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CHEMICAL AND PHARMACOLOGICAL PROPERTIES OF THE POTENT, SLOW CONTRACTING SUBSTANCE (KININ) IN WASP VENOM

BY

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The venom of the common wasp (*Vespa vulgaris*) has recently been shown to contain high concentrations of histamine, of 5-hydroxytryptamine, and also of a highly potent material which produces a characteristic delayed, slow contraction of the isolated guinea-pig ileum (Jaques and Schachter, 1954a). In the present work, further chemical and pharmacological properties of this unidentified substance have been studied. The results of these experiments indicate that the active material is of a polypeptide nature, and that in addition to its action on guinea-pig intestine it has a marked ability to contract rabbit intestine and to lower the arterial blood pressure of the rabbit and cat. Since all these actions were retained without relative loss of any activity during purification, it appears that a single substance accounts for all the pharmacological effects observed. If several substances are involved they must be so similar as to be isolated to the same degree during purification.

This substance in wasp venom is readily distinguished from quick contracting substances such as acetylcholine, histamine, and 5-hydroxytryptamine. It is, however, similar in many respects to substance P (Euler and Gaddum, 1931; Pernow, 1953), and to bradykinin (Rocha e Silva, Beraldo, and Rosenfeld, 1949), both of which are slow contracting polypeptides.

Since this substance in wasp venom cannot as yet be identified with any substance hitherto described, it is tentatively designated as wasp (or venom) kinin, or simply as kinin.

METHODS

Assay on the guinea-pig ileum was performed in a 15 ml. bath in the presence of atropine (0.2 μg.). Mepyramine (0.4 μg.) was added as well when assaying kinin activity. Desensitization to 5-hydroxytryptamine was accomplished by prolonged exposure of the preparation to this substance itself. Rabbit intestine was arranged in the same way and atropine (0.2 μg.) only was added throughout the experiment. Such a preparation does not respond significantly to histamine or 5-hydroxytryptamine.

Cats were anaesthetized with chloralose (80 mg./kg.) intravenously, preceded by ether, and rabbits with intravenous pentobarbitone sodium (30 mg./kg.) plus ether, if necessary. Arterial blood pressure was recorded with a mercury manometer from the common carotid artery, and intravenous injections were made through a cannula in the femoral vein. Cats were injected with atropine (0.5 mg./kg.) and mepyramine (1 mg./kg.) and rabbits with atropine (0.5 mg./kg.). Such preparations were insensitive to the maximal amounts of histamine and 5-hydroxytryptamine present in the crude venom injected.

Wasp Venom.—Wasp venom was obtained from the common wasp (*Vespa vulgaris*). Wasps were caught alive, frozen at $-10°$ C., thawed out, and the entire venom apparatus removed by simply pulling gently on the sting with fine forceps. Large numbers of such entire apparatuses were dried over P_2O_5, finely ground and stored at $-10°$ C. This material, referred to as wasp powder, has been kept at $-10°$ C. for months with no apparent loss of activity. Approximately 30% of the wasp powder was soluble in saline, which is the same value as that found by Jaques and Schachter (1954a). Throughout this investigation, expressed weights of venom are 30% of the weight of the dry venom powder.

Bee Venom (Apis mellifica).—Bee venom apparatus was removed in the same manner as described for wasps, but dried and stored as intact venom apparatus in sealed glass tubes at $-10°$ C. Approximately 45% of the bee venom apparatus was found to be soluble in saline; hence expressed values for weights of bee venom are 45% of the weight of dry venom apparatus.

Paper Chromatography.—Ascending chromatograms were run on Whatman's No. 1 filter paper with the n-butanol-acetic acid-water solvent described by Partridge (1948). In a few instances "Analar" phenol plus 5% water was used. The aqueous venom solution was applied to the paper as a strip 3–5 mm. wide and approximately 15 cm. long (at a concentration of about 1 mg./

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10 cm.). Chromatograms were run for about 16 hr. at room temperature, dried in a gentle current of air, cut into strips parallel to the origin, and the strips dried in a vacuum desiccator. The origin strip (strip O), was 1 cm. wide and comprised the area of application and a few mm. on either side, the adjacent strip was 1.5 cm. wide, and the remainder cut into 2.5 cm. strips. The components were eluted from the strips by maceration with distilled water or saline. Markers of histamine and 5-hydroxytryptamine (25 μg. each) were placed as spots outside the main line of venom. The marker strips and a narrow vertical section of the venom strip were sprayed with Pauly's reagent (diazotized sulphanilic acid) after drying.

Drugs.—Histamine was in the form of acid phosphate, 5-hydroxytryptamine as synthetic creatinine sulphate, and mepyramine as maleate. Weights of histamine and 5-hydroxytryptamine are expressed as base. Crystalline preparations of trypsin and chymotrypsin derived from bovine pancreas (Armour Laboratories) were employed in all experiments.

RESULTS

Properties of Kinin Using the Isolated Guinea-pig Ileum as Test Preparation

The addition of 10 μg. of crude wasp venom to the isolated guinea-pig intestine (in the presence of atropine and mepyramine) regularly produced a delayed, slow contraction which reached its maximum in approximately 50 sec. The delay of contraction was less with highly sensitive intestinal preparations or with large amounts of kinin, but was usually 5–10 sec. Complete relaxation of the muscle usually occurred within 45–60 sec. after washing out the bath with Tyrode's solution. Intervals between contractions were kept at 4 or 4.5 min. The responses were quite constant, particularly with purified preparations.

Stability.—Kinin dissolves readily on addition of saline or water to dry wasp venom and the venom solution is slightly acidic (*p*H 5–7). Such solutions, however, gradually lose activity at room temperature. The loss of kinin activity of venom solutions in saline, at room temperature for 24 hr., has varied from 15 to 80% in different experiments. Stability is increased at 4° C., but even under these conditions 50% or more of the activity may be lost in 72 hr. We were, therefore, early faced with the practical problem of stabilizing kinin in aqueous or saline solutions of venom for at least 24 hr. under working conditions. Assuming that its destruction might be enzymic in part, we tested the effect of heating freshly prepared venom solutions in a boiling water bath for 3–5 min. This procedure effected considerable stabilization of the active substance so that on no occasion was there a significant loss of activity for 24 hr. at room temperature, nor for 72 hr. at +4° C. Heating in this way does not destroy any kinin, since heated preparations possess activity equal to that of freshly dissolved venom. Heated solutions could be frozen and kept for weeks at −10° C. without loss of activity. Preliminary heating of aqueous or saline venom solutions in a boiling water bath for 3–5 min. was always performed when material was required for biological testing or paper chromatography.

Kinin was found to be heat stable in neutral and slightly acid solutions, less stable at low *p*H and very unstable at high *p*H. Thus, heating venom solutions (50 μg./ml.) in a boiling water bath for 10 min. at *p*H 6, in 0.5N-HCl, and in 0.5N-NaOH resulted in the destruction of 0, 15, and 90–100% of the activity, respectively. Results of these tests performed on the isolated guinea-pig ileum are shown in Fig. 1.

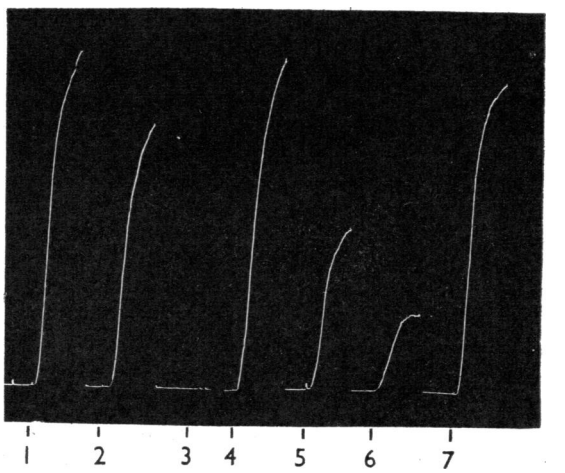

FIG. 1.—Stability of kinin. Contractions (15 sec.) of isolated guinea-pig ileum produced by wasp venom, heated in acid and alkali, for 10 min. in boiling water bath. 0.2 μg. atropine and 0.4 μg. mepyramine in bath throughout. 1 and 7, 10.0 μg. untreated venom. 2, 10.0 μg. heated in 0.5N-HCl. 3, 20.0 μg. heated in 0.5N-NaOH. 4, 10.0 μg. heated at *p*H 6. 5, 7.0 μg. unheated. 6, 3.5 μg. unheated.

Dialysability.—Kinin dialysed through cellophane, but at a slow rate. Wasp venom, in concentrations of 0.5–1.0 mg./ml., was dialysed at room temperature for 24 hr. against an equal volume of saline. At the end of this period the histamine concentration of the dialysate was equal to that of the residue, but the concentrations of kinin in dialysates in three experiments were only 4, 10, and 14% of the residues, respectively. The dialysate produced the characteristic delayed, slow contraction of the guinea-pig ileum in the presence of atropine and mepyramine,

and this persisted after 5-hydroxytryptamine desensitization (Fig. 2). Dialysis against larger volumes of saline with several changes of dialysate, even at +4° C., results in the loss of moderate amounts of

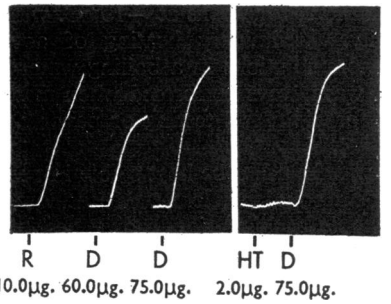

FIG. 2.—Dialysability of kinin. Effects of residue and dialysate of dialysed wasp venom on the guinea-pig ileum. Atropine (0.2 μg.) and mepyramine (0.4 μg.) present throughout. R= residue in cellophane sac. D=dialysate. The intestine was desensitized to 5-hydroxytryptamine between the panels. 5-Hydroxytryptamine was not washed out in the right-hand panel as indicated by unbroken tracing. The amounts are expressed as weights of venom, on the assumption that all the venom is present in the residue and in the dialysate.

kinin. In two experiments, venom (500 μg./ml.) was dialysed against 10 volumes of saline at +4° C. for 20 hr. (dialysate changed three times), and this resulted in the loss of approximately 30% of kinin from the cellophane sac as compared with non-dialysed control samples kept under the same conditions. Histamine and 5-hydroxytryptamine were, however, undetectable in the dialysis residue as judged by the absence of a quick contracting effect on the guinea-pig ileum. Thus, since the slow dialysability of kinin permits removal of freely dialysable substances from crude venom with only a partial loss of kinin, restricted dialysis may be useful in purification.

Solubility.—Kinin is insoluble in alcohol (95%), acetone (95%), and anhydrous ether. Volumes of 1.0 ml. of these reagents were added individually to separate tubes containing dry wasp powder (300–500 μg. venom), and, after thorough mixing, the solutions were centrifuged and the supernatant fluids removed. This procedure was performed three times with additional 1.0 ml. volumes of each solvent. The alcohol, acetone, and ether insoluble residues were dried, dissolved in saline, and tested on the guinea-pig ileum. In each case the slow contracting activity was recovered from the insoluble residue. Preliminary extraction with these fat solvents resulted in recoveries of up to twice the original amounts of kinin. It seems, therefore, that preliminary removal of lipids facilitates extraction of kinin. Considerable amounts of histamine and 5-hydroxytryptamine were found in the alcohol and acetone extracts, particularly in the former, but no traces of kinin; the activity of these extracts was completely abolished by mepyramine, plus desensitization of the intestine to 5-hydroxytryptamine.

Kinin is soluble in phenol (95%), trichloracetic acid (5%) and ammonium sulphate (60% saturated). Volumes of 1.0 ml. of these reagents were added individually to separate tubes containing dry wasp powder (0.5–1.0 mg. venom), thoroughly mixed, and centrifuged, and the supernatant fluids were removed. This procedure was performed three times with 1.0 ml. of each solvent. The materials insoluble in phenol and trichloracetic acid were freed of traces of solvent by washing with 10 ml. ether, and the ether was discarded. The three dried residues were dissolved in saline and tested on the guinea-pig ileum; in each instance the residue contained less than 5% of the kinin activity originally present in the venom. Considerable amounts of kinin, however, could be recovered from the phenol, trichloracetic acid or ammonium sulphate extracts. Kinin was precipitated from the phenol solution of dry venom by addition of 10 volumes of ether, precipitation being allowed to occur for one hour at room temperature. The precipitate was washed with ether, dried, dissolved in saline and tested for kinin activity on the guinea-pig ileum. Approximately 70% of the kinin activity in the venom was thus recovered from the phenol extract. Kinin was also recovered from the solution of trichloracetic acid by removing the latter by additions of two volumes of ether, the process being repeated five times; the remaining aqueous phase was then dialysed (+4° C. for 20 hr., against 10 volumes of saline), the residue of which contained 75% of the original kinin. The ammonium sulphate solution of venom was simply subjected to a more effective dialysis (+4° C. for 20 hr., against 10 volumes saline changed twice) because of the high concentrations of this salt. The residue in the sac still contained about 75% of the original activity. Kinin is, therefore, soluble in protein precipitating agents.

Rapid Destruction by Crystalline Preparations of Trypsin and Chymotrypsin.—Kinin was readily destroyed by incubating venom with small amounts of crystalline trypsin or chymotrypsin. In four separate experiments, incubation of venom (25 or 50 μg./ml.), for 30 min. at 35° C., with crystalline preparations of either of these enzymes (10–25 μg./ml.), resulted in a loss of 50–70% of kinin activity (Fig. 3). This is in contrast to the reported inability of crystalline trypsin to destroy bradykinin (Werle, Kehl, and Koebke, 1950; Rocha e Silva,

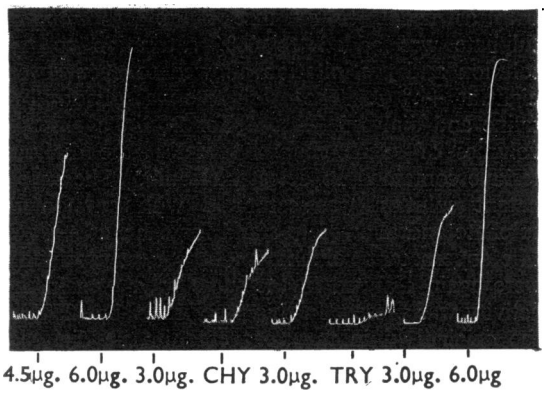

FIG. 3.—Destruction of kinin in venom by crystalline preparations of trypsin and chymotrypsin. Venom (25.0 μg./ml.) incubated at 37° for 30 min. with the crystalline enzymes (10.0 μg./ml.). Atropine (0.2 μg.) and mepyramine (0.4 μg.) in bath throughout. The amounts in μg. indicate untreated venom. CHY and TRY represent the addition to the bath of 6.0 μg. of venom incubated with chymotrypsin and trypsin respectively.

1951). We also demonstrated that the same crystalline trypsin preparation failed to destroy a preparation of crude bradykinin,* although it readily destroyed kinin under the same conditions.

Paper Chromatography of Wasp Venom and Elution of Kinin from Chromatograms

Crude wasp venom (heated, as described), chromatographed in the butanol-acetic acid solvent and sprayed with Pauly's reagent always showed two distinct reactions, corresponding in colour and position to the marker strips of histamine and 5-hydroxytryptamine. In some instances the origin (from which kinin was regularly eluted) showed a slight pink reaction. This colour reaction at the origin was never very marked, and is not necessarily indicative of kinin, since other substances may remain at the point of application of the material. One may conclude, however, that kinin does not give marked colour reactions with this reagent, since in some instances it was eluted in the absence of a colour reaction. The amount of kinin eluted varied in different experiments from 10–40% of the applied material.

Crude wasp venom chromatographed in phenol (95%) and sprayed with Pauly's reagent again showed the colour reactions corresponding to histamine and 5-hydroxytryptamine, both of which could be readily eluted; we were, however, unable to recover kinin by elution from wasp venom chromatograms in this solvent.

Bee venom, chromatographed in the butanol-acetic acid solvent, regularly showed the presence of histamine, which was readily eluted, but 5-hydroxytryptamine was not detected by colour reactions or in any eluates of the paper. The origin, and adjacent region, showed a marked colour reaction, and this eluate produced the delayed, but rapidly desensitized, contraction of the ileum which we observed with crude bee venom, as described later. Kinin was not detected in the bee venom eluates.

Histamine (commercial histamine acid phosphate), or the histamine present in wasp and bee venom, regularly appeared on the butanol-acetic acid chromatograms as two distinct separated spots on spraying with Pauly's reagent. Eluates corresponding to these spots were indistinguishable when tested on the guinea-pig ileum. Whether this is due to different histamine derivatives in commercial histamine and in tissue extracts, or to factors related to the chromatographic procedure, requires further investigation. 5-Hydroxytryptamine always appeared as a single well-defined spot.

Other Pharmacological Actions of Kinin

In addition to producing the delayed, slow contraction of the isolated guinea-pig ileum, crude wasp venom also contracted the isolated rabbit jejunum or ileum, markedly lowered the arterial blood pressure of the rabbit, and to a lesser degree that of the cat. The maximal amounts of histamine or 5-hydroxytryptamine present in the crude kinin solutions had little or no effect on the test preparations under the conditions of testing. Furthermore, as described later, purified kinin preparations free of histamine or 5-hydroxytryptamine, retained, without relative loss, all the pharmacological properties of crude kinin.

The addition of 20–50 μg. of venom to the isolated rabbit jejunum (in the presence of atropine) produced a very characteristic effect, in that the resulting increase in tone was regularly preceded by a brief initial depression of activity. The action of kinin on the rabbit intestine was not so constant as that on the guinea-pig ileum.

The intravenous injection of venom (5–15 μg./kg.) produced a marked immediate fall of the arterial blood pressure in the anaesthetized rabbit previously injected with atropine (0.5 mg./kg.). This effect was characterized by a secondary depression of the blood pressure, usually lasting several minutes, which developed as recovery of the arterial pressure appeared to be occurring.

The intravenous injection of wasp venom (10–25 μg./kg.) also produced an immediate fall of arterial blood pressure in the anaesthetized cat previously injected with atropine (0.5 mg./kg.) and

* Kindly supplied by Dr. M. Rocha e Silva.

mepyramine (1 mg./kg.). The rabbit was more sensitive to kinin in this respect than the cat.

These actions of crude kinin were, in each instance, abolished by heating the solutions in 0.5N-NaOH in a boiling water bath for 10 min. and greatly reduced or abolished by incubation with trypsin or chymotrypsin at 37° C. for 30 min.

Parallel Pharmacological Actions of Crude and Purified Kinin

The above experiments with crude wasp venom suggested that its actions on isolated guinea-pig and rabbit intestines, and on rabbit and cat arterial blood pressure, were produced by either a single or very similar substances. The probability that all these pharmacological effects are due to a single or extremely similar substances was strongly supported by the experiments described below, which demonstrated the qualitatively and quantitatively parallel actions of crude and purified kinin on the different test preparations.

Purified Kinin, No. 1.—This material was obtained by elution of kinin from paper chromatograms of crude venom. For this purpose, 9.0 mg. wasp venom was dissolved in several drops of water, and heated in a boiling water bath for 4 min.; the soluble material was deposited as a narrow strip approximately 15 cm. long, along the base of the paper. The base line eluates from five papers were pooled and most of it frozen at once at —10° C. for pharmacological testing. All the pharmacological tests were carried out within 24 hr. It was found that 0.05 ml. of kinin eluate corresponded to 10 μg. of crude venom as tested on the guinea-pig ileum. This same ratio of activity was also found in tests on the isolated rabbit intestine, and rabbit and cat arterial blood pressure (Fig. 4). Furthermore, the type of response produced by eluate and crude venom on all preparations was identical in every detail, and the activity of the eluate was again destroyed by heating in 0.5N-NaOH or by incubation with trypsin.

Purified Kinin, No. 2.—This preparation was obtained from chromatogram eluates of a dialysed ammonium sulphate extract of venom. For this purpose 15.0 mg. venom was extracted with 0.25 ml. water three times and the pooled extract heated in a water bath as usual, to stabilize kinin. Ammonium sulphate was added to 60% saturation to this solution and precipitation allowed to occur overnight at +4° C. The precipitate was dissolved in saline and dialysed (+4° C. for 20 hr. against 10 volumes of saline changed twice); the dialysed residue contained no detectable kinin. The supernatant solution was saturated with ammonium sulphate and allowed to stand for 6 hr. at +4° C. The supernatant solution and the new precipitate were again dialysed in the same way. Again, no activity was present in this precipitate, but 25% of the original kinin was recovered in the dialysed, saturated ammonium sulphate solution. Histamine and 5-hydroxytryptamine were undetectable in the dialysed ammonium sulphate solution either by chromatography or by tests for its ability to cause a quick contraction of the guinea-pig ileum. It did, however, produce a delayed slow contraction of the guinea-pig ileum, increased the tone and amplitude of contractions of the rabbit intestine after an initial inhibition, produced the characteristic biphasic depression of the arterial blood pressure of the rabbit and lowered that of the cat. Kinin, eluted from the origin of paper chromatograms of

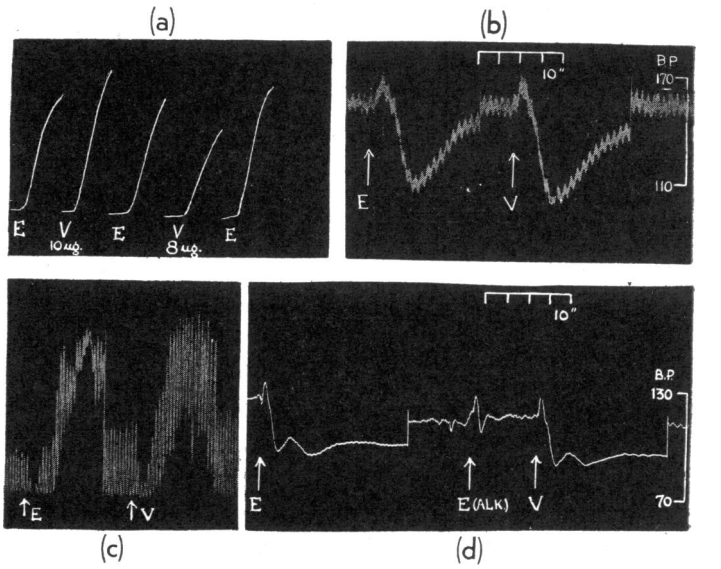

FIG. 4.—Parallel actions of crude wasp venom and eluate from origin of paper chromatogram on different test preparations. E, eluate from origin. V, crude venom. (*a*) Isolated guinea-pig ileum (atropine and mepyramine present). Contraction time, 50 sec. E, 0.05 ml. eluate which is approximately equivalent in activity to 10.0 μg. of venom. (*b*) Cat (3.5 kg.) arterial blood pressure. Animal previously injected with atropine (0.5 mg./kg.) and mepyramine (1.0 mg./kg.). Interval between injections, 5 min. E, 0.35 ml. eluate. V, 70.0 μg. venom. (*c*) Isolated rabbit intestine in the presence of atropine (0.2 μg.). E, 0.18 ml. eluate. V, 35.0 μg. venom. Interval between tests, 10 min. The initial depression of rhythmic activity is present with both preparations. (*d*) Rabbit arterial blood pressure. Rabbit previously injected with atropine (0.5 μg./kg.). Interval between injections, 5 min. E, 0.2 ml. eluate. E (ALK), 0.2 ml. eluate heated in 0.5N-NaOH in boiling water bath for 5 min. V, 40.0 μg. wasp venom.

this material, possessed the same properties, and amounts of the starting material and eluate found to be equipotent on the guinea-pig ileum elicited equivalent contractions of the rabbit jejunum (Fig. 5*b*). Fig. 5*a* shows the elution of kinin from the origin of this chromatogram and the absence of activity in the remaining eluates as tested on the guinea-pig ileum. The activity of this eluate was again destroyed by heating in alkali and by incubation with trypsin.

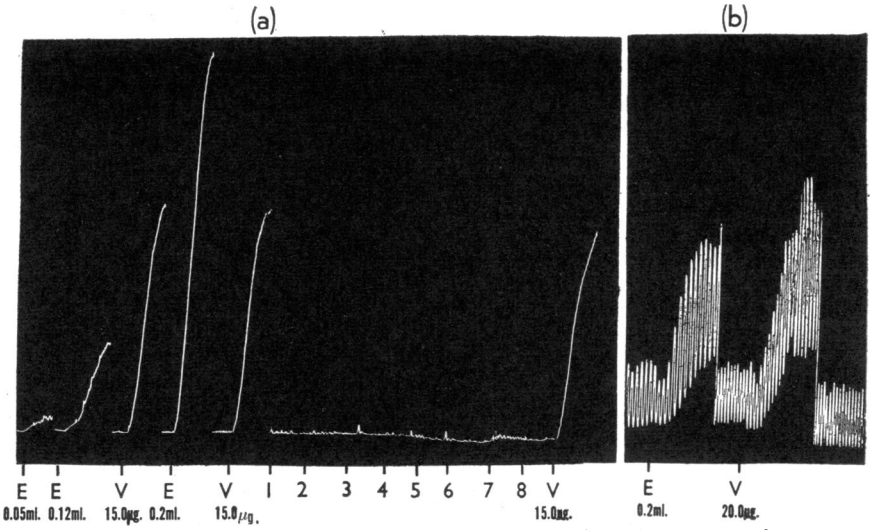

FIG. 5 (*a*).—Activity of paper chromatogram eluates of dialysed ammonium sulphate extract of wasp venom on the isolated guinea-pig ileum. Atropine (0.2 μg.) and mepyramine (0.4 μg.) present throughout. E, saline eluate from origin of chromatogram. V, dialysed ammonium sulphate extract of venom (amounts expressed in terms of equivalent activity in crude venom). 1–8, eluates from remaining strips of paper chromatogram. Approximately 20.0 μg. of the chromatographed venom was equivalent to 0.2 ml. eluate. (*b*) Activity of same eluate and equivalent amount of the ammonium sulphate extract on the isolated rabbit jejunum in the presence of 0.2 μg. atropine. 0.2 ml. eluate again produces an equivalent and similar reaction to 20.0 μg. of the chromatographed venom. The inhibition of the rhythmic activity is evident but not very marked in this preparation.

Purified Kinin, No. 3.—This was prepared by extracting 1.5 mg. venom with 1 ml. of alcohol three times. The dried residue, dissolved in a few drops of water, was chromatographed. Once again kinin was eluted only from the 1.0 cm. wide strip of paper around the line of origin, and the original material and eluate which evoked equivalent responses of the guinea-pig ileum also produced equivalent depressions of the cat's arterial blood pressure.

Experiments with Bee Venom

The addition of bee venom (5–10 μg.) to the untreated isolated guinea-pig ileum produced a rapid contraction which could be abolished by mepyramine; this effect is due to histamine. Quantitative assay of the histamine content of bee venom yielded values of 8–12 mg./g. venom. The addition of larger amounts (10–40 μg.) of venom to the isolated ileum in the presence of mepyramine produced a delayed slow contraction; this effect, however, was reduced after the first contraction and unobtainable after several additions of venom, although the preparation still responded to wasp kinin. The substance in bee venom, therefore, differs from kinin, and is perhaps an enzyme which acts by releasing substances from the muscle itself, thus producing rapid desensitization.

Bee venom was chromatographed in butanol-acetic acid solvent in the same manner as described for wasp venom, using 1–5 mg. of bee venom in different experiments. Narrow vertical strips of the chromatographed venom were sprayed with Pauly's reagent, and the remainder was cut in horizontal strips, eluted and tested on the guinea-pig ileum. On spraying with Pauly's reagent, several distinct colour reactions developed, one corresponding to the marker for histamine, and another intense orange-red colour at the origin and extending just beyond it. Elution of the strip corresponding to histamine proved it to have the pharmacological properties of histamine, and the histamine concentration in bee venom calculated from several eluates was approximately 10 mg./g. venom. The eluate from the origin contained the material producing the delayed, rapidly desensitized contraction of the guinea-pig ileum. No significant pharmacological activity was present in the remaining eluates.

Thus, unlike wasp venom, bee venom contains little or no 5-hydroxytryptamine or kinin. It does, however, contain high concentrations of histamine, and also another substance, possibly an enzyme, which produces a delayed, rapidly desensitized contraction of the guinea-pig ileum.

DISCUSSION

The chemical properties of kinin suggest that this potent substance is a polypeptide, or at least requires the integrity of a peptide linkage for its pharmacological actions. Since kinin retains all its pharmacological properties after partial purification

without relative loss of any of its actions, it seems that a single substance produces all the effects. If more than one substance is involved, they must be closely related compounds. The characteristic property of kinin, namely, the delayed slow contraction of the guinea-pig ileum, has been described for a number of substances; for example, the unidentified SRS (slow reacting substance) described by Feldberg, Holden, and Kellaway (1938) and thought to be a lecithin derivative; substance P (Euler and Gaddum, 1931; Gaddum and Schild, 1934; Pernow, 1953); trypsin (Rocha e Silva, 1939; Gaddum, 1953); substance R (Gaddum, 1953); bradykinin (Rocha e Silva, Beraldo, and Rosenfeld, 1949); and kallidin, which is believed to be identical with bradykinin (Werle and Berek, 1950). Of these substances, substance P, bradykinin and kallidin are known to be polypeptides, and all have many properties in common with kinin. It is, however, at present, impossible to identify kinin definitely with any of these compounds, since some of its properties appear to be different. Thus, substance P, chromatographed in butanol-acetic acid solvent, has an R_F value of 0.37 (Pernow, 1953), whereas crude or purified kinin was in all but one instance eluted only from the origin. In this single instance, a small proportion of eluted kinin was also in the adjacent strip. Also, bradykinin and kallidin are not destroyed by crystalline trypsin (Werle, Kehl, and Koebke, 1950; Rocha e Silva, 1951), whereas kinin readily is. Another possible difference to bradykinin is raised by the observation that purified bradykinin has an R_F value of 0.52 on paper chromatography in butanol-acetic acid solvent, although impure preparations moved little or not at all from the origin (Andrade, Diniz, and Rocha e Silva, 1953).

It is possible, however, that some of the apparent differences between one or other of these compounds may be related to the degree of purity of the preparation; or, that with relatively large molecules of this nature, minor chemical differences (possibly produced in purification) have little influence on pharmacological action. In view of their complex structure, further studies on highly purified preparations are necessary to determine whether any of these potent smooth muscle stimulating and hypotensive polypeptides, viz., substance P, bradykinin, kallidin, and kinin, are identical.

The experiments of Jaques and Schachter (1954) suggested that the potency of the slow contractor in wasp venom approached that of histamine on the isolated guinea-pig ileum. Since the present work indicates that kinin is a slowly dialysable peptide, it would appear to be more potent than histamine on a molar basis. The co-existence of a potent polypeptide in wasp venom with two widely distributed and biologically active substances such as histamine and 5-hydroxytryptamine suggests that kinin may likewise be widespread in nature. The presence of histamine, 5-hydroxytryptamine, and substance P in gastro-intestinal tissues (cf. Pernow, 1953) is thus an interesting parallel, particularly if substance P and kinin are identical. It is also of interest that a slow contracting substance has been found in plasma during anaphylaxis (Beraldo, 1950), and following administration of Compound 48/80 (Paton, 1951) or thalassine (Jaques and Schachter, 1954b).

Little or no kinin was detected in bee venom, although the concentration of histamine was of the same order as in wasp venom. Bee venom, however, did contain a potent material which caused a delayed, slow contraction of the guinea-pig ileum (in the presence of atropine and mepyramine), which rapidly desensitized to the venom itself. Our results show that this material may be readily eluted from paper chromatograms. Its potent action suggests that it may be involved in the release of histamine and other substances, caused by bee venom (Feldberg and Kellaway, 1937).

Summary

1. The substance in wasp venom, previously shown to produce a delayed, slow contraction of the guinea-pig ileum, has been further analysed. The results demonstrate that this substance, tentatively designated as " kinin," is a slowly dialysable peptide. Its similarity and possible identity with various slow contracting polypeptides is discussed.

2. Kinin causes contraction of the isolated rabbit jejunum, which is preceded by a characteristic brief depression of the rhythmic activity of the intestine. It is an extremely potent hypotensive agent on intravenous injection in rabbits; it also lowers the arterial blood pressure of the cat. The evidence indicates that kinin, in some respects, is a more potent pharmacological agent than histamine on a molar basis.

3. The stability of kinin in crude wasp venom is increased by heating the solution in a boiling water bath for several minutes. Kinin is very stable at neutral or slightly acid *p*H, relatively stable in 0.5N-HCl, and unstable in 0.5N-NaOH. It is soluble in water, 95% phenol, 5% trichloracetic acid, and in 60% ammonium sulphate; it is insoluble in 95% alcohol, 95% acetone, and in anhydrous ether. It dialyses slowly through cellophane and is readily destroyed by crystalline trypsin or chymotrypsin.

4. Kinin was further purified by paper chromatography in butanol-acetic acid solvent. It was regularly eluted (10–40%) from the origin of chromatograms. Purified eluates possessed all the properties of crude kinin, indicating that it is either a single substance or a mixture of similar substances.

5. Bee venom, unlike wasp venom, contains little or no 5-hydroxytryptamine or kinin. It does, however, contain a substance producing a delayed, slow contraction of the mepyramine treated guinea-pig ileum, with rapid desensitization. This substance, possibly an enzyme, is readily eluted from the origin of paper chromatograms.

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