AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 20, NUMBER 2, JUNE 1991

BLACKWELL SCIENTIFIC PUBLICATIONS
Oxford London Edinburgh Boston Melbourne
Berlin Paris Vienna

ISSN 0189-2913

Afr. J. Med. med. Sci. (1991) 20, 75–82.

Erythrocyte membrane ouabain-sensitive Na^+,K^+-ATPase of hypertensive Nigerians

O. O. OLORUNSOGO, S. O. A. LAWAL*, A. O. FALASE* AND W. G. OKUNADE
*Laboratory for Biomembrane Research, Department of Biochemistry and *Department of Medicine, College of Medicine, University of Ibadan, Ibadan, Nigeria*

Summary

An assessment of the ATPase functions of erythrocyte membrane of newly identified subjects having essential hypertension shows that Na^+,K^+-ATPase activity is higher in normal membranes than in membranes of individuals with essential hypertension. A study of the dependence of the enzyme on ATP in the presence of non-limiting concentrations of Na^+ (120 mM) and Mg^{2+} (3 mM) shows that the pump in the membranes of hypertensive individuals, like that of normal humans, is easily saturable by ATP (≥2 μM). Analysis of the results of kinetic studies on the enzyme, in the presence of 5 mM K^+, using the Hanes plot, reveals that, although the affinity (K_m) of the pump for ATP is unaffected in essential hypertension, its maximum velocity (V_{max}) is lower than in normal membranes.

Even though the reason for a reduced sodium pump function in essential hypertension is not yet clear, it may not be unconnected with the presence of an endogenous inhibitor or with genetic or diet-induced membrane defects, as previously proposed by other workers in this area of research.

Résumé

Une évaluation des fonctions ATPase des membranes de plasma erythrocyte de sujets nouvellement identifiés comme atteints d'une hypertension essentielle montre que l'activité spécifique de Na^+,K^+-ATPase est aussi plus élevée dans membranes normaux que dans les membranes d'individus hypertensifs. Une étude de la dépendance de l'enzyme sur l'ATP dans des concentrations non-limitantes de Na^+ (120 mM) et de Mg^{2+} (3 mM) montre que la pompe dans les membranes d'individus hypertensifs, comme celle des sujets normaux, est facilement saturable par l'ATP (≥2 μM). Une analyse des résultats obtenus des études cinétiques sur l'enzyme, en présence de 5 mM K^+, en se servant du plan Hanes, révèle que bien que l'affinité (K_m) de la pompe pour l'ATP soit intacte dans l'hypertension essentielle, sa vélocité optimale (V_{max}) est inférieure dans des membranes normaux.

Bien que la raison pour la fonction réduite de la pompe du sodium dans l'hypertension essentielle n'ait pas encore été établie, celle-ci peut être liée à la présence d'un dispositif de blocage endogène ou aux défauts de nature génétique du membrane ou provoqués par le régime comme elle a été proposée par d'autres chercheurs.

Correspondence: Dr O. O. Olorunsogo, Laboratory for Biomembrane Research, Department of Biochemistry, University of Ibadan, Ibadan, Nigeria.

Introduction

The Na^+,K^+-ATPase (EC.3.6.1.3.), a plasma membrane integral protein which reversibly couples the hydrolysis of ATP to a flow of Na^+ and K^+ against their electrochemical gradients, is responsible in all animal cells for the maintenance of several transmembrane ionic concentration gradients (recently reviewed [1]). Consequently, the ATPase plays a pivotal role in the regulation of a wide variety of physiological processes.

Numerous studies have, however, indicated that the sodium ion content of the erythrocytes of individuals with essential hypertension is higher than in normal and healthy persons [2–4]. In particular, Aderounmu *et al.* [5] have

shown that erythrocyte sodium ion content is elevated in Nigerians having essential hypertension. Furthermore, an abnormal transport of Na^+ and K^+ ions across the membrane of various tissues has been reported in hypertensive patients and in hypertensive animal models [6,7]. Clearly, sodium plays a role in the pathogenesis of essential hypertension.

Based on this notion, a circulating digitalis-like compound and/or a natriuretic hormone in blood has been suggested to be responsible for inhibiting the sodium pump function in patients with essential hypertension [8–13]. Several other possibilities, such as a generalized membrane defect, as well as specific membrane defects probably involving the sodium pump and the sodium-potassium co-transport, are being investigated [14–16]. Specifically, Meyer *et al.* [17] have demonstrated the presence of an inhibitor called endaline in about one-third of patients with sustained and moderate hypertension. Furthermore, a hypothalamic inhibitory factor (HIF) which inhibits the sodium pump in nanomolar amounts has been established in essential hypertension [18,19].

Although a plasma membrane Na^+,K^+-ATPase digitalis-like inhibitor has been demonstrated in the blood of Nigerians having essential hypertension [20], there is no information on the status of the sodium pump in this pathological condition in Blacks. The aim of this study was to fill that gap. In this report, we present evidence to show that the activity of the sodium pump is subnormal in individuals having essential hypertension. In particular, the maximum velocity of the enzyme is significantly lower in hypertensive individuals than in normal individuals. These differences may be attributed either to the presence of an endogenous inhibitor or to a generalized or specific membrane defect, probably of a genetic or dietary origin.

Materials and methods

All reagents were of the highest purity available. ATP (vanadium free), ethyleneglycol-bis-(2-aminoethyl ether) N,N,N^1,N^1-tetraacetic acid (EGTA), 4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid (HEPES) and fatty-acid-free bovine serum albumin were purchased from Sigma Chemical Co., St Louis, MO, U.S.A.

Preparation of haemoglobin-free erythrocyte ghost membranes

Blood samples were obtained from 25 individuals who were newly identified as having essential hypertension. The subjects were not receiving any medication or dietary therapy at the time of collection of blood. A set of 25 normotensive individuals who have no family history of hypertension were drawn from healthy volunteers. Haemoglobin-free erythrocyte plasma membranes were prepared by haemolysis in 1 mM EDTA, as previously described by Jarret & Penniston [21]. Packed red cells, obtained from whole blood samples by centrifugation at 3900 g_{max} for 5 min, were washed three times in five volumes of an isotonic solution containing 130 mM KCl, 20 mM Hepes (pH 7.4) by centrifuging at 5000 g_{max} for 5 min. The supernatant and a whitish-grey layer of white cells on top of the pellet were removed by aspiration. The washed red cells were later haemolysed in 5 volumes of 1 mM EDTA (Na salt) and 10 mM Tris-HCl (pH 7.4), and centrifuged at 18,000 g_{max} for 10 min to collect the membrane fraction. To prevent excessive resuspension of the membrane pellet at the end of each centrifugation step, centrifugation was hereafter performed with the centrifuge brake turned off. The membrane fraction obtained was washed three more times with at least 20 volumes of the lysing buffer in order to remove haemoglobin, calmodulin and other proteins. The white ghost membranes obtained after washing the lysed cells with at least 10 volumes of 10 mM Hepes (pH 7.4) were collected by centrifugation at 18,000 g_{max} for 10 min, finally resuspended in 100 mM NaCl, 25 mM KCl, 50 mM Hepes (pH 7.4), 500 μM $MgCl_2$ and 1 mM EGTA and stored at −40°C or used immediately. Addition of 25 mM KCl was omitted in some preparations.

Estimation of membrane protein

Membrane protein concentration was determined according to a modification of the method of Lowry *et al.* [22]. Membrane preparations were treated with 0.05% (w/v) deoxycholic acid and then precipitated with 10% TCA (final concentration) at room temperature. Fatty-acid-free bovine serum albumin was used as a standard.

Assay of Na^+, K^+-ATPase

Activity was determined by a slight modification of the method of Jones *et al.* [23]. Ouabain-sensitive Na^+,K^+-ATPase activity was determined in a reaction medium containing, in final concentrations, 50 mM Hepes, pH 7.5, 3 mM $MgCl_2$, 1 mM EGTA, 120 mM NaCl, 25 mM KCl, 20 mg membrane protein per ml and 3 mM Na_2 ATP, with or without ouabain (1 mM). The inorganic phosphate liberated on the hydrolysis of the γ-phosphodiester bond of ATP was estimated by the method of Stewart [24]. The activity that was inhibitable by 1 mM ouabain was taken as the Na^+,K^+-ATPase activity and was expressed as μmol inorganic phosphate liberated per mg membrane protein per h.

Determination of $(Ca^{2+}+Mg^{2+})$-ATPase and Mg^{2+}-ATPase activities

$(Ca^{2+}+Mg^{2+})$-ATPase was assayed by following the rate of release of inorganic phosphate from the γ-position of ATP as previously described [25]. The assay medium was made up of 120 mM KCl, 50 mM Hepes, pH 7.4, 5 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM EDTA and 50–100 μg erythrocyte membrane protein in a total volume of 800 μl. The mixture was pre-incubated for 5 min at 37°C with constant shaking, prior to the addition of ATP (2 mM, final concentration). The determination was run in duplicates with or without 120 nM calmodulin. The inorganic phosphate liberated was determined colorimetrically by standard procedure using ammonium molybdate and ascorbic acid. Blanks were run to correct for non-enzymic hydrolysis of ATP. Mg^{2+}-ATPase was assayed by the same protocol, except that 0.5 mM EGTA was added to the assay medium in place of $CaCl_2$. To obtain $(Ca^{2+}+Mg^{2+})$-ATPase activity, Mg^{2+}-ATPase activity was subtracted from the total activity observed in the absence of EGTA (i.e. in the presence of calcium). Activity was expressed as μmol inorganic phosphate liberated per mg membrane protein per h.

Results

Haemoglobin-deficient and calmodulin-free erythrocyte membranes were isolated from 25 hypertensive subjects and 25 healthy individuals who have no family history of essential hypertension. Table 1 summarizes the results obtained by a determination of the activities of some ATPase functions associated with the red cell membranes. Our data show that Mg^{2+}-ATPase activity of hypertensive membrane is about 1½ times higher than that of normal erythrocyte membranes. The reason for this is obscure as no known function has been ascribed to this ATPase action. Furthermore, the basal $(Ca^{2+}+Mg^{2+})$-ATPase activity of these membranes is identical in control and in hypertensive individuals, but the extent of stimulation by calmodulin, its endogenous positive modulator, is higher in normal membranes than in membranes of hypertensive individuals. These data are in agreement with previous reports from our laboratory and from other workers [26,27].

The Na^+,K^+-ATPase activity of the erythro-

Table 1. Erythrocyte plasma membrane ATPase activity of hypertensive humans

Enzyme	Membrane ATPase activity (μmol Pi mg protein^{-1} h^{-1})	
	Normal	Hypertensive
Mg^{2+}-ATPase	0.31 ± 0.07	0.50 ± 0.12
$(Ca^{2+}+Mg^{2+})$-ATPase		
Basal	0.73 ± 0.06	0.69 ± 0.07
Calmodulin-stimulated	2.34 ± 0.31	1.12 ± 0.11
	(3.2)*	(1.6)*
Na^+,K^+-ATPase	0.96 ± 0.07	0.76 ± 0.08

Values are means ± standard deviation for at least eight different experiments.
*Stimulation factor by calmodulin.

cyte plasma membranes of normal and hypertensive individuals is also shown in Table 1. On the whole, the activity of the pump in the membranes of hypertensive individuals is at least 20% lower than that of normal membranes, although the activity of the pump in three and two other hypertensive subjects was about 30 and 35%, lower, respectively, than the average activity obtained for normal membranes (results not shown). Surprisingly, the pump activity of the membrane of three other hypertensive individuals falls within the values obtained for normal membranes.

Figure 1 shows the ATP-dependence of the erythrocyte membrane Na^+,K^+-ATPase of healthy and hypertensive individuals in the presence of non-limiting concentrations of Na^+ (120 mM) and Mg^{2+} (3 mM), with or without 5 mM K^+. The results show that the pump of membranes isolated from healthy individuals is easily saturable, in the absence of K^+, by ATP concentrations ≥ 2 μM. The pump protein thus exhibits, in the absence of K^+, the Michaelis–Menten profile that is characterized in this case by a high apparent affinity for ATP as well as a low maximum velocity, even in the presence of highly saturating concentrations of ATP. The profile seen for the pump protein of the erythrocyte membranes of hypertensive individuals does not differ as such from that of healthy individuals. These results suggest that the affinities of the enzyme for Na^+ and ATP do not deviate from those seen in the erythrocyte plasma membranes of healthy individuals.

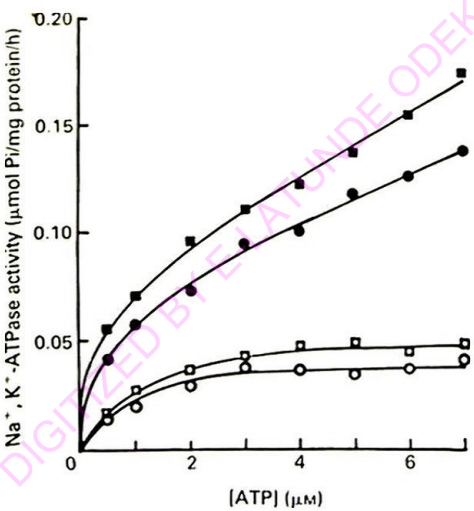

Fig. 1. ATP-dependence of erythrocyte plasma membrane Na^+,K^+-ATPase of healthy (squares) and hypertensive (circles) individuals. The ATPase was assayed in the absence (open symbols) and presence (filled symbols) of 5 mM KCl.

The sensitivity of the erythrocyte plasma membrane Na^+,K^+-ATPase of hypertensive and control humans to 5 mM K^+ is also shown in Fig. 1. The results indicate that the inclusion of 5 mM K^+ in the reaction medium increases the activity of the pump several-fold. In particular, the profile of the plot of ATPase activity against ATP concentration in the presence of 5 mM K^+ and non-limiting concentrations of Na^+ and Mg^{2+} does not follow a rectangular hyperbola as should be expected for a Michaelis type of reaction. This profile however, suggests that the mechanism of catalysis by the sodium pump is quite different from that of a one-substrate-one-enzyme reaction. Clearly, the results demonstrate that the sensitivity of the pump protein to 5 mM K^+ is lower in the membranes of hypertensive individuals than in healthy individuals, over a wide range of ATP concentrations. Consequently, the apparent affinity for ATP and the maximum velocity of the enzyme of the membranes of hypertensive individuals, in the presence of 5 mM K^+ and non-limiting concentrations of Na^+ and Mg^{2+}, may differ significantly from those of membranes of normal subjects.

Figure 2 is the Hanes plot for the Na^+,K^+-ATPase activity of the erythrocyte plasma membranes of control and hypertensive individuals at 5 mM K^+ under conditions of non-limiting concentrations of Na^+ and Mg^{2+} ions and in the presence of high concentrations of ATP. The intercept of the lines on the ordinate is the ratio K_m/V_{max}, while the slope of each line is $1/V_{max}$. It is thus possible from this type of plot to determine the apparent K_m for ATP and the maximum velocity of the sodium pump in the membranes of normal and hypertensive individuals.

Table 2 summarizes the values for the apparent affinity of the enzyme for ATP and its maximum velocity based on the Hanes plot represented in Fig. 2. The data show that the affinity (K_m) of the pump protein for ATP in the presence of 5 mM K^+ and non-limiting amounts of Na^+ and Mg^{2+} is unaffected in

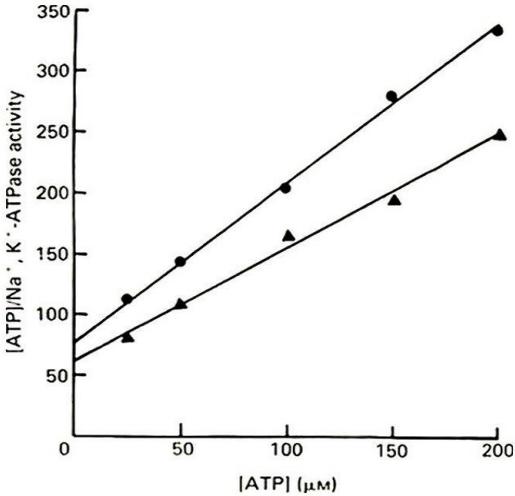

Fig. 2. Single reciprocal or Hanes plot for erythrocyte plasma membrane Na^+,K^+-ATPase of healthy (triangles) and hypertensive (circles) individuals. Assay was run in the presence of non-limiting concentration of Na^+, Mg^{2+} and K^+.

Discussion

Several studies in the area of Na^+ metabolism in essential hypertension have indicated that an excess of Na^+ intake is a major environmental factor contributing to the onset of increased peripheral arterial tone and arterial vascular resistance in humans. In erythrocytes, the transport systems involved in the regulation of intracellular Na^+ concentration include the ouabain-sensitive sodium pump, the furosemide (or bumetamide)-sensitive Na^+,K^+-co-transport system, which catalyses the inward and outward movement of Na^+ and K^+, and the Na^+ countertransport which catalyses a one-to-one exchange of internal Na^+ for external Na^+. Of the three systems, the sodium pump is the most widely studied and the best understood [28].

Although opinions differ as to what is the likely nature of the defect in sodium transport during essential hypertension, the sodium pump function remains the target for investigation, whether or not a generalized or specific

Table 2. Some kinetic parameters of the erythrocyte plasma membrane Na^+,K^+-ATPase of hypertensive individuals

Membranes	V_{max} (μmol Pi mg protein^{-1} h^{-1})	K_m (μmol ATP)
Normal	0.95 ± 0.06	59.2 ± 4.5
Hypertensive	0.75 ±0.10	57.5 ± 5.2
	($P < 0.02$)	NS

Each value is a mean of 15 different estimations ± standard deviation.
Significance was determined by Student's *t*-test.
NS = not significant.

essential hypertension, although in one hypertensive individual the K_m for ATP is about 25% lower than the mean value obtained for membranes of healthy individuals. However, the maximum velocity of the pump protein is about 25% higher in membranes of normal individuals than in the membranes of hypertensive humans. Furthermore, the V_{max} of the pump is not significantly different in the erythrocyte plasma membranes of two hypertensive individuals when compared with the values obtained for normal membranes.

membrane defect occurs in this pathological state. In this study, an assessment of the Na^+, K^+-ATPase of the erythrocyte plasma membrane of Nigerians having essential hypertension shows that this ATPase activity is lower than in healthy individuals (Table 1). It seems likely that a reduced Na^+,K^+-ATPase activity could be due to an inhibition of the pump by an endogenous compound that has been sequestered into the membrane, especially if the compound is lipid in nature, as reported previously [17], and/or a generalized or specific

defect in the membrane involving the nature of the bilayer [14]. The former possibility, however, seems more likely because of the demonstration of a digitalis-like inhibitor of the sodium pump in the blood of Nigerian hypertensives [20].

In order to gain insight into the differences or similarities between the kinetic properties of the pump in health and in essential hypertension, the membrane-bound enzyme was assayed under conditions of limiting concentrations of ATP (≤10 μM) and non-limiting amounts of Na^+ and Mg^{2+} because the enzyme exhibits different affinities for ATP when exposed to Na^+ or K^+ [29]. Our results show that the enzyme in the membranes of normal and hypertepsive individuals is easily saturable by ATP (about 2 μM) in the absence of potassium (Fig. 1) thus indicating that there is probably no difference in the affinities of the enzymes for Na^+ and ATP under these conditions, since the high-affinity binding site for ATP is only available when three sodium ions are bound on the cytoplasmic face of the enzyme [1].

In contrast, the sensitivity of the control enzyme to K^+ (5 mM) is quite different from that of the membranes of hypertensive individuals (Fig. 1). Results of several studies have shown that the low affinity sites for ATP are exposed only in the presence of K^+, which on binding brings about a dephosphorylation of the enzyme before K^+ can be transported inside [1]. It is not surprising, therefore, to find that the K_m (ATP) for the normal and hypertensive enzymes are identical in the presence of K^+ (5 mM), as this amount of the cation will be enough to cause the binding of ATP to its low affinity site (Fig. 1). The finding that the maximum velocity of the pump protein in hypertensive humans is lower than in normal membranes suggests that the ATPase of hypertensive membranes may require more potassium ions in order to attain the same maximum velocity as the normal enzyme. This observation is in favour of the possibility of an inhibitor whose inhibitory effect, like that of ouabain, may be abolished or minimized in the presence of an excess or high concentrations of K^+.

A number of alterations of the red cell membrane have, however, been reported in patients with essential hypertension [14]. Based on the observations that the purified enzyme requires certain acidic phospholipids for maximum activity [30] and that the activity of membrane-bound allosteric enzymes, of which the Na^+,K^+-ATPase is an example, are modified by changes in membrane lipid composition [31], it seems likely that membrane defects, probably of dietary or genetic origin, may be involved in the pathogenesis of essential hypertension. The finding by Cooper [32] that cholesterol enrichment of red cell membrane results in an inhibition of sodium-potassium cotransport, as well as the observation that a safflower seed diet increased membrane linoleic acid content [33], thus causing significant increase of sodium pump activity and a decrease in blood pressure, provide a direct evidence that an altered sodium pump activity could result from changes in membrane lipid composition in essential hypertension.

Thus, the observed decrease in apparent V_{max} of sodium pump in essential hypertension (Table 2) could be due either to changes in the composition of the annular lipid domain (phospholipids in the immediate environment) of this integral membrane protein or to an inhibition by, for example, a circulating digitalis-like factor, the natriuretic hormone endaline, and/or the hypothalamic inhibitor factor [13,17–20]. Although elevated intracellular free Ca^{2+} concentration in essential hypertension is frequently linked to an enhanced intracellular sodium level, reports of diminished stimulation of the erythrocyte membrane $(Ca^{2+}+Mg^{2+})$-ATPase by calmodulin [26,27] may not be unrelated to the same type of membrane defects that affect the sodium pump function in essential hypertension.

Therefore, for a thorough understanding of the exact mechanism of pump inhibition in essential hypertension there is a need to re-examine in great detail the structure and asymmetry of the lipid and protein components of the two halves of the erythrocyte bilayer in essential hypertension. It is only after this that an assessment of the various alternatives so far proposed for the involvement of sodium transport in the pathogenesis of essential hypertension may become meaningful.

References

1. Skou JC. The Na^+,K^+-pump — Overview. Methods Enzymol 1988;156:1–28.
2. Tobian L, Binion JT. Tissue cations and water in

arterial hypertension. Circulation 1952;5:754–8.
3. Lijnen P, M'buyamba-kabangu JR, Fagard RH, Groeseneken DR, Staessen JA, Amery AK. Intracellular concentration and transmembrane fluxes of sodium and potassium in erythrocytes of white normal male subjects with and without a family history of hypertension. J Hypert 1984; 2:25–30.
4. Gudmundsson O, Anderson O, Herlitz H *et al.* Blood pressure, intra-erythrocyte content, and transmembrane fluxes of sodium during normal and high salt intake in subjects with and without a family history of hypertension: evidence against a sodium transport inhibitor. J Cardiovasc Pharmacol 1984;6:S35–41.
5. Aderounmu FA, Salako LA. Abnormal cation composition and transport in erythrocytes from hypertensive patients. Eur J Clin Invest 1979;9: 369–75.
6. Jones AW. Altered ion transport in vascular smooth muscle from spontaneously hypertensive rats. Influence of aldosterone, norepinephrine, and angiotensin. Cir Res 1973;33:563–72.
7. Losse H, Zidek W, Vetter H. Intracellular sodium and calcium in vascular smooth muscle of spontaneously hypertensive rats. J Cardiovasc Pharmacol 1984;6:S32–4.
8. Blaustein MP, Ashida T, Goldman WF, Wier WG, Hamlyn JM. Sodium/calcium exchange in vascular smooth muscle: A link between sodium metabolism and hypertension. Ann N Y Acad Sci 1986;488:199–216.
9. Blaustein MP, Hamlyn JM. Role of natriuretic factor in essential hypertension. Ann Int Med 1983;98 (Suppl.):785–92.
10. Blaustein MP, Hamlyn JM. Sodium transport inhibition, cell calcium and hypertension. The natriuretic hormone — Na^+/Ca^{2+} exchange — hypertension hypothesis. Am J Med 1984;77:45–59.
11. Haddy FJ, Hamnani MB. The role of a humoral sodium-potassium pump inhibitor in low-renin hypertension. Fed Proc 1983;42:2673–80.
12. MacGregor GA, De-Wardener HE. A circulating sodium transport inhibitor and essential hypertension. J Cardiovasc Pharmacol 1984;6: S55–60.
13. De-Wardener HE, Clarkson EM. Concept of natriuretic hormone. Physiol Rev 1985;65:658–759.
14. Postnov VV, Onlov SN. Cell membrane alteration as a source of primary hypertension. J Hypert 1984;2:1–6.
15. Garay RP, Dagher G, Pernollet MG, Devynck MA, Meyer P. Inherited defect in Na^+,K^+ cotransport system in erythrocytes from essential hypertensive patients. Nature 1980;284: 281–3.
16. Garay R, Rosati C, Meyer P. Na^+ transport in primary hypertension. An N Y Acad Sci 1986; 488:187–95.
17. Cloix JF, Devynck MA, Meyer P. Chemical and clinical studies of endogenous digitalis-like factor in hypertension. Ann N Y Acad Sci 1986;488:217–27.
18. Haupert GT Jr, Carilli CT, Cantley LC. Hypothalamic sodium transport inhibitor is a high-affinity reversible inhibitor of Na^+-K^+-ATPase. Am J Physiol 1984;247:F919–24.
19. Carilli CT, Berne M, Cantley LC, Haupert GT Jr. Hypothalamic factor inhibits the (Na, K)-ATPase from the extracellular surface. Mechanism of inhibition. J Biol Chem 1985;260: 1027–31.
20. Osotimehin BO, Lawal SOA, Iyun AO *et al.* Plasma levels of digitalis-like substance in Nigerians with essential hypertension. Afr J Med Med Sci 1988;17:231–5.
21. Jarret HW, Penniston JT. Purification of the Ca^{2+}-stimulated ATPase activator from human erythrocytes: its membership in the class of Ca^{2+}-binding modulator proteins. J Biol Chem 1978;253:4076–682.
22. Lowry OH, Rosebrough NJ, Farr AD, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265–75.
23. Jones LR, Besch Jr HR, Flemming JW, McConnaugtey MM, Watanabe AM. Separation of vesicles of cardiac sarcolemma from vesicles of cardiac sarcoplasmic reticulum: comparative biochemical analysis of component activities. J Biol Chem 1979;254:530–9.
24. Stewart DJ. Sensitive automated methods for phosphate and Na^++K^+-ATPase. Anal Biochem 1974;62:349–64.
25. Olorunsogo OO, Villalobo A, Wang KKW, Roufogalis BD. The effect of calmodulin on the interaction of carbodimides with the purified human erythrocyte (Ca^{2+}+Mg^{2+})-ATPase. Biochim Biophys Acta 1988;945:33–40.
26. Olorunsogo OO, Okudolo BE, Lawal SOA, Falase AO. Erythrocyte membrane Ca^{2+} + pumping ATPase of hypertensive humans: reduced stimulation by calmodulin. Biosci Rep 1985;5:525–31.
27. Vezzoli G, Elli AA, Tripodi G, Bianchi G, Carafoli E. Calcium ATPase in erythrocytes of spontaneously hypertensive rats of the Milan strain. J Hypert 1985;3:645–8.
28. Fleischer S, Fleischer B. ATP-driven pumps and related transport: the Na^+,K^+-pump. Methods Enzymol 1988;156:1–611.
29. Jorgensen PL, Anderson JP. Structural basis for E-E conformation transitions in Na^+,K^+-pump and Ca-pump proteins. J Membr Biol 1988;103:95–120.

30. Roelofsen B, Van Deenen LIM. Lipid requirements of membrane bound ATPase studies on human erythrocyte ghosts. Eur J Biochem 1973;40:245–57.
31. Farias RN, Bloj B, Morero RD, Sineriz F, Trucco RE. Regulation of allosteric membrane-bound enzymes through changes in membrane lipid composition. Biochim Biophys Acta 1975;415:231–51.
32. Cooper RA. Abnormalities of cell membrane fluidity in the pathogenesis of disease. N Engl J Med 1977;297:371–7.
33. Heagerty AM, Ollerenshaw JO, Jackson J, Bing RF, Thurston H, Swaler JD. Linoleic acid, leucocyte sodium transport, free calcium and blood pressure. Br Heart J 1985;54:635–6.

(*Accepted* 17 *July* 1989)