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A COMPARATIVE STUDY OF KININ, KALLIDIN, AND BRADYKININ

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Partially purified kinin, a polypeptide in wasp venom, has been found to be a potent smooth-muscle stimulating and hypotensive agent. Such a preparation was 10 to 100 times more effective than histamine in enhancing capillary permeability on intradermal injection, and 10 times more effective than acetylcholine in evoking pain on a cutaneous blister base. Some differences between the actions of salivary kallikrein and trypsin in releasing kallidin or bradykinin have been observed, and some modifications of previous methods of preparing crude kallidin and bradykinin are suggested. Kallidin and bradykinin are effective enhancers of capillary permeability in the guinea-pig and rabbit. Chemical and pharmacological tests failed to differentiate between kallidin and bradykinin which must be, therefore, closely similar compounds. The possible role of kallidin and bradykinin in physiological or pathological conditions is discussed.

Kinin (Jaques and Schachter, 1954; Schachter and Thain, 1954), kallidin (Werle, Götze and Keppler, 1937; Werle and Berek, 1950; Werle, 1955) and bradykinin (Rocha e Silva, Beraldo and Rosenfeld, 1949; Andrade, Diniz and Rocha e Silva, 1953; Rocha e Silva, 1955) are potent polypeptides, with many properties in common. All are known to produce a characteristic delayed slow contraction of the isolated guinea-pig ileum, and to lower markedly the arterial blood pressure of the rabbit and cat on intravenous injection. They are stable in neutral or acidic, but not in alkaline, solution; they are insoluble in anhydrous acetone or ether, and are rapidly inactivated by chymotrypsin. Kinin occurs in large quantities, together with histamine and 5-hydroxytryptamine, in the venom of the common wasp (*V. vulgaris*); kallidin and bradykinin are released from the α_2 globulin fraction of serum by the action of kallikrein and trypsin (or *B. jararaca* venom) respectively. In view of their similar properties it was of interest to extend previous studies of these three substances and to compare their properties under the same conditions. Preliminary results of such a comparison have been reported to the Physiological Society (Holdstock, Mathias and Schachter, 1956).

In the present experiments kinin has been further purified and characterized chemically and pharmacologically. It has been found to be extremely effective in enhancing capillary permeability and in evoking cutaneous pain on application to a blister base. Kallidin and bradykinin were also shown to increase capillary permeability. The different methods of preparing kallidin and bradykinin were compared, and some modifications of previous methods are described.

Despite their many common properties, kinin has been definitely distinguished from kallidin and bradykinin. The latter two substances, however, were not successfully distinguished by pharmacological or chemical tests, and must be closely similar, or possibly identical, compounds.

METHODS

Isolated Smooth Muscle and Arterial Blood Pressure.—Preparations of guinea-pig ileum, rabbit intestine, and rat uterus were suspended in Tyrode solution in an 18 ml. bath. Guinea-pig and rabbit intestine were kept at 34 to 36° C. and rat uterus (anoestrous) at 26 to 28° C., the latter temperature being required to eliminate spontaneous activity in this preparation. Atropine (0.2 μg.) and mepyramine (0.4 μg.) were added to the bath before each test, except when assaying for histamine, in which case mepyramine was omitted. Usually the polypeptide was in contact with the tissue for 60 sec.

Dogs were anaesthetized by intravenous injection of a mixture of chloralose (50 mg./kg.) and urethane (500 mg./kg.); rabbits received pentobarbitone sodium (30 mg./kg.) intravenously, supplemented by ether. The substances tested were injected into the femoral vein and the arterial blood pressure was recorded with a mercury manometer from a carotid artery.

Paper Chromatography. — Chromatograms, usually ascending, were run on Whatman No. 1 filter paper for 16 to 18 hr. at room temperature, in the N-butanol/acetic acid/water solvent described by Partridge (1948). Solutions were applied in narrow strips approximately 1 to 2 cm. long. Mixtures, such as kinin and bradykinin (or kallidin and bradykinin), were chromatographed after superimposing applications of the different substances, the solutions being allowed to dry between applications. After chromatography, the paper was dried in air and cut into parallel horizontal strips, strip 0 being 0.5 cm. on either side of the line of application, strips 1 to 10 measuring 1 cm. each, and the remaining strips, up to the solvent front, 2 cm. each. Each strip was eluted with 1.5 ml. Tyrode solution, and 0.2 ml. or more was tested on the isolated guinea-pig ileum or rat uterus. The amounts of kinin applied to a 2 cm. strip for chromatography varied from 25 to 100 units, and that of kallidin or bradykinin from 3 to 6 units.

Kinin was also chromatographed in ethyl methyl ketone/pyridine/water (60 : 15 : 25-homogeneous solvent) and in amyl alcohol/pyridine/water (35 : 35 : 30-organic layer).

Paper Electrophoresis.—This was carried out in the apparatus of Markham and Smith (1951) using electrolyte solutions of either N/1 acetic acid (*p*H 2.5) or M/20 sodium phosphate (*p*H 8.1) in the terminal compartments. The solution to be tested was applied as a narrow strip, 2 cm. long, to the middle of the filter paper (Whatman No. 1), which was immersed in carbon tetrachloride in the middle compartment. Electrophoresis was carried out for approximately 4.5 hr. at 220 V. D.C. The current in the experiments at *p*H 2.5 and at *p*H 8.1 was 0.3 to 0.5 mA and 1.8 to 3.0 mA, respectively. The paper was subsequently dried and cut into anodal and cathodal strips.

Capillary Permeability.—Guinea-pigs or rabbits were injected intravenously with a 5% solution of pontamine sky blue 6 BX (E. Gurr) in saline (1.2 ml./kg.), into the saphenous or marginal ear vein respectively, and the injection of the test substance was then made either intradermally (guinea-pig, 0.1 ml.; rabbit, 0.2 ml.) or intravenously. Details of this procedure have been described by Miles and Miles (1952).

Cutaneous Pain.—Superficial blisters (approximately 5 mm. diameter) were produced on the flexor surface of the forearm in three subjects, either by burning the skin with a heated spatula or by the application of a cantharidin plaster as described by Armstrong, Dry, Keele and Markham (1953). The test solutions (made up in Tyrode solution and kept at room temperature) were gently applied to the blister surface, left in contact for 1 to 2.5 min., and then washed off with Tyrode solution. Five minute intervals were allowed between washing off a solution and the next application. The intensity of the pain was arbitrarily graded by the subject into 5 " degrees." The subjective assessment of pain was recorded by a voltmeter modified by extending the pointer and increasing the torque of its movement by an elastic band, so that its pointer wrote on a smoked drum. The subject selected one of six of a series of push buttons (according to the absence or " degree " of pain) which switched in 0, 2, 4, 6, 8 or 10 V. derived from a low voltage source. The selection of any one push button automatically disconnected the previous circuit. The deflexion of the pointer increased linearly with the voltage.

Serum and Serum Globulin.—Ox blood was collected at slaughter, defibrinated, centrifuged at 3,000 rev./min. for 20 min. and the serum dialysed for 36 to 48 hr. at 4° C. (against 10 to 20 volumes of distilled water, changed 2 to 3 times). When heated serum was used, serum was heated for 3 hr. at 56 to 58° C. to destroy the kallidin- and bradykinin-inactivating peptidase (Werle, Götze and Keppler, 1937), centrifuged again, and then dialysed as described. It was then used immediately or frozen at −4° C. for future use.

Crude ox serum globulin was prepared from heated, dialysed serum (as described above) by addition of an equal volume of saturated ammonium sulphate, and precipitation was allowed to occur for about 1 hr. at room temperature. After centrifugation, the precipitate was dissolved in a volume of distilled water equal to one-half to two-thirds the original volume of serum. The globulin solution was then dialysed against running tap water overnight, at room temperature; the final volume of dialysed globulin solution was approximately equal to the original volume of serum. Globulin solution prepared in this way is referred to as " heated globulin."

Human Salivary Kallikrein.—Human mixed saliva was collected from normal individuals over a period of several hours and pooled. After centrifugation, the supernatant was slowly filtered with suction and the filtrate dialysed for 24 hr. (against 10 volumes distilled water changed 3 times) at 4° C. Four volumes of acetone (Analar) were then added slowly, and the mixture allowed to stand at room temperature for 20 min. for precipitation to occur. The material was again centrifuged, the supernatant solution discarded, and the residue dried over phosphorus pentoxide. This residue, which contained some water-insoluble material, possessed 100% of the kallikrein activity of the original saliva, as measured by its ability to release kallidin. The residue adhered firmly to the centrifuge tube as a thin coating and was not suitable for weighing, but, if dissolved in water and freeze-dried, it yielded a light, white, flocculent material, which was readily weighable. This method of preparing kallikrein differed slightly from that of Werle and Roden (1936), who precipitated it from saliva with 50% acetone. We increased the acetone concentration to 80%, since in our experience 50% acetone precipitated only a small fraction of the kallikrein in some instances; also, freeze-drying provided an excellent dry preparation.

Drugs and Other Materials.—Histamine was used as acid phosphate, mepyramine as maleate, and atropine as sulphate. Weights of histamine and mepyramine are expressed as base.

Trypsin and chymotrypsin were crystalline preparations (Armour) containing less than 50% $MgSO_4$. In later experiments, a salt-free preparation of crystalline trypsin was used.

A preparation of substance P from horse intestine was kindly provided by Professor J. H. Gaddum, and it contained 13.8 units/mg.

Units of Kinin, Kallidin and Bradykinin.—The unit of kinin or of kallidin was an arbitrarily selected weight of material (prepared according to the methods given below) which caused a strong contraction of the guinea-pig ileum. Such a contraction was produced by 1.0 units of kinin or 0.25 to 0.5 units of kallidin. The units are related to the weights of materials in the following sections.

Preparation of Kinin, Kallidin, and Bradykinin

Kinin.—Kinin was prepared from isolated, dried venom sacs (separated from the sting, duct, etc.) of the common wasp, *Vespa vulgaris*, as previously described (Schachter and Thain, 1954). Crude kinin preparations, which were used as standards, were prepared by extracting approximately 100 dried isolated venom sacs with 10 ml. 95% ethanol, 5 to 7 times. The residue, free of histamine and 5-hydroxytryptamine was dried and kept in a desiccator over P_2O_5. Different batches so prepared contained 1 unit of kinin in 5 to 20 μg. of the powder; such preparations are referred to as crude kinin. Some kinin was also extracted by washing with large amounts of ethanol, but most of the activity remained in the residue.

A more purified preparation of kinin was prepared by extracting 36 mg. venom sacs (approximately 100 sacs) with 2 ml. glacial acetic acid four times. The acetic acid extract was mixed with 15 volumes of anhydrous ether and kept at 4° C. for 2 hr., centrifuged, and the residue washed five times with 95% ethanol. The residue, which was practically invisible and spread over the centrifuge tube, was taken up in distilled water and freeze-dried, yielding 2.8 mg. of a white flocculent powder, which contained 1 unit of kinin in 0.4 μg. This preparation is very hygroscopic on exposure to air, but has retained its activity at room temperature in a desiccator for 9 months. Extraction and purification of kinin in this way resulted in recovery of 80 to 100% of the original activity. This preparation is referred to as purified, or partially purified, kinin.

Kallidin.—At first kallidin was prepared according to the method of Werle, Ehrlicher and Koebke (1951) with slight modifications. Heated, dialysed ox serum was incubated for 15 min. at 35° C. with a dry preparation of human salivary kallikrein (1 ml. serum to kallikrein equivalent of 1 ml. saliva) dissolved in a minimal volume of water. The reaction was terminated by the addition of 1/10 of the volume of N/1 H_2SO_4 to the mixture. This mixture was dialysed overnight against running distilled water. The total dialysate was approximately 20 times the volume of the dialysand and was neutralized by addition of saturated $Ba(OH)_2$, using a *p*H meter to determine the end point. The total dialysate was evaporated to a small volume under reduced pressure in a slow stream of nitrogen. The glass evaporator incorporated a water-cooled, double coil condenser. The dried residue (unsuitable for weighing) was dissolved in a small volume of distilled water, centrifuged to remove insoluble material, and freeze-dried, yielding a light, greyish, readily weighable powder. A powder prepared by the above procedure contained 1 unit of kallidin in 500 μg.; the total recovery of crude kallidin was 240 mg./100 ml. serum (4.8 units/ml. serum).

Kallidin was also prepared from serum by extraction with boiling ethyl alcohol as used in the preparation of bradykinin by Rocha e Silva *et al.* (1949). Heated, dialysed ox serum was incubated with kallikrein as described above, and the mixture added to twice its volume of boiling ethanol for 5 min. and then centrifuged. The supernatant solution was reduced to small volume in an evaporator as above, dried, and the residue ground to a fine homogeneous powder. This preparation was relatively inactive, containing only 1 unit in 10 mg. powder, but activity was increased to 535 μg./unit by extracting the powder with glacial acetic acid and precipitating the active material with 10 volumes of ether at 4° C. The final recovery of kallidin after these procedures was 3.4 units/ml. serum. The reason for the low activity of the boiling ethanol extract would appear to be that the precipitation of serum proteins by ethanol is greatly reduced in dialysed salt-free serum. Thus, boiling ethanol extraction of kallidin prepared in the same way, but from non-dialysed serum, yielded a preparation of 550 μg./unit without further purification, the recovery being 17.6 units/ml. serum. Extraction of kallidin with boiling ethanol yielded a suitable dry powder without freeze-drying.

The most effective and simple method of preparing crude kallidin was found to be the incubation of salivary kallikrein with " heated globulin " (see above) and subsequent extraction with boiling ethanol. " Heated globulin " solution was incubated for 15 min. at 35° C. with a dry preparation of human salivary kallikrein (dissolved in a minimal volume of H_2O); the concentration of kallikrein/ml. of globulin solution corresponded to that in 1 ml. pooled human saliva. The kallidin was then extracted with boiling ethanol and the alcoholic extract reduced in volume as above, and dried. This preparation contained 1 unit in 75 μg. of dry powder and the yield was 7.6 units/ml. serum. Purification of this preparation by extraction with glacial acetic acid and precipitation with ether produced more than a 3-fold increase in activity, namely 1 unit in 24 μg., but the recovery was only about 10%.

Bradykinin.—Bradykinin was prepared by the action of crystalline trypsin on ox serum globulin as described by Rocha e Silva *et al.* (1949), but with some modifications which increased the yield. Since the observations of Werle *et al.* (1937) indicated that the yield of kallidin from serum is increased if the kallidin-inactivating peptidase is destroyed by pre-heating the serum (we confirmed this observation), we compared the release of bradykinin by trypsin from heated and unheated serum, and from globulin prepared from heated or unheated serum. In all instances the concentration of kallidin reached a greater peak with the heated substrate, the greatest concentration occurring with " heated globulin."

We therefore employed " heated globulin " for the preparation of bradykinin as well as for the preparation of kallidin. Approximately 400 ml. of " heated globulin " solution (from 400 ml. dialysed ox serum) was incubated with salt-free crystalline trypsin (400 μg./ml.) for 20 min., the bradykinin extracted with boiling ethanol, and taken to dryness as described for kallidin. The total recovery of dry material was 1.8 g. which contained 1 unit of activity/140 μg.; the yield of bradykinin was 32 units/ml. This recovery is more than 50 times greater than that calculated from the figures of Rocha e Silva *et al.* (1949), and was probably due, in part, to the reduced inactivation of bradykinin by " heated globulin." Further purification by extraction with glacial acetic acid and precipitation with ether yielded a preparation containing 1 unit/100 μg., but the total recovery was only 51%.

Two preparations of bradykinin prepared by the action of *B. jararaca* venom on ox globulin were kindly provided by Dr. M. Rocha e Silva. They contained 1 unit in 100 μg., and 25 μg., respectively.

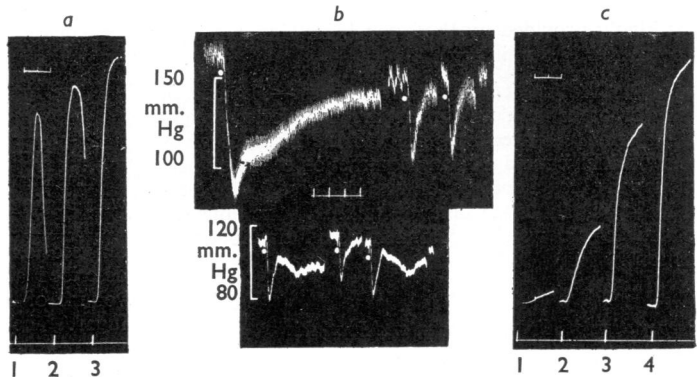

FIG. 1.—Effects of partially purified kinin (0.4 μg. = 1 unit) on different preparations. (*a*) Isolated rat uterus, 18 ml. bath, atropine (0.01 μg./ml.), 28° C. At 1, 2, and 3, addition of 0.1, 0.25, and 0.5 μg. kinin respectively. (*b*) Arterial blood pressure. Above: Dog, 15 kg., atropine (0.1 mg./kg.) and mepyramine (1.0 mg./kg.). At white dots, from left to right, 2.7, 0.7, and 0.7 μg./kg. kinin were administered intravenously. Below: Rabbit 2.8 kg., atropine (0.1 mg./kg.). At first and last dots, kinin, 1.0 μg./kg.; at middle dot, kallidin, 2 units/kg., were given intravenously. (*c*) Isolated guinea-pig ileum, atropine (0.01 μg./ml.) and mepyramine (0.02 μg./ml.), 35° C. At 1, 2, 3, and 4, addition of 0.1, 0.4, 0.7, and 1.0 μg. kinin respectively. Time, 30 sec.

RESULTS

Properties of Partially Purified Kinin

Effects on Smooth Muscle and Arterial Blood Pressure.—Kinin has previously been shown to contract smooth muscle and to lower the arterial blood pressure (Schachter and Thain, 1954). These properties have been confirmed, and the pharmacological studies extended with more purified material. The isolated rat uterus was the most sensitive preparation tested, but the guinea-pig ileum is the most suitable for assay, since a graded response is obtainable over a convenient range of concentrations. The order of sensitivity of the different preparations was: rat uterus > guinea-pig intestine > rabbit intestine. Rat uterus responded markedly to purified kinin in concentrations of less than 0.005 μg./ml. and the guinea-pig ileum to less than 0.025 μg./ml. (Fig. 1).

Purified kinin lowered the arterial blood pressure of the rabbit and dog on intravenous injection of 1.0 μg./kg., or less. This effect was undiminished on repeated injection and unaffected by treatment of the animal with atropine and mepyramine (Fig. 1). The hypotensive action of kinin, therefore, is not mediated through release of histamine. Also, on a weight basis, the purified preparation is far more effective than histamine in lowering the blood pressure of the rabbit or dog.

Cutaneous Pain.—Since some polypeptides have been shown to produce pain on application to a blister base on human skin (Armstrong, Keele, Jepson and Stewart, 1954), kinin was tested in similar experiments on 3 subjects. All subjects experienced moderate pain on application of concentrations of 100 μg./ml. of the purified preparation to a blister base; concentrations of 1 mg./ml. produced a more severe and protracted reaction. One subject, on whom lower concentrations were tested, reacted to solutions containing 20 μg./ml. but not to those with 10 μg./ml. Kinin was more effective than equal concentrations of acetylcholine in all subjects. The response differed from that to acetylcholine in that there was regularly a delay of 30 to 90 seconds before the subject responded; also, the pain reaction was generally more protracted than it was to acetylcholine (Fig. 2). The sensation of itchiness frequently occurred with the lower concentrations of kinin, or when the pain reaction decreased on prolonged contact with the solution.

Capillary Permeability.—The ability of kinin to increase capillary permeability to circulating dye was found to be one of its most striking pharmacological properties. Purified kinin was at least 10 times more effective than histamine in causing increased permeability to pontamine sky blue in the guinea-pig, and at least 100 times more effective

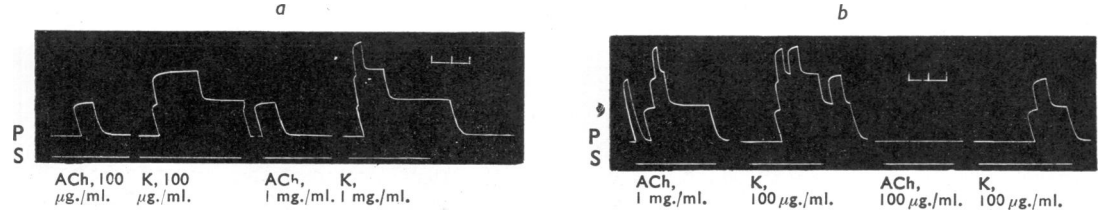

FIG. 2.—Records of pain production by kinin (0.4 μg. = 1 unit) on application to blister base on human skin. The pain response is recorded as an upward deflexion and is graded in arbitrary steps. (*a*) and (*b*) are experiments on two different subjects. P, pain response; S, stimulus (solution applied to blister base); K, kinin; ACh, acetylcholine. Time, 30 sec.

in similar experiments in the rabbit (Fig. 3). All animals blued effectively to concentrations of 0.1 μg./ml., and in some instances significant reactions were still detectable with less than 0.001 μg./ml. In both species the reaction generally appeared more quickly than did that to histamine, and, in general, the degree of blueness was greater than for histamine "lesions" of equal size.

On intravenous injection in the guinea-pig, kinin produced a generalized mild blueing reaction involving the entire skin, and also the visceral organs (Fig. 4). This differs from the regional blueing of the head and neck, presumably due to regional variations in histamine distribution in skin, caused by intravenous injections of the histamine liberator, 48/80 (Feldberg and Miles, 1953). The blueing of the internal organs of the guinea-pig produced by intravenous injections of purified kinin (5 to 25 μg./kg.) is in contrast to the negative or doubtful effects following the injection of equal amounts of histamine.

Paper Electrophoresis.—Crude preparations of kinin were found to migrate to the cathode, at approximately the same rate, whether at *p*H 2.6 or 8.1. In one experiment, approximately 200 units of crude kinin were electrophoresed (see Methods) using N/1 acetic acid (*p*H 2.6) as electrolyte. After 4 hr. of electrophoresis (0.3 to 0.5 mA) the paper was dried and cut into strips numbered 0 to 6 on each side of the line of application, and each strip eluted with 2 ml. saline. Strip 0 measured 0.5 cm. on either side of the line of application, and strips 1 to 6 were each 2.5 cm. wide. Large amounts of kinin were recovered in cathode strips 1 to 3, of which approximately 80% was in strip 2 (Fig. 5). No activity was detectable in the remaining eluates using guinea-pig ileum as test object.

Crude kinin was again similarly electrophoresed except that the electrolyte was M/20 sodium phosphate (*p*H 8.1). In this experiment the current varied from 2 to 3 mA and electrophoresis lasted 5 hr. The paper was dried and divided as before. Large amounts of kinin were recovered from the eluates of cathode strips 2 and 3 only, strip 2 having about twice the activity of strip 1. The anodal strips again possessed no pharmacological activity.

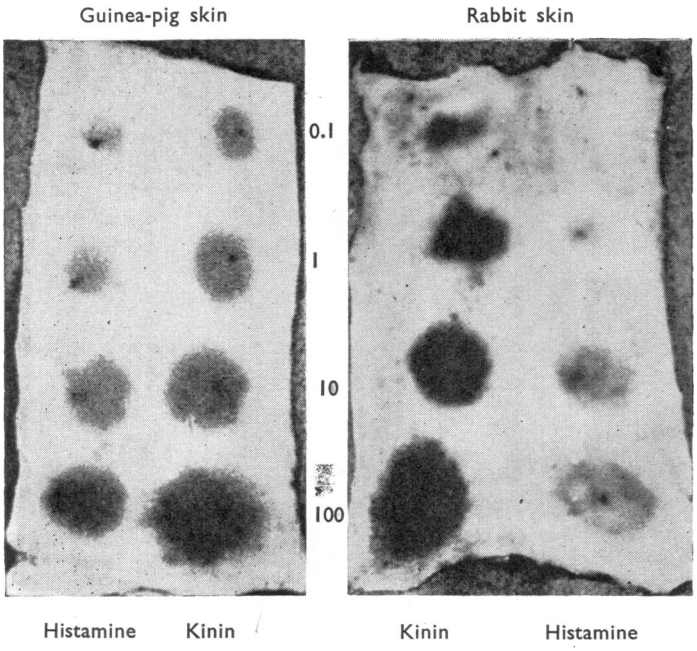

FIG. 3.—Effect of intradermal injections of kinin (0.4 μg. = 1 unit) and histamine on capillary permeability to a circulating dye in the guinea-pig and rabbit. The animals were previously injected with 5% pontamine blue (1.2 ml./kg.) intravenously. The dark areas are sites of intradermally injected kinin and histamine (volume: 0.1 ml. in the guinea-pig, 0.2 ml. in the rabbit) in concentrations of 0.1, 1, 10, and 100 μg./ml. from above downwards as indicated by the numerals between the two photographs. Note greater effectiveness of kinin.

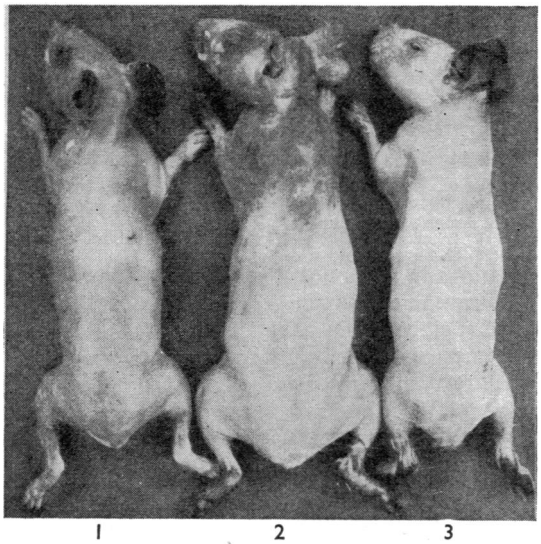

FIG. 4.—Effect of intravenous injections of kinin (0.4 μg. = 1 unit) and compound 48/80 on capillary permeability in the guinea-pig. All animals were previously injected with pontamine blue intravenously, and are depicted above 20 min. after the intravenous injection of: 1, kinin (50 units/kg.); 2, 48/80 (5 mg./kg.); 3, dye alone.

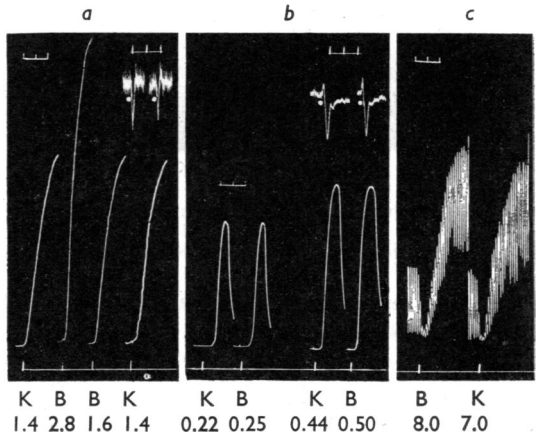

FIG. 6.—Comparison of the pharmacological activity of kallidin and bradykinin. K, kallidin; B, bradykinin. Numerals below K and B refer to units added to 18 ml. organ bath. Time, 30 sec. *a*, Isolated guinea-pig ileum, 35° C. Above: arterial blood pressure. Dog, 15 kg., chloralose-urethane. At white dots: left, 4.8 units bradykinin; right, 4.2 units kallidin. *b*, Isolated rat uterus, 28° C. Above: arterial blood pressure. Rabbit, 2.0 kg. Left, 4.8 units bradykinin; right, 4.2 units kallidin. *c*, Isolated rabbit jejunum, 35° C.

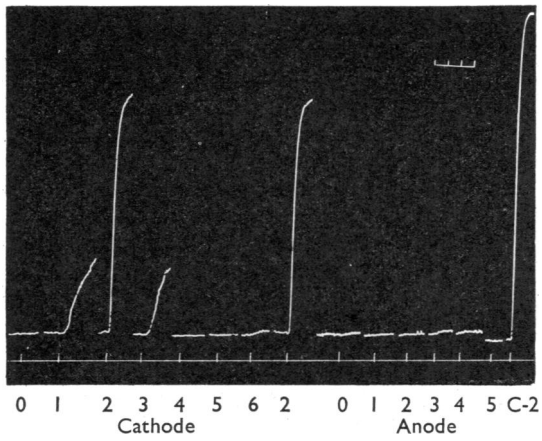

FIG. 5.—Paper electrophoresis of kinin in N/1 acetic acid. Effects on isolated guinea-pig ileum produced by 0.1 ml. eluates of cathodal and anodal strips. C-2 is eluate of cathode strip 2. For details see text. Time, 30 sec.

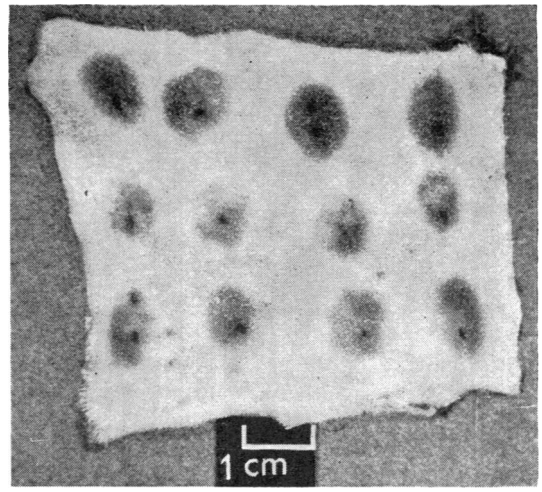

FIG. 7.—Comparison of effects of intradermal injections of kinin (top row), kallidin (middle row) and bradykinin (bottom row) in increasing capillary permeability to circulating pontamine blue in the guinea-pig. Each substance was injected at 4 sites in the same concentration. The concentrations of the substances injected were of equivalent activity in contracting the isolated guinea-pig ileum; kinin (top row) is therefore relatively more effective than kallidin and bradykinin in increasing capillary permeability.

Paper Chromatography.—Previous observations that crude kinin failed to move, or moved only slightly, from the application in ascending butanol-acetic acid chromatograms (Schachter and Thain, 1954) were confirmed. Furthermore, the same result was obtained with the most highly purified kinin in descending as well as ascending chromatograms in butanol-acetic acid, ketone-pyridine, or amyl alcohol-pyridine solvents.

Comparison of Kallidin and Bradykinin

Paper Chromatography.—Bradykinin and kallidin were first chromatographed separately in butanol-acetic acid solvent, and the eluates tested on the guinea-pig ileum. The R_F values of bradykinin in different experiments were 0.25, 0.26, 0.34, 0.35, and 0.37 respectively; those of kallidin were 0.28, 0.30, 0.32, and 0.35. In all instances, elution of the chromatogram yielded only a single pharmacologically active " peak " which was fairly well defined, occupying 2.5 cm. or less of a horizontal strip. In four of these experiments the bradykinin preparation was one prepared by the action of *B. jararaca* venom on globulin; in the experiment yielding an R_F value of 0.35 the bradykinin was prepared by the action of trypsin on " heated globulin." The kallidin preparations were those prepared by the action of human salivary kallikrein on heated serum, and subsequent recovery of kallidin in the dialysate (see Methods).

Similar experiments were also carried out but with the applications of bradykinin (prepared as above with either venom or trypsin) and kallidin (prepared as above) superimposed on each other on the paper and chromatographed. In all instances the pharmacological activity was again eluted as a single " peak." The R_F values of the mixture of kallidin and bradykinin did not differ significantly from those in which they were chromatographed singly.

Paper Electrophoresis.—Kallidin (heated serum+kallikrein, dialysis) and bradykinin (globulin+*B. jararaca* venom) were subjected to paper electrophoresis for 4 hr. at 0.3 to 0.5 mA in N/1 acetic acid as electrolyte. Since both substances migrated to the cathode at similar rates, attempts at separation by this method were not pursued. In both instances activity was found in the cathode strip approximately 3 cm. from the application. The anodal eluates were inactive when tested on the rat uterus or guinea-pig ileum.

Parallel Assays.—Bradykinin (globulin+*B. jararaca* venom) was assayed against kallidin (heated ox serum+kallikrein, dialysis) on 5 different test preparations. Their activities were qualitatively and quantitatively parallel in all instances. Assayed on the guinea-pig ileum 1 unit of kallidin was equivalent to 1.1 units of bradykinin. The same ratio of activity was found for the rat uterus, rabbit intestine, and rabbit or dog arterial blood pressure (Fig. 6). A similar comparison was made of their effects on capillary permeability in the guinea-pig, using kallidin which was prepared somewhat differently (heated globulin+kallikrein, ethanol extraction). Again, they were indistinguishable in their ability to increase capillary permeability to a circulating dye. However, concentrations of kinin which were equivalent to those of kallidin and bradykinin in contracting the guinea-pig ileum were considerably more effective in increasing capillary permeability in this species (Fig. 7).

Specific Desensitization.—As we observed with kinin, the guinea-pig ileum could not be desensitized by prolonged contact with high concentrations of kallidin or bradykinin. Pharmacological distinction of these polypeptides by this method is therefore impossible.

Proteolytic Enzymes.—Incubation of kallidin or bradykinin (0.5 to 1.0 unit/ml.) with an equal volume of human serum or of crystalline chymotrypsin (100 μg./ml.) for 20 min. at 35° C. resulted in the loss of 80 to 100% of activity. Activity was unaffected by incubation with crystalline trypsin under the same conditions. Similar results were obtained with the different preparations of kallidin and bradykinin.

Distinction of Kinin from Kallidin and Bradykinin

Paper Chromatography.—A distinct separation of kinin from mixtures containing kallidin or bradykinin was regularly and easily obtained by chromatography in butanol-acetic acid solvent. Kinin was regularly eluted from the origin of the chromatogram, whereas the others were obtained from a strip with an R_F of approximately 0.35. The result of a typical experiment is shown in Fig. 8. Kinin was readily distinguished from substance P in the same way, since the latter was recovered from the paper at an R_F of 0.32, which agrees with the value obtained by Pernow (1953).

Proteolytic Enzymes.—Kinin differs from kallidin or bradykinin in its greater susceptibility to inactivation by crystalline trypsin. Thus, the incubation of kinin (1 unit/ml.), irrespective of its state of purity, with crystalline trypsin (50 μg./ml.) for 20 min. at 35° C., resulted in the loss of 70 to 100% of its activity.

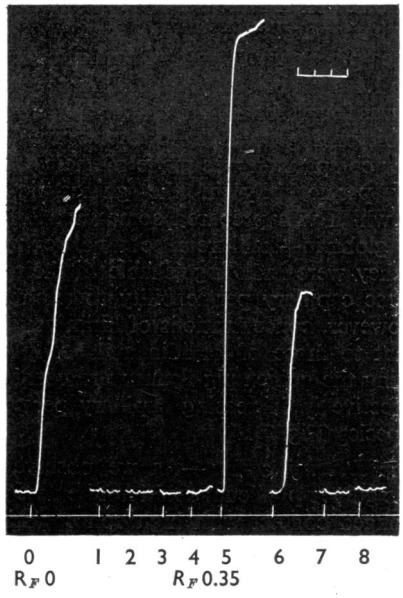

FIG. 8.—Contractions of isolated guinea-pig ileum showing separation of kinin from bradykinin by paper chromatography in butanol-acetic acid solvent. Atropine (0.01 μg./ml.) and mepyramine (0.02 μg./ml.) were present in the 18 ml. bath, 35° C. Time, 30 sec. 0 to 8 are the responses to eluates of horizontal chromatogram strips after running a mixture of kinin and bradykinin as described in the text.

Rabbit Blood Pressure.—A qualitative difference between the effects of kinin and kallidin or bradykinin was the distinct secondary depression of the arterial blood pressure consistently seen only with kinin (Fig. 1*b*). This characteristic has been previously noted with cruder preparations of kinin (Schachter and Thain, 1954).

Comparison of the Release of Kallidin and Bradykinin

Both human salivary kallikrein and bovine pancreatic trypsin released higher concentrations of kallidin and bradykinin, respectively, from serum or globulin which had been previously heated. With heated serum or globulin, the concentration of the smooth muscle stimulant increased for about 20 min. of incubation, and then decreased slowly, so that high concentrations were still present even after 1 hr. With unheated substrates, however, the concentration of kallidin or of bradykinin decreased considerably after 5 to 10 min. of incubation. The more effective release from heated substrates was presumably due to the inactivation of serum peptidase by mild heating (56° C. for 2 to 3 hr.), which does not affect kallidinogen or bradykininogen (Werle *et al.*, 1937). Fig. 9 shows the greater concentrations of bradykinin obtained by the action of trypsin on heated, than on unheated, globulin.

It was also observed that the optimal conditions for the release of kallidin were not identical with those for the release of bradykinin. Thus, the ratio of concentrations of kallidin after 20 min. incubation at 35° C. of salivary kallikrein with heated serum and "heated globulin," respectively, was approximately 1.5/1; with trypsin, however, under the same conditions, the ratio of bradykinin concentrations was approximately 1/4. This reversal of the ratio suggests a difference between the actions of kallikrein and trypsin, the exact nature of which requires further analysis.

Since kallikrein has not been obtained in a pure state, it is not possible to compare quantitatively its effectiveness in releasing kallidin with that of trypsin in releasing bradykinin. However, kallikrein must be a far more potent agent than trypsin in this respect, since incubation of a very crude preparation of human salivary kallikrein, in a concentration of less than 1.0 μg./ml., produced large amounts of kallidin from 0.5 ml. heated ox serum. Crude kallikrein was at least 10 times as effective as crystalline trypsin in this respect.

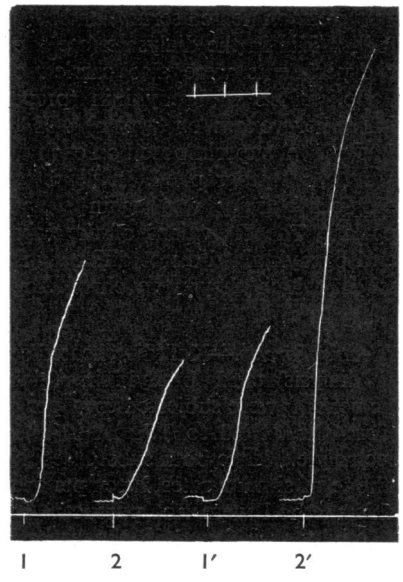

FIG. 9.—Contractions of isolated guinea-pig ileum in response to bradykinin released by crystalline trypsin from unheated and "heated globulin." 1 and 2: 0.5 ml. globulin solution + 0.1 ml. trypsin (1 mg./ml.) incubated for 2 and 2½ min. respectively. 1′ and 2′: as in 1 and 2 but using "heated globulin." 18 ml. bath, atropine (0.01 μg./ml.) and mepyramine (0.02 μg./ml.), 35° C. Time, 30 sec.

Discussion

The present experiments confirm previous observations indicating the pharmacological similarity of kinin, kallidin and bradykinin. It has, however, proved possible to distinguish kinin from these other compounds by paper chromatography and by differential sensitivity to inactivation by trypsin. Since kallidin and bradykinin were indistinguishable by all the comparative tests, they must be closely similar compounds. None the less, our experiments cannot be regarded as proving them to be identical, since it is possible for polypeptides to be indistinguishable in their pharmacological and chemical properties and yet differ in their chemical composition. Thus, pure vasopressins of bovine and porcine origin differ only in that the former contains lysine, and the latter arginine, as its basic amino acid (du Vigneaud, Lawler and Popenoe, 1953). A final answer regarding the distinction of bradykinin from kallidin must, therefore, await studies on pure preparations.

A number of the observations *in vitro* suggests the possibility that the activation of serum kallikreinogen, or the release of kallidin, might occur *in vivo*. For example, serum kallikreinogen is activated *in vitro* by changes in *p*H (Werle, 1934), by papain (Kraut, Frey and Werle, 1933), and by trypsin (Werle, Forell and Maier, 1955); also, substances resembling kallidin and bradykinin are released when serum comes into contact with glass (Armstrong, Keele, Jepson and Stewart, 1954) or when it is diluted (Schachter, 1956). On the basis of the activation *in vitro* of kallikreinogen by *p*H changes, experiments were carried out on reactive hyperaemia by Frey (1930), who obtained indirect evidence suggesting that serum kallikreinogen was activated during circulatory arrest and accounted for the subsequent hyperaemia. However, the release of kallidin into the venous outflow of the dog's hind limb could not be demonstrated after 10 min. of arrest of the arterial supply (Schachter, unpublished).

Ungar and Parrot (1936) suggested that the vasodilatation of the submaxillary gland and tongue during stimulation of the lingual nerve was due to the secretion of salivary kallikrein into the blood circulation. Hilton and Lewis (1955a and b) have recently demonstrated that a " bradykinin-releasing enzyme " appears in the effluent of the Tyrode-perfused submaxillary gland during stimulation of the chorda tympani nerve, and have suggested that vasodilatation in the salivary gland is due to this " enzyme " being secreted into the capillaries of the tissue during salivary secretion. There is no evidence that the " bradykinin-releasing enzyme " in saliva described by these authors differs from salivary kallikrein. Their observations do, however, support the possibility that such a mechanism operates to produce local vasodilatation in the secreting salivary gland. The physiological role of pancreatic kallikrein remains completely obscure, since it exists in the pancreas in an inactive form (Werle, 1937), and, even if it were secreted into the blood stream, it would be pharmacologically inert. On the other hand, it is activated in the duodenum by trypsin (Werle and Urhahn, 1940), but there it has no known digestive or other function. Pancreatic trypsin also exists in an inactive form in the pancreas, and it is similarly difficult to envisage how its possible slow release into the blood circulation during pancreatic activity could result in the release of bradykinin.

The ability of these polypeptides to evoke pain, to enhance permeability, and to cause vasodilatation would make them extremely effective mediators of inflammatory reactions, but definite evidence of their contribution to any type of injury reaction has not yet been presented. Therefore, despite the pharmacological potency of kallidin and bradykinin, and despite the fact that endogenous mechanisms exist for their potential elaboration in high concentrations, their role in physiological or pathological processes remains obscure.

Wasp venom is able to release histamine, and also contains high concentrations of histamine, 5-hydroxytryptamine, kinin (Jaques and Schachter, 1954; Schachter and Thain, 1954) and hyaluronidase (Jaques, 1955). Since the present experiments demonstrate the potent pain-producing and capillary permeability enhancing properties of kinin, there remains little, if anything, to be accounted for in the local reactions to a wasp sting. It does not follow, however, that the occasional severe generalized reaction of humans to a wasp sting (Brown, 1944) is due to these agents in the venom. Although it is possible that the intravenous injection of the contents of one wasp gland would produce a reaction of this nature in any individual, it is also possible that these rare reactions are truly anaphylactic, that is, due to previous sensitization to venom protein.

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