PAPERS AND SHORT REPORTS

Evidence for a raised concentration of a circulating sodium transport inhibitor in essential hypertension

GRAHAM A MACGREGOR, STEPHEN FENTON, JAMSHID ALAGHBAND-ZADEH, NIRMALA MARKANDU, JOSEPH E ROULSTON, HUGH E DE WARDENER

Abstract

A cytochemical technique that measures the ability of plasma to stimulate guinea-pig renal glucose-6-phosphate dehydrogenase (G6PD) activity in vitro, which is a marker of its ability to inhibit $\text{Na}^+\text{-K}^+$ -adenosinetriphosphatase ($\text{Na}^+\text{-K}^+\text{-ATPase}$), was used in 19 patients with essential hypertension and 23 normotensive, healthy subjects. The ability of plasma to stimulate G6PD was significantly greater in the hypertensive patients when they were taking their normal sodium diet than in the normotensive subjects, and was significantly correlated with blood pressure. The ability of plasma to stimulate G6PD was inversely correlated with plasma renin activity in the hypertensive patients and increased with age and sodium intake in the normotensive subjects.

These results support the hypothesis that essential hypertension, and also perhaps the increase in blood pressure with age in communities that consume large quantities of salt, is in part due to an increase in a circulating concentration of an inhibitor of $\text{Na}^+\text{-K}^+\text{-ATPase}$.

Introduction

We have suggested that essential hypertension is, in part, due to an increase in a circulating sodium transport inhibitor.¹ There is evidence in normal rats,² dogs,³ and man^{4,5} that plasma contains an inhibitor of $\text{Na}^+\text{-K}^+\text{-adenosinetriphosphatase}$ ($\text{Na}^+\text{-K}^+\text{-ATPase}$), the concentration of which is directly related to the state of sodium balance. We made this observation in normal man⁵ using a cytochemical technique that measures $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in guinea-pig renal slices.⁶ This is a new and difficult technique and is still being developed. However, inhibition of $\text{Na}^+\text{-K}^+\text{-ATPase}$ —for example, with ouabain—is associated with stimulation of renal glucose-6-phosphate dehydrogenase (G6PD) activity,⁷ and the cytochemical technique used to stimulate G6PD activity in guinea-pig kidney is well established.^{8,9} We therefore measured the plasma's ability to stimulate renal G6PD activity in normotensive subjects and in patients with essential hypertension to test the hypothesis that in essential hypertension there is a raised concentration of a $\text{Na}^+\text{-K}^+\text{-ATPase}$ inhibitor.

Subjects and methods

Nineteen unselected patients with essential hypertension, whose diastolic pressure after two months of observation was between 90 and 130 mm Hg, and 23 normotensive, healthy subjects, whose diastolic pressure after one to two months of observation was between 60 and 90 mm Hg, were included in the study. All subjects gave their informed consent. Sixteen of the 19 patients with essential hypertension had not received previous treatment. The three other patients had had their treatment stopped for at least two months before the study. Patients and controls 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 (11 women, eight men; 12 white, seven black) was 45 (range 22–63) years. The 23 normotensive subjects (three women, 20 men; all white) had a mean age of 38 (range 20–67) years. In all subjects measurements were made of blood pressure, the ability of plasma to stimulate guinea-pig renal G6PD activity, plasma renin activity, and 24-hour urinary sodium excretion (on two occasions) when they were taking

Department of Medicine, Charing Cross Hospital Medical School, London W6 8RF

GRAHAM A MACGREGOR, MRCP, senior lecturer and honorary consultant physician

STEPHEN FENTON, BSc, biochemist

NIRMALA MARKANDU, SRN, research assistant

JOSEPH E ROULSTON, BSc, senior biochemist (present appointment: lecturer and honorary senior biochemist, Department of Clinical Chemistry, Royal Infirmary, Edinburgh)

HUGH E DE WARDENER, FRCP, professor of medicine

Department of Chemical Pathology, Charing Cross Hospital Medical School

JAMSHID ALAGHBAND-ZADEH, MRCP, senior lecturer

their normal diet. Mean urinary sodium excretion in the 19 hypertensive patients taking their normal diet was 162 ± 16 mmol (mEq)/day; that in the 23 controls was not significantly different, being 146 ± 14 mmol/day. Mean blood pressure in the hypertensive subjects was 163/108 mm Hg and in the controls 119/74 mm Hg.

Twelve of the normal subjects, aged 20–25 years, and five of the patients with high blood pressure were also studied on the fifth day of taking a high-sodium diet, which consisted of their normal intake plus 200 mmol Slow Sodium (Ciba), and on the fifth day of taking a low-sodium diet (10 mmol/day); all studies were done with the subjects attending as outpatients. Blood pressure was measured at the same time of day, in the same room, by the same nurse using semi-

FIG 1—Ability of plasma to stimulate guinea-pig renal glucose-6-phosphate dehydrogenase (G6PD) activity in vitro in normotensive subjects and hypertensive patients taking their normal diet.

automatic sphygmomanometers (Arteriosonde 1217¹⁰) free from observer bias. The blood pressure used was the mean of five readings taken at one- or two-minute intervals 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 minutes between 10 00 am and noon. Plasma renin activity was measured by radioimmunoassay.¹¹ The normal range for plasma renin activity in normotensive subjects sitting upright between 10 00 am and noon and receiving a sodium intake of 100–200 mmol/day is 0.4–1.9 mmol/l/h (0.5–2.5 ng/ml/h).

The cytochemical technique used to measure G6PD activity in sections from slices of guinea-pig kidney was that originally described by Chambers *et al*⁸ and subsequently modified by Fenton *et al*.⁹ Its adaptation to measure the changes in G6PD activity caused by a purified natriuretic extract obtained from human urine inhibits that Na^+/K^+ -ATPase activity and the specificity of this response have been described by Zadeh *et al*.¹² Using this adapted method and alternate sections from slices of guinea-pig kidney exposed for from two to eight minutes to a dilution of either natriuretic extract or normal human plasma, S Fenton *et al* (unpublished results) found that the rise in G6PD activity is associated with a simultaneous fall in Na^+/K^+ -ATPase activity (measured by the technique of Chayen *et al*).⁶ The maximum rise in G6PD activity occurred at two minutes and the maximum fall in Na^+/K^+ -ATPase activity at four minutes. The time course of the changes induced by the natriuretic extract and the plasma was similar. In addition, Zadeh *et al*¹² and S Fenton *et al* showed that the rises in G6PD activity induced by a wide range of concentrations of natriuretic extract and plasma are parallel. The identity of these

changes suggests that the substance responsible for the changes in the Na^+/K^+ -ATPase and G6PD activity is common to both the natriuretic extract and the plasma. The natriuretic extract was, therefore, used as a standard. The maximum stimulation of renal G6PD activity was obtained with 384 ng/l (S Fenton *et al*). This amount was assigned an arbitrary value of one unit of G6PD-stimulating activity/ml, and the ability of plasma to stimulate G6PD activity was expressed in these units. Results were expressed as means $\pm$ SEM. Statistical analysis was performed using Student's *t* test, and for correlations the method of least squares.

Results

The mean ability of plasma to stimulate G6PD in the 19 hypertensive patients when they were taking their normal sodium diet was 195 ± 52 units/ml. This was significantly higher than that in the 23 controls, in whom it was 22.5 ± 5.8 units/ml ($p < 0.001$; fig 1). When the two groups were combined there was a significant correlation between systolic blood pressure, mean blood pressure, and diastolic

FIG 2—Ability of plasma to stimulate guinea-pig renal glucose-6-phosphate dehydrogenase (G6PD) activity in vitro plotted against diastolic pressure in 42 subjects taking their normal diet.

pressure and the ability of plasma to stimulate G6PD activity ($r = 0.61$; $r = 0.71$; $r = 0.69$; $p < 0.001$, respectively; fig 2). These correlations were still significant when the younger controls were excluded from the analysis ($r = 0.43$; $r = 0.54$; $r = 0.50$; $p < 0.02$; $p < 0.005$; $p < 0.01$, respectively).

The patients were divided into two groups. Seven patients had plasma renin activity below the normal range (mean 0.19 ± 0.05 mmol/l/h (0.25 ± 0.06 ng/ml/h); mean age 49 (range 34–61) years; mean blood pressure 168/112 mm Hg). The 12 other patients had plasma renin activity within the normal range (mean 1.48 ± 0.18 mmol/l/h (1.93 ± 0.24 ng/ml/l); mean age 42 (range 22–63) years; mean blood pressure 160/107 mm Hg). The ability of plasma to stimulate G6PD in the group with low plasma renin activity was 400 ± 97 units/ml, which was significantly greater ($p < 0.001$) than that in the group with normal plasma renin activity (74.9 ± 9.2 units/ml). There was a significant inverse correlation in the 19 patients between the ability of plasma to stimulate G6PD and plasma renin activity ($r = -0.61$, $p < 0.001$). There was no such correlation in the controls ($r = 0.34$, $p < 0.1$). The plasma's ability to stimulate G6PD in the patients with normal plasma renin activity was significantly greater than that in the 11 older controls (mean age 54 (range 40–67) years), in whom it was 34.5 ± 10.4 units/ml ($p < 0.01$); this, in turn, was significantly greater than that in the 12 younger controls (mean age 23 (range 20–25) years), in whom it was 11.5 ± 3.4 units/ml ($p < 0.05$). In the controls there was a significant correlation between age and the ability of plasma to stimulate G6PD ($r = 0.58$, $p < 0.005$), but this correlation was not significant in the patients ($r = 0.27$, $p > 0.5$).

When the 12 white and seven black patients with hypertension were compared the mean ability of plasma to stimulate G6PD was 158 ± 62 units/ml in the white patients and 257 ± 81 units/ml in the black

($p < 0.05$). Blood pressure, age, and plasma renin activity were not significantly different between these two groups. The differences between hypertensive patients and normotensive subjects were still significant when the black patients were excluded from the analysis (white patients 158 : 68 units/ml, controls 22.9 : 5.6 units/ml; $p < 0.01$).

In the group of 12 young controls, in whom measurements were made while they were taking high, normal, and low-sodium diets, the ability of plasma to stimulate G6PD with the 10 mmol diet was 1.8 : 1.0 units/ml; with the normal diet it was 11.5 : 4.8 units/ml and with the high-sodium diet 32.7 : 12.5 units/ml. The differences between the diets were all highly significant ($p < 0.001$). In the five hypertensive subjects (mean age 50 (range 34–63) years) in whom similar measurements were made and of whom four had low plasma renin activity, the ability of plasma to stimulate G6PD was 380 : 96 units/ml with the low-sodium diet, 468 : 143 units/ml with the normal diet, and 980 : 303 units/ml with the high-sodium diet.

Discussion

These results show that the ability of plasma to stimulate G6PD is increased in patients with essential hypertension, and that this ability is directly related to arterial pressure. Several findings suggest that the plasma's ability to stimulate G6PD is a marker of its capacity to inhibit $\text{Na}^+\text{-K}^+\text{-ATPase}$. Firstly, inhibition of $\text{Na}^+\text{-K}^+\text{-ATPase}$ by various agents including ouabain stimulates G6PD activity.⁷ Secondly, by using the cytochemical technique described above S Fenton *et al* showed that the inhibition of $\text{Na}^+\text{-K}^+\text{-ATPase}$ induced by plasma in slices of guinea-pig kidney is associated with a simultaneous increase in G6PD activity (unpublished results). Thirdly, plasma from the same normal subjects taking low- and high-sodium diets inhibited $\text{Na}^+\text{-K}^+\text{-ATPase}$ ⁵ and stimulated G6PD in a manner that was directly related to the sodium intake; the plasma's ability to inhibit $\text{Na}^+\text{-K}^+\text{-ATPase}$ and to stimulate G6PD when the subjects were taking the high-sodium intake was about 25 and 17 times greater, respectively, than that when they were taking the low-sodium intake. Finally, the ability of the plasma from the hypertensive patients to stimulate G6PD activity was also related to sodium intake.

These observations, which suggest that the plasma of patients with essential hypertension contains a raised concentration of a $\text{Na}^+\text{-K}^+\text{-ATPase}$ inhibitor, support the hypothesis¹ that essential hypertension is due, in part, to an increase in a circulating sodium transport inhibitor secreted in response to an inherited impairment of the kidney's ability to excrete sodium. If sodium intake is greater than about 50 mmol/day there will be a tendency in these patients for sodium and water retention and an increase in extracellular volume. This will increase the concentration of a circulating $\text{Na}^+\text{-K}^+\text{-ATPase}$ inhibitor, which, by inhibiting tubular reabsorption of sodium, increases urinary sodium excretion, thereby restoring sodium balance towards normal. This increase in a circulating sodium transport inhibitor inhibits sodium transport in other cells, in particular red and white cells but more importantly smooth-muscle cells of the arteriole. In smooth-muscle cells, by the mechanism that Blaustein¹³ outlined, an increase in a circulating sodium transport inhibitor will increase the tone of the cells and eventually cause an increase in peripheral resistance and a rise in blood pressure. The recent findings of Poston *et al*¹⁴ support the hypothesis: they incubated white cells from normotensive subjects either in plasma from other normotensive subjects or in plasma from hypertensive patients. It was shown that when the cells from normotensive subjects were incubated in the plasma of hypertensive patients the white cells acquired the same impairment of ouabain sodium transport as the white cells from hypertensive patients. The sodium transport of white cells from normotensive subjects incubated in plasma from other normotensive subjects did not change.

The observation that in hypertensive patients the plasma's ability to stimulate G6PD rises as plasma renin activity falls suggests that the changes in both are due to an expansion of

effective blood volume. These results are supported by the work of Edmondson and MacGregor,¹⁵ who showed that the impairment of white-cell sodium transport was greater in patients with an impaired response of the renin system to a low-salt diet compared with patients who had a normal response. As ouabain directly inhibits renin secretion¹⁶ the fall in plasma renin activity in essential hypertension might be due, in part, to the rise in the plasma's ability to inhibit $\text{Na}^+\text{-K}^+\text{-ATPase}$. The finding that the ability of plasma to stimulate G6PD rises with age is interesting in view of the fall in glomerular filtration rate that also occurs with age.¹⁷ As there is no evidence, however, that salt intake diminishes with age, sodium balance must be maintained by an inhibition of tubular reabsorption of sodium. The finding that the plasma's ability to stimulate G6PD and therefore to inhibit $\text{Na}^+\text{-K}^+\text{-ATPase}$ increases with age suggests that this is one mechanism by which this inhibition is made. The rise in blood pressure that occurs with age in communities with a high salt intake¹⁸ may therefore, as in essential hypertension, also be due in part to an increase in a circulating $\text{Na}^+\text{-K}^+\text{-ATPase}$ inhibitor.

We thank Dr E M Clarkson for providing the natriuretic extract, and Diana Elder, metabolic ward dietitian. This study was supported by the Wellcome Trust and the National Kidney Research Fund.

References

- de Wardener HE, MacGregor GA. Dahl's hypothesis that a saluretic substance may be responsible for a sustained rise in arterial pressure: its possible role in essential hypertension. *Kidney Int* 1980;18:1–9.
- Gonick HC, Kramer HJ, Paul W, Lu R. Circulating inhibitor of sodium-potassium activated adenosine triphosphate after expansion of extra cellular fluid volume in rats. *Clin Sci Mol Med* 1977;53:329–34.
- Gruber KA, Whitaker JM, Buckalew VM Jr. Endogenous digitalis-like substance in plasma of volume-expanded dogs. *Nature* 1980;287:743–5.
- Poston L, Wilkinson SP, Sewell R, Williams R. Inhibition of leucocyte sodium transport during mineralocorticoid escape. *Clin Sci* 1980;58:9p.
- de Wardener HE, Clarkson EM, Bitensky L, MacGregor GA, Alaghband-Zadeh J, Chayen J. Effect of sodium intake on ability of human plasma to inhibit renal $\text{Na}^+\text{-K}^+\text{-adenosine triphosphatase}$ in vitro. *Lancet* 1981; i:411–2.
- Chayen J, Frost TB, Dodds RA, *et al*. The use of hidden metal-capture reagent for the measurement of $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity: a new concept in cytochemistry. *Histochemistry* (in press).
- Dikstein S. Stimulability, adenosine triphosphatases and their control by cellular redox processes. *Naturwissenschaften* 1971;58:439–43.
- Chambers DJ, Dunham J, Zanelli JM, Parsons JA, Bitensky L, Chayen J. A sensitive bioassay of PTH in plasma. *Clin Endocrinol* 1978;9:375–9.
- Fenton S, Somers S, Heath DA. Preliminary studies with the sensitive cytochemical assay for PTH. *Clin Endocrinol* 1978;9:381–4.
- George CF, Lewis PJ, Petrie A. Clinical experience with use of ultrasound sphygmomanometer. *Br Heart J* 1975;37:804–7.
- Roulston JE, MacGregor GA. Measurement of plasma renin activity by radioimmunoassay after prolonged cold storage. *Clin Chim Acta* 1978; 88:45–8.
- Alaghband-Zadeh J, Fenton S, Clarkson EM, MacGregor GA, de Wardener HE. Preliminary studies for the possible detection in plasma of the small molecular weight urinary natriuretic material. In: Lichardus B, Schrier RW, Ponc J. *Hormonal regulation of sodium excretion*. Amsterdam: Elsevier, 1980:341–8.
- Blaustein MP. Sodium ions, calcium ions, blood pressure regulation and hypertension: a reassessment and a hypothesis. *Am J Physiol* 1977; 232:C165–73.
- Poston L, Sewell RB, Wilkinson SP, *et al*. Evidence for a circulating sodium transport inhibitor in essential hypertension. *Br Med J* 1981;282:847–9.
- Edmondson RPS, MacGregor GA. Leucocyte cation transport in essential hypertension: its relation to the renin-angiotensin system. *Br Med J* 1981;282:1267–9.
- Churchill PC. Possible mechanism of the inhibitory effect of ouabain on renin secretion from rat renal cortical slices. *J Physiol* 1979;294:123–34.
- Shock NW, Davies DF. Age changes in glomerular filtration rate, effective renal plasma flow and tubular secretory capacity in adult males. *J Clin Invest* 1950;29:496–507.
- Miall WE, Lovell HG. Relationship between change of blood pressure and age. *Br Med J* 1967;ii:660–4.

(Accepted 12 August 1981)