Defect in the Excretion of a Vasoactive Polypeptide Fraction
A Possible Genetic Marker of Primary Hypertension

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SUMMARY A polypeptide fraction isolated from the urine of normotensive subjects lowers the blood pressure (BP) in a rabbit bioassay (mean BP decrease 33.8% ± 0.6%, SEM). Patients with primary hypertension exhibit reduced or no activity (mean BP decrease 8.8% ± 1.2%). In contrast, patients with secondary forms of hypertension show activity like normotensives (mean BP decrease 33.4% ± 1.0%). The results of the bioassay in the two patient groups correlate well with the family incidence of hypertension (68% and 37% for primary and secondary hypertension respectively). Cases with borderline hypertension fall into two groups; a larger one with vasoactivity in the bioassay and lower family incidence of hypertension; and a smaller group reacting like patients with primary hypertension. Only the latter group may represent an initial stage of primary hypertension. In normotensive children and young men, an inactive fraction was found in 31% and 28% respectively. These inactive groups had twice the family incidence of hypertension compared to the groups with vasoactivity. These results suggest the existence of a possible genetic marker of primary hypertension and may offer the possibility to detect the disease before its manifestation. (Hypertension 3: 574-579, 1981)

KEY WORDS • primary hypertension • borderline hypertension • urinary vasoactive fraction • vasoactive fraction in normal population • familial hypertension • familial vasoactivity

A genetic origin of primary (essential) hypertension has been proposed since 1923.1-2 This has usually been based on epidemiologic studies of the family history obtained by interviewing family members. However, no biochemical markers of the postulated genetic abnormality have been established.

The urine of normotensive subjects contains significant amounts of low molecular weight kinins and kallikrein.8 In addition, we have previously reported the existence of a vasoactive polypeptide fraction in the urine of normotensive human beings irrespective of age and sex.4,8 This fraction was shown to dilate the vessels of the general circulation and to retain activity in an isolated vessel preparation, while it was inactive in the pulmonary circulation, the isolated heart, or intestinal preparation.5,7 As reported earlier, a similar, crude polypeptide fraction isolated from the urine of patients with primary hypertension did not lower the blood pressure (BP) in the bioassay.5,8 In view of these findings, we attempted to isolate and characterize the polypeptide fraction in a greater number of patients with different types of hypertension. In addition, we carried out studies on samples of the normal population. Since altered kallikrein excretion has been reported in patients with hypertension,5,8 we also measured the kallikrein content in the urinary polypeptide fraction under study.
POSSIBLE GENETIC MARKER OF PRIMARY HYPERTENSION/Wollheim et al. 575

Subjects and Methods

Urine from control subjects and patients was collected and processed either as 24-hour urine or as representative pooled fractions of the day and night urine. The patient urines were always analyzed in a strictly double-blind fashion. Three groups of patients with arterial hypertension as defined by the WHO criteria were studied: those with primary hypertension, secondary hypertension, and borderline hypertension (table I). The patients were advised to avoid, if possible, antihypertensive medication during the last 3 days prior to urine collection. Control urines were collected from 19 healthy volunteers. Finally, 674 young men, 18–25 years old, were examined during their military service, as well as 252 children, 2–15 years old, hospitalized for various diseases; both groups were normotensive at the time of investigation. A family history of hypertension was assessed by examination of the charts. In a first group of military personnel, a simple interview was carried out; in a larger second group, the subjects' families were asked to answer a questionnaire. In the investigation of children, the parents were interviewed and their BP was measured.

Usually 1 liter of urine was concentrated by vacuum distillation (40°C, approximately 3 atmospheres, final volume of the distillate 1/5 of the original volume). The distillate was dialyzed against running distilled water for 12 to 18 hours, and precipitated with acetone (urine/acetone 1/2, v/v). (Alternatively, ethanol or ammonium-sulphate can be used, but was not in this study). The watersoluble part of the pellet was lyophilized and subjected to column chromatography using 0.012 M pyridine, pH 7.15, as the eluent. The concentration of kallikrein in the polypeptide Fraction A from normotensives was tested on 12 control subjects, after the distillation step; 0.7–8.0 mg (mean 3.0 ± 0.5 SEM), which was not significantly different from the results of the repeated tests. From their military service, as well as the mean BP were monitored (mean BP range, 80 to 115 mm Hg). The criteria for vasodilating activity were the maximal lowering of the BP by at least 20% for more than 3 minutes (see Results). This way of expressing the results was chosen to minimize the influence of differences in the resting BP of the rabbits. However, expression of the results in absolute terms gives similar figures (29.7 ± 1.7 mm Hg corresponds to 28.8% ± 1.5%, mean ± SEM of 20 experiments). Usually, 5 μg per kg of rabbit weight of the final lyophilisate desolved in 0.1 ml H2O was injected intravenously. As a standard to test the sensitivity of the rabbits, a known preparation from control subjects was injected in the beginning and the end of the assay. When a given vasoactive polypeptide fraction was tested in the same animal, the following example of a dose response characteristic was obtained. A rabbit weighing 3.5 kg received 0.1, 0.5, 1.0, 5.0, 10, 50, and 100 μg, which resulted in a BP lowering of 13% lasting 2 minutes, 26% lasting 3 minutes, 28% lasting 5 minutes, 28% lasting 5 minutes, 35% lasting 8 minutes, 44% lasting 8 minutes, and 47% lasting 8 minutes, respectively.

The concentration of kallikrein in the polypeptide Fraction A was also measured in some of the groups by the method of Trautschold et al., which assesses the esterolytic activity using benzoyl-L-arginine-ethyl-ester.

The results of the bioassay are expressed as mean ± SEM. Statistical comparison for the difference in BP decrease of the patients with primary and secondary hypertension was made by the nonparametric Wilcoxon, Mann and Whitney U test. For testing the association between BP decrease and positive family history, the chi-square test for 2 × 2 contingency tables was used.

Results

Bioassays in Controls

To test the variability of the bioassay, two given Fractions A from normotensives were tested on 12 and 14 rabbits. The mean BP-lowering activity was 35.2% ± 2.1% and 38.4% ± 3.0%, respectively. Fraction A from 19 male and female controls collected repeatedly over a period of up to 5 years (291 urines) lowered the BP in the bioassay in 95% of cases by 20% or more, 33.8% ± 0.6% mean ± SEM (fig. 1). It is of interest that, when only the first urine sample of these 19 subjects was evaluated, the BP decreased 35.8% ± 2.4% (mean ± SEM), which was not significantly different from the results of the repeated tests.

For vasodilating activity, 20% BP-lowering activity for more than 3 minutes was chosen as a minimum
value. Such fractions will be referred to as VA+; while those without or with less than 20% activity will be referred to as VA−.

Vasoactivity of Fraction A from Patients with Different Types of Hypertension

The results of the bioassay of urine extracts from patients with primary hypertension and secondary hypertension are shown in figure 2. Fraction A from 76 patients with primary hypertension was found to be VA−; the BP decreased 8.8% ± 1.2%. In contrast, the urine extracts from 71 patients with secondary hypertension matched for age and sex with the group of primary hypertension (table 1) were VA+; the BP decreased 33.4% ± 1.0%. The difference between these two groups of patients is highly significant (p < 0.0001). The 75 cases with the clinical picture of borderline hypertension showed a nonhomogeneous response in the bioassay: 54 cases were VA+, BP decreased 33.8% ± 1.5%; 21 cases were VA−, BP decreased 14.9% ± 1.9%.

Distinction of the Vasoactive Principle in Fraction A from Other Depressor Systems

Kallikrein

The kallikrein activity was measured in Fraction A of normotensive controls and some of the patients with primary and secondary hypertension. In 74 control urines, 0.01 mg of Fraction A contained 1.31 ± 0.12 kallikrein mU (mean ± SEM). In 54 cases of primary hypertension, the value was 0.40 ± 0.04 kallikrein mU; in 29 cases of secondary hypertension, 1.41 ± 0.17 kallikrein mU. When the BP-lowering activity of Fraction A was correlated with the concentration of kallikrein found in the same material, no striking correlations were observed (controls: r = 0.36, n = 74; primary hypertension: r = 0.07, n = 54; secondary hypertension: r = 0.40, n = 29; the three groups combined: r = 0.27, n = 157).

Prostaglandins

To exclude the possibility that Fraction A exerts its BP-lowering activity by altering endogenous prostaglandin activity, studies were performed with indomethacin treatment of rabbits prior to the bioassay. Indomethacin was given i.v. either in two injections (3 mg/kg at 15 minutes prior to the bioassay and repeated 60 minutes later) or as a single 6 mg/kg injection. In another series, rabbits were given 8 mg/kg indomethacin in five daily oral doses. The BP-lowering activity of Fraction A from normotensive controls in indomethacin-treated animals remained unaltered.

Correlation of Bioassay Results (VA+ or VA−) to Family History of Hypertension

The 19 healthy controls were selected from families without a history of hypertension. As shown in figure 3, patients with secondary hypertension (VA+) reported a family history of hypertension in 37%.
FIGURE 2. Distribution of per cent blood pressure (BP) decrease in rabbit bioassay of urine preparations from hypertensive patients. Upper Panel. Primary hypertension (76 cases). Mean BP decreased 8.8% ± 1.2%. Lower Panel. Secondary hypertension (71 cases). Mean BP decreased 33.4% ± 1.0%. Statistical significance, $p < 0.0001$.

FIGURE 3. Correlation between vasoactivity (VA) of the polypeptide fraction and incidence of family history of hypertension. Normotensive controls, $n = 19$; secondary hypertension, $n = 71$; primary hypertension, $n = 76$; borderline hypertension $VA^+$, $n = 54$; borderline hypertension $VA^-$, $n = 21$; soldiers $VA^+$, $n = 485$; soldiers $VA^-$, $n = 189$; children $VA^+$, $n = 175$; children $VA^-$, $n = 77$. 
Patients with primary hypertension (VA⁻) showed a family history of hypertension in 68%. Patients with borderline hypertension categorized as VA⁺ had a family history of hypertension in 39%, while patients categorized as VA⁻ had a family history of hypertension in 62%.

In view of the apparent correlation between inactivity in the bioassay and family history of hypertension in the various patient groups, it was of interest to compare the occurrence of a family history of hypertension and vasoactivity of the polypeptide fraction in samples of the normotensive young population. For these studies, two groups of young men during their military service and a group of children hospitalized for various nonhypertensive diseases were investigated. On the first occasion, 290 soldiers were examined (doctoral thesis of M. Arnold) and on the second 384 soldiers (doctoral thesis of D. Bülow). The results of the two investigations were similar and were therefore combined. As shown in figure 3, of the total group, 485 subjects were VA⁺ (BP decreased 29.5% ± 0.5%) and reported a family history of hypertension in 160 cases (33%). In the group of 189 VA⁻ subjects (BP decreased 7.0% ± 0.6%) 115 reported a family history of hypertension (61%). Finally, of the 252 children examined (doctoral thesis of G. Bülow), 175 were VA⁺ (BP decreased 39.9% ± 1.3%); 47 of these were found to have family history of hypertension (27%). In contrast, of the 77 VA⁻ children (BP decreased 8.3% ± 1.1%) 52 were found to have a family history of hypertension (68%). The correlation between VA⁺, VA⁻ respectively and family history of hypertension was highly significant in the group of primary and secondary hypertension, in soldiers and children (p < 0.0001). The group of borderline hypertension had a p < 0.1, probably due to the small number of patients.

**Discussion**

Our results demonstrate that the urine of healthy subjects irrespective of sex and age contains a polypeptide fraction that lowers the BP upon injection into anesthetized rabbits. Although the amount of the active fraction excreted in the urine of control subjects was not quantified, no correlation was found between the 24-hour urine volume and the excreted amounts of Fraction A in urines where this was examined (r = 0.22, n = 40). The polypeptide nature of the substances in the fraction is indicated by its physicochemical behavior. The molecular weight of the active substance remains to be determined. Based on the insolubility of the active principle in acetone and ethanol, it is clearly not a lipoprotein or a prostaglandin-like substance. Furthermore, the BP-lowering activity of the polypeptide fraction does not appear to be mediated by endogenous prostaglandins, since inhibition of prostaglandin synthesis by indomethacin did not alter the BP-lowering activity.

It was important to distinguish the vasoactive polypeptide fraction from the components of the kallikrein-bradykinin-system that also lower BP. Both kallikrein and bradykinin are found in the urine. The measurements of kallikrein in the polypeptide Fraction A from control subjects and patients with primary and secondary hypertension allowed two conclusions. First, the observed values of kallikrein are far too small to explain the BP-lowering activity of Fraction A. Thus, to obtain the same BP-lowering as with 10 μg of Fraction A, 0.2 kallikrein units of purified pig pancreas kallikrein are needed (not shown). This is 150 times the measured concentration of kallikrein in 10 μg of Fraction A. Second, in none of the examined groups was there a striking correlation between the measured kallikrein concentration and the BP effects of the same specimens in the bioassay. It is thus unlikely that the vasoactive principle in Fraction A is kallikrein. However, a definitive distinction from kallikrein is not possible until further purification of the substances in the urine fraction has been achieved.

It is suggested that the potent vasodilating polypeptide Fraction A contains a humoral factor of importance for BP regulation. The interaction of this new humoral principle with the known depressor and pressor systems remains to be clarified. Likewise, the production site of the vasoactive fraction is not yet known. Since patients with reduced renal function were shown to excrete an active fraction, the kidney appears an unlikely site of origin. This question can only be resolved when a semiquantitative assay allows the measurement of the active principle in plasma and tissues. It was previously shown, by the use of a more crude preparation, that the fraction seems to act directly on the vessels of the general circulation. A possible mode of action was suggested from experi-

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**Table 1. Characteristics of Three Patient Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cases</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Mean ± SEM</td>
<td>F</td>
</tr>
<tr>
<td>Primary hypertension</td>
<td>76</td>
<td>19-78</td>
<td>43.9 ± 1.5</td>
<td>39</td>
</tr>
<tr>
<td>Secondary hypertension</td>
<td>71*</td>
<td>17-71</td>
<td>42.6 ± 1.6</td>
<td>37</td>
</tr>
<tr>
<td>Borderline hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VA⁺</td>
<td>54</td>
<td>15-54</td>
<td>32.9 ± 1.3</td>
<td>5</td>
</tr>
<tr>
<td>VA⁻</td>
<td>21</td>
<td>17-42</td>
<td>28.4 ± 1.6</td>
<td>5</td>
</tr>
</tbody>
</table>

*Renal hypertension, n = 50; endocrine, n = 6 (2 pheochromocytoma, 4 primary hyperaldosteronism); contraceptive pills, n = 15.
†VA = vasodilating activity (see Results, Section 2).
POSSIBLE GENETIC MARKER OF PRIMARY HYPERTENSION/Wollheim et al.

ments in rabbits in which the more crude fraction inhibited the pressor reflex from the carotis sinus. Patients with primary hypertension excrete a polypeptide fraction with weak or no vasodilating activity. However, as judged by the protein separation techniques used here, the inactive fraction did not show any clear differences from the active fraction. At present, it is unclear whether the lack of BP-lowering activity is due to a primary change of the active molecule or to a secondary inactivation of the molecule. The elevated BP per se does not result in a loss of the fraction's vasoactivity since patients with secondary hypertension, comparable in age and severity of the disease with the group of primary hypertensives, excreted a vasoactive fraction.

The different behavior in the bioassay was paralleled by a marked difference in the incidence of family history of hypertension in the two groups of patients (fig. 3). Such a parallelism between the excretion of a polypeptide fraction lacking vasoactivity and occurrence of family history of hypertension was again observed in patients with borderline hypertension (VA: family history of hypertension in 62%). However, the larger part of this patient group who excreted a vasoactive fraction only reported a family history in 39% of cases. Based on these findings, it may be speculated that only the smaller part of patients with borderline hypertension represent the initial stage of primary hypertension, while the larger group may represent a form of reactive hypertension. If this were the case, differences in the prognosis and therapy of these patients become evident.

Since vasodilating activity normally excreted in the urine is defective in patients with primary hypertension, the possibility that this defect represents a genetic marker should be considered. The importance of an early detection of primary hypertension is generally accepted. Because of the good correlation between genetic predisposition and excretion of a defective polypeptide fraction in the patient material, it was of particular interest to investigate groups of young normotensive subjects. Indeed, the results obtained in children and young military personnel again show good correlation between the results of the bioassay and occurrence of family history of hypertension. A prospective study of these subjects should demonstrate whether it may be possible, by use of this test, to detect primary hypertension long before its manifestation.

Acknowledgments

The authors thank Professors H. P. Wolff and A. Distler, University of Mainz, for providing urine samples and charts of their patients; Professors E. Wecker and E. Helmreich, University of Würzburg, for stimulating discussions; Dipl. Phys. E. Moll, Unit of Nuclear Medicine, University of Würzburg, for help with the calculation of statistics; and S. Görlich, for excellent secretarial assistance. Kallikrein was kindly provided by Professor G. L. Haberland, Bayer AG, Wuppertal; indomethacin by Merck, Sharp & Dohme, München.

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Defect in the excretion of a vasoactive polypeptide fraction A possible genetic marker of primary hypertension.

E Wollheim, S Peterknecht, C Dees, A Wiener and C B Wollheim

Hypertension. 1981;3:574-579
doi: 10.1161/01.HYP.3.5.574

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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