Brit. J. Pharmacol. (1958), **13**, 326.

THE CHROMATOGRAPHIC BEHAVIOUR OF WASP VENOM KININ, KALLIDIN AND BRADYKININ

BY

A. P. MATHIAS AND M. SCHACHTER

From the Departments of Biochemistry and Physiology, University College, London

(RECEIVED MAY 21, 1958)

Wasp venom kinin which has hitherto appeared to be homogeneous can be resolved by ion-exchange chromatography into a single major and two minor components. These are indistinguishable by their action on smooth muscle and by their rapid inactivation by chymotrypsin. All three components of wasp kinin are chromatographically different from kallidin or bradykinin. The close similarity of the latter compounds is confirmed by their identical behaviour on an ion-exchange resin.

Wasp venom kinin, kallidin, and bradykinin are substances with many chemical and pharmacological properties in common. Wasp kinin occurs with histamine and 5-hydroxytryptamine in the venom of the common European wasp, *Vespa vulgaris* (Jaques and Schachter, 1954; Schachter and Thain, 1954). Kallidin and bradykin (termed plasma kinins by some authors) are derived by the actions of kallikrein (Werle, 1955), or of snake venoms and trypsin (Rocha e Silva, 1955) on an α_2 globulin of blood plasma. All three substances appear to be polypeptides. They produce a characteristic slow contraction of isolated smooth muscle, are vasodilator agents, increase capillary permeability and cause pain when applied to the exposed blister base of human skin (Holdstock, Mathias, and Schachter, 1957). The present experiments are concerned with the chromatographic behaviour of wasp kinin and of kallidin and bradykinin.

METHODS

Preparation of Wasp Kinin, Kallidin, and Bradykinin.—Wasp venom kinin was prepared by extracting dried wasp venom glands with acetic acid; the kinin was precipitated by the addition of ether, washed repeatedly with small amounts of ethanol, taken up in water and freeze-dried. In several instances the material was, in addition, dialysed against 10 to 20 volumes of water in cellophane sacs with continuous shaking (dialysate changed hourly for 3 hr.). Bradykinin and kallidin were prepared by the action of crystalline trypsin and human salivary kallikrein respectively on heated ox serum globulin. Details of these procedures are described by Holdstock *et al.* (1957). The bradykinin preparation contained 1 unit (Rocha e Silva, Beraldo and Rosenfeld, 1949) in 140 μg. of dry material; 120 μg. kallidin was equivalent in activity on the guinea-pig ileum to 1 unit of bradykinin.

Chromatography. — Ascending paper chromatograms were run at room temperature on Whatman No. 1 paper and the eluates tested on the isolated rat uterus and guinea-pig ileum as previously described (Holdstock *et al.*, 1957). The solvents used were: (*a*) water (56%)/ammonium sulphate (20%)/ "cellosolve" (24%) as described by Martin and Porter (1951); "cellosolve" in ethylene glycol mono-ethyl ether; (*b*) ethanol/0.2N-HCl; and (*c*) *n*-butanol/acetic acid/water (Partridge, 1948). The chlorine-starch-iodide spray reaction was performed as described by Rydon and Smith (1952) except that the time of exposure of the paper to chlorine was shortened from 10 min. to 1 min. The azocarmine test was carried out according to Turba and Enenkel (1950).

By the methods outlined by Hirs, Moore, and Stein (1953), we found that wasp kinin was completely absorbed on the resin, Amberlite (XE-64), from solution in a phosphate buffer (Gomori, 1955) at *p*H 6. At higher *p*H values, however, kinin partitioned between the buffer and resin and a *p*H of 7.7 was considered satisfactory for ion-exchange chromatography. The resin was prepared as described by Hirs *et al.* (1953), and equilibrated with 0.1M phosphate buffer (*p*H 7.7) containing thymol, and packed in a glass cylinder as a column 0.9×25 cm. Wasp kinin (300 to 500 μg.) was dissolved in 0.5 ml. of the buffer (0.05 ml. was kept beside the column and 0.05 ml. frozen, as controls), slowly added to the column and washed on with a small amount of buffer. Elution was continued with the same buffer at a flow rate of approximately 3 ml./hr. The eluate was collected in fractions of 0.94 ml. which were frozen

at regular intervals. The tubes were later thawed and tested for pharmacological activity. Bradykinin (9.0 mg.) and kallidin (8.0 mg.) were chromatographed in the same way.

Isolated Smooth Muscle Preparations.—Rat uterus or guinea-pig ileum was suspended in an 18 ml. bath at 26 to 28° and 34 to 36° respectively. Atropine and mepyramine were present in the bath throughout with the isolated guinea-pig ileum, and atropine and lysergic acid diethylamide (LSD) with the rat uterus; the final concentration of each of these drugs in the bath was 10^{-4} g./l.

Drugs and Other Reagents.—Atropine was used as sulphate, mepyramine as maleate, and (+)-lysergic acid diethylamide as tartrate. Trypsin and chymotrypsin were crystalline, salt-free preparations (Armour).

Results

Paper Chromatography

The R_F of wasp kinin has been either zero or very low in all solvents hitherto employed for its chromatography on paper (Holdstock *et al.*, 1957). In the present experiments, however, we have succeeded in obtaining a high R_F value in two solvents. These were ammonium sulphate-cellosolve and ethanol-hydrochloric acid (see Methods). Most experiments were carried out with the former. Pharmacological testing of eluates on the isolated rat uterus or guinea-pig ileum revealed activity only in a single well-defined band with an R_F approximately 0.45 in both solvent systems. The region on the paper which corresponded to the eluted pharmacological activity was devoid of ultra-violet absorbing or fluorescent material. It did, however, react very faintly with ninhydrin, strongly with chlorine-starch-iodide reagent and particularly strongly with azocarmine. In fact, this was the only area stained by azocarmine with chromatographed wasp kinin prepared as described above and dialysed for a limited time. There were, however, at least two pharmacologically inactive bands of higher R_F values showing positive reactions to

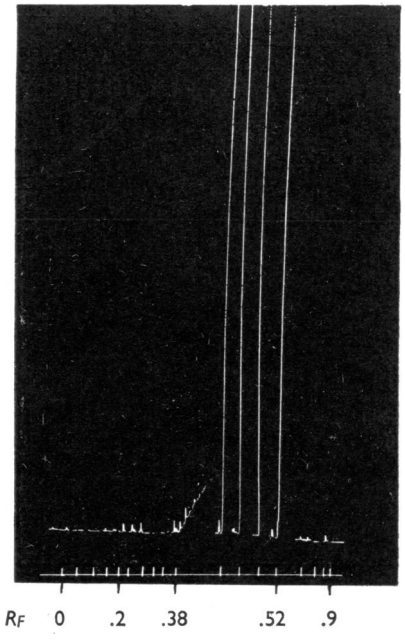

FIG. 1.—Contractions of isolated guinea-pig ileum in the presence of atropine and mepyramine (each 10^{-4} g./l.) to eluates of two-dimensional paper chromatogram of wasp kinin. The R_F value are those in the second solvent $(NH_4)_2SO_4$-cellosolve.

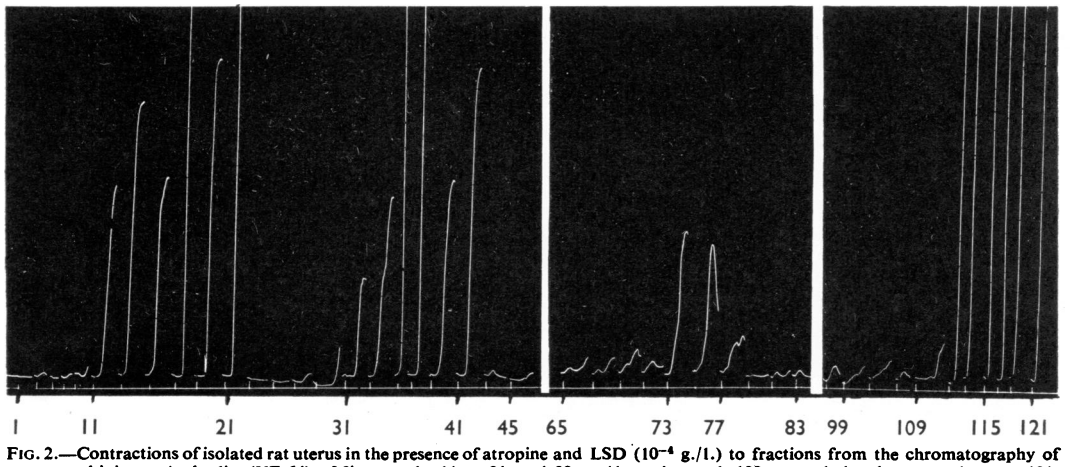

FIG. 2.—Contractions of isolated rat uterus in the presence of atropine and LSD (10^{-4} g./l.) to fractions from the chromatography of wasp kinin on Amberlite (XE-64). Minor peaks 11 to 21 and 29 to 41; major peak 109 onwards has been cut short at 121. Trace activity (in this experiment only) in fractions 73 to 77.

ninhydrin and to chlorine-starch-iodide. Two-dimensional chromatograms were also run using butanol-acetic acid as the first solvent and ammonium sulphate-cellosolve as the second. Elution of the paper yielded only a single pharmacologically active area in the position expected from unidimensional chromatography (Fig. 1) with a recovery of 30 to 40%. This area reacted strongly with azocarmine. The positive reaction of the pharmacologically active material with the chlorine-starch-iodide and azocarmine reagents supports the view that it is a polypeptide.

Bradykinin and kallidin could not be separated by paper chromatography in the ammonium sulphate-cellosolve solvent. These substances moved somewhat faster than wasp kinin with an R_F value of approximately 0.5. Recoveries were approximately 25%.

Ion-exchange Chromatography

Five experiments in which wasp kinin of varying purity was applied to the ion-exchange resin Amberlite (XE-64) yielded identical results. In all experiments there were two minor (but definite) peaks of activity emerging from the column rapidly and close to one another, and a delayed major peak (Fig. 2). The major peak which appeared in fractions 100 to 110 and persisted beyond fraction 140 contained approximately 85

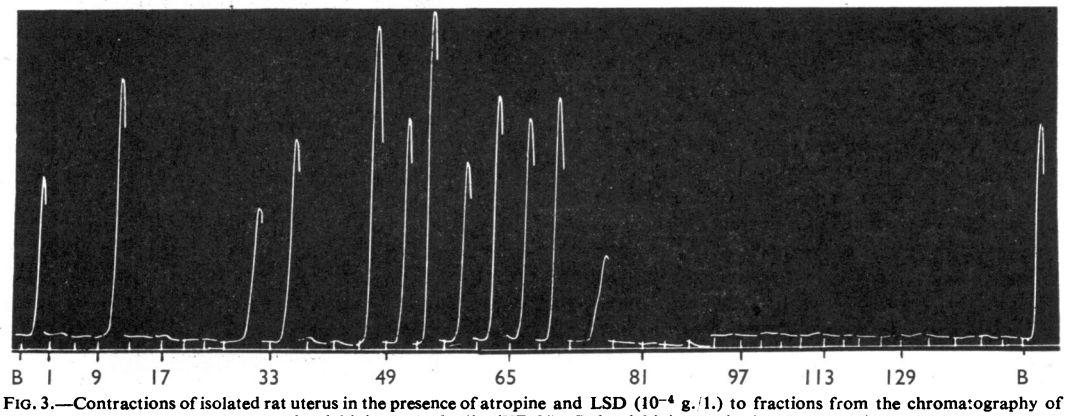

FIG. 3.—Contractions of isolated rat uterus in the presence of atropine and LSD (10^{-4} g./l.) to fractions from the chromatography of bradykinin on Amberlite (XE-64). B, bradykinin standard.

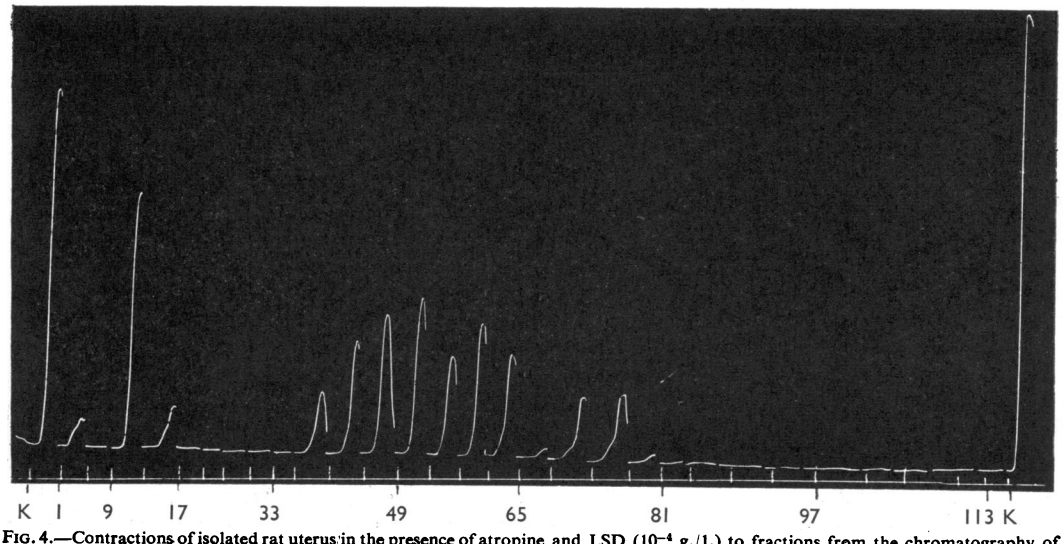

FIG. 4.—Contractions of isolated rat uterus in the presence of atropine and LSD (10^{-4} g./l.) to fractions from the chromatography of kallidin on Amberlite (XE-64). K, kallidin standard.

to 90% of the activity recovered in the eluate. The total recovery of activity was approximately 30% at room temperature and 50% or more when chromatography was carried out at 4°. All three peaks possessed the same relative activity on both the guinea-pig ileum and rat uterus preparations. Also, the three activities were completely destroyed by incubation for 20 min. at 37° with crystalline chymotrypsin and much reduced by trypsin (25 μg./ml.). The fractions corresponding to each of the three peaks were bulked, dialysed for a limited time against water to reduce the concentration of buffer salts, and each of the three peaks rechromatographed individually. We failed, however, to recover activity in this way and it would appear that wasp kinin(s) becomes less stable in a purified state. Attempts to remove the buffer salts with the resin "Biodeminrolit" (Permutit) proved unsuccessful because kinin was also completely adsorbed on the resin.

Kallidin and bradykinin were also chromatographed at room temperature in the same way. The behaviour of these two substances on the resin was almost identical. The main bradykinin peak emerged in fractions 44 to 72 (Fig. 3) and that of kallidin in 38 to 73 (Fig. 4). In the case of bradykinin there was also a minor activity in fractions 29 to 33. Both kallidin and bradykinin show a very narrow band of activity in tube 9 which corresponds to the hold-up volume of the column. This activity is probably due to traces of the major active material washed out by impurities which pass freely through the column. The recoveries of bradykinin and kallidin were approximately 25% and 15% respectively.

Part of the expenses of this work were defrayed by a grant from the Central Research Fund of the University of London which is gratefully acknowledged.

References

Gomori, G. (1955). In *Methods in Enzymology*, **1**, 143. New York: Academic Press.

Hirs, C. H. W., Moore, S., and Stein, W. H. (1953). *J. biol. Chem.*, **200**, 493.

Holdstock, D. J., Mathias, A. P., and Schachter, M. (1957). *Brit. J. Pharmacol.*, **12**, 149.

Jaques, R., and Schachter, M. (1954). Ibid., **9**, 53.

Martin, A. J. P., and Porter, R. R. (1951). *Biochem. J.*, **49**, 215.

Partridge, S. M. (1948). Ibid., **42**, 238.

Rocha e Silva, M., Beraldo, W. T., and Rosenfeld, G. (1949). *Amer. J. Physiol.*, **156**, 261.

—— (1955). In *Polypeptides which Stimulate Plain Muscle*, J. H. Gaddum, ed. Edinburgh: Livingstone.

Rydon, H. N., and Smith, P. W. G. (1952). *Nature, Lond.*, **169**, 922.

Schachter, M., and Thain, E. M. (1954). *Brit. J. Pharmacol.*, **9**, 352.

Turba, F., and Enenkel, H. J. (1950). *Naturwissenschaften*, **37**, 93.

Werle, E. (1955). In *Polypeptides which Stimulate Plain Muscle*, J. H. Gaddum, ed. Edinburgh: Livingstone.