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RESPONSES OF SKIN BLOOD VESSELS TO BRADYKININ, HISTAMINE AND 5-HYDROXYTRYPTAMINE

BY MALCOLM GREAVES AND SAM SHUSTER
From the University Department of Dermatology, Newcastle upon Tyne

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SUMMARY

1. The responses of human cutaneous blood vessels to intradermal injection of bradykinin, histamine and 5-hydroxytryptamine (serotonin) are studied in order to evaluate the ability of these agents to mediate the vascular changes of sustained acute inflammation in the skin.

2. Bradykinin produces erythema, owing to a direct effect on blood vessels, and wealing. Dose–response studies indicate that bradykinin is more potent than serotonin or histamine in respect of wealing.

3. The response to serotonin differs qualitatively as well as quantitatively according to dose. High doses cause wealing and erythema with the characteristics of an axon reflex flare, but low doses produce erythema by a local effect without wealing.

4. Using the technique of arterial occlusion, the occurrence of tachyphylaxis in respect of wealing was demonstrated with histamine and serotonin, but not with bradykinin. This evidence suggests that of the three agents, only bradykinin can mediate increased vascular permeability in sustained acute inflammation.

5. The specificity of tachyphylaxis and the failure of anti-histamine to antagonize bradykinin wealing suggest that bradykinin and histamine act on separate blood vessel receptors.

6. Corticosteroids do not inhibit wealing due to a wide range of doses of bradykinin. The anti-inflammatory activity of corticosteroids may therefore be due to reduced formation of kinins.

INTRODUCTION

Mediation of the vascular components of sustained acute inflammation in the skin has been attributed to several naturally occurring vaso-active substances. Two of these, histamine and 5-hydroxytryptamine (serotonin) have long been the subjects of intensive study. The evidence for and against their participation in inflammation has been discussed in recent reviews, notably those of Wilhelm (1962), Spector & Willoughby (1963) and Rocha e Silva (1964). Recently the discovery that short chain polypeptides, typified by the nonapeptide bradykinin, are highly potent in respect

of production of vasodilation and increased vascular permeability (Elliott, Horton & Lewis, 1960; Konzett & Sturmer, 1960; Schachter, 1962; Lewis, 1962) has led to recognition of the possibility that kinin formation may account for some of the signs and symptoms of inflammation in the skin (Greaves & Shuster, 1964).

One method of evaluating the ability of these and other vaso-active substances to bring about the changes of sustained acute inflamation is to study the responses of cutaneous blood vessels to them. In the present investigation detailed studies of vascular responses of the skin to bradykinin were carried out and compared with results of similar experiments using histamine and serotonin.

METHODS

The subjects studied were young healthy adults of both sexes and young adults of both sexes with skin lesions sufficiently localized to allow testing of clinically normal skin.

The following vaso-active agents were injected: bradykinin (synthetic, Sandoz), 1 mg/ml., histamine acid phosphate, 1 mg/ml. and serotonin creatinine sulphate (Sandoz), 10 mg/ml.

All doses of vaso-active agents were injected intradermally in volume 0·1 ml. using a tuberculin syringe and no. 15 needle. All experiments were performed on the flexor aspect of the forearm.

A weal was defined using the criteria of Lewis (1927). Only the central area, usually circular, was measured, no account being taken of pseudopod-like projections or satellite weals. Two diameters at right angles were measured 10 min after the time of injection, unless otherwise stated. The mean of these values is directly proportional to the square root of the area of the weal.

In experiments designed to demonstrate the presence or absence of tachyphylaxis in respect of wealing for any agent the following method based on that of Lewis (1927) was adopted. A sphygmomanometer cuff is placed on one upper arm of the subject and inflated above arterial pressure. The agent is injected intradermally when, because of arterial occlusion, no weal appears. Occlusion is terminated after 30 min following which little or no trace of wealing is seen compared with a control injection of the same dose of the agent into the same forearm in the presence of restored circulation. After 5 min either the same or another vaso-active agent is injected into the precise site of the previous injection and the size of any resulting weal is measured. Correction is made for the presence of any residual weal at the site of the first injection by subtracting its mean diameter from the mean for the weal due to the second injection. This corrected value is compared with the value for the control weal.

RESULTS

Isosmotic saline vehicle

The response of the skin to 0·1 ml. aqueous 0·154 M-NaCl was studied in seven subjects. In each a well-defined bleb of mean diameter $9{\cdot}0 \pm 6$ mm S.D. was raised. In all subjects no trace of the bleb remained at 10 min.

The bradykinin preparation used in the present experiments contains 5 mg Chloretone for every 100 μg bradykinin. In three subjects injection of 0·1 ml. of a solution of 5 mg Chloretone in 1·0 ml. 0·154 M-NaCl produced a response identical with that of 0·154 M-NaCl, no wealing being present at 10 min.

Bradykinin

Intradermal injection of bradykinin is followed by three responses: pain, a weal, and in some subjects, erythema.

Pain. Sixteen of nineteen subjects given 10 μg bradykinin complained of pain, usually described as burning in quality and lasting 10–45 sec. Pain was less frequent in response to lower doses. No itching was experienced by any of the subjects given doses ranging from 0·1 to 10 μg.

Weal. In all of nineteen subjects, 10 μg bradykinin induced weal formation of mean diameter 15·6 $\pm$ 3·4 S.D. The weals persisted for 4–5 hr. The dose–response regression for wealing with 0·1–10 μg bradykinin has a low gradient (Fig. 1). Furthermore, the regression of the plot does not pass through zero on the ordinate. The explanation for this (Fig. 1) is the saline solvent which produces a significant bleb at 5 min.

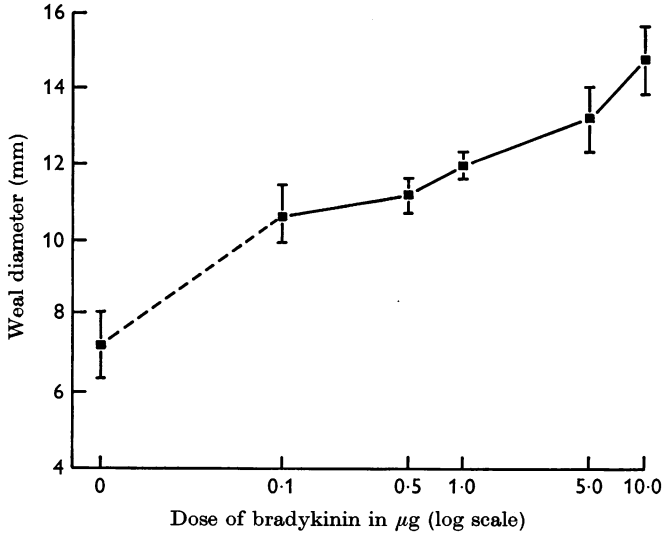

Fig. 1. Dose–response plot for wealing reaction to bradykinin. For each dose the mean and standard error of responses in ten subjects is given. Weal diameters measured at 5 min. Zero value represents the mean bleb diameter of an equal volume of isosmotic saline solvent injected at 5 min in thirteen young adult subjects of both sexes.

Erythema. Twelve of the nineteen subjects who developed wealing in response to 10 μg bradykinin, developed erythema of mean diameter 21·1 $\pm$ 8·2 mm S.D. Bradykinin erythema is not mediated by an axon reflex since spread of erythema was sharply limited by a tight elastic band applied round the forearm immediately proximal to the site of injection of 10 μg bradykinin.

Serotonin

Serotonin induces changes in the skin which differ qualitatively as well as quantitatively with the dose injected.

Erythema. Injection of 100–1000 μg serotonin is followed after 10 sec by a gradually extending bright red flare. If 100 μg serotonin is injected into skin immediately distal to a tightly constricting elastic band the flare is not confined to the skin distal to the band, suggesting that the flare, like the histamine flare, is mediated by an axon reflex. Using lower doses of serotonin it is apparent that erythema due to this agent is of two kinds. Injection of 0·1 μg serotonin causes erythema near the injection site of a darker shade than the flare and which is sharply confined by an elastic band, thus suggesting that it is a local effect and not mediated by an axon reflex. No bright red flare occurred with this dose of serotonin.

Wealing. Ten subjects were each given 1, 10, 100, and 1000 μg serotonin intradermally. The linear relationshp between log dose and magnitude of the weal is shown in Fig. 2. All ten subjects given 100 μg developed

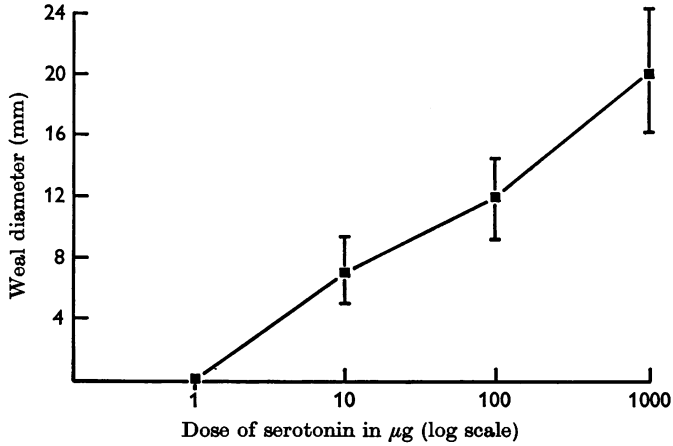

Fig. 2. Dose–response plot for wealing reaction to serotonin. For each dose the mean and S.D. of responses in ten subjects is given.

weals, pseudopods and satellite weals being frequently seen. Wealing was less constant with 10 μg and failed to occur in response to 1 μg. It was also of interest that doses which failed to induce wealing usually failed to induce flare formation although the dull red erythematous halo still appeared.

Pain. Pain, which was experienced by all subjects given 10–1000 μg serotonin was dull in quality and lasted as long as 3–4 hr in some subjects, persisting in all cases long after inflammation had subsided. Marked

hyperalgesia of the skin and deeper tissues of the forearm was noted and was particularly intense in the presence of venous constriction.

Venous constriction. Injection of 100–1000 μg serotonin into the flexor surface of the forearm caused intense constriction of the superficial veins of the forearm in some subjects. These changes lasted 10–20 min and were associated with intense aching of the forearm.

Tachyphylaxis

The presence or absence of tachyphylaxis in respect of increased vascular permeability was determined for bradykinin, histamine and serotonin.

Bradykinin. In eight subjects 10 μg bradykinin was injected intradermally into a forearm which had been rendered completely ischaemic. No weal appeared although erythema could be seen at the site of the injection. Thirty minutes later the circulation was restored and, after a further 5 min, no wealing could be detected at the site of the first injection in six subjects. In two, small weals developed which were diminished in size compared with control weals. A second dose of 10 μg bradykinin was then injected in the same site as the first. In all eight subjects a weal then appeared which was of the same order of magnitude as the control weal. Detailed results of this experiment are given in Table 1.

TABLE 1. Absence of tachyphylaxis to second bradykinin injection 35 min after the first

	Weal response (mm) Subject no.								Mean and S.D. of responses for group (mm)
Injection of bradykinin 10 μg	1	2	3	4	5	6	7	8	
Control injection, no occlusion	15	16	21	12·5	13	13	12·5	13	14·5 ± 2·9
First injection residual weal after termination of occlusion	12	8·5	0	0	0	0	0	0	—
Second injection, reinjection at same site as first injection	20	17·5	20	11	10·5	14	13	18	—
Corrected response to second injection	8	9	20	11	10·5	14	13	18	12·9 ± 4·2

Weal response to second dose of 10 μg bradykinin, injected at the same site as the first 5 min after termination of arterial occlusion of 30 min duration.

In the eight subjects the mean weal diameter for the second injection of bradykinin was 12·9 ± 4·2 mm S.D. compared with 14·5 ± 2·9 mm S.D. for the control injection. The difference between the means is of low significance ($n = 7$, $t = 1{\cdot}3$, and $0{\cdot}3 > P > 0{\cdot}2$). No evidence of tachyphylaxis with bradykinin was therefore detected.

In those six subjects where a weal failed to appear on release of the cuff after 30 min arterial occlusion it could be contended that bradykinin from the first injection was no longer present, and that if it had persisted

tachyphylaxis might have been demonstrable. The following experiment carried out in four of these subjects demonstrates that bradykinin from the first injection is still present 2·5–5 min before restoration of circulation. The cuff was inflated above arterial blood pressure and 10 μg bradykinin injected 30, 25, 20, 15, 10, and 5 min before release. In two of the subjects the 5 and 15 min injections were omitted and instead bradykinin was

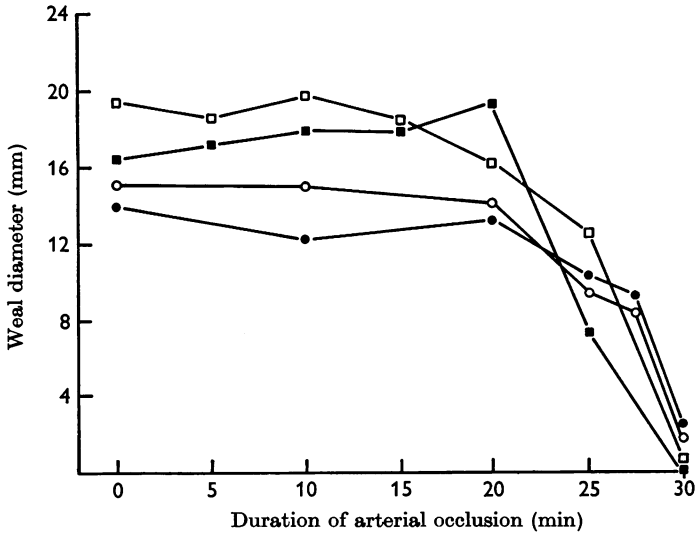

Fig. 3. Weal response to 10 μg bradykinin 5 min after termination of arterial occlusion of duration 0–30 min.

TABLE 2. Absence of tachyphylaxis to second injection of bradykinin 30–32·5 min after the first

	Mean weal diameter (mm)		
Subject	Control	First injection	Second injection (corrected)
1	19	7	16
2	15	7	13·5
3	14	5	17
4	14·5	7	13

Weal response to second dose of 10 μg bradykinin injected at the same site as the first 5 min after termination of arterial occlusion of 25–27·5 min duration.

given 27·5 min before restoration of circulation. The results, which are plotted in Fig. 3, show that even after 25–27 min occlusion bradykinin is still present since moderate wealing appeared, although this was abolished at 30 min. When in the same four subjects bradykinin was now injected into the weal which appeared after 25–27·5 min occlusion, the area of wealing increased and was, after subtracting the mean diameter of the

residual weal, approximately equal to the control weal due to the same dose of bradykinin (Table 2).

Histamine. In the same eight subjects who were tested in the bradykinin experiments, the wealing response to 100 μg histamine injected during arterial occlusion was absent or reduced after 30 min ischaemia, although the presence of erythema and an axon reflex flare indicated persistence of histamine at the original injection site. The presence of tachyphylaxis was confirmed since, by contrast with bradykinin, injection of the same dose of histamine at the site of the original injection 5 min after removal of the cuff failed to cause wealing in six subjects, and in the remaining two wealing was markedly reduced compared with a control injection (Table 3).

After correction for any residual wealing due to the first injection the mean diameter for the second injection in the eight subjects was $2{\cdot}1 \pm 3{\cdot}9$ mm S.D. compared with the corresponding control value of $20{\cdot}4 \pm 3{\cdot}2$ mm S.D. This difference is highly significant ($n = 7$, $t = 9{\cdot}75$ and $P < 0{\cdot}001$).

Thus, unlike bradykinin, histamine induces tachyphylaxis in the skin in respect of increased vascular permeability.

TABLE 3. Tachyphylaxis to second injection of histamine 35 min after the first

	Weal response (mm) Subject no.								Mean and S.D. of responses for group (mm)
Injection of histamine 100 μg	1	2	3	4	5	6	7	8	
Control injection, no occlusion	20	25	25	23·5	16·5	16·5	18	18·5	$20{\cdot}4 \pm 3{\cdot}2$
First injection residual weal after termination of occlusion	0	0	0	0	5	0	0	0	—
Second injection, reinjection at same site as first injection	0	0	7·5	0	5	0	9	0	—
Corrected response to second injection	0	0	7·5	0	0	0	9	0	$2{\cdot}1 \pm 3{\cdot}9$

Weal response to second dose of 100 μg histamine injected at the same site as the first 5 min after termination of arterial occlusion of 30 min duration.

Serotonin. As with histamine, injection of serotonin is followed by a state of refractoriness in respect of wealing. In three out of four subjects 30 min arterial occlusion inhibited wealing due to 100 μg serotonin although marked erythema and a flare appeared, indicating that serotonin from the original injection was still present. In the fourth, wealing was greatly reduced compared with the corresponding control. A second injection of 100 μg serotonin in the same site as the first was followed by reduced or absent wealing compared with control injections (Table 4).

TABLE 4. Tachyphylaxis to second injection of serotonin 35 min after the first

Injection of serotonin 100 μg	Weal response (mm) Subject no. 1	2	3	4	Mean and standard deviation for group (mm)
Control injection, no occlusion	17·5	7	11	14	12·4±4·5
First injection residual wheal after termination of occlusion	0	0	0	5	—
Second injection reinjection at same site as first injection	0	0	0	9	—
Corrected response to second injection	0	0	0	4	1·0±4·3

Weal response to second dose of 100 μg serotonin injected at the same site as the first 5 min after termination of arterial occlusion of 30 min duration.

Specificity of tachyphylaxis

Six subjects were simultaneously injected at separate sites in the same arm with 10 μg bradykinin and 100 μg histamine during arterial occlusion, which was then terminated after 30 min. No wealing developed due to either agent in any of the subjects. The same dose of bradykinin was then injected into skin previously injected with histamine and likewise histamine was injected into the bradykinin site. Both second injections produced wealing similar in size to control weals (Table 5).

Thus, bradykinin does not induce tachyphylaxis to histamine and histamine does not induce tachyphylaxis to bradykinin.

TABLE 5. Specificity of tachyphylaxis

Subject no.	Weal response to second injection (mm): First injection bradykinin 10 μg Second injection histamine 100 μg	First injection histamine 100 μg. Second injection bradykinin 10 μg
1	15	12·5
2	14	12·5
3	—*	10
4	10	15
5	10	12
6	14	12

Weal response to second dose of 10 μg bradykinin or 100 μg histamine injected at the same site as the first dose of, respectively, histamine or bradykinin, 5 min after termination of arterial occlusion of 30 min duration. * Experiment omitted.

Corticosteroids

The effect of two corticosteroids, prednisone and hydrocortisone, on wealing due to 0·1, 0·5, 1·0, 5, and 10 μg bradykinin was studied. These doses were given in six subjects before and 8 hr after subcutaneous in-

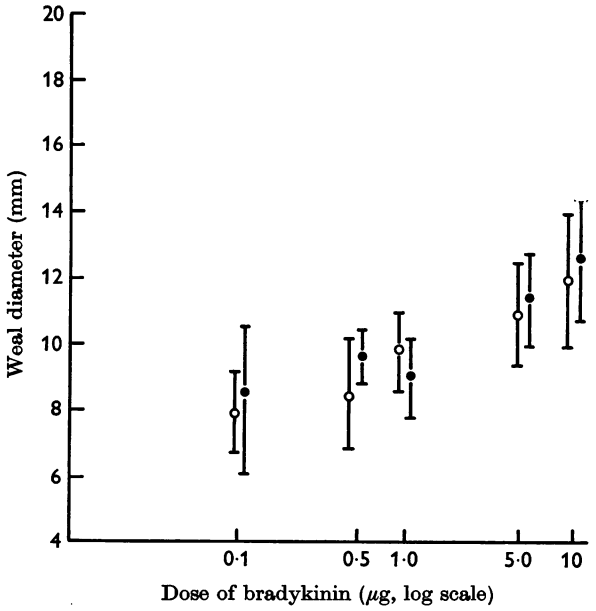

Fig. 4. Dose–response plots for bradykinin weal before (basal) and 2 hr after the last of eight 10 mg doses of prednisone. For each dose the mean and S.D. of responses in seven subjects is given. ●, Basal; ○, oral prednisone for 48 hr.

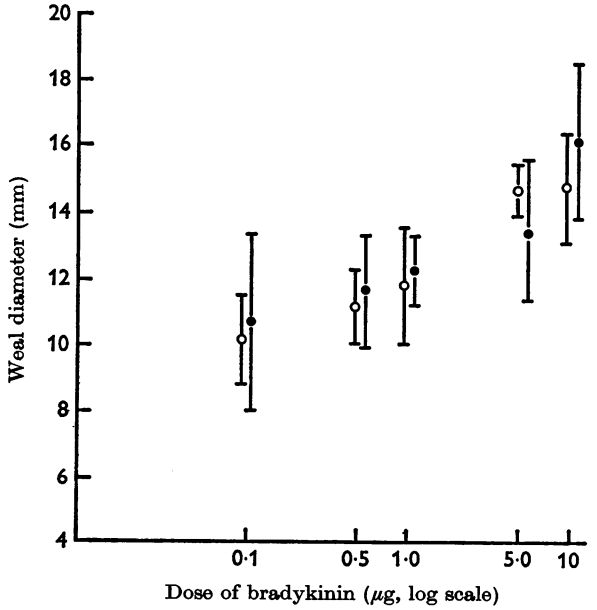

Fig. 5. Dose–response plots for bradykinin weal before (basal, ●) and 8 hr after (○) an injection of 100 mg hydrocortisone in six subjects. Basal values obtained by injecting a volume of 0·154 M saline equal to the hydrocortisone. For each dose the mean and S.D. of responses in six subjects is given.

jection of 100 mg hydrocortisone, and in seven subjects before the first and 2 hr after the last of eight 10 mg doses of prednisone taken orally every 6 hr. Control experiments were carried out using the same doses of bradykinin, in which instead of hydrocortisone, an equal volume of 0·154 M saline was injected. The resulting dose–response plots are shown in Figs. 4 and 5. Corticosteroids failed to modify significantly the wealing responses to 10 μg bradykinin and to lower doses.

Antihistamine

Doses of the antihistamine chlorpheniramine which inhibited weal formation due to intradermally injected histamine, failed to inhibit the weal response to bradykinin (Table 6).

Eight subjects in whom 10 μg bradykinin induced weal formation were given 10 mg chlorpheniramine by intramuscular injection. Two hours later the same dose of bradykinin was repeated intradermally. There was no significant difference between the size of the bradykinin weals before and after chlorpheniramine ($P > 0{\cdot}4$). That chlorpheniramine was reaching the skin in concentrations sufficient to antagonize exogenous histamine wealing was shown by significant inhibition of wealing due to intradermal injection of 100 μg histamine given at the same time as the test dose of bradykinin in each subject ($P < 0{\cdot}01$).

TABLE 6. Effect of chlorpheniramine on bradykinin and histamine weals

	Weal response (mm)			
	Control		Chlorpheniramine	
Subject	Histamine 100 μg	Bradykinin 10 μg	Histamine 100 μg	Bradykinin 10 μg
1	13·5	14	9	15
2	18	14·5	17	17
3	18	19·5	12·5	17·5
4	22	12	18	11·5
5	23	20	15·5	13·5
6	13·5	7·5	9·5	10
7	11·5	16	9	14
8	19	16	16	14·5
Mean	17·3	15·1	13·3	14·1

10 μg bradykinin and 100 μg histamine given before and 2 hr after intramuscular injection of 10 mg chlorpheniramine.

DISCUSSION

The results of the present investigation indicate that bradykinin can mediate sustained acute inflammation in the skin. Bradykinin produces erythema, increased vascular permeability and pain, and it has proved to be more potent in respect of whealing than serotonin and histamine. Furthermore, histamine and serotonin, but not bradykinin, exhibit

tachyphylaxis in respect of increased vascular permeability and therefore cannot mediate sustained inflammation.

The appearance of erythema, but no axon reflex flare, in response to bradykinin in ten out of twelve subjects is in agreement with the findings of Herxheimer & Schachter (1959) using purified bradykinin. It is of interest to compare the erythema responses of bradykinin and serotonin. Bradykinin and lower doses of serotonin both produce erythematous reactions which are presumably due to a direct effect on the blood vessels since spread is limited by a tight elastic band. Higher doses of serotonin but not bradykinin produce in addition an axon reflex flare. Similar results with serotonin were observed by Demis, Davis & Lawler (1960). The dual nature of the vasodilator response to serotonin is of great interest in view of evidence that, in higher doses, serotonin may behave as a histamine liberator (Feldberg & Smith, 1953; Rowley & Benditt, 1956; Frank, Rapp, Biro & Glickman, 1964).

Although short-lived pain was felt in the majority of subjects with higher concentrations of bradykinin, no subject noticed itching in response to the wide range of bradykinin doses used. Absence of itching contrasts with the findings of Cormia & Dougherty (1960) who do not state the dose range within which itching occurred in their subjects. These workers used a crude bradykinin preparation and itching may have been in response to impurities.

The readiness with which wealing could be demonstrated in response to low as well as high concentrations of bradykinin in the present study is in striking contrast with the lack of agreement on this point in earlier published studies. Wealing in human skin was first reported by Herxheimer & Schachter (1959). Soon afterwards Cormia & Dougherty (1960) reported the failure of doses of crude bradykinin up to 100 mg/ml. to produce weals. The absence of wealing with bradykinin was also noted by Elliott, Lewis & Horton (1960).

The slope of the dose–response plot for wealing with bradykinin was less than both for serotonin (Fig. 2) and for histamine (Bain, 1951). Regression of the dose–response plot does not intersect the ordinate at zero, evidently because the bleb effect of the saline solvent is still significant at 5 min.

The shape of the dose–response plot for wealing with serotonin supports the view of Miles & Miles (1952) that the diameter of areas of increased vascular permeability in response to injections of vaso-active agents is linearly related to log dose. That the potency of bradykinin in respect of weal formation is greater than that of serotonin is readily seen by comparison of dose–response plots for the two agents (Figs. 1 and 2).

The method of testing for tachyphylaxis in respect of wealing described in the present study deserves more widespread use in evaluating vaso-

active agents. A clear distinction can be drawn between agents such as histamine and serotonin which exhibit tachyphylaxis and therefore are likely to be capable of mediating only short-lived inflammatory reactions and bradykinin which shows no evidence of tachyphylaxis. The validity of the test depends on being able to show that the vaso-active agent is present during the period of ischaemia. This possibility does not require exploration in the case of histamine and serotonin, both of which showed activity at the site of injection after restoration of circulation, in the form of erythema and a flare.

The persistence of bradykinin in the ischaemic arm during 30 min occlusion is readily understandable in view of the inhibitory effect of small reductions in pH on kininase activity (Edery & Lewis, 1962). The demonstration of wealing from the first injection of bradykinin on removal of the cuff after 25–27·5 min occlusion indicates persistence of bradykinin up to this time, when, as demonstrated by a second injection of bradykinin, no tachyphylaxis is present. The possibility, however, remains that with bradykinin as well as with histamine and serotonin vaso-activity persists after disappearance of the agent.

Collier, Holgate, Schachter & Shorley (1960) and Frimmer & Krych (1963) found no evidence of inhibition of bradykinin activity by corticosteroids. Our evidence supports this and is contrary to the findings of Frank, Rapp, Biro & Glickman (1964) since wealing due to a wide range of doses of bradykinin was not inhibited by oral or parenteral corticosteroids. Corticosteroids inhibit the delayed inflammatory response to streptokinase, a powerful activator of kinin-forming enzymes and the mechanism of anti-inflammatory activity of these compounds may therefore lie not in the action of kinins on blood vessels, but on enzymic formation of kinin (Greaves & Shuster, 1963; Greaves, 1965). A similar conclusion has recently been reached by Cline & Melmon (1966) who demonstrated inhibition by corticosteroids of kinin formation *in vitro*.

Rocha e Silva & Garcia Leme (1963) and Lish & McKinney (1963) have found that some effects of bradykinin can be antagonized by antihistamines. The present experiments show in contrast that the responses of cutaneous blood vessels to bradykinin are unaffected by a dose of antihistamine which will mitigate the response to injected histamine. In its effect on skin blood vessels bradykinin differs from histamine in the absence of an axon reflex flare, and the absence and specificity of tachyphylaxis suggests that the receptor sites for cutaneous vascular responses to bradykinin and histamine are separate.

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