The African Journal of Medicine and Medical Sciences

Volume 17
1988

BLACKWELL SCIENTIFIC PUBLICATIONS
Oxford London Edinburgh Boston Palo Alto Melbourne

Afr. J. Med. med. Sci. (1988) 17, 231–235.

Plasma levels of digitalis-like substance in Nigerians with essential hypertension

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Summary

A plasma Na^+-K^+ ATPase inhibitor, which is estimated by a technique in which it competes with ouabain for binding on red cells, was measured in three groups of individuals: (a) normotensive subjects without a family history of hypertension, (b) normotensive subjects with a family history of hypertension, (c) untreated essential hypertensive subjects. The mean value of the inhibitor in group (b) subjects was significantly higher than the mean value in group (a). The mean value in group (c) subjects was also significantly higher than in group (a) subjects. However, the means of the values in groups (b) and (c) were not significantly different. There was a significant positive correlation between the levels of the inhibitor and the urinary Na^+ excretion in all subjects. However, there was no correlation between the inhibitor levels and mean arterial pressure. The relevance of these results to the pathophysiology of hypertension in the black African subject is discussed.

Résumé

L'activité d'un inhibiteur de triphosphatase d'adénosine sodique et potassique dans le plasma sanguin a été estimé selon une technique mesurant l'affinité de bondage compétitif sur les corpuscules rouges entre l'inhibiteur et l'ouabaine, chez trois groupes d'individus qui incluent: (a) Les gens normotensifs sans histoire familiale d'hypertension arterielle, (b) Les gens normotensifs avec histoire familiale d'hypertension, (c) Les gens d'hypertension. Les taux moyens d'inhibiteur dans le groupe (b) et le groupe (c) sont significativement supérieurs à celui du groupe (a). Néanmoins il n'y a pas de différence signifiante entre le groupe (b) et le groupe (c). Il y a une signifiante correlation positive entre les taux d'inhibiteur et de natrium urinaire dans tous les groupes. Cependant il n'y a pas de correlation entre les taux d'inhibiteur et la tension artérielle moyenne. L'implication de ces résultats sur la pathophysiologie de l'hypertension artérielle chez les Africains noirs est discuté.

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Introduction

A plasma Na^+-K^+ ATPase inhibitor (i.e. putative natriuretic factor) has been suggested to be of consequence in the pathogenesis of hypertension [1–3]. Physiological studies in experimental animals have demonstrated that the serum levels of the factor respond briskly to changes in extracellular volume [4,5], and studies in patients with pathological expansion of plasma volume, e.g. acromegaly, have confirmed its elevation [6]. Since the suggestion in previous studies is that volume factors may play a significant role in the pathogenesis of essential hypertension in the black subject [7–9], we decided to study Na^+-K^+ ATPase activity in our patients in an attempt to provide the much needed information for further evaluation of this initial impression. We believe that this effort may assist in the understanding of the biology of this important disease in this environment.

Patients and methods

Fifty-two subjects were recruited into the study after careful clinical and biochemical assessment. These comprised three groups.

(a) Twenty control subjects without a family history of hypertension. There were 12 males and eight females in this subgroup, with an age range of 19–68 years and a mean of 43 ± 12 years (s.d.). The mean arterial pressure (MAP) ranged from 70 to 93.3 with a mean of 83.3 ± 6 (s.d.).

(b) Thirteen control subjects with a definite family history of hypertension, i.e. at least one parent had been medically attended on account of essential hypertension. There were seven males and six females in this subgroup, with an age range of 22–71 years and a mean of 45 ± 7 years (s.d.). The MAP ranged from 76.3 to 100 with a mean of 86.6 ± 11 (s.d.).

(c) Nineteen subjects who presented with essential hypertension. This subgroup consisted of 10 males and nine females, with an age of 23–75 years and a mean of 43.5 ± 12 years (s.d.). The MAP ranged from 110 to 160 with a mean of 133.3 ± 10 (s.d.).

None of our subjects had: (i) a serum creatinine value higher than 0.9 mg/100 ml, (ii) obesity as adjudged by their heights and weights, (iii) exposure to any form of medication.

Blood pressure measurements

The blood pressure was measured in the supine position after adequate rest taking the fifth phase of the Koroktoff sound as the diastolic pressure. All blood pressures were recorded from the right arm by the same observer (B.O.).

Preparation of plasma samples

Venous blood was collected with heparin as an anticoagulant in ice-chilled tubes. The samples were centrifuged initially at 3000 × *g* to separate the plasma. The red cells and buffy coat were discarded and the plasma boiled immediately in a water-bath for 15 min. The protein coagulum was broken into small fragments and the mixture was separated and stored at −80°C for processing.

^{3}H-Ouabain binding to erythrocytes

The binding experiments were performed according to the methods described by Devynck *et al.* [3]. Fresh red blood cells, obtained from subjects who did not have a family history of hypertension, were washed using a Tris-HCl buffer and made up to an haematocrit of between 1% and 3%. The cells were then incubated for 5 h at 37°C in the presence of five different concentrations of ^{3}H-ouabain (ranging from 2×10^{-9} to 2.5×10^{-8} M). Non-specific (non-saturable) binding was also assessed by running parallel assays in the presence of excess unlabelled ouabain (10^{-4} M).

The differential binding in the absence and presence of excess cold ouabain was taken as specific binding. Bound radioactivity was separated from free radioactivity on a Millipore filtration system using Whatman GFC filters. The filters were subsequently dried and the radioactivity counted.

Binding parameters, apparent affinity and number of binding sites were determined by computer analysis of Scatchard plots. In studying our subjects, binding experiments were carried out in the presence of plasma extracts and the K_D values were derived. The ability of the plasma extracts to compete with ouabain was quantified by the K_D^1/K_D ratio where K_D^1 and K_D represent the apparent affinities of ouabain for the sites in the presence and absence of plasma, respectively.

Determination of electrolytes and creatinine

Serum and urinary Na^+ were estimated by the usual flame photometric methods, while serum bicarbonate and creatinine were by the standard autoanalyser methods. All the estimations had adequate internal and external controls.

Statistical analysis

This was performed using Student's *t*-test, and significance levels were checked for in standard statistical tables using 95% confidence limits.

Results

Even though there were obvious differences in the scatter of the K_D ratios in the three patient

groups, there was a great deal of overlap between one group and the other (Fig. 1). The values in group A subjects (controls without family history) ranged from 0.52 to 1.28 with a mean value of 1.03 ± 0.19 (s.d.), while the values in group B (controls with family history) ranged from 1.00 to 1.60 with a mean of 1.32 ± 0.17 s.d. The values in group B subjects were significantly higher than those in group A individuals ($P < 0.001$). The K_D values in group C subjects were even higher than in the two previous groups. The values ranged from 1.08 to 2.65 with a mean of 1.63 ± 0.45 (s.d.). This mean was significantly higher than the mean of the group A ratios ($P < 0.001$). When compared with the mean of the group B subjects, there was a slight difference but not as remarkable as the difference between groups A and C.

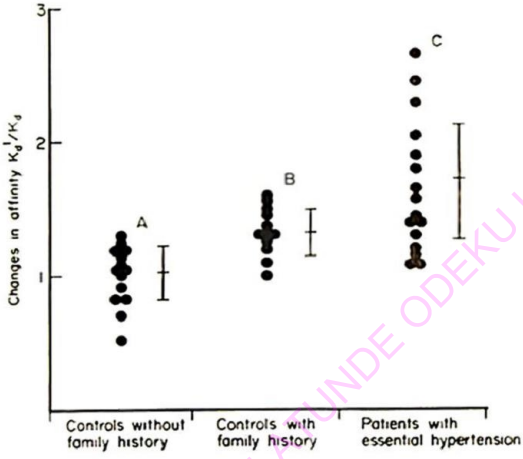

Fig. 1. Scattergram showing the K_D ratios in the three groups studied. (A) controls without family history, (B) controls with family history, (C) patients with essential hypertension.

Thus it would appear that the values in the hypertensives and the controls with a family history are continuous (i.e. like they are derived from the same population) whereas the values from the subjects without family history are discrete and separate.

There was a positive correlation between 24-h urinary Na (UNa) and K_D ratios in all the three groups of subjects (group A: $r = 0.71$, $P < 0.05$; group B: $r = 0.73$, $P < 0.05$; group C: $r = 0.69$, $P < 0.05$).

Discussion

We have attempted to employ the new tool of indirect measurements of Na^+-K^+-ATPase inhibitor to enable us to elucidate the role of volume expansion in the pathophysiology of high blood pressure in the African black subject.

Previous physiological and pharmacological studies seem to suggest that the volume component is important in the pathology of hypertension in the black subject as evidenced by: (i) a high prevalence of low plasma-renin activity in most studies in black subjects [7,10]; (ii) the ineffectiveness of beta-blockers and the seeming need for a natriuretic agent for effective antihypertensive therapy [11–13]; (iii) the racial differences that have been demonstrated in plasma volume measurements in comparative studies [8,14]; and (iv) the peculiarities of Na^+-K^+ exchange across cell membranes in *in-vitro* studies [15–17].

In contrast to these observations, there are studies that suggest that the basic differences in pathophysiology might relate essentially to the differences in the intake of Na^+ and K^+ since the urinary Na^+/K^+ ratio in black subjects is usually larger than in Caucasian subjects [18, 19]. This observation supports the viewpoint of Sever *et al.* [20] that the difference in the prevalence of blood pressure is related to socio-economic conditions, since food items containing K^+ are usually more expensive and blacks in the areas studied belong to the low socio-economic groups.

In our previous studies, we have also shown that most of our hypertensive patients belong to the low renin subset [21]. However, in our plasma volume studies, the majority of our patients had contracted plasma volume compared with controls [22]. These results, we felt, were not consistent, so we decided to investigate the volume component using another tool — the assessment of a circulating Na^+-K^+ ATPase inhibitor — since this has been demonstrated to be a sensitive index of the blood volume. Significant increases of the inhibitor factor have been reported in response to acute plasma volume expansion in both man and experimental animals [4,5]. Clinical studies of patients with acromegaly who have demonstrably elevated plasma volumes have shown a significant decrease in the inhibitory factor after

effective treatment of the primary disease [6].

The results of our present studies indicate that the inhibitor values in our hypertensive subjects are slightly higher than in the two control group of subjects, but there is a great deal of overlap in the scatter of the values. Indeed, 50% of the hypertensive patients had values within the same range as the controls. The probable implication of this observation is that the volume component of the disease is not predominant in most of our patients after all. However, there is a suggestion that it might be contributory in some of them.

We attempted to relate inhibitor factor values to the chronicity of illness. This is in response to a previous postulate, which suggests that in the early phases of hypertensive disease there is probably an increase in plasma volume that gradually contracts as the disease becomes established [23]. We encountered considerable difficulty with this as it is virtually impossible to put a definite time-point to the onset of hypertension. All the patients were recruited fresh on first consultation and that was when they were made aware of their disease. It might be possible at a later date to design animal model experiments to study the dynamics of the inhibitor factor in response to the progress of hypertension.

Our results are similar to those obtained in the same laboratories in Caucasian subjects [3]. They strengthen the evidence of those who argue that there are no racial differences in the plasma volume of hypertensives [24,25]. Furthermore, the study of Na^+-K^+ ATpase inhibitor by Macgregor *et al.* [1], using a different detection system, obtained similar results, showing that there were no differences between the blacks and the whites in their series. However, Gruber *et al.* [2,26] showed distinct racial differences in their studies.

We believe that our data are likely to be a true reflection of the pathophysiology of the disease in black subjects, as our patients denied a prior exposure to any form of medication that might affect the results of the binding studies. Thus, working on this premise, it would seem that an increase in plasma volume does not predominate in the sustenance of blood pressure in our hypertensive subjects. It is possible that it plays an important role in the initiation of disease but once it is established other mechanisms come into operation.

Acknowledgments

We acknowledge the support of the Nigerian Ministry of Science and Technology Lagos. B. Osotimehin was a recipient of a French Medical Fellowship.

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(*Accepted* 22 *September* 1987)