mean ratio at 1 hour of the PGE_1 tests performed at the same time was 0.99±0.08 and for tests on two consecutive days the ratio was 0.98±0.06. The values obtained at other times after the injection, as well as those obtained with lower doses of kallikrein (1 U) and PGE_1 (0.1 μg), did not differ from those given here.

RESULTS

Effect of antihistamines. The edematous kallikrein infiltration present 5 hours after the injection was reduced by both mepyramine and cyproheptadine, as seen from Table I, where the mean ratios from all patients are given. Patients with chronic urticaria had abnormally increased reactions to kallikrein. In this group the inhibition caused by antihistamines was highly significant at all times of observation (Table II). In the other patients the inhibition after antihistamines was less significant, but the tendency to inhibition was obvious.

The influence of antihistamines on the erythematous reaction induced by 0.1 and 0.5 μg PGE_1 was inconsistent. A slight decrease of the erythematous reaction was seen 20 min after the injection (Table III). The erythema produced at 1 hour by 0.5 μg of PGE_1 was reduced after cyproheptadine, but not after mepyramine. The erythema induced by 0.1 μg of PGE_1 was, however, reduced by mepyramine. The influence on the reaction in eleven patients with chronic urticaria tested with PGE_1 did not deviate from that seen in the twenty-one other patients.

Both mepyramine and cyproheptadine strongly inhibited the axon-reflex mediated flare seen after intradermal injection of histamine (Table IV). After mepyramine the histamine weal was also significantly reduced ($p<0.01$). Cyproheptadine did not influence the size of the wheal. Neither mepyramine nor cyproheptadine changed the wheal seen after bradykinin. Whether or not they influenced the erythema is uncertain, but a marked inhibition of the flare was seen in those patients who developed an axon-reflex mediated flare ($p<0.001$).

Effect of acetylsalicylic acid. The infiltrations induced by 4 U of kallikrein were not significantly reduced by aspirin. The reactions to 2 U of kallikrein were, however, markedly decreased (Table I). Only one patient with chronic urticaria was given the full dose of aspirin. His urticaria worsened on the second day of aspirin consump-

Table II. *Influence of antihistamines and prednisone on the reaction to kallikrein in (a) patients with chronic urticaria; (b) patients with various other dermatoses*

Drugs		No. of patients	Mean values ± S.E. of the mean of individual ratios for size of kallikrein reaction after/before treatment Kallikrein 2 U			
			0.3 h	1 h	2 h	5 h
Mepyramine + Cyproheptadine	(*a*)	11	0.54 ± 0.05^{d}	0.51 ± 0.13^{d}	0.46 ± 0.12^{d}	0.40 ± 0.06^{d}
	(*b*)	18	0.76 ± 0.09^{b}	0.81 ± 0.09	0.85 ± 0.14	0.70 ± 0.11^{b}
Prednisone (repeated doses)	(*a*)	7	0.99 ± 0.21	0.70 ± 0.15	0.74 ± 0.20	0.42 ± 0.13^{c}
	(*b*)	8	1.12 ± 0.15	0.99 ± 0.18	0.89 ± 0.16	0.68 ± 0.12^{a}

[a] $p < 0.05$. [b] $p < 0.02$. [c] $p < 0.01$. [d] $p < 0.001$.

tion, but his reaction to kallikrein remained the same. Four patients with chronic urticaria were given a single dose of 1 g of aspirin without any obvious influence on the reactivity to kallikrein.

The reactions to PGE_1, histamine and bradykinin were not influenced by treatment with aspirin (Tables III and IV).

Effect of prednisone. After repeated doses of 50 mg of prednisone, the kallikrein infiltrations present 5 hours after injection were diminished. The patients with chronic urticaria showed a more pronounced reduction of their kallikrein reactivity than the other patients (Table II). A single dose of 50 mg prednisone caused no significant change of the intradermal reaction to kallikrein; but none of the patients given this dose of prednisone had chronic urticaria.

No effect on the response to histamine or bradykinin was detected after treatment with prednisone (Table IV) and the response to PGE_1 was only slightly diminished ($p < 0.05$) (Table III).

DISCUSSION

Effects on reactions to kallikrein. There are few substances available with a specific inhibitory effect on the kallikrein-kinin system that are also suitable for therapeutic use. A kallikrein inhibitor purified from cattle parotid gland and lung (Trasylol®, Bayer AG, Leverkusen, Germany) normalized the reactions to kallikrein in patients with chronic urticaria. This cannot be recommended for repeated use at present, however, as there seems to be a risk of serious, allergic side-effects (11). No specific antagonists to kinins are available, although some antihistamines are, to some extent, also anti-bradykinins. From animal and in vitro experiments, inconsistent results have been reported about the effects of antihistamines, acetyl-salicylic acid and corticosteroids on the kallikrein-kinin system (6). It is difficult, therefore, to draw conclusions on the effect of these drugs on the kallikrein-kinin system that are valid for humans. These difficulties are also partly de-

Table III. *Influence of antihistamines, acetylsalicylic acid and prednisone on cutaneous reaction to intradermally injected prostaglandin E_1*

Drugs	No. of patients	Mean values ± S.E. of the mean of individual ratios for size of reaction to PGE, after/before treatment			
		PGE_1 0.1 μg		PGE_1 0.5 μg	
		0.3 h	1 h	0.3 h	1 h
Mepyramine	20	0.84 ± 0.08	0.81 ± 0.07^{b}	0.85 ± 0.08	1.00 ± 0.08
Cyproheptadine	12	0.84 ± 0.09	0.85 ± 0.09	0.89 ± 0.03^{c}	0.80 ± 0.05^{c}
Acetylsalicylic acid	13	1.00 ± 0.11	0.99 ± 0.08	1.16 ± 0.13	1.08 ± 0.12
Prednisone (repeated doses)	13	1.03 ± 0.14	0.74 ± 0.10^{a}	1.04 ± 0.12	0.89 ± 0.09
Prednisone (single dose)	13	—	—	1.14 ± 0.15	0.99 ± 0.08

[a] $p < 0.05$. [b] $p < 0.02$. [c] $p < 0.01$.

Table IV. *Influence of antihistamines, acetylsalicylic acid and prednisone on cutaneous reactions to histamine and bradykinin*

Drug	No. of patients	Mean values ± S.E. of the mean of individual ratios for size of reaction to histamine and bradykinin after/before treatment		
		Histamine		Bradykinin
		Weal 0.3 h	Flare 0.5 h	Weal 0.3 h
Mepyramine	20	0.78 ± 0.07^a	0.60 ± 0.06^b	1.07 ± 0.20
Cyproheptadine	10	0.91 ± 0.24	0.37 ± 0.09^b	1.20 ± 0.18
Acetylsalicylic acid	13	1.17 ± 0.14	0.91 ± 0.08	1.21 ± 0.15
Prednisone (repeated doses)	13	1.33 ± 0.19	0.78 ± 0.21	1.10 ± 0.17

[a] $p < 0.01$. [b] $p < 0.001$.

pendent upon the incompleteness of our knowledge about the factors involved in the activation of kallikrein in man. For instance, the significance of histamine and histamine release for this process is not clear. In dogs injected intra-arterially with histamine or the histamine releasing compound 48/80 an increased kinin-forming activity was detected in lymph (4). Our findings of delayed whealing after intradermal injection of histamine in patients with increased reactions to kallikrein also indicate that kallikrein may be activated by histamine (9). On the other hand, simultaneous injections of histamine and kallikrein produced the same reaction as kallikrein alone in patients with normal sensitivity to kallikrein; nor did pretreatment with compound 48/80 influence the reaction to kallikrein (15). A reduction of the kallikrein-induced edema was, however, obtained after treatment with both mepyramine and cyproheptadine. Mepyramine is known to be one of the more specific antihistamines and is probably devoid of an antibradykinin effect whereas cyproheptadine can, in vitro, inhibit both histamine and bradykinin (19). None of these antihistamines influenced the wealing reaction to bradykinin. The reduction of the response to kallikrein after antihistamines might indicate either that a histamine release occurs parallel with the formation of kinins or that the antihistamines have other effects, not associated with their antihistamine properties, which result in subsequent reduction of the kallikrein-induced edema. The probability of kinins being able to release histamine and vice versa has been discussed by Melmon & Cline (13). The presence of an axon-reflex mediated flare after the injection of bradykinin in many normal subjects might also indicate that kinins could induce a histamine release (9).

The strong reaction to kallikrein found in most patients with chronic urticaria still was increased after antihistamines although it was markedly less when compared with that before antihistamines. The therapeutic effect of antihistamine in chronic urticaria was, however, usually poor (14). In view of the inhibition of the kallikrein reaction, a better effect of antihistamines on the clinical symptoms should, perhaps, have been anticipated. A possible explanation for this discrepancy might be that the role of histamine in the activation of kallikrein or formation of kinins is not dominant, or that the kinins formed are different from those produced by injection of kallikrein.

The effect of acetylsalicylic acid on experimentally induced inflammatory states has been reported by several authors. Administration of salicylate was found to suppress the inflammatory edema produced by thermal injury in rats, and rubefacients in man and guinea pigs (6). In these tests antihistamines had no inhibitory effect. Northover & Subramanian observed an inhibitory action by salicylates on the activities of kallikrein in vitro (17). They also noted that a marked inhibition of the local reaction to intradermally injected kallikrein in rabbits occurred on systemic administration of analgesic-antipyretic drugs. Their findings could not be confirmed by Hebborn & Shaw (7) or Lewis (12). They obtained no effect with acetylsalicylic acid on the activity of kallikrein, but nearly complete inhibition after administration of Trasylol®. Davies et al. also demonstrated that aspirin and various other anti-inflammatory drugs had no effect on kinin forma-

tion in vitro (2). They could not preclude an in vivo effect from these drugs, however, either from metabolites or through inhibition of some other factor relevant for activation of kallikrein.

In the current investigation aspirin was found to reduce the effects of kallikrein in patients without urticaria, but did not change the reactions to histamine or bradykinin. The mechanism of this inhibitory action is not clear. There is evidence that salicylates exert their anti-inflammatory action by inhibiting venular constriction and by decreasing capillary and especially venular permeability (18). It has also been proposed that salicylates act by releasing corticosteroids, by blocking the destruction of the steroids or by acting synergistically with steroids (3). Another factor of importance for the inhibitory action of both aspirin and corticosteroids on the size of the test might be that they inhibit the spreading of the injected kallikrein.

A marked inhibition of the kallikrein reaction was observed during treatment with prednisone, and this was especially pronounced in patients with chronic urticaria. Opinions on the effects of corticosteroids on the kallikrein-kinin system differ. Cline & Melmon found that cortisol prevented release of kinin from substrate by granulocytes or contact with glass (1). Whether the steroid influenced the substrate or the kallikrein activation was not clear. They also discussed the probability of interference with the action of the Hageman factor. Their findings could not be confirmed by Eisen et al. (5). They found little, if any, inhibition of kinin formation by corticosteroids. The results of the present study indicated, however, that the reaction to kallikrein in vivo was inhibited by prednisone, especially in patients with chronic urticaria, all of whom had abnormally increased reactions to kallikrein.

Effects on reactions to Prostaglandin E_1. The prostaglandins E_1 and E_2 are potent local vasodilators of the small arteries when injected intradermally. The effect on the skin seems to be similar to the reaction described as antidromic vasodilatation and it was therefore suggested that the prostaglandins E might be mediators of this reaction (10). It is possible that part of the vasodilatation occurring in inflammatory reactions might be of this antidromic type (20).

Little is known about the influence of various pharmacologic agents and drugs on the cutaneous reactions to PGE. In a previous study it was found that large doses of epinephrine were needed to blanch or prevent an erythematous reaction to PGE (10). It was also found that treatment with antihistamines had no inhibitory effect on the production of the PGE erythema, whereas in the present study a slight inhibition could be observed during antihistamine treatment. Different antihistamines, doses and ways of administration were used and the dose of PGE was larger (5 μg) than in the present study. It was also previously shown that pretreatment of the skin with the compound 48/80 did not influence the response to 0.1 and 1 μg of PGE. This, as well as the slight influence of antihistamines, may indicate that the role of histamine is not essential for the formation of an erythematous response to PGE_1. The slight inhibition obtained from antihistamines on the PGE_1 erythema in this study might possibly be due to an inhibition of a concomitant axon-reflex mediated flare. It could not, however, be a main component of the erythema as the histamine-induced flare—in contrast to the PGE erythema—was strongly inhibited by antihistamines.

After the administration of salicylates the reaction to PGE_1 was essentially the same as before treatment. The effect on the hyperalgesia experienced at the site of PGE_1 erythema was difficult to evaluate. The lack of effect on the erythematous response might be explained if it is correct that salicylates have no vasoconstrictor ability, but rather inhibit inflammatory reactions by decreasing capillary and venular permeability and by inhibiting venous constriction (18). The decrease in the reaction to PGE_1 after repeated doses of prednisone is not of the same magnitude as that seen after local treatment with fluocinolone acetonide. The latter produced blanching of the skin and inhibition of the axon-reflex mediated flare (8) and diminished the reaction to PGE_1 both initially and one to two hours after injection (10). The differences obtained between systemic and local treatment might be explained by the strong vasoconstrictive properties of fluocinolone. It is also likely that the local concentration of active steroid is higher after fluocinolone treatment.

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