Plasma Protein Changes in Primary Hypertension in Humans and Rats

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SUMMARY To determine whether plasma protein changes may be associated with primary hypertension, we analyzed plasma proteins from essential hypertensive (EHT) patients and genetically hypertensive rats using two-dimensional electrophoresis. An additional plasma protein, having a molecular weight of 13,000 daltons and an isoelectric point of 4.5, was found in 82% of the patients with borderline or moderate hypertension (n = 29) and in all permanently hypertensive patients (n = 12). This protein was detected in 36% of normotensive (NT) subjects (n = 50). In the latter, the influence of family history, sex, and secondary hypertension were studied. Plasma proteins were also studied in spontaneously hypertensive rats (SHR). In all plasma from young male (n = 10) and female (n = 6) SHR, two additional proteins (molecular weight = 16,000 daltons, pH = 4.7 and 5.1) were detectable. These plasma proteins were not detectable in male Wistar Kyoto rats (WKY) and in 50% of female WKY, and their frequency was 10% (n = 10) and 0% (n = 3) in normal male WKY and in male WKY rendered hypertensive by methylprednisolone, respectively. We conclude that these alterations of plasma proteins may be considered a biochemical feature of primary hypertension. (Hypertension 5: 128-134, 1983)

KEY WORDS • essential hypertension • spontaneous hypertension • plasma proteins • two-dimensional electrophoresis

RECENTLY, several different groups of investigators examined cellular features characteristic of primary hypertension. Most of these studies were concerned with blood cells that are easily accessible to clinical investigations. Changes in transmembrane fluxes of Na\(^+\), K\(^+\), and Li\(^+\),\(^{1,16}\) alterations in calcium binding to inner sites of plasma membranes,\(^{17,18}\) and differences in phosphoinositide content and metabolism\(^{19-21}\) have been described in erythrocytes from both essential hypertensive (EHT) humans and genetically spontaneous hypertensive rats (SHR). Other circulating cells were also found to have altered properties in EHT including increased intracellular Na\(^+\) content in lymphocytes\(^{22-24}\) and altered serotonin and noradrenaline handling in platelets.\(^{25-27}\)

The significance of these changes, and particularly their possible pathogenic interest, is poorly understood. However, as some of them appear genetically transmitted and characteristic of families with a high propensity to develop EHT,\(^{13}\) it has been suggested that they may be of interest for the identification of genetically hypertension-prone subjects.

In addition, some recent investigation have indicated that some biochemical changes also characteristic of primary hypertension could equally affect plasma proteins both in rats\(^{28}\) and humans.\(^{29}\) In the latter, Nardi et al.\(^{29}\) have shown that plasma of EHT subjects differs from that of normotensives (NT) by the presence of an excess of a protein with an apparent molecular weight (MW) of 14,500 daltons. The function of this protein and its degree of significance in EHT remain unknown.

The development of a new two-dimensional electrophoresis method, which markedly improves the analysis of a complex mixture of proteins,\(^{30}\) led us to reinvestigate the possibility that plasma proteins can be altered in human and rat hypertension. Additional acidic plasma proteins were indeed demonstrated in subjects with EHT and in the SHR. The present study includes a further characterization of plasma proteins in primary hypertension and a description of the frequency of abnormal protein patterns in human EHT and their relatives.
Material and Methods

Subjects

The analysis was performed on plasma samples from 106 Caucasian subjects. All of them had a free dietary Na\(^+\) intake. Venous blood was sampled between 9:30 and 10:30 a.m. and collected in chilled heparinized tubes. Blood pressure was recorded with subjects in the sitting position using a mercury manometer according to the recommendations of the World Health Organization (WHO). Subjects were divided into several groups as described below.

Essential Hypertensive Patients

In 41 hypertensive patients (26 males and 15 females, aged 13–84 years, mean 41.4 ± 2.7 years), the diagnosis of EHT was established after careful clinical investigation, including intravenous pyelogram. None of the patients had plasma creatinine levels above 100 \(\mu\)mole/liter or marked alterations (hemorrhage, papilledema) of the retina. Electrocardiogram (ECG) changes suggestive of left ventricular hypertrophy and/or coronary insufficiency were present in 60% of the patients. Among these patients, only three had received antihypertensive treatment before blood sampling; all other patients were untreated. Daily urinary Na\(^+\) excretion varied between 100 and 205 mmole/24 hours. Plasma renin activity, determined by the method of Haber et al.,\(^3\) ranged between 0.4 and 2.3 ng/ml/hour (normal values: 1.5 ± 0.5 ng/ml/hour). These patients were divided into two subgroups according to the severity of the hypertension.

EHT Group 1. This group included 29 EHT patients (18 males and 11 females, aged 13–47 years, mean 32.9 ± 2.0 years) with borderline or moderate hypertension. Patients with borderline hypertension had a transient elevation in blood pressure (above 160/95 mm Hg on others). Some patients had a moderate permanent hypertension not exceeding 15 mm Hg above the normal values. The average blood pressure of the group was 160.5 ± 3.1/95.6 ± 10 mm Hg.

EHT Group 2. This group included 12 EHT patients (eight men and four women, aged 52–84 years, mean 62.8 ± 3.3 years). In these patients, blood pressure was permanently elevated: 183.8 ± 4.8/100.1 ± 3.2 mm Hg.

Normotensive Subjects

This group comprised 50 healthy volunteers (19 males and 31 females, aged 11–64 years, mean 29.9 ± 1.8 years). The average blood pressure was 122.5 ± 3.5/77.1 ± 1.7 mm Hg. The normotensive (NT) subjects could be also divided into two subgroups according to the familial blood pressure of these subjects.

Normotensive Group with No Family History of Hypertension. This group included 23 NT subjects (10 men and 13 women, aged 22–44 years, mean 30.3 ± 1.2) who were carefully selected on the basis of two criteria: 1) systolic and diastolic blood pressures below 140 and 85 mm Hg, respectively; and 2) blood pressures of relatives were never found to be above 160/95 mm Hg. In addition, no case of hypertension was reported among their second-degree relatives. The average blood pressure of these NT was 120.3 ± 2.2/78.3 ± 1.5 mm Hg.

Normotensive Group with a Family History of Hypertension. This group included 27 NT subjects (9 males and 18 females, aged 11–64 years, mean 29.1 ± 2.3) who had at least one parent with high blood pressure. The average blood pressure of these NT was 125.3 ± 4.0/76.0 ± 1.8 mm Hg.

Patients with Chronic Renal Failure

We also studied 15 patients with advanced chronic renal insufficiency, 12 of whom were undergoing intermittent hemodialysis. Five patients (mean age 43.6 ± 2.7 years, four males, one female) were slightly hypertensive (blood pressures (BP) between 175/105 and 180/85 mm Hg). The elevation of their blood pressure was considered to be at least in part a consequence of the underlying bilateral renal disease. Ten patients (mean age 38.6 ± 2.2 years, 6 males, 4 females) were NT when blood was sampled (BP between 110/70 and 130/85 mm Hg).

Rats

Okamoto SHR and their NT controls, Wistar Kyoto rats (WKY), derived from the National Institutes of Health (NIH) stock, were supplied by IFFA-CREDO (France). SHR and WKY rats were studied at 3–4 weeks of age before establishment of significant high blood pressure, in order to eliminate the influence of the elevation of blood pressure per se. The average systolic arterial pressure (recorded by tail sphygmomanometry) was 105 ± 4 and 95 ± 4 mm Hg (\(n = 10\)) for SHR and WKY, respectively.

Normal male WKY rats (150–200 g), supplied by IFFA CREDO, were rendered hypertensive by an intramuscular injection of 20 mg/kg of methylprednisolone according to Krakoff et al.\(^3\) and Elijovich and Krakoff.\(^4\) After 9 days, systolic arterial pressure of these glucocorticoid hypertensive rats was 149.9 ± 4.9 mm Hg (\(n = 19\)) as compared to 102.8 ± 3.7 for control rats (\(n = 12\)); blood sampling was performed after BP measurement.

In all groups, blood was collected by cardiac puncture on stunned rats and immediately transferred into chilled, heparinized tubes.

Treatment of Plasma

Blood was centrifuged for 10 minutes at 4°C at 1500 \(\times g\) immediately after collection. Plasma was immediately mixed with sodium dodecyl sulfate (SDS), Nonidet NP40, \(\beta\)-mercaptoethanol, and urea; final concentrations were 1%, 8%, 5%, and 9 M, respectively. The samples were analyzed on the same day or stored at \(-30°C\) until analysis. The time between bleeding and plasma treatment did not exceed 20 minutes.
Electrophoresis

Detergent-treated plasma samples were subjected to two-dimensional electrophoresis according to O'Farrell. In the first dimension the pH-gradient was formed using 2% (wt/vol) 3–10 phamalyte (Pharmacia Fine Chemical, Uppsala, Sweden) in 5% acrylamide gels. The electrofocusing was developed in 14.5 × 0.15 cm (i.d.) gels at 400 V overnight. The second dimension was run according to the discontinuous system of Laemmli. The slab acrylamide gels comprised a 13% running gel overlaid with 3% stacking gel. These gels (18 × 20 × 0.15 cm) were run under 11.5 mA until the tracking dye (bromophenol blue) reached the bottom of the gel. Proteins were revealed by staining with 0.25% Coomassie brilliant blue, 40% methanol, and 10% acetic acid solution for 5–6 hours at 22°C. Destaining was obtained at 4°C in the same solution free of Coomassie blue.

Reagents

Acrylamide and bis acrylamide (twice-crystalized) were obtained from Serva (Heidelberg, Germany); N,N,N',N'-tetra-methyl-ethylenediamine (TEMED), ammonium persulfate, urea, Nonidet NP40, and β-mercaptoethanol were purchased as reagent grade from Sigma Chemical Company (St. Louis, Missouri). Ampholytes were purchased from Pharmacia (Uppsala, Sweden). Other chemical products were obtained from local sources as reagent grade. Plasma renin activity was measured using a kit from the CEA (France).

Results

Subjects

Typical two-dimensional protein patterns of plasma from NT and EHT subjects are shown in figure 1. To visualize the less abundant proteins an overloading of plasma protein was necessary. Thus, some gels often showed vertical streaks near the site of the albumin’s isoelectric point. The 250 or so visible spots corresponded to proteins with an apparent molecular weight ranging between 10,000 and 120,000 daltons and an isoelectric point (pH) between 3 and about 8 pH units. Use of detergents throughout the whole procedure resulted in a dissociation of plasma protein conformation with conservation of primary structure. Some of these spots were established to correspond to known proteins; i.e., albumin (a), IgG light chains (b), IgG

![Figure 1](http://hyper.ahajournals.org/Downloaded)
heavy chains (c), arginine-rich lipoprotein (d), $\alpha_{\text{2}}$ macroglubin (e), fibrinogen $\gamma$-chain (f).

Reproducibility

The intraassay variability was tested on fresh denatured plasma and up to 2 months of conservation at $-30^\circ\text{C}$. The interassay variability was determined on different samples of plasma obtained from same subject. When these samples were examined in separate assays, each spot on one gel was seen unambiguously to correspond to a single spot on the other gel. However, small fluctuations in the position of the spots, of the order of magnitude of 2–3 mm, and small variations in the form of the albumin spot, were observed. Such variations were due to the fact that the isoelectric focusing gels were run at different times and, occasionally, there was a slight variation at the basic end of the isoelectric focusing gel. However, this variability was negligible as this study was focused on proteins with a molecular weight below 20,000 daltons. Within this range of molecular weight, only few spots were visualized and fluctuation of the albumin spot was of no consequence.

Comparison Between Normotensive and Essential Hypertensive Subjects

Highly sophisticated methods, such as the use of a computerized densitometer, may be necessary to compare samples. However, a clear difference was easily demonstrated in the present study consisting of an additional protein in the EHT plasma that was not present in the NT plasma (fig. 1). This protein had an apparent mol wt of 13,000 daltons and pH of 4.5; it has been named 4.5-protein, for convenience.

The overall frequency of the 4.5-protein as a function of mean blood pressure, without distinction of age, sex, or etiology, is represented in figure 2 (solid line). The 4.5-protein was detected in 28 of 38 subjects (74%) whose mean blood pressure exceeded 110 mm Hg, and in only 18 of 51 subjects (35%) with a mean blood pressure lower than 100 mm Hg. When only EHT patients were considered, the frequency of the 4.5-protein was never lower than 80% (dotted line) whatever the mean blood pressure, the sex, and the age of the patient.

The presence of the 4.5-protein in plasma was analyzed according to the etiology of hypertension, and the family history of high blood pressure in the NT (fig. 3). The frequency of 4.5-protein in EHT patients was 82% in Group 1 and 100% in Group 2. In EHT Group 1, the 4.5-protein frequency was 89% for men and 73% for women. The frequency of the 4.5-protein in both groups of EHT was significantly higher ($p < 0.001$, $X^2$ test) than other groups. In patients with chronic renal failure, the frequency of the 4.5-protein was low and not significantly different from the NT group, whatever their blood pressure. In NT subjects, the 4.5-protein was detectable with a frequency of 36%. The 4.5-protein frequency was 26% in NT with no known family history of hypertension and 44% in NT with a family history of hypertension.

In the NT group, the frequency of the 4.5-protein was analyzed according to the sex of the subjects. When NT with no known family history of hypertension were considered, the 4.5-protein could not be detected in the plasma of any of the 10 men, whereas it was observed in 46% of the plasma from women (fig. 4). However, a similar frequency of the 4.5-protein (44%) was observed in subjects from both sexes who had at least one relative with elevated blood pressure.

Rats

Typical two-dimensional protein patterns of WKY and SHR rat plasma are shown in figure 5. Approximately 250 spots of proteins of a mol wt between 10,000 and 120,000 daltons and pH between 3 and about 8 pH units were again observed. The intraassay and interassay reproducibilities were tested as for human plasma and were satisfactory.

The sole difference between WKY and SHR rat plasma, which was observed with very high reproducibility, was the presence of two proteins in SHR plasma that were virtually undetectable in WKY plasma. The frequency of detection of these proteins is repre-
FIGURE 3. The frequency of the detection of the 4.5-protein in plasma of EHT patients (left), of NT subjects (middle), and of uremic patients (right). Shaded bars indicate borderline EHT (left) and permanent EHT (right). Open bars indicate NT without known family EHT (left) and NT with known family history of EHT (right). Dotted bars indicate secondary hypertensive uremic patients (left) and normotensive uremic patients (right). The number of each group is given in the corresponding bar. ** = p < 0.001 vs others in X² test.

FIGURE 4. Frequency of the detection of the 4.5-protein in the NT group with and without known family history of EHT. The number of each group is given in the corresponding bar. ** = p < 0.01 vs genetics + males in X² test.

Figure 5. In plasma from male (n = 10) and female (n = 6) SHR, the frequency was 100% as compared to 0% for male WKY (n = 10) and 50% for female WKY (n = 6). In normal Wistar rat plasma, the frequency of these proteins was 10% (n = 10) and 0% (n = 3) in glucocorticoid hypertensive rats.

Discussion

Two-dimensional analysis coupling electrofocusing and electrophoresis according to O'Farrell is one of the most accurate methods for plasma protein studies. The two-dimensional gels obtained in the present study on human plasma proteins were similar to those previously reported by Anderson and Anderson. However, the proteins detected in the low mol wt range appeared less numerous in our investigation. This difference may have resulted from the use of different ampholytes and of different denaturing conditions. However, the use of potent denaturing agents was found, in agreement with O'Farrell, to be necessary to improve inter- and intraassay reproducibility on fresh- and deep-frozen plasma samples from NT and EHT.

The present analysis was restricted to plasma proteins with a mol wt of less than 45,000 daltons as they do not require the use of a computerized densitometer. The main finding of the present clinical study was the presence of a protein having a mol wt of 13,000 and a pH of 4.5 in the plasma of most EHT, and its apparent absence in the majority of NT. The threshold of sensitivity of the Coomassie detection for low mol wt proteins is about 0.1 μg by spot, which suggests that the absence of detection is not synonymous with a lack of this circulating protein and that lesser amounts of this protein may exist in NT subjects. When the 4.5-protein was detectable, the fact that the gels were loaded with 1 mg of plasma proteins allowed us to estimate that the 4.5-protein represents at least 0.01% of total plasma proteins.

Comparison of the frequency of the 4.5-protein in EHT, NT, and secondary hypertensives suggests that it may be a biochemical feature characteristic of the first group, but the significance is hampered by a substantial overlap between hypertensives and NT. The 4.5-protein was detected in offspring of hypertensive parents; as the protein is associated with primary hypertension in adults, one may wonder whether these children having the 4.5-protein are genetically hypertension-prone. Nevertheless, such a hypothesis, which stems from data obtained in male subjects, cannot be...
proved in females, where the protein can be detected even in the absence of hypertension in the parents.

The data reported in the present study is in agreement with the previous report of Nardi et al.\textsuperscript{29} These authors, using an unidimensional electrophoresis, suggested that a 14,500 dalton band of proteins was more important in plasma from EHT than in that of NT. No indication of the influence of sex and family history was given. The relationship between Nardi’s protein and ours remains to be demonstrated.

Given its low molecular weight, the presence of the 4.5-protein in urine appears possible. However, the application of two-dimensional electrophoresis to urine requires both concentration and desalting of the urine before analysis. These two additional steps could produce some protein alterations that could render detection of the 4.5-protein in the urine doubtful.

Densitometry is essential to determine the concentration of the 4.5-protein. Thus, we could not correlate it with the severity of hypertension or evaluate whether its concentration could be altered with antihypertensive drug treatment.

In SHR, two plasma proteins that were virtually undetectable in control WKY were constantly demonstrated. It is possible that these proteins were present in trace amounts in WKY, but computerized densitometric analysis would be needed to certify this. These two “additional” proteins in SHR represent, as described above for human plasma, at least 0.01% of the total plasma proteins. The chemical characteristics of these proteins (molecular weight and isoelectric points) are slightly different from those of EHT. The constancy of these two proteins in the plasma of SHR and their low incidence in WKY indicates that they may be considered a biochemical feature of genetic hypertension. This interpretation is strengthened by the absence of these proteins in normal Wistar rats and rats rendered hypertensive by glucocorticoid administration. If these two “additional” proteins are biochemical markers of genetic hypertension, they should be present in the plasma of other genetically hypertensive rat strains. This hypothesis is presently under investigation. In rats, we are investigating whether the frequency of detection of these two proteins in NT is greater in females than in males, as was found in humans.
The present investigation brings no information concerning the nature and the function of the 4.5-protein in EHT and the two additional proteins observed in SHR. As strong denaturing conditions were used for the treatment of plasma and during electrophoresis, further studies are necessary to know whether these proteins are in their native form or if they represent a moiety of circulating protein complexes.

The high frequency of the 4.5-protein observed in EHT patients and conversely its low frequency in secondary hypertensive patients and in NT, the high frequency of the two-additional proteins in SHR and their low frequency in WKY rats, led us to consider these plasma proteins as a biochemical feature of primary hypertension. Although the nature and role are entirely unknown, the measurement of the 4.5-protein could already have some clinical interest for the detection of children who may be genetically prone to the development of hypertension with age.

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References

17. Postnov YV, Orlov SN, Pokudin NI: Decrease in calcium binding by the red blood cell membrane in spontaneously hypertensive rats and in essential hypertension. Pfuiers Arch 379: 191, 1979
Plasma protein changes in primary hypertension in humans and rats.
J F Cloix, M A Devynck, J L Brentano and P Meyer

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