Plasma Digitalislike Activity in Essential Hypertension or End-Stage Renal Disease

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SUMMARY Plasma extracts from 119 subjects showed a digitalislike activity, as evidenced by the ability of these extracts to inhibit ouabain binding to the Na⁺-K⁺ pump. High levels of the digitalislike compound were found in 18 of 54 untreated hypertensive subjects, 7 of 21 normotensive subjects with a family history of hypertension, and 10 of 14 patients with end-stage renal failure. Dialysis significantly reduced the activity of this compound. These results suggest 1) that endogenous digitalislike factor is not directly linked to hypertension but rather is related to sodium balance and 2) that it neither originates nor is activated by renal tissue, as it was present in four of six anephric patients. (Hypertension 8: 632-638, 1986)

KEY WORDS • Na⁺-K⁺-ATPase • ouabain • essential hypertension • chronic renal failure • dialysis • hypertensive heredity

THE renal regulation of sodium and water homeostasis is mediated by intrarenal, physical, and hormonal factors and by extrarenal hormones. The latter include factors retaining Na⁺ and water, such as aldosterone and antidiuretic hormone, and factors eliminating Na⁺ in the urine while the Na⁺ balance is positive. The existence of a humoral natriuretic factor inhibiting renal tubular Na⁺ reabsorption was inferred from various experiments in the 1960s showing that the natriuresis following acute and chronic Na⁺ loading could not be explained by changes in aldosterone, vasopressin, or the glomerular filtration rate.1 More recent work has shown that, after a Na⁺ load, plasma and urine indeed contain one or more substances that inhibit renal Na⁺,K⁺–adenosine triphosphatase (ATPase), the enzymatic expression of the Na⁺-K⁺ pump,2 displace ouabain from its receptor sites,2 and compete for digoxin antibodies.3

Although little is known about the physiological role of the natriuretic hormone with digitalislike properties and its structure has not yet been elucidated, several results imply that this substance may have a pathogenic or physiological role in both hypertension (for review, see Reference 4) and chronic renal insufficiency. In animal Na⁺–dependent hypertension, a circulating Na⁺-K⁺ pump inhibitor appears to be responsible for a reduction in ouabain-sensitive cation fluxes in blood vessels.5 In essential hypertensive humans, cytochemical6 or spectrophotometric7 methods showed an increase in plasma Na⁺-K⁺-ATPase transport inhibitor. This finding was reinforced by a study showing that the leukocyte ouabain-sensitive Na⁺ efflux was reduced in hypertensive patients.8 Blaustein9 suggested that the resulting increase in intracellular Na⁺ enhanced the free Ca²⁺ concentration, which in turn raised vascular resistance and blood pressure.9 It was therefore proposed that a Na⁺-K⁺ pump inhibitor might play an important role in the pathogenesis of human essential hypertension.

A natriuretic Na⁺ transport inhibitor has also been described in the plasma and urine of patients with renal insufficiency.10–12 The presence of this substance might explain the decrease in ouabain-sensitive cation fluxes13,14 and the plasma digoxinlike immunoreactivity15 found in patients with this disorder. Such an inhibitor might play a role in the progressive rise in Na⁺ excretion that accompanies the fall in the glomerular filtration rate.10 Therefore, in the present study the ability of plasma extracts to inhibit [³H]ouabain binding was examined in two series of patients with either essential hypertension or renal impairment.
Subjects and Methods

The digitalislike properties of plasma extracts were measured in 119 subjects who were placed into one of three groups: normotensive subjects, essential hypertensive subjects, and patients with renal failure.

Normotensive Subjects

The normotensive group comprised 42 white, healthy volunteers divided into two subgroups of 21 each based on the presence or absence of a family history of hypertension. All normotensive subjects were on an unrestricted sodium diet. Eight women and 13 men, ranging in age from 22 to 53 years (mean, 32.1 ± 2.0 years), had no known family history of hypertension among their first or second degree relatives. Their mean blood pressure was 91 ± 2 mm Hg. Nine women and 12 men, ranging in age from 23 to 55 years (mean, 34.4 ± 1.7 years), had at least one first or second degree relative with sustained high blood pressure. Their mean blood pressure was 96.2 ± 2.2 mm Hg.

Essential Hypertensive Patients

Essential hypertension was diagnosed after complete investigation in 54 white subjects (29 men and 25 women) ranging in age from 19 to 76 years (mean, 44 ± 2 years). On repeated examination, all had sustained hypertension with diastolic blood pressure greater than 160 mm Hg. Their mean blood pressure was 122.7 ± 1.7 mm Hg. None of these patients had plasma creatinine levels above 110 μM, retinal hemorrhages, or papilledema. All hypertensive subjects were on an unrestricted sodium diet. None had undergone blood transfusion or digitalis therapy for at least 2 months before the study, indicating that, when present, ouabain binding inhibition by plasma extracts was secondary to endogenous factor (or factors).

<table>
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<th>Age (yr)</th>
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<th>MAP (mm Hg)</th>
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Mean ± SEM 47.8 ± 4.4  59 ± 4  56.9 ± 4.1  96.7 ± 2.6  91.7 ± 3.9

BD = before dialysis; AD = after dialysis; MAP = mean arterial pressure.
The inhibition of [H]ouabain binding to the digitalis sites of the red blood cells was also measured as a function of the concentration of the added plasma extracts and was compared with the effect of their K⁺ content. These experiments were performed using 2.10⁻⁹ M [H]ouabain and various volumes of plasma extracts. Binding was measured at equilibrium as previously described. With all plasma extracts tested, the inhibition increased with the amount of plasma extracts as did K⁺ ions and ouabain (Figure 1).

All values are expressed as means ± SEM. Nonparametric U-Mann and Whitney test was used to compare the groups.

### Results

The ability of plasma extracts to interact with the Na⁺-K⁺ pump was quantified by the relative change they induced in the apparent affinity of ouabain for the digitalis site of the Na⁺-K⁺ pump (KD ratio). The mean KD ratio in the hypertensive subject was 0.80 ± 0.2, which was significantly higher than that seen in normotensive control subjects with no known family history of hypertension (0.21 ± 0.04). Of the 54 hypertensive subjects, 18 had values above the upper limit of those found in the controls (Figure 2). Interestingly, a high level of the endogenous digitalis-like factor was also found in certain normotensive subjects with a family history of hypertension (see Figure 2). The mean KD ratio in this group was 0.67 ± 0.14.

In the hypertensive patients, the inhibition of ouabain binding was independent of age, sex, blood pressure, and plasma renin activity, whereas it was slightly but significantly correlated with urinary sodium excretion (Figure 3). After antihypertensive treatment, the relative changes in mean blood pressure and ouabain binding inhibition did not correlate.

In patients with nondialyzed renal failure, ouabain binding inhibition did not differ from that of the normotensive control group with no family history of hypertension (0.35 ± 0.03; Figure 4).
Before dialysis, plasma extracts from patients with end-stage renal failure exhibited a higher $K_D$ ratio than did the normotensive controls, and this effect was independent of the presence or absence of the kidneys. The elevation of ouabain binding inhibition observed in end-stage renal failure was similar to that observed in untreated hypertensive subjects (see Figure 4). After dialysis, the values of the $K_D$ ratio in anephric and nonanephric subjects (0.34 ± 0.1 and 0.21 ± 0.05, respectively) were similar to those of the normotensive controls (0.21 ± 0.02). These values were thus significantly reduced by dialysis (Figure 5).

**Discussion**

The main result of the present investigation is the finding that plasma extracts from some subjects with essential hypertension or end-stage renal disease contain substances with digitalislike properties, as indicated by the ability of these extracts to inhibit ouabain binding to red blood cells. Ouabain binding to its receptor sites, located on the external side of the Na⁺-K⁺ pump, is a highly specific process reflecting structural complementarity between the ligand and the receptor. Binding may be reduced by allosteric changes such as those induced by $K^+$ ions. Under our experimental conditions, however, $K^+$ ions were too diluted to account for the observed inhibition. The inhibition of ouabain binding exerted by plasma extracts therefore reflects the effect of endogenous plasma component (or components) with digitalislike properties.

The data obtained in hypertensive subjects confirm and extend a previous observation from our laboratory. The endogenous digitalislike activity measured in the present investigation may also bear some relationship to the Na⁺ transport inhibitor detected in hypertensive subjects by cytochemical or spectrophotometric methods and by ouabain-sensitive rubidium flux measurement in leukocytes. Furthermore, the decrease in the affinity of ouabain binding to human red blood cells has been reported to be correlated to the inhibition of Na⁺,K⁺-ATPase.

The present results concerning hypertensive subjects differ from other data in some aspects. High levels of endogenous digitalislike activity were present in 30% of our subjects, a smaller proportion than that reported by others. Furthermore, we found no correlation between the level of this factor and blood pressure or plasma renin activity. These discrepancies may be related to differences in sex, severity of the disease, or ethnic characteristics. For instance, all patients studied by Hamlyn et al. were men and 70% were black.
Figure 2. Individual values for changes in the apparent affinity of ouabain for its binding sites induced by deproteinized plasma in normotensive subjects with (+) and without (−) a family history of hypertension and untreated essential hypertensive subjects. Binding inhibition is expressed by the ratio \( (K_D' - K_D)/K_D \), where \( K_D' \) indicates the apparent affinity of ouabain for erythrocytes in the presence of plasma and \( K_D \) indicates the same affinity in its absence. Bars represent means ± SEM. NS = not significant.

Figure 3. Correlation between urinary Na⁺ excretion and the ability of plasma extracts from essential hypertensive subjects to inhibit ouabain binding to the digoxis sites of the erythrocytes.

whereas our study included women and concerned only whites. It is also possible that high circulating levels of this factor, reflecting some aspect of the Na⁺ balance, are present only in salt-sensitive essential hypertension.

Other investigators have drawn attention to the possible outcome of changes in cellular Na⁺ metabolism in subjects from hypertensive families. These changes include an increase in intracellular Na⁺, an increase in the net Na⁺ efflux from erythrocytes, and a reduction of leukocyte Na⁺ effluxes in the absence or presence of plasma. The presence of an endogenous inhibitor in normotensive subjects with a family history of hypertension indicates either a genetic transmission or some peculiar environmental influence. A genetic influence on the release of the natriuretic factor has been proposed by De Wardener and MacGregor. A genetic perturbation of Na⁺ metabolism was suggested by Grim et al. who showed a blunted natriuretic response to saline load in normotensive first degree relatives of hypertensive subjects. It is possible that the secretion of the endogenous digitalislike factor is either under direct genetic control or is triggered by an unknown genetic abnormality of Na⁺ metabolism.

The presence of this factor in patients with end-stage renal disease agrees with previous data indicating an increased erythrocyte Na⁺ content, a reduced ouabain-sensitive Na⁺ efflux rate, and a plasma digoxin immunoreactivity in such patients. Considerable evidence supports the hypothesis that renal Na⁺ excretion is partly regulated by a humoral agent released or activated by extracellular fluid volume expansion. Increased amounts of endogenous digitalislike substance are found in the plasma of volume-expanded humans, dogs, and rats. End-stage renal patients also have a chronic volemic expansion, which may be responsible for an increase in an endogenous digitalislike compound.

In the present study, ouabain binding in normotensive controls with no family history of hypertension was the same as that in patients with little or no extra-
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FIGURE 4. Inhibition of ouabain binding to erythrocytes induced by plasma extracts from normotensive subjects with no family history of hypertension (controls), essential hypertensive subjects (EHT), and subjects with renal insufficiency who either required or did not require dialysis. Six of the former were anephric. Bars represent the SEM. Stars indicate a significant difference compared with values in controls (p < 0.05).

FIGURE 5. Ouabain binding inhibition induced by plasma extracts from six anephric (*) and eight nonanephric () patients with end-stage renal disease before (PRE) and after (POST) dialysis. There was a significant difference between values before and after hemodialysis (p < 0.01).

Cellular fluid expansion whose renal insufficiency did not necessitate dialysis and in two dialyzed subjects with a residual diuresis. Krzesinski and Rorive13 suggested that the decrease in ouabain-sensitive Na+ efflux observed before dialysis was related to a volume-dependent plasma factor. The present results are in agreement with this hypothesis.

Endogenous digitalislike factor does not appear to be linked directly to hypertension, since 1) it only increased in 30% of our hypertensive subjects, 2) all normotensive patients with end-stage renal disease and 30% of the normotensive subjects with a familial hypertensive heredity exhibited higher values than did the normotensive subjects with no family history of hypertension, and 3) there was no correlation between the changes in ouabain binding inhibition and arterial blood pressure induced by antihypertensive therapy.

The existence of a relationship between endogenous digitalislike factor and Na+ balance is supported by the correlation between ouabain binding inhibition and Na+ balance and the decrease in the inhibition of ouabain binding after dialysis. However, we cannot exclude the possibility that this factor is dialyzable, which would explain why it decreases with dialysis.

The investigation of anephric patients allows discussion of the origin of this factor. Some authors obtained a small molecular weight fraction from bovine27 and rat hypothalamus28 that inhibits Na+,K+-ATPase and interacts with ouabain for binding sites on Na+,K+-ATPase. Others claim that a high molecular weight natriuretic substance originates from the kidney.29,30 Our results suggest that the endogenous digitalislike compound detected in our patients neither originates in nor is activated by renal tissue, as high levels of it were present in four of six anephric patients.
References

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G Deray, M G Pernollet, M A Devynck, J Zingraff, A Touam, J Rosenfeld and P Meyer

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