An increase in a circulating inhibitor of Na\textsuperscript{+},K\textsuperscript{+}-dependent ATPase: a possible link between salt intake and the development of essential hypertension

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Summary
1. The plasma's ability to stimulate guinea-pig renal glucose 6-phosphate dehydrogenase (G6PD) in vitro was measured by a cytochemical technique in 23 normotensive subjects and 19 patients with hypertension, all of whom were studied on their normal sodium intake. The ability of plasma to stimulate renal G6PD was significantly ($P < 0.001$) increased in the hypertensive patients (mean $195 \pm 52$ units/ml) compared with the normotensive subjects (mean $22.2 \pm 5.8$ units/ml). In all 42 individuals, there was a significant correlation between diastolic pressure and the ability of plasma to stimulate G6PD ($r = 0.69$, $P < 0.001$).

2. The ability of plasma to stimulate G6PD was greatest in the hypertensive patients with values of plasma renin activity below the normal range. In the normotensive subjects the ability of plasma to stimulate G6PD was significantly greater in the older subjects.

3. As the ability of plasma to stimulate G6PD reflects its ability to inhibit Na\textsuperscript{+},K\textsuperscript{+}-dependent ATPase, these results suggest that patients with essential hypertension have an increase in a circulating inhibitor of Na\textsuperscript{+},K\textsuperscript{+}-ATPase. The results support the hypothesis that a rise in a circulating sodium transport inhibitor may, in part, be responsible for the rise in blood pressure in essential hypertension, and may form the link between salt intake, abnormalities of sodium transport and a rise in blood pressure.

Key words: Na\textsuperscript{+},K\textsuperscript{+}-dependent adenosine triphosphatase, glucose 6-phosphate dehydrogenase, plasma renin activity, sodium transport.

Introduction
We have suggested that, in line with an original suggestion by Dahl, Knudsen & Iwai [1] and the work of Haddy & Overbeck [2] and that of Blaustein [3], the rise in blood pressure in essential hypertension may, in part, be due to an increase in the concentration of a circulating sodium transport inhibitor [4]. There is evidence in the normal rat [5], dog [6] and man [7, 8] that the plasma contains an inhibitor of Na\textsuperscript{+},K\textsuperscript{+}-ATPase, the concentration of which is directly related to the state of sodium balance. One of the studies in normal man [8] used a cytochemical technique which measures Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity in guinea pig renal slices [9]. The technique to measure Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity in guinea pig slices is new and is still being developed whereas the technique to measure the activity of glucose 6-phosphate dehydrogenase (G6PD) in the same system is well established [10, 11], and has been used in this laboratory for some time. We have therefore measured the ability of plasma to stimulate guinea pig renal glucose 6-phosphate dehydrogenase in normotensive subjects and hypertensive patients as a marker of the plasma's ability to inhibit Na\textsuperscript{+},K\textsuperscript{+}-ATPase.

Methods
Normotensive, healthy subjects whose diastolic pressure after a 1–2 month period of observation was between 60 and 90 mmHg, and unselected

Abbreviation: G6PD, glucose 6-phosphate dehydrogenase.
patients with essential hypertension whose diastolic pressure after a 2-month period of observation was between 90 and 130 mmHg, were included in the study. All subjects gave their informed consent. 16 of the 19 patients had not had previous therapy; in the three patients who had, it was stopped for at least 2 months before the study. Patients and normotensive subjects were excluded if they were taking any drug, including the oral contraceptive pill, or had evidence of renal failure, heart failure or cerebrovascular disease. The mean age of the 19 patients with essential hypertension was 45 years (range 22–63 years). There were 11 females and eight males; 12 Caucasians, seven negroes. Of the 23 normotensive subjects, 12 of them were aged 20–25 years (mean age 23 years) and 11 older normal subjects (mean age 54 years; range 40–67 years). Measurements were made of blood pressure, the ability of plasma to stimulate guinea pig renal G6PD activity, plasma renin activity and two 24 h urinary sodium when on their normal diet. Blood pressure was measured at the same time of day, in the same room by the same nurse using observer bias-free semi-automatic sphygmomanometers (Arteriosonde) [12]. Blood pressure used was the mean value of five readings at 1–2 min intervals measured in the supine position. Blood for measurement of the plasma’s ability to stimulate G6PD and plasma renin activity was taken after the subject had been sitting upright for 10 min between 10.00 hours and 12.00 hours. Plasma renin activity was measured by radioimmunoassay [13]. The normal range under these conditions for the assay is 0.5 to 2.5 ng h⁻¹ ml⁻¹. The cytochemical technique used to measure G6PD activity in sections from slices of guinea pig kidney is that originally described by Chambers et al. [10], and subsequently modified by Fenton et al., [11]. Its adaptation to measure changes in G6PD activity caused by a purified natriuretic extract obtained from human urine which inhibits Na⁺,K⁺-ATPase activity, and the specificity of this response, have been described by Alaghband-Zadeh et al. [14] and S. Fenton et al. (unpublished work). The natriuretic extract was used as a standard. The maximum stimulation of renal G6PD activity occurred at 2 min and was obtained with 384 pg/ml. This amount was assigned an arbitrary value of 1 unit of G6PD stimulating activity/ml, and the ability of plasma to stimulate G6PD activity was expressed in these units. Results are expressed as mean (±SEM). Statistical analysis was performed by Student’s t-test. For hormone values, log values were used. For correlations the method of least squares was used.

Results

The 19 hypertensive patients were split into two groups, one group with a plasma renin activity below the normal range and the second group with a plasma renin activity within the normal range. The ability of plasma to stimulate G6PD in the group with the low plasma renin activity was 400 ± 97 units/ml, which was significantly greater ($P < 0.001$) than in the group with normal plasma renin activity, in whom it was 74.9 ± 9.2 units/ml (Fig. 1). Taking all 19 hypertensive patients, there was a significant inverse correlation between the ability of plasma to stimulate G6PD and plasma renin activity ($r = -0.61 \ P < 0.001$). In the 11 older, normal subjects, the ability of plasma to stimulate G6PD was 34.5 ± 10.5 units/ml, which was significantly less ($P < 0.01$) than the hypertensive patients with normal values of plasma renin activity. In the 12 younger, normotensive subjects, the ability of their plasma to stimulate G6PD was 11.5 ± 3.4 units/ml, which was significantly lower than that of the older, normotensive subjects ($P < 0.05$). Taking all the normotensive subjects, there was a significant correlation between age and the ability of plasma to stimulate G6PD ($r = 0.58 \ P < 0.005$), but this
Discussion

The justification for proposing that the plasma's capacity to stimulate G6PD is a marker of its plasma's ability to inhibit Na\(^+\), K\(^+\)-ATPase. The finding that the ability of plasma to stimulate G6PD rises with age in normotensive subjects is interesting in view of the fall in glomerular filtration rate that also occurs with age [17]. As there is no evidence that salt intake diminishes with age, sodium balance must be maintained by inhibiting tubular reabsorption of sodium. The demonstration that the plasma's ability to stimulate G6PD and therefore to inhibit Na\(^+\), K\(^+\)-ATPase increases with age suggests that this is one mechanism by which this adjustment is made. It also follows that the rise in blood pressure that tends to occur with age in high sodium-eating communities [18] may also be, in part, due to a rise in the concentration of a circulating Na\(^+\), K\(^+\)-ATPase inhibitor.

The results of these observations support the increasing evidence [19, 20] that the plasma of patients with essential hypertension contains an increased concentration of an inhibitor of Na\(^+\), K\(^+\)-ATPase and provide evidence for the hypothesis [4] that this inhibitor may, in part, be responsible for the rise in blood pressure that occurs in essential hypertension, and may be the link between an inherited difficulty in the kidney's ability to excrete sodium, salt intake, abnormalities of sodium transport and the development of high blood pressure.

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References


