COUNTERCURRENT DISTRIBUTION OF THE SUPERNATANT OF INCUBATED GLOMERULI

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Received 23 March 1970

1. Introduction

Isolated rat glomeruli produce a vasoactive polypeptide *in vitro* which we have tentatively identified by pharmacological tests as angiotensin I [1]. Two questions remained unanswered: first, was it really angiotensin I or another closely related but not identical polypeptide; and second, was the pressor activity due entirely to angiotensin I or was more than one short lasting pressor substance secreted by the glomeruli? In order to answer these two questions, we performed a countercurrent distribution of the concentrated supernatant of incubated rat glomeruli.

The results confirm that (a) angiotensin I is the main pressor substance and (b) definite amounts of angiotensin II and an unidentified polypeptide are also found.

2. Materials and methods

The supernatant of incubated glomeruli from 10 rat kidneys was obtained by a method described elsewhere [1] and concentrated one hundred times by freeze-drying. This material was subjected to countercurrent distribution with a phase volume of 1.8 ml. The solvent mixture consisted of equal volumes of 2-butanol and aqueous buffer composed of 0.1 M Na phosphate and 3.0 M NaCl, pH 7. After 40 transfers, all the tubes were acidified by the addition of 0.15 ml concentrated HCl and shaken. Time was allowed for re-equilibration and the butanol phase removed and dried. Samples were diluted with saline and assayed for their pressor activity in nephrectomized rats treated with pentolinium.

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3. Results and discussion

Three peaks, A, B, and C, were observed (fig. 1). Their respective distribution coefficients in five experiments were A: 0.17–0.25; B: 0.66–0.90 and C: 5.40–12.0. All of them lost their activity upon incubation with trypsin. On silica gel chromatography, the R_f of peaks A and B were identical to that of angiotensin II standards, while peak C was different (fig. 2). The material of peaks A and B caused strong contraction of a preparation of isolated guinea pig ileum. Peak A contracted a rat uterus preparation, but peak B did not elicit any response, unless a dose 20 times higher was used. However, upon incubation with fresh rat plasma peak B was active on the uterus. Our results then confirm those reported by Skeggs et al. [3]. Peak A corresponds to angiotensin II, and accounted for 5 to 25 percent of the total pressor activity of the starting material. Peak B, corresponding to angiotensin I accounted for 54 to 76 percent of the total activity and peak C, which elicited erratic responses on the guinea pig ileum and contracted the rat uterus preparations, accounted for 11 to 20 percent of the total activity.

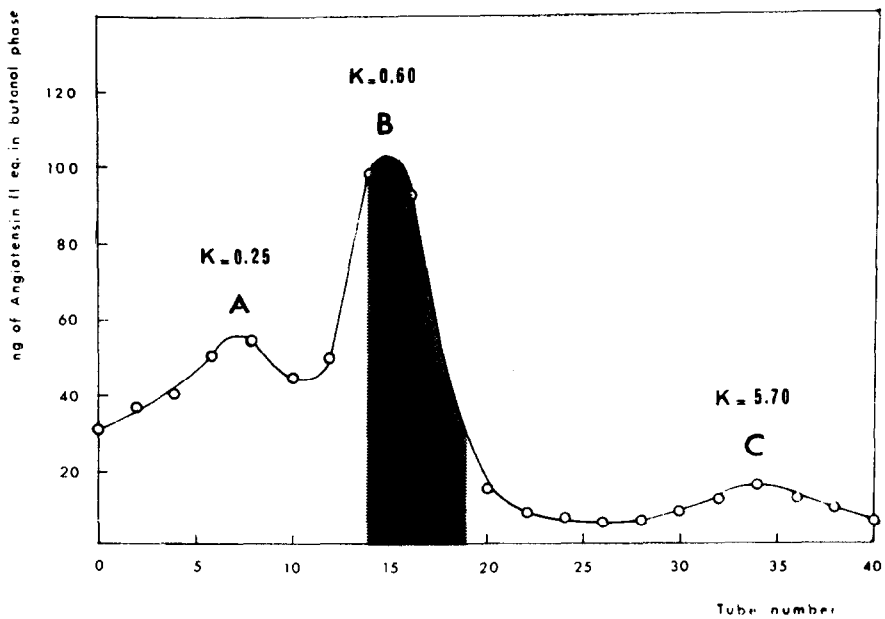

Fig. 1. Distribution patterns of the concentrated supernatant of incubated rat glomeruli. Tubes 14 to 18 were pooled and incubated with fresh rat plasma.

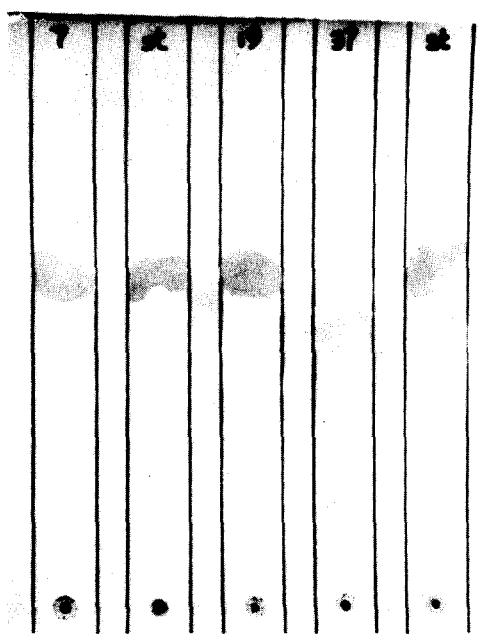

Fig. 2. Silica gel thin-layer chromatographic mobilities of peaks A, B, and C in a mixture of 1-butanol/water. (Numbers refer to fractions in fig. 1).

In order to confirm that peak B really was angiotensin I, tubes 14 to 18 were pooled and incubated with fresh rat plasma for one minute at 37°. A new countercurrent distribution was performed and two active peaks were again found with partition coefficients identical to the A and B peaks of the initial countercurrent experiment. As expected, almost all the pressor activity, 77 percent of the original peak B, was found in the new peak A, and only 23 percent remained in peak B. These experiments show that (a) angiotensin I is the main vasoactive polypeptide produced *in vitro* by rat isolated glomeruli, (b) it is almost certain that our preparation of isolated glomeruli had a converting enzyme [4] because we always detected some angiotensin II in the supernatants; since we have shown previously that our preparation is not contaminated with blood plasma, the enzyme must be of tissue origin; and (c) the isolated glomeruli secreted a third short lasting pressor substance which is destroyed by trypsin and contracts the rat uterus without addition of plasma.

A substance similar to angiotensin I has also been isolated in the whole non-incubated kidney and in several other tissues of the rat [5].

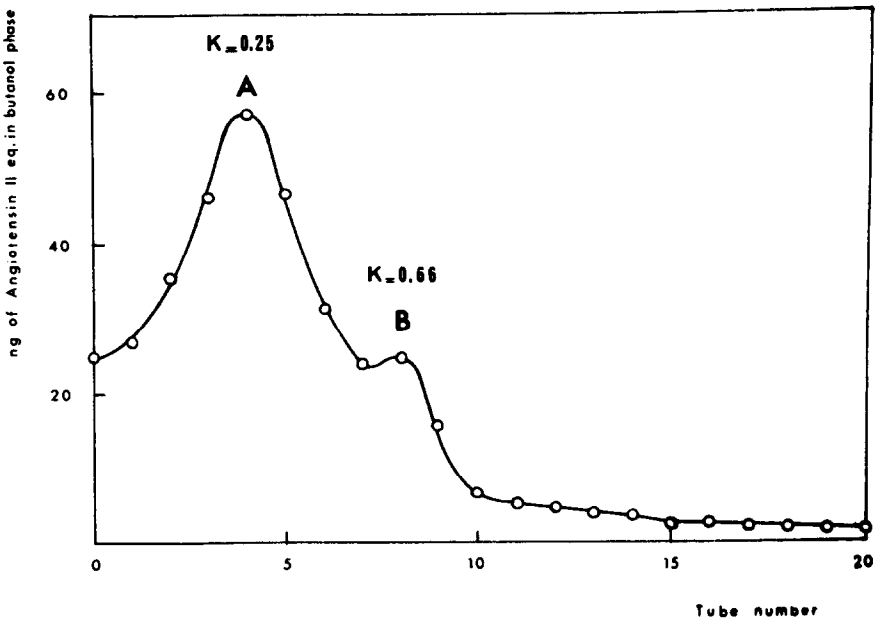

Fig. 3. Countercurrent distribution of peak B (fig. 1) after incubation with fresh rat plasma.

Acknowledgements

We are grateful to Professor Alejandro C.Paladini for encouragement and helpful discussion and Miss Susana I.Brocca for the excellent technical assistance.

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