THE ASSAY OF HYPERTENSIN FROM THE ARTERIAL BLOOD OF NORMOTENSIVE AND HYPERTENSIVE HUMAN BEINGS

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Human essential hypertension, as well as experimental renal hypertension, has been considered by many investigators to be the result of the release into the circulation of a pressor substance elaborated by the kidneys. The confirmation of this theory would appear to require the isolation and identification of a pressor material from the blood of human beings with hypertension which is of renal origin and is present in sufficient quantities to be the cause of the elevation of the blood pressure. Prominent among the pressor substances suspected of being the chemical mediator of hypertension is the powerful vasoconstrictor substance, hypertensin, which is produced by the action of the renal enzyme renin upon its plasma substrate α-2-globulin. Hypertensin has been isolated from the circulating blood of dogs with benign and malignant experimental renal hypertension by use of an artificial kidney. The methods employed in these experiments were not adequate to prove or disprove that hypertensin was the effective agent in elevating the blood pressure of the hypertensive animals (1, 2). A direct method for the isolation and assay of hypertensin from blood was therefore developed (3). This study is concerned with the application of this method to the isolation and assay of hypertensin in the blood of human beings with normal blood pressures and those with benign and malignant hypertension.

Methods

Collection of Blood Samples.—The patient's right femoral area was shaved. 30 to 60 minutes prior to the procedure, 0.1 to 0.2 gm. of short-acting barbiturate was administered by mouth unless contraindicated. The area of skin previously shaved was prepared in the usual manner with merthiolate and draped with sterile towels. The skin and subcutaneous tissues down to and around the femoral artery were infiltrated with 1 per cent procaine. A Cournand 18-gauge arterial puncture needle was introduced through the skin and subcutaneous tissues 2 to 3 cm. below the right inguinal ligament and advanced in a cephalodorsal direction into the femoral artery. The stylet was withdrawn and the needle quickly connected to a short polyethylene catheter with an internal diameter of 0.047 inch, which had been inserted through a rubber stopper into an Erlenmeyer flask containing 1 liter of 95 per cent ethanol. A partial vacuum was maintained in the flask so that the blood ran rapidly from the artery

into the alcohol, minimizing any enzymatic action of renin on its substrate α-2-globulin to form hypertensin as well as preventing the destruction of preexisting hypertensin by hypertensinase present in the blood. The Erlenmeyer flask containing the ethanol was first weighed on a torsion balance and then 265 gm. of arterial blood representing 250 ml. was collected for each sample. It took on the average about 4 to 6 minutes to collect each sample. At the end of the collection, the needle was quickly withdrawn from the artery and firm pressure was maintained over the point of puncture for 5 to 10 minutes. No hematomata or other untoward effects were noted in any case. During the collection of the blood, the Erlenmeyer flask was alternately agitated and weighed so that there was an immediate mixture of the alcohol and blood, thereby rapidly inactivating and precipitating the enzymes along with most of the protein.

After thorough mixing, the blood and alcohol mixture was filtered on a Buchner funnel. The precipitated proteins were washed on the funnel with 1 liter of 80 per cent alcohol. The alcoholic filtrate and the washings were combined and stored in deepfreeze until chemically processed.

Purification of Extracts.—The alcoholic filtrates were submitted to a chemical procedure which has recently been described in detail (3). Briefly the alcoholic solution was acidified, concentrated by vacuum evaporation, the residual denatured protein removed by centrifugation, and the concentrate extracted with ether to remove lipids. The solution was then further evaporated to the point of saturation with sodium chloride. The hypertensin was extracted into butanol from the acidified saturated salt solution and then was adsorbed from it onto a column of alumina by passage of the butanol extract through it. Impurities were eliminated by washing the column with 85 per cent alcohol. The active material in greatly purified form was then removed from the column by elution with 50 per cent alcohol. This alcoholic solution was then concentrated by vacuum evaporation to a final volume of 1 ml. The pH was adjusted to neutrality and the extract stored in the frozen state until assayed.

The blood samples were processed in groups of 4, all operations being conducted in parallel. One control sample was included in each group and consisted of a sample of dog or human blood to which a known amount of hypertensin had been added. This procedure permitted close control of the percentage of hypertensin recovered in all experiments.

Previously reported experiments showed that the method recovered between 40 per cent and 65 per cent of hypertensin added to individual blood samples. The average recovery was 50 per cent. The same percentage of recovery was obtained in control samples throughout the present set of experiments. No difference in the recovery of hypertensin was noted between human and dog blood.

Extracts of blood prepared in this manner were found to contain 0.22 m.eq. of Na, 0.0011 m.eq. of K, 2.71 mg. of total N_2, and 28.5 mg. of total solids as computed for a single preparation from 250 ml. of blood.

Inactivation Experiments.—The effect of trypsin on the pressor activity of extracts was determined in all cases in which sufficient active material was available after assay. The details of the procedure used have been previously described (3).

Assay Methods.—The pressor effect of the preparation was assayed by intravenous injection into rats and compared to that produced by an injection of a standardized hypertensin solution. All assays are in terms of Goldblatt units. A detailed description of the assay methods has been previously published (3).

EXPERIMENTAL

Arterial blood for hypertensin assay was obtained from three groups of patients: (*a*) normotensive controls; (*b*) hypertensive cardiovascular disease,

benign phase; and (*c*) hypertensive cardiovascular disease, malignant phase.

Normotensive Controls.—These patients were in the hospital for conditions unrelated to cardiovascular disease and had no history of hypertension or renal disease. Their average blood pressures were well below 150 mm. of mercury systolic and 100 mm. of mercury diastolic. Their eyegrounds were normal, the heart was normal in size by physical examination and x-ray, and there was

TABLE I

Assay of Hypertensin in the Blood of Patients with Normal Blood Pressure

Patient	Age	Sex	Systolic blood pressure	Diastolic blood pressure	Hypertensin found
			mm. of Hg	*mm. of Hg*	*units per liter*
V. D. D.	26	M	120	65	0.00
L. G. B.	64	M	120	80	0.00
J. G.	23	M	118	74	0.00
D. A. A.	20	M	130	80	0.00
M. F. B.	24	M	124	68	0.00
M. F. H.	32	M	114	60	0.00
R. R. R.	26	M	114	90	0.00
J. A.	25	M	130	90	0.00
S. F. S.	28	M	120	60	0.00
O. D. W.	26	M	130	74	0.00
R. C. P.	26	M	130	80	0.01
W. L. F.	37	M	120	70	0.02
J. G. B.	32	M	119	65	0.02
J. W. H.	53	M	115	80	0.02
W. T. W.	45	M	119	85	0.02
T. G. H.	33	M	116	80	0.02
G. H.	30	M	125	80	0.02
H. W. E.	27	M	135	84	0.02
P. J. L.	41	M	118	80	0.02
P. C. F.	41	M	125	75	0.02
J. J.	36	M	126	79	0.03
S. W.	31	M	120	82	0.03
C. C. G.	29	M	124	70	0.05
F. B.	28	M	108	78	0.05

no evidence of arteriosclerosis. Renal function was normal as indicated by routine urinalyses.

Assay of the extracts of the blood samples from this group, which consisted of 24 patients, yielded values which ranged from 0.00 to 0.05 units per liter of blood. The average value obtained was 0.014 unit per liter. The results are shown in Table I.

Hypertensive Cardiovascular Disease, Benign Phase.—These patients, for the most part, were admitted to the hospital because of their hypertensive

cardiovascular disease. However, in a few cases, hypertension was discovered in patients who had been admitted for some other reason. The average blood pressures of these individuals were above 145 mm. of mercury systolic and 100 mm. of mercury diastolic. Their cardiac status varied from that of individuals with no evidence of abnormality by history, physical examination, EKG, and x-ray to that of patients who had been admitted with varying degrees of congestive heart failure and cardiac enlargement. Ophthalmoscopic examinations revealed no more than grade II hypertensive retinopathy. Most

TABLE II

Assay of Hypertensin in the Blood of Patients with Benign Essential Hypertension

Patient	Age	Sex	Systolic blood pressure	Diastolic blood pressure	Hypertensin found
			mm. of Hg	*mm. of Hg*	*units per liter*
A. J. L.	36	M	204	135	0.00
A. N.	56	M	173	111	0.00
A. P.	62	M	202	103	0.00
E. E. A.	59	M	187	112	0.00
A. J. L.	36	M	204	135	0.02
F. P. T.	55	M	210	142	0.02
C. A.	57	M	164	105	0.02
F. A. B.	62	M	165	116	0.02
R. L. W.	43	M	201	134	0.02
H. P.	55	M	198	130	0.03
A. F. B.	44	M	146	100	0.03
M. S.	51	M	212	129	0.03
H. E. G.	59	M	156	120	0.04
L. A. G.	63	M	195	115	0.04
F. S.	37	M	170	106	0.05
A. F. B.	44	M	146	100	0.05
C. D. K.	57	M	174	106	0.06
C. M.	54	M	206	125	0.06

of these patients showed only grade I changes. Renal function was normal in most instances as indicated by routine urinalyses and the usual clinical renal function studies. In a few of these patients, there was minimal impairment of renal function as indicated by slight albuminuria, slight limitation of the concentrating ability of the kidneys as well as minimal depressions of urea clearance and P.S.P. excretion.

The concentration of hypertensin found in the blood of this group, consisting of 18 patients, ranged between 0.00 and 0.06 units per liter. The average concentration was 0.028 unit per liter. The results are shown in Table II.

Hypertensive Cardiovascular Disease, Malignant Phase.—These patients had been admitted to the hospital because of the severity or rapid progression of

their hypertensive cardiovascular disease. Their mean blood pressures ranged well over 150 mm. of mercury systolic and 100 mm. of mercury diastolic. Ophthalmoscopic examination revealed grade III to grade IV hypertensive retinopathy. These patients all showed varying degrees of cardiac enlargement, and many of them had some degree of congestive failure at some time during their hospital course. Renal function was markedly impaired in these patients as indicated by albuminuria, hematuria, oliguria, a low fixed specific gravity of the urine, and increasing nitrogen retention. There was no history of primary renal disease in any case.

Most of the patients in this group have since died, and of those who were autopsied, everyone showed varying degrees of necrotizing arteriolitis, consistent with the clinical diagnosis of the malignant phase of essential hypertension. The one patient with benign hypertension, who died and was autopsied, had no evidence of necrotizing arteriolitis. The cause of death in this case was myocardial infarction with mural thrombosis and multiple emboli.

TABLE III

Assay of Hypertensin in the Blood of Patients with Malignant Essential Hypertension

Patient	Age	Sex	Systolic blood pressure	Diastolic blood pressure	Hypertensin found
			mm. of Hg	*mm. of Hg*	*units per liter*
C. S.	32	M	227	150	0.08
L. A. S.	33	M	202	146	0.10
F. J. W.	32	M	205	149	0.11
R. J. H.	51	M	225	133	0.11
A. H. A.	62	M	220	120	0.31
					0.24
J. A. G.	38	M	206	134	0.24
G. T.	54	M	203	148	0.24
M. J.	48	F	300	160	0.40
L. G.	47	F	289	159	0.40
L. T.	42	F	247	149	0.43

Concentrations of hypertensin ranging between 0.08 and 0.43 units per liter were found in this group of 10 patients. The average value was 0.27 unit per liter. The results are tabulated in Table III.

DISCUSSION

The pressor material obtained from arterial blood in this study was identified as hypertensin by the following means. The active pressor material from 10 patients with malignant hypertension, 6 with benign hypertension, and 2 normotensives was tested for susceptibility to destruction by trypsin. In all cases tested, the activity was completely destroyed, thus eliminating all other

known pressor materials with the exception of ACTH and pitressin. These two possibilities were excluded by comparison of the contour of the pressor response obtained, as well as by failure to recover these substances by the method used when reasonable amounts of them were added to blood samples (3).

The average concentration of hypertensin found in the blood of patients with malignant hypertension was approximately 20 times the average concentration found in the blood of the patients serving as controls. All values obtained in the malignantly hypertensive group are greater than the largest normal value found. This is, therefore, a significant difference and hence the inference seems justified that hypertensin contributes in some degree to the maintenance of elevated blood pressure in patients with malignant hypertension. This finding is in keeping with the concept of the renal origin of hypertension set forth by Goldblatt and his coworkers (4).

The average concentration of hypertensin found in the blood of patients with benign hypertension was twice that found in the "normal" group. The degree of overlapping is marked but the difference is statistically significant when subjected to the *t*-test ($P = 0.03$ at the 5 per cent level). It should be noted, however, that the concentration of hypertensin found in both the benign and normal groups is very low. Therefore, it would be unwise to draw specific conclusions as to the participation of the renin-hypertensin mechanism in causing the elevation of blood pressure in benign essential hypertension. It is possible that during some portions of each day, the patients may secrete moderate amounts of this material which could not be detected by a single sampling; for the method as thus far developed does not lend itself to multiple determinations at intervals throughout the day. It would also seem possible that after prolonged and continuous constriction of the arterioles, due to hypertensin, that only minimal amounts of it would be needed to maintain an elevated blood pressure.

Experiments designed to determine whether prolonged infusions of hypertensin over weeks and months will maintain a sustained rise in blood pressure, even though declining amounts of hypertensin are injected, are planned for the future. This experiment must be done to clarify the point. It is certainly important to ascertain whether the elevation of blood pressure occurring in benign essential hypertension is of renal origin, and is due to the renin-hypertensin mechanism.

SUMMARY

Hypertensin has been assayed in the blood of patients with normal blood pressure and in those with essential hypertension in both the benign and malignant phases.

250 ml. samples of arterial blood were obtained, chemically purified, and

concentrated to a volume of 1 ml. These extracts were then assayed in anesthetized rats.

The concentrations of hypertensin in the blood of patients with the malignant phase of essential hypertension were found to be greatly increased. The concentrations of hypertensin found in patients with benign hypertension had a moderate degree of overlapping with those found in the normotensive group, but the mean concentration of hypertensin in the former group was twice that of the controls. Although these results are statistically significant, the amounts of hypertensin recovered in the benign group are so small that no conclusions can be drawn as to its effectiveness in producing vasoconstriction in these patients.

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