PAPERS AND SHORT REPORTS

Reversal by verapamil of defect in sodium transport in leucocytes in essential hypertension

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Abstract

The effect of treatment with verapamil on cell sodium transport was studied in the leucocytes of patients with essential hypertension. Previously described abnormalities of sodium efflux rate constant and intracellular sodium content were confirmed, the component of the sodium efflux rate constant sensitive to ouabain being lower and the intracellular sodium content higher in the patients compared with controls. Verapamil reversed these abnormalities and reduced blood pressure.

Introduction

Widespread but conflicting reports have been made of abnormalities of cell sodium transport in patients with essential hypertension. These defects have included abnormalities of the sodium pump, of sodium and potassium cotransport, and of sodium and lithium countertransport. In essential hypertension the sodium content of leucocytes is generally agreed to be high¹⁻³ as a result of reduced activity of the sodium pump.^{1-3,4} If the composition of leucocyte cations is representative of that of other cells, raised intracellular sodium contents might also be found in vascular smooth muscle in essential hypertension.

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This might lead to increased contractility and peripheral resistance by reducing the efflux of calcium, which is linked to the influx of sodium, with a consequent increase in cell calcium content.⁵ Because of this possible link between intracellular sodium and calcium and essential hypertension we investigated the transport of sodium and the electrolyte content in leucocytes from patients with essential hypertension before and during treatment with the calcium channel blocker verapamil.

Patients and methods

We studied 18 patients with essential hypertension (eight women, 10 men, 14 of whom were white; mean age 53 (range 24-67)) and 17 controls (six women, 11 men, of whom all were white; mean age 39 (range 19-65); mean blood pressure 124/79 mm Hg). Blood pressure was measured with either an Arteriosonde or a Riester sphygmomanometer and recorded as the mean of at least three readings. Fourteen of the patients had not received any treatment before the study. Of the remaining four, all of whom had stopped treatment more than one month before the study, two had been receiving thiazide diuretics. Patients underwent tests before and after receiving verapamil for four to 11 weeks at a mean daily dose of 400 (range 240-480) mg.

Leucocytes were isolated from heparinised whole blood (100 ml) and the intracellular sodium content, sodium influx rate constant, and sodium efflux rate constant estimated by the method of Hilton and Patrick.⁶ Briefly, erythrocytes were sedimented with dextran and leucocytes isolated from the supernatant. Residual erythrocytes were removed by hypotonic lysis and the leucocytes then incubated for 20 minutes with sodium-22 at 37°C in Earle's buffer (pH 7.4). After being washed the cells were resuspended in buffer with and without ouabain (final concentration 10 µmol/l). Aliquots were removed at timed intervals over 14 minutes, centrifuged at 0°C, and counted for residual radioactivity. The protein content of each sample was measured by the method of Lowry⁷ and the logarithm of radioactivity/mg protein plotted against time. The sodium efflux rate constant was calculated as the slope of the linear regression. The component sensitive to ouabain was taken as the difference in rate constants in the presence and absence of ouabain.

Sodium influx into leucocytes was estimated from the uptake of sodium-22 over eight minutes with an appropriate correction for simultaneous efflux. The intracellular sodium content was calculated in the same sample after drying, weighing, and extraction in 1 M

nitric acid, the sodium being measured by flame photometry. Statistical analysis was by Student's *t* test for paired findings. Results are given as means (SEM).

Results

Treatment with verapamil produced a significant fall in blood pressure (mean blood pressure before treatment 174/108, after treatment 147 (4)/89 (2) mm Hg; $p < 0.001$). As described previously³ the component of the leucocyte sodium efflux rate constant sensitive to ouabain was lower and the intracellular sodium content higher in the patients with essential hypertension before treatment than in the controls (table). After treatment with verapamil for at least one month the sodium efflux rate constant sensitive to ouabain in the patients had increased and the leucocyte intracellular sodium content (figure) fallen to values not significantly different from those found in controls (table). Treatment also resulted in

Leucocyte sodium content in 14 patients with essential hypertension before and after treatment with verapamil. Vertical bars indicate mean (SEM) values.

Conversion: SI to traditional units—Sodium: 1 mmol = 1 mEq.

reduced influx of sodium into leucocytes. Weight was closely monitored in 10 of the 18 patients with hypertension but did not show any significant fall. When cell samples were too small to permit a full study priority was given to determining the sodium efflux rate constant.

Discussion

The finding that the sodium content of leucocytes is increased in patients with essential hypertension has led to the suggestion that, if a comparable change takes place in vascular smooth muscle cells, this might be responsible for the increased peripheral resistance seen in essential hypertension. This suggestion stems from the postulated link between the cell contents of sodium and calcium.⁵ Smooth muscle fibres are permeable to calcium, and a large electrochemical gradient for calcium favouring inward movement is normally maintained across their plasma membranes. They must, therefore, be capable of extruding calcium; part of this efflux of calcium ions may be in exchange for the sodium ions that are entering the cell down an electrochemical gradient. It is postulated that the net effect of an increased intracellular sodium content is to reduce that part of calcium efflux that is linked to sodium influx, thus leading to a rise in the intracellular calcium content and a chronic increase in vascular tone. Although such a sodium-calcium exchange mechanism is present in cardiac muscle,⁸ it has yet to be shown in leucocytes or smooth muscle.

Calcium antagonists are increasingly being used in the management of essential hypertension and are thought to lower peripheral vascular resistance by reducing the free calcium concentration in smooth muscle cells. This effect may be achieved both by blocking the influx of calcium and by altering the internal distribution of calcium ions. There are several possible mechanisms whereby a fall in free cell calcium concentrations might reduce cell sodium content. Firstly, it has been observed in red cells that a raised intracellular calcium content directly affects the cell membrane by increasing its permeability to sodium.⁹ If the reverse is also true, lowering the cell calcium content should reduce the intracellular sodium content. Secondly, any reduction in internal calcium content will diminish the activity of the postulated calcium-sodium exchange mechanism, leading to a fall in influx of sodium. Thirdly, calcium ions inhibit the sodium pump.¹⁰ A reduced intracellular calcium content may therefore increase the sodium efflux rate constant, which would contribute to a reduction in leucocyte sodium content.

In this study treatment with verapamil was found to reverse the abnormality of sodium transport that occurs in essential hypertension. This reversal may be due to changes in cell calcium content as described. Alternatively, it may represent a direct and hitherto unrecognised primary action of the drug on the cell independent of any calcium blocking action.

The defect in leucocyte sodium transport is also reversed when patients with hypertension are treated with thiazide diuretics, but not when they are treated with other hypotensive agents.¹¹ It has been postulated that this represents an indirect action of the drug whereby it reduces blood volume and thereby produces a fall in the concentration of a circulating inhibitor of the sodium pump. We have not excluded such an effect of verapamil, but a diuretic action has not been reported, and weight loss was not evident in the patients studied.

Whether the effects of verapamil on the transport of sodium in cells are linked to the mechanism of its hypotensive action is

Mean (SEM) sodium influx and efflux rate constants, intracellular sodium content, and sodium influx in patients with essential hypertension before and during treatment with verapamil

	Controls (n = 17)	n	Patients		p
			Before treatment	After treatment	
Total sodium efflux rate constant (/hour)	3.66 (0.07)	18	3.32 (0.12)	3.70 (0.11)	<0.05
Ouabain sensitive sodium efflux rate constant (/hour)	3.10 (0.08)	18	2.77 (0.11)	3.13 (0.12)	<0.002
Ouabain insensitive sodium efflux rate constant (/hour)	0.56 (0.06)	18	0.55 (0.06)	0.57 (0.07)	NS
Intracellular sodium content (mmol/kg cell dry weight)	50 (4)	14	61 (3)	48 (2)	<0.001
Sodium influx (mmol/kg cell dry weight)	182 (17)	13	208 (13)	158 (10)	<0.005

Conversion: SI to traditional units—Sodium: 1 mmol = 1 mEq.

not clear, but current in vitro investigations into the effect of the drug on leucocytes may help to distinguish between a direct and indirect mode of action. With sodium increasingly being implicated in the genesis of hypertension and the undoubted role of calcium in the control of peripheral vascular resistance the reduction of cell sodium content by a calcium antagonist is of considerable interest. It remains to be seen whether other calcium antagonists have similar effects.

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Malabsorption of protein bound vitamin B₁₂

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Abstract

Patients with subnormal serum vitamin B₁₂ concentrations were tested for absorption of protein bound vitamin B₁₂ and compared with controls. Absorption of the protein bound vitamin appeared to decrease with increasing age in healthy subjects. Differences between the result of this test and the result of the Schilling test in patients who had undergone gastric surgery were confirmed; such differences were also seen in some patients who had iron deficiency anaemia, an excessive alcohol intake, or folate deficiency. Defective absorption was also found in six patients with an adequate dietary intake of vitamin B₁₂, normal Schilling test results, low serum vitamin concentrations, and tissue changes responding to treatment with vitamin B₁₂.

Malabsorption of the vitamin from protein bound sources, which is not detected by the Schilling test, may produce vitamin B₁₂ deficiency of clinical importance.

Introduction

One of the standard procedures used to investigate a low serum concentration of vitamin B₁₂ (cobalamin) is the Schilling test. Introduced in 1953, this measures ability to absorb cobalamin from an aqueous solution.¹ In more recent years, however, it has been suggested that in certain cases there is a lack of correlation between the results of the Schilling test and the cobalamin state of the patient. For example, after partial gastrectomy true tissue cobalamin deficiency may develop despite a normal Schilling test result.² In such instances it has been proposed that there is malabsorption of the vitamin that is not shown by the test. To show this malabsorption several tests of absorption of protein bound cobalamin (which are claimed to approximate more closely to the physiological process) have been used in small numbers of patients.³⁻⁵ In one such test the vitamin is bound to chicken serum.

The aim of the present study was to assess the absorption of

protein bound cobalamin in patients with low serum cobalamin concentrations. The test was performed on a control group of volunteers and on patients suffering from a variety of conditions. All but three patients had low serum concentrations of cobalamin.

Methods and subjects

LABORATORY TECHNIQUES

Blood counts were done with a Coulter S-Plus. Serum cobalamin concentrations were determined by radioisotope assay with the technique of saturation analysis; pure human intrinsic factor served as the binder (reference range 170-900 ng/l). This method has been used for 10 years in the haematology department of this hospital and has been validated by comparison with euglena assay. With this assay nearly all patients with pernicious anaemia show serum concentrations below 50 ng/l. Serum and erythrocyte folate concentrations were measured microbiologically with *Lactobacillus casei* (reference ranges: serum folate 3-12 µg/l, erythrocyte folate 160-600 µg/l). In selected patients gastric juice collected over one hour was analysed for acid and intrinsic factor after stimulation with pentagastrin. Serum gastrin concentrations were assayed with the radioimmunoassay Gastrin-17 kit (reference range 50-250 ng/l) as an indirect measurement of gastric acidity.

The test of absorption of protein bound cobalamin was done with cyanocobalamin, labelled with cobalt-57, bound to chicken serum (Tissue Culture Services, Slough). Individual doses were prepared by mixing 4 ml sterile chicken serum with the standard Schilling test dose of 1 µg (0.037 MBq; 1 µCi) cobalamin in aqueous solution (30 ml). Total and rapid binding of the vitamin was shown in sample doses by the addition of albumin coated charcoal and centrifugation; there was no loss of isotope counts from the supernatant. The chicken serum itself contained only a very small amount of cobalamin. The oral dose of protein bound vitamin was given together with an intramuscular injection of 1 mg unlabelled cobalamin, and urinary excretion of the labelled vitamin was measured over the next 24 hours. The isotope excreted in 4 ml aliquots of urine was counted for 100 minutes in a Beckman Biogamma counter. The 95% confidence limits at an excretion level of 0.3% were ±0.044%.

Schilling tests were also performed. Urinary excretion of the isotope over 24 hours (normal 10% or more) was measured. The 95% confidence limits at an excretion level of 10% were ±1.2%.

SUBJECTS

We studied 49 subjects. Fifteen served as controls, and the remainder comprised six patients with pernicious anaemia, six who had undergone gastrectomy, four with chronic iron deficiency anaemia, eight with folate deficiency, four alcohol abusers, and six others.

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