Increased activity of a factor ("transferangiotensin") favoring the binding of angiotensin II to arterial wall in hypertension

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Increased activity of a factor ("transferangiotensin") favoring the binding of angiotensin II to arterial wall in hypertension. A plasma protein component with an apparent mol wt of 700,000 increases angiotensin II binding by macromolecules in arterial wall extracts. Its activity is increased in several types of hypertension, most prominently in essential hypertension.

Augmentation de l'activité d'un facteur ("transferangiotensine") qui favorise la liaison de l'angiotensine-II à la paroi artérielle au cours de l'hypertension. Une protéine plasmatique, de poids moléculaire apparent de l'ordre de 700,000, augmente la liason de l'angiotensine-II à des macromolécules des extraits de parois artérielles. Son activité est augmentée dans différents types d'hypertensions, surtout dans l'hypertension essentielle.

The experimental findings of Mizukoshi and Michaelakis [1] have shown the existence, in plasma from patients with essential hypertension, of one or more factors that potentiates the action of angiotensin II and norepinephrine. The hypertensive effect of angiotensin II or norepinephrine appeared to be increased when plasma from patients with essential hypertension was administered simultaneously to bilaterally nephrectomized rats.

The effect of angiotensin II is probably mediated via its interaction with specific binding sites ("receptors") that are located in the arterial wall and other target cells [2]. Quantitative estimation of the affinity and number of arterial wall receptors has been difficult. One such attempt at quantification has used immunological methods [3].

Several laboratories have reported the presence of angiotensin-binding macromolecules in plasma [4–7]. Although there is no evidence to suggest that norepi-

nephrine is also bound by these binders, and no direct evidence of their biological significance, we have nevertheless elected to examine the plasma of normal and hypertensive humans for the presence of factors which might affect angiotensin's interaction with arteries. It is the purpose of this paper to describe some of the characteristics of such a factor which favors the binding of angiotensin II by arterial wall. The activity of this plasma compound is apparently increased in several forms of arterial hypertension, but mainly in that which is said to be "essential" in type. Since its action appears to occur through an increased transfer of angiotensin II to the arterial wall receptor, we propose that it should be named "transferangiotensin" (TA).

Methods

General reagents and apparatus. Val-5-angiotensin II, Asp^1-β-amide was purchased from Ciba (Basel, Switzerland). Angiotensin I and its antibody were obtained from Schwarz/Mann (Orangeburg, NY). Antialdosterone-γ-lactone antibody was purchased from Antibodies Allied (Davies, California). ^{3}H-aldosterone (1α, 2α) with a specific activity of 35 Ci/mmole and sodium iodide, 80 to 140 mCi/ml (^{125}I) in NaOH solution, were acquired from the Amersham Radiochemical Center (England). Lysozyme, with an activity of 15,000 u/mg; pronase, containing 70,000 PUK U/g; and activated charcoal were purchased from Merck and Co. (Darmstadt, West Germany). Dextran T-70, Sephadex G-25 and G-200 and Sepharose 6-B were purchased from Pharmacia (Sweden). Ion exchange paper chromatography sheets,

type P87, were obtained from Whatman (England). Cellulose acetate strips for electrophoresis were purchased from Atom (Barcelona). Dowex-1 ion exchange resin, 200–400 mesh, was obtained from Sigma (St. Louis, MO). Human albumin, γ-globulin (IgG) and fibrinogen were the gift of Laboratorios Hubber (Barcelona). Anti-angiotensin II antibody was developed according to the procedure of Goodfriend, Ball and Farley [8].

Spectrophotometric readings were performed on a spectrophotometer (Hitachi-Perkin Elmer, type 139). Centrifugation was performed in a refrigerated centrifuge (Sorvall RC-2B). Radioactivity was counted in a liquid scintillation counter (Nuclear Chicago, Isocap-300). Collections during chromatography were performed with a fraction-cutter (Shandon-MBI). Electrophoresis was carried out in an electrophoresis apparatus (Atom-500).

Plasma samples. Plasma was obtained from normal subjects and hypertensive patients by taking 20 ml of peripheral venous blood in the presence of 0.05 ml of 20% sodium edetate (EDTA), pH 7.4. The samples were chilled and centrifuged at 4°C for ten minutes at 6,000 rpm on a rotor (SE-2 Sorvall). The supernatants were stored at −20°C, until assay.

Etiology of hypertension. Five clinical indexes were used to describe the hypertensive patients: *1)* history and physical examination; *2)* levels of plasma renin activity, as described by the radioimmunoassay method of Haber et al [9] and modified according to Pinto and Arellano [10]; *3)* urinary excretion of aldosterone using the radioimmunoassay procedure of Farmer et al [11]; *4)* urinary excretion of 17-hydroxy [12] and 17-ketosteroids [13]; and *5)* urinary excretion of vanillylmandelic acid [14]. These determinations were performed while the patients were maintained on a normal sodium diet and were receiving no drug therapy.

Iodination and purification of angiotensin II. Angiotensin II was labeled by a modification of the method of Hunter and Greenwood [15]. ^{125}I-sodium iodide, 5 mCi in 50 μl of NaOH solution, pH 8 to 11, was mixed with 20 μl of 0.3 M potassium phosphate buffer (pH 7.5), 15 μl of Chloroamine T (3.6 mg/ml in 0.3 M phosphate buffer, pH 7.5) and 4 μg of angiotensin II. The mixture was incubated at room temperature for 15 sec. The reaction was stopped with 75 μl of sodium metabisulfite (2.4 mg/ml), in 50 mM potassium phosphate buffer (pH 7.5). The solution was acidified by adding 2 μl of 4 M acetic acid.

Purification of labeled angiotensin II was performed on a 10×0.2 cm column (Dowex-1), equilibrated by thorough washing with 10 mM HCl. The sample was eluted in 0.5 ml fractions with water. To each fraction,

20 μl of 0.5 M sodium phosphate buffer (pH 7.5) and 0.2% casein were added. Radioactive samples were pooled together and stored at −20°C until required. The purification procedure was tested by spotting 10^5 cpm on ion exchange paper followed by descending chromatography on the system pyridine-acetic acid-water-ethanol (65:27:278:400) and autoradiographed according to the method of Goodfriend et al [8]. The mass of radioactive peptide was determined by the procedure of Jones [16]. Specific activity varied from 0.15 to 0.22 mCi/μg.

Preparation of the arterial wall "receptor". Arterial wall receptor was obtained from albino rabbits of 1.5 kg body wt, housed and fed under routine conditions. After being fasted overnight, the animals were killed by decapitation. The aorta was dissected from the point of its origin in the heart to the iliac bifurcation and washed free of blood in cold 0.9% sodium chloride in 5 mM sodium phosphate buffer (pH 7.4). The aorta was suspended in 30 ml of the same buffer and homogenized with a blender (Mini-Pimer) at 4°C, for three minutes; the suspension was then homogenized further with a homogenizer (Afora, Barcelona). The suspension was centrifuged at 30,000 ×g for 30 min. The supernatant was stored at −20°C until needed. Before each assay, the arterial extract was diluted with saline-phosphate buffer to a final concentration of 5 mg/ml of protein, as determined by the biuret method [17].

Individual experiments were performed with the extract of aortas from different rabbits, and repeated utilizing at least three different preparations.

Angiotensin binding assay systems. Angiotensin binding was measured by three different systems: *a)* capability of the plasma to affect angiotensin binding by arterial wall, *b)* binding by arterial wall and *c)* binding by plasma itself.

a. "Transferangiotensin" activity (potentiation of arterial binding). The transferangiotensin assay system involved the measurement of angiotensin bound by the arterial wall when blood plasma was added.

Polystyrene tubes contained in a final volume of 1 ml: 10 μmoles of sodium phosphate buffer (pH 7.4), 50 to 60 μg of rabbit arterial wall extract, 10 μg of lysozyme, approximately 10,000 cpm of ^{125}I-angiotensin II and varying amounts of plasma (usually 2 to 50 μl) from either normal or hypertensive patients. In control tubes, either arterial wall extract, plasma, or both, were omitted.

After incubation at 4°C for 24 hr, the separation of free from bound angiotensin II was performed by adding 1 ml of a suspension of 5% activated charcoal and 0.5% Dextran in 5 mM sodium phosphate buffer (pH 7.4). After thorough mixing, the incubation

mixtures were allowed to stand at 4°C for 15 min, then centrifuged for ten minutes at 6,000 rpm in a rotor (Sorvall SE-2). Supernatants were counted in 15 ml of scintillation fluid [18]. Radioactivity in the supernatant after coated charcoal adsorption was taken as the measure of angiotensin II bound to the macromolecules in either the arterial wall extract or plasma.

Charcoal concentration (between 5 and 0.32%) had no significant effect on transferangiotensin activity. Specificity of the plasma as a source for transferangiotensin was shown by replacing it with the same amount of protein in a liver extract.

b. Transferangiotensin unit definition. A unit is defined as the amount of plasma needed to increase by 1% the angiotensin bound by the arterial wall extract, after blanking out the bound radioactivity by the plasma and the arterial wall as separately assayed.

c. Binding by arterial wall. The assay system contained the same ingredients already mentioned, but plasma was left out. Increasing amounts of unlabeled angiotensin II (from 0 to 200 ng) were added to the assay system. Control tubes contained no arterial extract.

d. Binding by plasma. This was assessed as already described using an aliquot of plasma (50 μl). A curve of displacement of labeled angiotensin II by unlabeled angiotensin II was determined by adding increasing amounts of unlabeled angiotensin to the tubes containing plasma and a constant amount of labeled angiotensin.

e. Physical-chemical properties of "transferangiotensin". These experiments were performed with a pool of plasma from hypertensive patients. *1. Optimum pH.* The effect of the pH on the ability of plasma to potentiate binding activity to arterial extract was tested by replacing the phosphate buffer with 10 μmoles of Tris-HCl buffer at different pH's from 5 to 9. The reaction was carried out as described. *2. Dialysis.* One ml of hypertensive plasma was dialyzed at 4°C in cellophane tubing (Visking) against two changes (2 liters each) of 5 mM sodium phosphate buffer (pH 7.4). The dialysate was evaporated to dryness at 35°C in a flash evaporator (Büchi). The residue was dissolved in 0.5 ml of dialysis buffer. "Transferangiotensin" activity was assayed before and after dialysis and in the dissolved dialysate. *3. Temperature.* One ml of hypertensive plasma was heated at 100°C for five minutes and the precipitate was removed by centrifugation. *4. Extract with solvents.* One ml of hypertensive plasma was extracted with 10 ml of ether. The ether layer was removed and evaporated to dryness under nitrogen. The residue was dissolved in 1 ml of 5 mM sodium phosphate buffer (pH 7.4) and the "transferangiotensin" activity assayed. *5. Protein*

digestion. One ml of hypertensive plasma was incubated for 72 hr at 4°C in the presence of 100 μg of pronase, 10 μmoles of sodium borate buffer (pH 7.5) and 0.5 μmoles of calcium chloride. The reaction was terminated by adding 10 μmoles of sodium EDTA (pH 7.4). In subsequent tests the same amount of EDTA was added to control assay tubes. *6. Metal and ion effects.* To test the effects of metals in the assay system, 10 μmoles of sodium EDTA (pH 7.4) were added. Specific ion effects were tested by adding increasing amounts of sodium chloride, potassium chloride or calcium chloride, varying from 0 to 1 μmole per assay tube.

f. Characterization of the potentiating factor. Characterization of components involved in potentiating angiotensin binding was performed by gel chromatography and electrophoresis. *1. Fractionation of plasma angiotensin binders.* Columns of Sephadex G-25, G-200 and Sepharose 6-B, 35×1.8 cm in size, were thoroughly washed with 0.1 M sodium phosphate buffer (pH 7.4). The samples were applied to the columns with a Sephadex applicator. Elution was performed at room temperature with the phosphate buffer at a rate of 0.5 ml/min. Radioactivity was counted in each 1-ml fraction. The samples applied to the columns contained in a final volume of 1 ml: 10 μmoles of phosphate buffer (pH 7.4), 10 μg of lysozyme, 2×10^6 cpm of ^{125}I-angiotensin II, 0.2 ml of either normal or pooled hypertensive plasma and 0.6 mg of protein from rabbit arterial wall. In six experiments, pooled plasma was replaced by individual plasma samples from subjects 4, 5 and 6 (controls) and patients 7, 8 and 9. Incubation mixtures without plasma were also prepared, incubated at 4°C for 24 hr and then applied to the column. Some control samples were applied without prior incubation. For calculation of mol wt, the columns were standardized utilizing human albumin, γ-globulin and fibrinogen. *2. Characterization of "transferangiotensin" activity.* Hypertensive plasma samples of 15 ml were lyophilized, and the residue dissolved in 1.5 ml of water. These samples were applied to Sephadex G-200 columns, 42×3.5 cm, equilibrated and eluted at room temperature with 10 mM sodium phosphate buffer (pH 7.4). Two-ml fractions were collected at a rate of 1 ml/min. Transferangiotensin activity was assayed in increasing volumes up to 0.4 ml aliquots from alternate fractions. Angiotensin binding activity in plasma was tested as already described, eluting with 10 mM phosphate in 2-ml fractions. *3. Electrophoretic mobility of angiotensin binders.* Two radioactive peaks from Sephadex G-200 column fractionation of incubation mixtures containing radioactive angiotensin II and hypertensive plasma were pooled and lyophilized. The residues were dis-

solved in 1 ml of water and 70 μl samples were spotted on cellulose acetate strips. Control strips received 35 μl of normal serum, or labeled angiotensin II alone. Electrophoresis was carried out at 200 v for three hours at 4°C, in 0.083 μ of barbital buffer (pH 8.4). When electrophoresis was finished, each strip was cut into 0.5-cm pieces, and its radioactivity was counted. Alternate cellulose acetate strips were stained with Ponceau red and scanned at 520 mμ, followed by the counting of radioactivity.

g. *Identification of the radioactivity bound by "transferangiotensin".* The first peak of radioactivity obtained from Sephadex G-200 columns developed as described above under "Fractionation of plasma angiotensin binders" and was tested with hypertensive plasma as a binder. An aliquot of 15×10^5 cpm of ^{125}I-angiotensin II bound by transferangiotensin was spotted on four cellulose acetate strips, and electrophoresis was carried out as already described. The ^{125}I peak released from the hypertensive plasma was eluted with 4 ml of water and lyophilized. The residue was dissolved in 1 ml of water and identified by performing two tests: *a)* An aliquot of 0.4 ml plus 15 μg of unlabeled carrier angiotensin II was spotted on ion exchange paper and was developed by chromatography, as described herein. Unlabeled angiotensin II was stained with ninhydrin [19] and radioactivity determined by counting 1-cm strips. *b)* Another aliquot (0.5 ml) was used as tracer angiotensin II in an antibody-binding test [8]. The separation of the free angiotensin II from antibody-bound angiotensin II was performed with 1 ml of 1% activated charcoal and 0.1% Dextran in 5 mM phosphate buffer (pH 7.4).

Results

Each result was repeated at least three times, except for the chromatographic analysis of plasma from individual subjects, which was performed only once.

Activity of "transferangiotensin". "Transferangiotensin" activity in 14 patients with essential hypertension ranged from 430 to 2,800 U/ml (Tables 2 and 3). Pooled plasma from patients with essential hypertension had 1,010 U/ml (13 subjects). The level of "transferangiotensin" activity in pooled plasma from two patients with renal-vascular or renal parenchymal disease was 670 and 490 U/ml, respectively. "Transferangiotensin" appears to be present in normal plasma, but at lower levels of activity, from 105 to 410 U/ml (Table 1). Pooled plasma from six normal subjects had an activity of 360 U/ml.

No "transferangiotensin" activity was detected in extracts of rabbit liver homogenates.

Binding of angiotensin II. Angiotensin II binds to arterial wall (Fig. 1). Unlabeled angiotensin II com-

Table 1. "Transferangiotensin" activity in control plasma[a]

Subject No.	Transfer angiotensin activity U/ml
1	410
2	360
3	345
4	290
5	216
6	105

[a] Each value is the mean of three determinations.

Table 2. Clinical data

Patient No.	Duration of disease yr	Age yr	Blood pressure mmHg, systolic/ diastolic	Creatinine clearance ml/min/ 1.73 m^2	Optic fundus grade
Essential hypertension					
7	5	39	160/100	106	II
8	4	45	170/110	125	II
9	9	54	180/110	102	II-III
10	3	41	170/100	117	II
11	6	47	190/110	131	II-III
12	5	46	190/120	99	II-III
13	6	48	170/110	110	II
14	7	50	170/100	114	II
15	4	47	190/120	132	II-III
16	6	56	200/130	116	II-III
17	8	45	160/100	129	II
18	6	45	180/120	101	II
19	7	48	170/110	134	II
20	4	47	190/120	116	II
Renal vascular hypertension					
21	1	28	240/140	96	III
22	1.5	23	210/150	103	III
Renal parenchymal disease					
23	9	35	190/130	51	III-IV
24	8	29	180/120	26	III
25	6	32	190/120	62	III-IV

petes with [125]I- angiotensin II for this binding, but angiotensin I does not. Fifty μg of protein from arterial wall extract binds an average of 13% of radioactive angiotensin II (22 experiments), with a range from 10 to 42%. There was no correlation between feeding or housing conditions of the animals and binding efficiency.

Plasma, itself, bound a mean of 8% of the ^{125}I-angiotensin II added, ranging from 7 to 12%. Competition with labeled angiotensin II by unlabeled polypeptide was found (Fig. 1).

Table 3. Analytical data[a]

Patient No.	Renin activity ng/ml/hr of angiotensin I	"Transfer angiotensin" U/ml	Urinary aldosterone μg/24 hr	Urinary V.M.A. mg/24 hr	Urinary 17-keto-steroids mg/24 hr	Urinary 17-hydroxy-steroids mg/24 hr
		Essential hypertension				
7	0.05	2,800	11	3.0	6.1	5.4
8	0.8	2,360	12	2.1	5.5	5.9
9	1.0	1,400	9	3.2	6.7	5.6
10	0.4	1,370	13	2.9	7.1	4.9
11	1.2	1,180	8	3.1	6.7	5.0
12	0.7	1,100	14	2.3	8.1	6.2
13	0.3	1,014	11	1.8	5.2	6.0
14	0.3	980	9	3.3	6.4	6.6
15	0.1	960	12	2.6	5.9	6.9
16	1.2	910	14	2.4	5.0	5.4
17	0.4	780	15	1.9	5.6	6.1
18	0.6	710	10	3.4	6.0	7.3
19	0.8	650	9	2.1	6.2	5.9
20	0.9	430	16	3.0	6.1	8.0
		Renal vascular hypertension				
21	39	790	17	3.6	7.6	5.6
22	61	510	15	3.1	7.3	8.1
		Renal parenchymal disease				
23	14	305	16	3.4	5.9	7.7
24	21	560	13	2.9	7.3	6.3
25	16	465	11	3.2	6.5	5.1

[a] Each value is the mean of two determinations performed on samples collected while patients were not receiving medication.

Physical-chemical properties. 1. Dialysis, pronase digestion, heating and solvent extraction. "Transfer-angiotensin" activity was partly destroyed by dialysis, heating at 100°C, extraction with solvents and pronase

Fig. 1. *Angiotensin II binding by plasma and arterial wall extract.* The experiment was performed as described in Methods. Arterial wall (●), plasma (▲).

digestion (Table 4). The activity that was lost by dialysis was not restored by adding concentrated dialysate to the incubation mixture. No residual pronase activity was found after its reaction was stopped.

2. Effect of EDTA and ions. Increased "transfer-angiotensin" activity (18 to 36%) was achieved by adding EDTA to the incubation mixtures. Calcium also enhanced "transferangiotensin" activity (24 to 43%). An effect of sodium or potassium salts was not observed.

3. Optimum pH. The optimum pH for "transfer-angiotensin" activity was 7.5, but a second optimum was also noticeable (Fig. 2).

Characterization. 1. Fractionation of plasma angiotensin binders. Elution profiles of plasma binders and labeled angiotensin II from Sephadex G-25 columns showed two peaks of radioactivity. The first peak migrated with the void volume, while the second corresponded to free angiotensin II. No difference in the peak positions was found when arterial wall extract was added.

Elution profiles from Sephadex G-200 columns showed significant differences, depending on the contents of the incubation mixtures. When hypertensive plasma and arterial wall extract were present, three
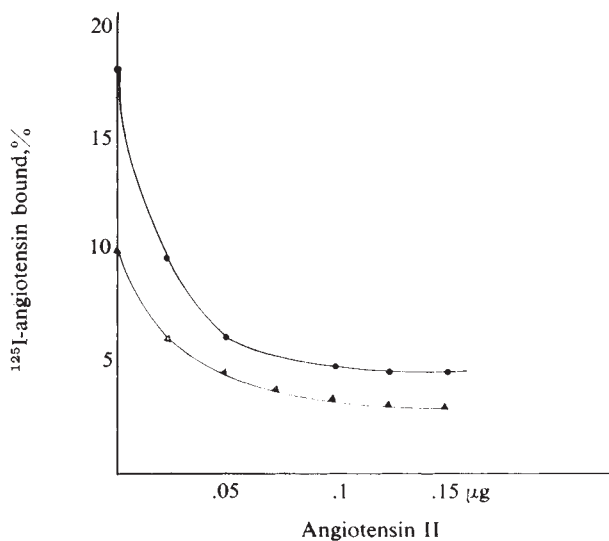
Table 4. Physical chemical properties of plasma angiotensin-binders[a]

Specimen	"Transfer-angiotensin" activity	Experimental range
Original plasma	100	100
After dialysis	32.3	−15.3–41
Dialysis plus 0.2 ml of dialysate	25.5	−11–48
Pronase digestion	64.5	12–71
Heating 100° × 5 min	62.6	2–63
Residue (0.2 ml) from ether extraction	8.4	0.3–13

[a] Essential hypertensive pooled plasma was divided into four 1-ml aliquots. The first aliquot was dialyzed as described in Methods, the dialysate evaporated to dryness and the residue dissolved in 0.5 ml. Assays of transferangiotensin activity were run before and after dialysis and after the retentate was combined with increasing amounts of the concentrated dialysate. The second aliquot was digested with Pronase as described in Methods. The third aliquot was extracted with ether and the residue dissolved in 1 ml of buffer. The fourth aliquot was heated to 100°C. Assays of transferangiotensin activity were performed before and after each procedure, and the results expressed as percentages of the starting activity.
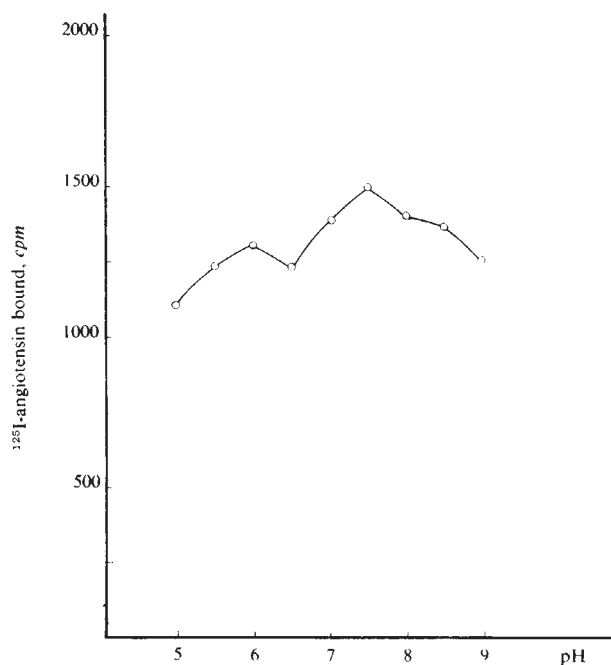
peaks of radioactivity were found (Fig. 3). The most prominent peak corresponded to the void volume. The second had an approximate mol wt of 50,000, while the third migrated with free angiotensin II. When plasma was omitted from the incubation mixture, only a small peak of radioactivity appeared in the void volume position. The profile of the plasma alone was similar to that of plasma plus arterial wall extract, but the void volume peak was smaller.

When Sepharose 6-B was used, three peaks of radioactivity appeared. The major peak migrated with the void volume, while the middle peak had an approximate mol wt of 700,000 and the smallest migrated with the combined 50,000 mol wt and free angiotensin II position. Chromatography on Sepharose 6-B of an incubation mixture containing arterial wall extract alone showed three peaks of radioactivity. The first peak migrated with the void volume, the second had a higher exclusion volume and the third migrated with the combined 50,000 mol wt and free angiotensin II position (Fig. 4).

Bound radioactivity was diminished 80% when the incubation period was omitted, but no major change in chromatographic profile was detected.

A similar profile was found when hypertensive plasma was replaced by normal plasma, although the amount of angiotensin II bound was much lower.

In brief, incubation of radioactive plasma resulted in the appearance of more radioactivity in lower mol wt fractions than when it was incubated with arterial wall extract alone. There was evidence for the disappearance of the second peak of the arterial extract on
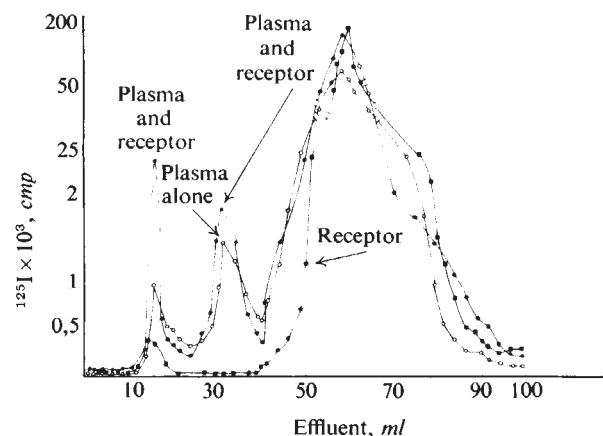

Fig. 2. *Optimum pH.* Hypertensive plasma was incubated with radioactive angiotensin, arterial wall extract, and 10 μmoles of Tris-HCl at different pH, as described in Methods.

Fig. 3. *Typical binding profiles on Sephadex G-200 columns.* Incubation, equilibration and elution were carried out as described in Methods. The elution profile of ^{125}I-angiotensin II incubated with plasma plus the arterial wall extract (●), with plasma alone (○) and with arterial wall extract alone (■) are shown.
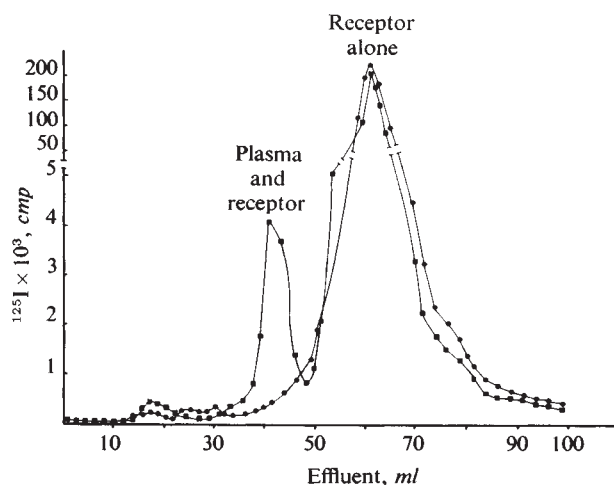
Fig. 4. *Typical angiotensin II binding profiles on Sepharose 6-B columns.* The experimental procedure was performed as described in Methods. The elution profile of ^{125}I-angiotensin II when incubated with plasma plus arterial wall extract (■) is compared with that from angiotensin plus arterial wall extract (●) alone.

Sepharose 6-B chromatography, when plasma was added to the incubation mixture (Fig. 4).

2. Characterization of "transferangiotensin" activity. Transferangiotensin activity elutes from Sepharose 6-B at a position corresponding to 700,000 mol wt. No transferangiotensin activity was found in the position of the 50,000 mol wt and smaller component (Fig. 5). Between 25 and 46% of the transferangiotensin activity applied to the column was lost.

3. Electrophoretic localization. A concentrate of the 700,000 mol wt component eluted from Sepharose 6-B migrated in advance of albumin on electro-
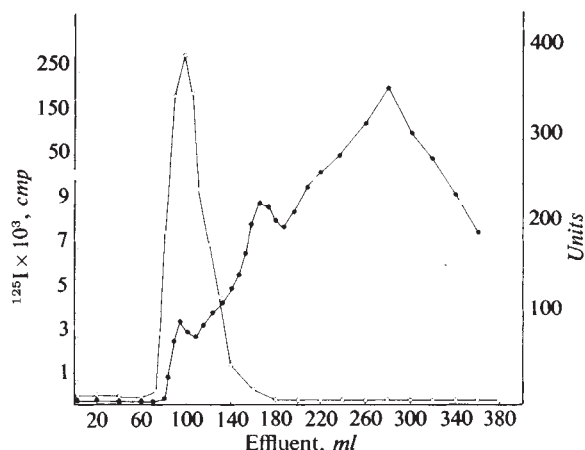

Fig. 5. *Identification of "transferangiotensin" activity.* Experimental conditions were performed as described in Methods. The plasma component possessing transferangiotensin activity (○) is compared with angiotensin binding activity (●).
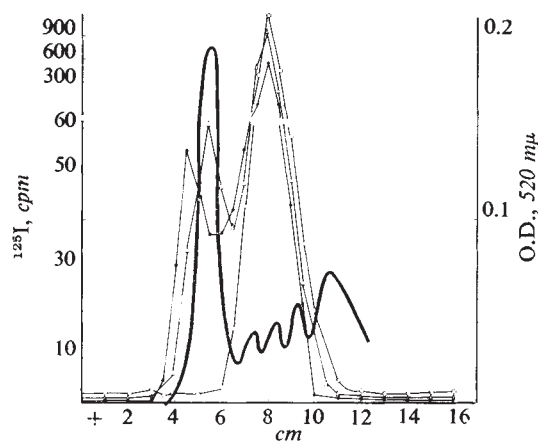

Fig. 6. *Electrophoretic localization.* The heavy (●) and light (△) plasma angiotensin II binders were separated by electrophoresis, and radioactivity migration compared with that of ^{125}I-angiotensin II (○) and normal serum (—) stained with Ponceau red.

phoresis. The 50,000 mol wt component migrated with albumin (Fig. 6).

4. Identification of the compounds bound by transferangiotensin. The radioactive compound bound by the 700,000 mol wt component had an electrophoretic migration corresponding to free angiotensin II. It co-chromatographed with angiotensin II, and it was displaced from anti-angiotensin II antibody by unlabeled angiotensin II.

Discussion

The data presented in this report relate to two phenomena: the binding of angiotensin II by human plasma macromolecules, and the ability of one of these binders to enhance the binding of angiotensin by macromolecules in an extract of rabbit aorta. The

Table 5. Comparative binding activity of sepharose 6-B fractions[a]

Source	Radioactivity, %	
	Plasma plus arterial wall extract	Arterial wall extract
First peak	0.5	0.2
Second peak	0	0.4
Third peak	1.6	0
Fourth peak	97.9	99.4

[a] Equilibration, elution and sampling were performed as described in Methods. The total recovered ^{125}I (in counts per minute, cpm) is designated 100. The percentage of cpm in the different peaks of the incubate containing plasma plus arterial wall extract is compared to that of the arterial wall extract alone.

plasma binders have apparent mol wts of 50,000 and 700,000. It was the heavier one that served to increase binding by arterial wall extracts.

The heavy factor which potentiates binding by the arterial wall extract is thermolabile, and it is probably protein in nature. It also migrates in advance of albumin during electrophoresis. We propose the name "transferangiotensin" in order to suggest a mechanism of action which involves the transfer of angiotensin from solution in plasma to receptors in target tissue. Transferangiotensin may also interact with a component(s) of the arterial wall, since there was disappearance of one binding peak in arterial wall extract when plasma was added.

The apparent discrepancy between the effects of calcium and the EDTA may indicate the need of calcium ions and the simultaneous presence in the assay system of a metal that inhibits the activity of transferangiotensin. The loss of transferangiotensin activity after dialysis, followed by failure to recover the loss by addition of dialysate, can be interpreted as the irreversible loss of a cofactor. There was less loss of angiotensin activity after gel filtration than after dialysis.

The differences in results of heating and pronase digestion may perhaps be explained by the protection afforded by concentrated protein solutions. Apparently, loss of activity by the digestion of angiotensin cannot be implicated because the enzymatic reaction was stopped and no residual pronase activity was found.

Angiotensin II bound by transferangiotensin does not seem to be transformed, because the released product chromatographs and competes for antibody with angiotensin II.

Lack of transferangiotensin activity in a binding fraction with a mol wt of 50,000 that migrates with albumin could be interpreted either as binding by albumin itself, or by a subunit from the heavier component.

The data do not bear on the kinetics of transferangiotensin activity, its specificity for other molecules such as norepinephrine or the mechanism of its interaction with a receptor. However, its increased activity in plasma from patients with benign essential hypertension does suggest that it may play a possible role in the pathogenesis of hypertension. The wide range of transferangiotensin activity (430 to 2,800 U/ml) among patients with essential hypertension also implies that the pathogenesis of this disease may well be multifaceted.

The relationship of transferangiotensin (or the 50,000 mol wt plasma binder as well) to those described by others is unknown [4–7], as is the relationship of

our arterial extract to the "receptors" that have been prepared from rabbit aorta by Devynck et al [20].

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