International Journal of Applied
and Natural Sciences (IJANS)
ISSN(P): 2319-4014; ISSN(E): 2319-4022
Vol. 3, Issue 1, Jan 2014, 25-32

International Academy of Science,
Engineering and Technology
Connecting Researchers; Nurturing Innovations

NA^+/K^+-ATPASE ACTIVITIES IN NORMOTENSIVE HUMAN SUBJECTS WITH AND WITHOUT FAMILY HISTORY OF HYPERTENSION IN SOUTH-WEST NIGERIA

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ABSTRACT

It is well known that there is an important genetic contribution to the development of essential hypertension. This study aims at knowing the erythrocyte Na^+/K^+-ATPase activities in individuals with (+FH) and without family history of hypertension (-FH) and to investigate the possible action of erythrocyte sodium pump in the pathophysiology of hypertension. Erythrocyte Na^+/K^+-ATPase activities were studied among 99 normotensive students of college of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, aged between 16 and 30 years. Sixty four were –FH while 35 were +FH. Other parameters studied were body mass index (BMI), waist/hip ratio, systolic and diastolic blood pressure.Na^+/K^+-ATPase activities in subjects with family history of hypertension were significantly reduced compaired with those without family history of hypertension ($P<0.0001$). No significant differences were found in BMI, waist/hip ratio, mean systolic and mean diastolic blood pressure, but subjects with family history of hypertension had higher systolic and diastolic blood pressure.Na^+/K^+-ATPase activities in +FH and –FH are familial and may underlie membrane cation transport in these subjects.

KEYWORDS: Blood Pressure, Erythrocyte Na^+/K^+-ATPase, Essential Hypertension

INTRODUCTION

Hypertension is a global cardiovascular disease where treatment remains a major financial burden. Emerging evidence identifies hypertension as a major cause of morbidity and mortality globally including sub-saharan Africa (Cappucio et al., 2004; Olatubosun et al., 2000; Cooper et al., 1997). There are indications that the burden of non-communicable diseases (NCDs) such as hypertension is increasing in epidemic proportion in Africa. According to World Health Report (2002), NCDs accounted for 22% of the total deaths in the region in the year 2000; cardiovascular diseases alone accounted for 9.2% of the total deaths, killing even more than malaria (WHO, 2002).

The pathogenesis of essential hypertension is poorly understood, although accumulating evidence suggest that genetic and environmental factors are of important relevance (Waeber and Brunner, 2001). One of the factors involved in the development of essential hypertension is the alteration of cellular sodium metabolism. It has also been suggested that biochemical and biophysical abnormalities of cell membranes (Kisters et al., 2000) may actively participate in the pathogenesis of hypertension (Tsuda et al., 1997), and that such abnormalities seem to be involved not only in vascular smooth muscle cells, but also in circulating blood cells (Manish et al., 1987).

Some studies have identified important aetiologic factors while others on mechanisms focused on genetic factors and ATPase activities but the findings are inconclusive.Therefore, this study aimed at determining possible influence of

family history of hypertension on erythrocyte membrane sodium pump transport in normotensive human subjects with and without family history of hypertension.

METHODS

The study population was made up of LadokeAkintolaUniversity of Technology, (LAUTECH) Ogbomoso, Oyo State, Nigeria, with and without history of hypertension. Classification of volunteers was achieved by an initial distribution of questionnaires to very many of them. These questionnaires were collected back personally. In cases where there were doubt of the volunteers parent(s) being hypertensive, or when the volunteer was not sure of his or her parents status, such volunteers were asked to make telephone calls to confirm in my presence. In this study, history of first degree relatives (parents) were considered, and subjects were subsequently classified accordingly.

All blood pressure (BP) was measured with Accussons mercury sphygmomanometer at the left arm. Subjects whore BP were > 120/180mmHG were excluded from the study. All subjects were registered at the university health centre after their comprehensive medical examination. Their medical records were checked to ensure that none had DM, history of cardiovascular disorder, sickle cell disease, and history of usage of anti-hypertensive medication and glomerulonephritis or any other renal pathology. All the subjects used for the study were certified medically fit by LAUTECH University Health Centre.

Ethical Consideration: Full ethical approval was obtained from the Ethics and Research committee of the College of Health Sciences, LAUTECH, Ogbomoso. Informed consent was also obtained from each subject. Sample size – Ninety nine subjects were studied. These included subjects whose either or both parents are hypertensive. Sixty four subjects had negative family history of hypertension (termed –FH) and 35 had positive family history (+FH). Five of these +FH had both parents hypertensive. The sample population consisted of 47 males and 52 females.

Chemicals: Adenosine triphosphate (sodium salt), Trizma HCL, and Trichloroacetic acid were purchased from sigma chemicals company, St. Louis, U. S. A. All other reagents were of purest grades commercially available.

Blood Samples: The different venous blood samples of antecubital vein (4ml) were collected by venipuncture into lithium heparinized sterile bottles form healthy consenting volunteers at the college of health sciences, LAUTECH, Ogbomoso. The samples were stored at 4^oC and used within 24hours of collection.

Preparation of Erythrocyte Membrane: the blood sample was centrifuged at 5000g for 10minutes and the plasma and buffy coat removed. The resultant precipitate was washed three times with 0.15mM NaH_2 Po_4 $2H_2O$ (pH 7.7). The erythrocyte membrane was gotten by further centrifugation at 5000g for 10minutes. The resultant precipitate was washed with 10mM Tris-HCL (pH 7.7) and suspended in 3ml of distilled water. The isolated membranes were stored at 4^oC and used within 24 hours of collection of blood sample.

Enzyme Assay: The assay of the enzyme activities follows the procedure of Hesketh et al (1978), and monitored the inorganic phosphate (Pi) released from ATP. For Na^+/K^+-ATPase, the reaction mixture contained 0.5ml each of 0.35M NaCL, 17.5mM KCL, 21.0mM $MgCl_2$, 10mM Tris – HCL (pH 7.4), and 8.0mM ATP Na_2.This was determined by addition of erythrocyte membrane preparation and the mixture incubated at 37^oC for 1 hour.

The reaction was terminated by the addition 0.8ml of ice-cold, 10% (w/v) trichloroacetic acid and the resultant mixture was kept for 20 minutes at 4^oC. To determine the Na^+/K^+-ATPase activity, the mixture was centrifuged for 5 minutes using a bench top centrifuge.

The concentration of phosphate in 1ml of the supernatant was measured by the method described by Fiske and Subbarow (1925) as adapted by Elekwa et al. (2005). In this method, 1.0ml of 2.5% ammonium molybdenum was added and 0.1ml of 2% ascorbic acid followed after 10 minutes. From the pilot study, it was discovered that the colour change was time dependent. Consequently, the method was modified to limit the time for the colour development to a specific period (<1 minute), and this period was adopted uniformly for the entire sample. The absorbance of the final mixture was measured at 725nm using *SP 830 plus* spectrophotometer (Metertech Inc. Taiwan).

Protein Determination: The protein determination from the membrane preparation was carried out using the principle of the biuret assay (Weichselbaum, 1946). Standard phosphate curve – The protocol for phosphate determination was according to the procedure of Stewart (1974).Other parameters determined were body mass index (BMI) and Waist/hip retro.

Data Analysis: Statistical analysis was performed using Graphpad Prism Version 4.0. Results were expressed as the mean±S.E.M. Comparison of the two groups was performed using student's t-test. A P-value of less than 0.05 was taken as statistically significant.

RESULTS

NA^+/K^+-ATPASE ACTIVITIES IN NORMOTENSIVE SUBJECTS WITH AND WITHOUT FAMILY HISTORY OF HYPERTENSION

Na^+/K^+-ATPase Activities (µmole Pi/mg protein/jr x 10^{-3}) were 13.46±1.242 (Positive Family history) and 16.72±1.831 (Negative family history). The value for subjects with family history of hypertension is significantly lower than that for those without family history of hypertension (P<0.0001) (figure 1)

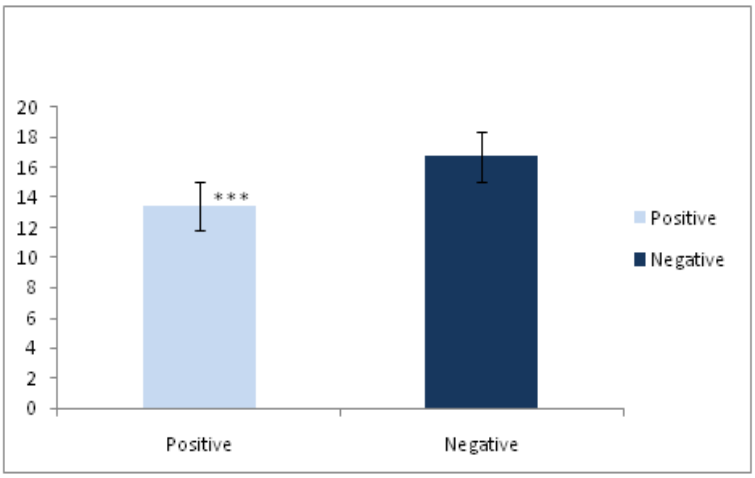

Figure 1: Showing Na^+/K^+-ATPase Activities Normotensive Subjects with and without Family History of Hypertension

Na^+/K^+-ATPASE ACTIVITIES IN SUBJECTS WITH ONLY ONE PARENT AND THOSE WITH BOTH PARENTS HYPERTENSIVE

Na^+/K^+-ATPase Activities (µmole Pi/mg Protein/hr x 10^{-3}) were 14.22±1.392 in subjects with both parents hypertensive is significantly lower than for subjects with either of their parents hypertensive (P<0.0298) (figure 2)

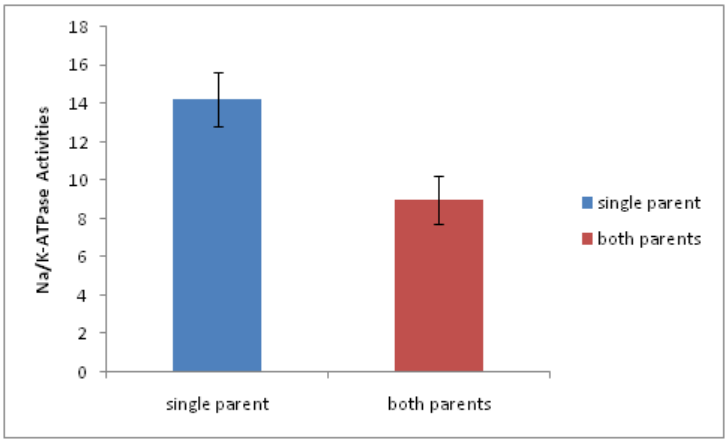

Figure 2: Showing Na^+/K^+-ATPase Activities in Subjects with Only One Parent and Those with Both Parents Hypertensive

Table 1: Showing Clinical Characteristics of Subjects

Variable	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Age (Years)	BMI	Waist/Hip Ratio
+FH(n=35)	115.14±1.38	77.54±1.04	23.34±0.42	21.49±0.56	0.83±0.01
-FH(n=64)	112.22±1.23	76.03±0.82	22.73±0.38	20.83±0.37	0.84±0.01
P-Value	0.14	0.27	0.31	0.31	0.56
GENDER					
Male (Positive) (n=20)	115.00±1.99	77.20±1.66	23.40±0.63	21.59±0.58	0.86±0.01
Male (Negative) (n=27)	115.77±1.38	79.23±0.91	23.69±0.55	20.42±0.42	0.88±0.01
P-Value	0.74	0.26	0.73	0.10	0.25
Female(Positive) (n=15)	115.33±1.92	78.00±1.07	23.27±0.53	21.39±1.01	0.80±0.01
Female (Negative) (n=37)	109.73±1.75	73.78±1.12	22.05±0.49	21.12±0.56	0.82±0.01
P-Value	0.07	*0.03	0.16	0.82	0.46

Data represent mean ± SEM for the variables

+FH = Subjects with Family History of Hypertension

-FH = Subjects without Family History of Hypertension

* = Significant.

DISCUSSIONS

Erythrocyte membrane ghost Na^+/K^+-ATPase activities were determined in this study. The demonstration that the arterial wall of hypertensive patients contained increased sodium and water (Tobian and Binion, 1952) suggested that elevated blood pressure might be the result of a global abnormality of electrolyte handling; hence, interests have been focused on more accessible human blood cells (Blaustein, 1984). The erythrocyte has a large number of membrane Na^+/K^+-ATPase pump sites and if defective sodium transport is implicated in causing essential hypertension, this cell provides a readily obtainable model of the study. While the erythrocyte cell does not contribute to increased peripheral resistance, it is likely that it reflects changes in electrolyte transport by vascular smooth muscle (Blaustein, 1984). Therefore, abnormalities in erythrocytes could provide a valuable marker for an underlying vascular abnormality. Essential hypertension is an important cause of morbidity and mortality among adults (Akinkugbe, 2003; Imam and Olorunfemi, 2002) and probably has its origin in childhood.Many abnormalities have been described in normotensive offsprings of hypertensive patients and these include alterations in transport mechanisms (Canessa et al., 1981;

Milnev et al., 1984). This study was therefore designed to investigate the influence of family history of hypertension on erythrocyte membrane Na^+/K^+-ATPase activities in normotensive subjects with family history of essential hypertension. Although various studies have been done on erythrocyte sodium pump activities in offsprings of hypertensive patients, reports have been conflicting; while reduced sodium pump activities in subjects with family history of hypertension has been reported (Svenson and Sigstrom, 1986; Cooper et al., 1983), reports of normal sodium pump activity have equally been made (Ravogli et al., 1990; Mongeau, 1984). In this study, the erythrocyte sodium pump activities were significantly reduced in normotensive subjects with family history of hypertensions compared with normotensive individuals without hypertensive history (figure 1).

It is possible that both genetic and environmental factors play a part in the pathogenesis of essential hypertension, no single underlying mechanism has been identified to explain the excessive increase in blood pressure. However, Blaustein (1977) hypothesized that changes in cell membranes as a result of genetic and environmental factors, or both, may account for the changes in sodium fluxes seen in essential hypertension. Furthermore, it has been said that the accumulation of intracellular sodium in the smooth muscle cells of resistance vessels resulting from altered sodium transport might interfere with sodium-calcium exchange at the cell membrane (Iwamoto et al., 2005). The increase in intracellular Ca^{++} in the smooth muscle could cause an increase in tone and contractility, and could explain the finding of raised peripheral resistance in essential hypertension.

Many reports have shown that essential hypertension is more common among the relatives of hypertensive patients. Studies in twins have shown that a monozygotic twin of a hypertensive subject has a substantially greater risk of being hypertensive than a dizygotic twin, suggesting an important role for heredity in aetiology of blood pressure (Mcllhaney et al., 1975; Levine et al., 1982). In this study, thirty five of the total ninety nine had family history of hypertension, but of those thirty five, thirty had either of their first-degree relative (father or mother) hypertensive, while only five had both parents hypertensive. The erythrocyte sodium pump activities of subjects that had both parents hypertensive were significantly reduced compared with their counterparts that had either of their parents hypertensive ($P<0.0289$) (figure 2). The risk of a child becoming hypertensive increases if both parents have essential hypertension (Miyao and Furusho, 1978). The finding in this study supported this hypothesis but stronger association would have been made if the sample size of subjects that had both parents hypertensive were larger than that used in this study.

There is no significant difference in the mean systolic and mean diastolic blood pressure between subjects with and without family history of hypertension (Table 1). However, subjects with family history of hypertension had higher mean systolic and mean diastolic blood pressure (Ravogli et al., 1990). Cooper et al. (1983) investigated high school children aged 15-18 years and found significant differences in sodium pump activity between children with and without family history of hypertension. Children with positive family history, however, also had significantly raised diastolic blood pressure (BP). Comparing positive and negative females in this study (Table 1), there is no significant difference between their systolic BP, although those with positive family history had higher systolic BP. Significant differences was in their diastolic BP.

CONCLUSIONS

The results from these study has shown that sodium pump activities is reduced in subjects with history of hypertension in their first-degree relatives compared with those with negative history of hypertension. The abnormality observed in red cell membrane pump activity possibly reflects changes in electrolyte transport in vascular smooth muscle. Thus, data suggest that abnormal red cell membrane sodium transport has a familial component; although it is not cause by

the hypertension, it may be the earliest pathophysiological step in its development, possibly allowing the identification of children at risk of essential hypertension.

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