Race and Sex Differences in Erythrocyte Na^+, K^+, and Na^+-K^+-Adenosine Triphosphatase

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Abstract

Several reports indicate that erythrocytes (RBCs) from blacks and men have higher sodium concentrations than those from whites and women. One possible mechanism to explain this finding is a difference in the activity of Na^+-K^+-ATPase. To explore this possibility, we have studied the Na^+ and K^+ kinetics of RBC Na^+-K^+-ATPase and RBC Na^+ and K^+ concentrations in 37 normotensive blacks and whites, both males and females. The maximal initial reaction velocity (V_{max}) values for RBC Na^+-K^+-ATPase were lower in blacks and men as compared with whites and women. Higher RBC Na^+ levels were observed in blacks and males vs. whites and females. Significant inverse correlations were noted between the Na^+-K^+-ATPase activity and RBC Na^+ concentrations. These findings indicate that cellular Na^+ homeostasis is different in blacks and men as compared with whites and women. Since higher RBC Na^+ concentrations have also been observed in patients with essential hypertension as compared with normotensive subjects, the higher intracellular Na^+ concentrations in blacks and men may contribute to the greater predisposition of these groups to essential hypertension.

Introduction

Several studies have shown that the Na^+ concentration in erythrocytes (RBCs)[1] from normotensive blacks is higher than that of their white counterparts (1–3) and that erythrocytes of normotensive men have a higher Na^+ concentration than those from women (4–7). Since essential hypertension is more common in blacks and men as compared with whites and women of premenopausal age (8–10), and because increased Na^+ concentration has frequently been demonstrated in blood cells of hypertensive patients (11–14), it is possible that the higher RBC Na^+ concentration in blacks and males reflects differences in the cellular regulation of Na^+ which increase the likelihood of developing hypertension. The nature of these differences may be related to a reduced activity of the Na^+-pump, the physiological correlate of the Na^+-K^+-ATPase (3, 15), altered function of facilitated transport processes for Na^+ and K^+ (16, 17), or increased permeability for these ions.

In the present study, we examined the RBC Na^+ and K^+ concentrations, the Na^+ and K^+ kinetics of RBC Na^+-K^+-ATPase activity, and several related parameters in normotensive blacks and whites of either sex. Our principal objective was to demonstrate differences in RBC Na^+ and K^+ regulation between normotensive blacks and males vs. their counterparts that might be linked to the higher incidence of hypertension in the former two groups.

Methods

Subjects were recruited from the faculty, student body, housestaff, and nurses of the University of Medicine and Dentistry of New Jersey. A total of 37 subjects were studied: 9 black women (age 32.0±2.1 yr, body weight: 62.6±3.3 kg; mean±SEM), 9 black men (27.3±0.4 yr, 80.3±4.4 kg), 9 white women (30.1±1.1 yr, 58.5±1.5 kg), and 10 white men (28.5±1.2 yr, 78.0±2.2 kg).

The subjects had no history of hypertension, renal disease, hemolytic anemia, or thyroid or neurological diseases. None of the black subjects had the sickle cell trait. No dietary restrictions were imposed. Potential subjects were excluded if they were taking diuretics, antihypertensive or thyroid medication, oral contraceptives, or estrogen. Pregnancy, massive obesity defined as a Quetelet Index[2] > 5 (18), systolic blood pressure (BP) > 140 mmHg, or diastolic BP > 90 mmHg excluded potential subjects from the study. BP was the average taken in both arms in a sitting position, using the Korotkoff first and fifth components.

Blood was drawn in the morning in a sitting position after the subject had ambulated for 1 h. For RBC Na^+-K^+-ATPase and intracellular electrolyte determinations, blood was drawn into heparinized tubes, while EDTA tubes were used for plasma renin activity (PRA) measurements. Preparation of RBC membrane fractions was begun within 45 min of blood collection. PRA was determined by a Renak (Hoffmann La-Roche, Nutley, NJ) radioimmunoassay kit. One blood sample for PRA measurement from a black man was lost. A PRA value from a white woman was excluded because it fell beyond 2 SDs from the mean. A 24-h urine collection was obtained and analyzed for Na^+ and K^+ using flame photometry. Three black women, one white man, and five black men did not collect their urine. Creatinine was determined by a modified picrate method (19).

RBC membranes were prepared as described by Sacks et al. (20). Briefly, following the removal of plasma and buffy coat, the erythrocytes were washed three times in 295 mosmol $MgCl_2$. A small aliquot of cells was removed for measurement of intracellular electrolytes. These cells were lysed with deionized water, and their Na^+ and K^+ concentrations were measured in an atomic absorption spectrophotometer. The remaining cells were lysed by suspension in 10 mM Tris-HCl (pH 7.4) for 20 min at 4°C. The membranes were collected and washed five times by repeated suspension in the Tris buffer and centrifugation at 48,000 *g* for 10-min periods. Membranes thus obtained were frozen overnight at −20°C.

For Na^+-K^+-ATPase assay, RBC membranes were thawed at room temperature. The incubation medium (1 ml) for the assay contained

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1. *Abbreviations used in this paper:* BP, blood pressure; PRA, plasma renin activity; RBC, erythrocyte; STIF, Na^+ transport inhibitory factor; VSMC, vascular smooth muscle cell.

2. Quetelet index = $\frac{\text{weight } (lb)}{\text{height}^2\ (in)} \times 100$.

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the following: NaCl, 5–100 mM; KCl, 0.5–10 mM; $MgCl_2$, 5 mM; EGTA, 1 mM; imidazole-HCl, 100 mM; ATP, 3 mM (pH 7.4). To determine the ouabain-insensitive ATPase activity, KCl was omitted and ouabain (1 mM) included in the incubation mixture. Membranes were incubated for 60 min at 37°C. Preliminary studies indicated that ATP hydrolysis was linear during this time interval provided that the protein content was <500 μg/ml. Na^+-K^+-ATPase activity was defined as the difference between inorganic phosphate (P_i) production with and without the presence of ouabain. All assays were performed in triplicate. P_i was measured as described by Fiske and Subbarow (21) and protein was determined by the Lowry method (22). The specific activity of the enzyme was expressed as nanomoles P_i generated per milligram protein per hour. For measurements of the kinetics of Na^+ activation of the enzyme, K^+ concentration was set at 10 mM. To determine the kinetics of K^+ activation of Na^+-K^+-ATPase, Na^+ concentration was set at 50 mM.

Equation 1 was used to analyze K^+ activation of Na^+-K^+-ATPase:

$$v = V_{max} \cdot \frac{[K]^n}{[K]^n + K_a^n} \quad (1)$$

where v equals initial reaction velocity, V_{max} equals maximal initial reaction velocity, K equals K^+ concentration in the medium, K_a equals equilibrium dissociation constant for K^+, and n equals the Hill coefficient (23). This equation is a modification of a Michaelis-Menten-like interaction between an activator (K^+) and an enzyme (Na^+-K^+-ATPase) that incorporates a cooperative mode of interaction between K^+ and the enzyme.

Na^+ appears to exert a dual effect on Na^+-K^+-ATPase. Below a medium concentration of 20 meq/liter, Na^+ stimulates the enzyme, whereas at Na^+ concentrations > 20 meq/liter, Na^+-K^+-ATPase activity declines. To describe this Na^+-enzyme interaction, a model introduced by Haldane (24) for enzyme substrate inhibition was applied. In this model, it is assumed that when the substrate (ATP) for Na^+-K^+-ATPase is above saturation level, the activator (Na^+) behaves as a substrate. Thus, the following reaction model can be utilized to explain the Na^+ inhibitory effect.

$$Na^+ + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{-}Na^+ \xrightarrow{k_2} E + Na^+$$
$$\qquad\qquad k_3 \Big\downarrow\Big\uparrow k_{-3}$$
$$\qquad\qquad Na^+{-}E{-}Na^+$$

In the model, as the reaction proceeds from left to right, the substrate is transformed into product. The mathematical solution of this model results in:

$$v = V_{max} \cdot \frac{[Na]}{[Na] + K_m + K_i \cdot [Na]^2} \quad (2)$$

where v equals initial reaction velocity, V_{max} equals maximal initial reaction velocity, [Na] equals Na^+ concentration in the medium, K_m equals the Michaelis-type equilibrium dissociation constant (function of k_1, k_{-1}, and k_2); K_i, equilibrium dissociation rate constant for binding of the second Na^+ to the enzyme (function of k_3 and k_{-3}).

One-way analysis of variance and calculations of correlation coefficients were applied in evaluating the data. Nonlinear regression analyses were used to describe Na^+ and K^+ effects on RBC Na^+-K^+-ATPase (25). The calculations were performed on an IBM 3033-U computer.

Results

Values of BP, Quetelet index, serum Na^+, K^+, and plasma creatinine concentrations are listed in Table I. Systolic BP was higher in blacks than in whites and higher in males than females. The source of these differences was primarily the low systolic BP in white females. It should be pointed out, however, that the systolic and diastolic BP values are within the normal range for adult subjects.

Table II presents the RBC, urinary electrolytes, and PRA values. Blacks demonstrated significantly higher intracellular Na^+ values as compared with whites ($P = 0.02$). The difference in erythrocyte Na^+ concentration between black and white females was also significant ($P = 0.005$) and that between black and white males was of borderline significance ($P = 0.07$). A difference of borderline significance in intracellular Na^+ concentration was observed between females and males ($P = 0.06$), the latter having a higher value. In addition, females demonstrated intracellular K^+ which was nearly significantly greater than that in males ($P = 0.07$).

The urinary Na^+ excretion was higher in males than in females ($P < 0.001$) independent of race. This is probably related to the higher Na^+ intake in the former group as indicated by the sex-related weight differences. Urinary K^+ excretion was significantly lower in blacks than in whites ($P < 0.005$) and lower in black females than in white females ($P < 0.02$).

Table I. Clinical and Laboratory Findings

	Blood pressure					
	Systolic*	Diastolic	Quetelet index	Serum Na^+	Serum K^+	Serum creatinine
	mmHg	*mmHg*		*meq/liter*	*meq/liter*	*mg/100 ml*
Black females (BF)	116.8±4.4 (9)	71.3±1.6 (9)	3.37±0.18 (9)	139.9±0.6 (9)	4.10±0.13 (9)	0.94±0.08 (9)
White females (WF)	106.9±2.7 (9)	72.6±2.7 (9)	3.25±0.1 (9)	140.0±0.33 (9)	4.33±0.20 (9)	0.88±0.03 (8)
Black males (BM)	124.8±3.4 (9)	76.9±2.6 (9)	3.39±0.23 (9)	141.1±0.7 (9)	4.10±0.09 (9)	1.00±0.05 (9)
White males (WM)	120.5±2.1 (10)	76±2.7 (10)	3.44±0.07 (10)	140.5±0.5 (10)	4.2±0.12 (10)	1.02±0.04 (9)

* BF vs. BM, $P < 0.05$. (BF + WF) vs. (BM + WM), $P < 0.001$. (BF + BM) vs. (WF + WM), $P < 0.05$.

Table II. RBC, Urine Electrolytes, and PRA

	Intracellular Na^{+}*	Intracellular K^{+}†	Intracellular Na^{+}/K^{+}	Urinary Na^{+} excretion§	Urinary K^{+} excretion‖	Urinary Na^{+}/K^{+}¶	PRA**
	meq/liter	*meq/liter*		*meq/24 h*	*meq/24 h*		*ng/ml plasma/h*
Black females (BF)	8.89±0.52 (9)	95.6±4.1 (9)	0.095±0.008 (9)	97.7±16.0 (6)	40.6±4.2 (6)	2.35±0.13 (6)	2.18±0.27 (9)
White females (WF)	7.59±0.40 (9)	90.0±4.2 (9)	0.085±0.006 (9)	88.6±5.90 (9)	67.4±8.4 (9)	1.45±0.17 (9)	3.84±0.61 (8)
Black males (BM)	10.19±1.00 (9)	87.1±3.3 (9)	0.117±0.012 (9)	167.1±36.9 (4)	59.0±1.1 (4)	2.86±0.39 (4)	3.01±0.61 (8)
White males (WM)	8.63±0.36 (10)	85.1±3.2 (10)	0.103±0.007 (10)	154.7±22.0 (9)	79.2±6.0 (9)	2.17±0.33 (9)	4.07±0.41 (10)

* (BF + BM) vs. (WF + WM), $P = 0.02$; WF vs. BF, $P < 0.005$; (BF + WF) vs. (BM + WM), $P = 0.06$; BM vs. WM, $P = 0.07$. † (BF + WF) vs. (BM + WM), $P = 0.07$. § (BF + WF) vs. (BM + WM), $P < 0.001$. ‖ (BF + BM) vs. (WF + WM), $P < 0.005$; BF vs. WF, $P < 0.02$. ¶ BF vs. WF, $P = 0.03$; (BF + BM) vs. (WF + WM), $P < 0.02$. ** (BF + BM) vs. (WF + WM), $P < 0.01$; BF vs. WF, $P < 0.02$.

The PRA was significantly lower in blacks than in whites ($P < 0.01$). In addition, as a group, black females had a lower PRA ($P < 0.02$) than their white counterparts.

K^{+} activation of erythrocyte Na^{+}-K^{+}-ATPase is depicted in Fig. 1. It is clear that blacks demonstrate a lower measured activity of Na^{+}-K^{+}-ATPase at each concentration of K^{+} in the medium. In addition, black females and males show lower activity of the enzyme as compared with their respective white counterparts. Analysis of the data according to equation 1 has revealed that a Hill coefficient larger than unity appears appropriate to describe the deviation of the results from what is expected by simple Michaelis-Menten kinetics (as shown by

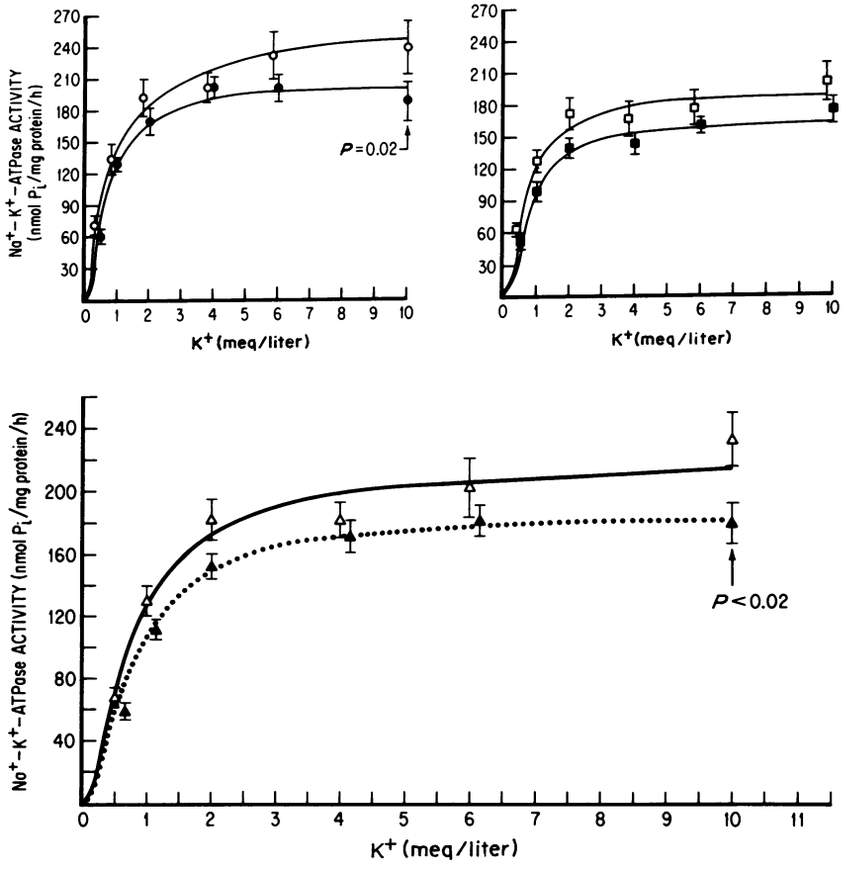

Figure 1. K^{+} activation of erythrocyte membrane Na^{+}-K^{+}-ATPase. The lower panel portrays the pooled results of erythrocyte Na^{+}-K^{+}-ATPase of black (males and females) (▲) and white (males and females) (△). The upper left inset represents a comparison between black females (●) and white females (○). The right upper inset describes the activation of the enzyme in black males (■) and white males (□). Individual points with significant differences are indicated. The overall kinetic variables of the activation and 95% confidence intervals are presented in Table III. The number of observations is equal to the number of subjects in the respective groups.

Table III. K^+ Activation of the Na^+-K^+-ATPase

	V_{max} (nmol P_i/mg protein/h)	K_a	Hill's coefficient (n)
	nmolP_i/mg protein/h	*meq/liter*	
All blacks	181.7 (168.4–195.0)	0.75 (0.62–0.89)	1.80 (1.15–2.46)
All whites	216.9 (190.8–243.0)	0.80 (0.58–1.03)	1.53 (0.76–2.30)
All females	221.3 (200.0–242.5)	0.80 (0.62–0.99)	1.66 (0.95–2.36)
All males	177.1 (161.4–192.9)	0.74 (0.58–0.89)	1.74 (0.98–2.50)
Black females	196.3 (179.4–213.2)	0.73 (0.57–0.88)	2.10 (1.11–3.10)
White females	263.5 (195.0–331.0)	1.01 (0.40–1.60)	1.17 (0.32–2.03)
Black males	171.0 (150.8–191.3)	0.81 (0.59–1.04)	1.41 (0.76–2.06)
White males	185.0 (163.0–207.0)	0.70 (0.50–0.90)	2.06 (0.71–3.41)

Numbers in brackets indicate 95% confidence interval.

the curves in the figure). Despite the uncertainties about the exact nature of the interaction between K^+ and the Na^+-K^+-ATPase (26, 27), this model has been previously employed with satisfactory results (28). The specific parameters of K^+ activation are presented in Table III. The pooled V_{max} values of the white group are 19.2% higher than those of blacks. Further analyses indicate that white females have substantially higher V_{max} values (34%) than black females. White males also demonstrate a slightly higher (8%) V_{max} than black males.

Na^+ activation of erythrocyte Na^+-K^+-ATPase is shown in Fig. 2. It is evident that the observed enzymatic activity declines at medium Na^+ concentrations higher than 20 meq/liter. This phenomenon was documented previously (29, 30). It is also clear that, irrespective of sex, blacks exhibit lower activity of the enzyme at the different medium Na^+ concentrations. The model used herein to analyze the data is relatively simple, but as seen in Fig. 2, satisfactory fitness is achieved between the observed data (symbols in the figure) and the computer predicted curves as per equation 2. The parameters describing the Na^+ dependency of the enzyme are presented in Table IV. They indicate a 21% higher V_{max} value for whites than for blacks. This difference arises primarily from that of black vs. white females (29%). The scatter in the data of the K_m and K_i is relatively wide and, therefore, firm conclusions related to these parameters cannot be obtained. Judged by the K_i and K_m values, it appears that black females demonstrate the least sensitivity for Na^+ inhibition and the highest sensitivity for Na^+ activation of the enzyme. On the other hand, black males exhibit the highest sensitivity for Na^+ inhibition and the lowest response to Na^+ activation of the enzyme.

It is noteworthy that the V_{max} values obtained from the Na^+ kinetics consistently exceed those from the K^+ kinetic studies. Apart from the possibility of theoretical inaccuracies in the two models that were chosen to attain a good fitness

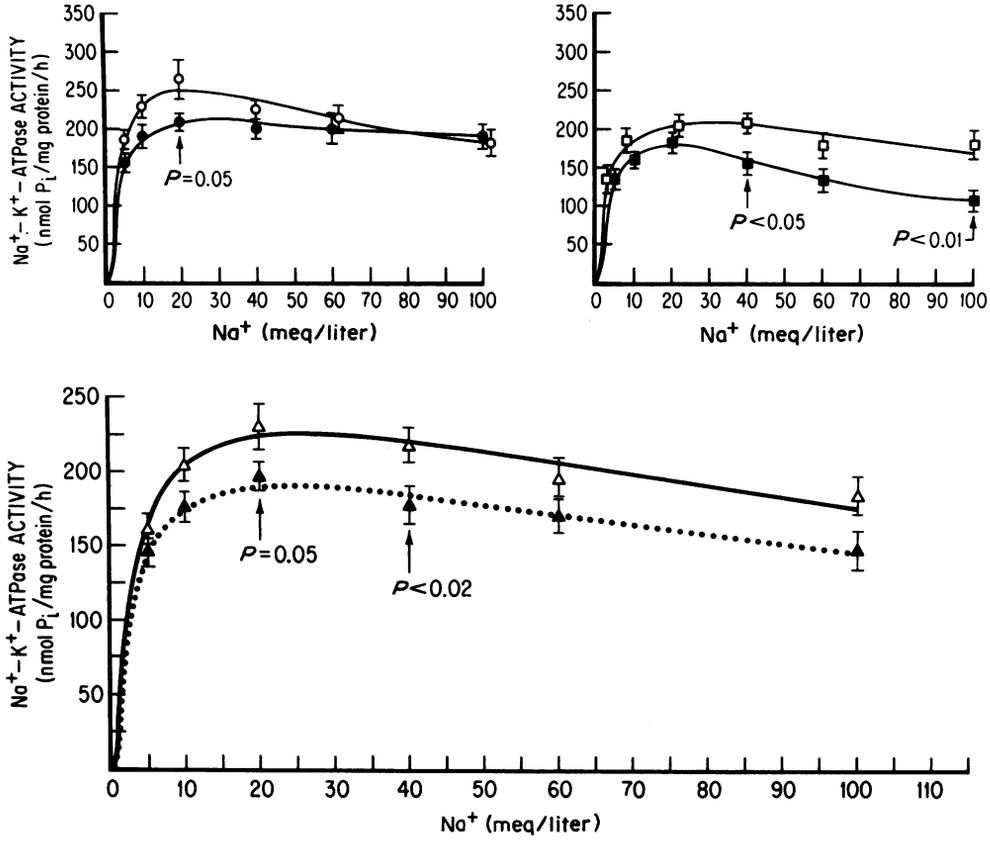

Figure 2. The Na^+ kinetics of erythrocyte Na^+-K^+-ATPase. The lower panel depicts overall activation of the enzyme in erythrocyte membranes derived from black (males and females) (▲) and white (males and females) (△). The left upper panel presents a comparison between black (●) and white (○) females and right upper panel depicts the activation of the enzyme in erythrocytes of black (■) and white (□) males. Individual points with significant differences are noted. The overall kinetic parameters of the activation (and inhibition) of the enzyme and 95% confidence intervals are presented in Table IV. The number of observations is equal to the number of subjects for each group.

Table IV. Na^+ activation of the Na^+-K^+-ATPase

	V_{max}	K_m	K_i
	nmol P_i/mg protein/h	*meq/liter*	*liter²/meq²*
All blacks	244.9 (181.7–308.1)	3.25 (0.36–6.10)	0.0065 (0.0008–0.0121)
All whites	298.7 (225.2–372.2)	3.97 (1.00–6.90)	0.0066 (0.0012–0.0120)
All females	289.9 (236.0–344.0)	3.22 (1.18–5.25)	0.0054 (0.0016–0.0092)
All males	267.8 (181.2–354.4)	4.52 (0.45–8.60)	0.0090 (0.0009–0.0170)
Black females	242.5 (188.5–296.7)	2.58 (0.17–4.99)	0.0025 (0.0010–0.0062)
White females	343.5 (239.2–447.7)	4.01 (0.42–7.61)	0.0087 (0.0013–0.0160)
Black males	272.0 (139.0–404.0)	4.90 (−1.20–11.19)	0.0150 (0.0007–0.0310)
White males	263.6 (167.0–359.0)	4.10 (−0.47–8.68)	0.0048 (0.0024–0.0120)

Numbers in brackets indicate 95% confidence interval.

with the data, the inhibitory effect of the relatively high Na^+ in the medium may also play a role in the discrepancy, i.e., at the 50 meq/liter medium Na^+ that was used in the K^+ kinetic experiments, an inhibition of the enzyme is already present. Thus, the V_{max} of the K^+ activation isotherm represents a somewhat suppressed enzyme function.

The intracellular Na^+ concentration was correlated with the activity of Na^+-K^+-ATPase at specific Na^+ (20 and 100 meq/liter) and K^+ (6 and 10 meq/liter) medium concentrations. The activities of the enzyme at the two K^+ concentrations represent V_{max} values. At 20 meq Na^+/liter, the activity of Na^+-K^+-ATPase exhibit a peak whereas that of the 100 meq Na^+/liter incorporates the inhibitory effect of the ion. At the four medium concentrations of the two ions, Na^+-K^+-ATPase activity showed an inverse relation with intracellular Na^+ concentration. The correlation coefficients between the intracellular Na^+ and the activity of the enzyme at medium concentrations of 6 and 10 meq K^+/liter are -0.41 ($P < 0.02$) and -0.35 ($P < 0.05$), respectively. The respective correlation coefficients for Na^+ activation of the enzyme and intracellular Na^+ at medium concentrations of 20 and 100 meq Na^+/liter are $r = -0.36$ ($P < 0.05$) and $r = -0.39$ ($P = 0.02$).

Discussion

It is well established that whites and premenopausal females have a strikingly lower incidence of essential hypertension as compared with blacks (8–10) and age-matched males (8). The mechanisms responsible for these differences are not clear. The free intracellular Na^+ concentration is thought to have a paramount role in the contractility of the vascular smooth muscle cell (VSMC); an increase in its concentration favors the contraction process (31, 32). Since human VSMCs are not readily available, blood cells and particular RBCs have been routinely used to examine the intracellular Na^+ homeostasis of hypertensive patients. The tacit assumption of such investigations is that abnormalities in cellular Na^+-K^+ homeostasis demonstrated in RBCs represent generalized defects and, therefore, they also occur in the vascular smooth muscle. Bearing this reservation in mind, it is of particular interest that in our present study erythrocytes of blacks and males demonstrated higher intracellular Na^+ concentrations than their counterpart groups, namely, whites and females. The limited data available support our observations of higher intracellular Na^+ concentration of normotensive blacks as compared with whites (1–3). Findings of lower erythrocyte Na^+ levels in females as compared with males have also been documented by several investigators (4–7), but not by others (2, 33, 34). In the latter reports, the age range of the subjects varied widely (2, 34) or was not documented (33). Because the intracellular Na^+ concentration seems to increase with age (7) and this increment is more pronounced among women (4), heterogeneity in the group age can easily mask sex-related differences. The age range in our investigation was relatively small (25–40 yr) and there were only minor differences in the mean ages among the groups.

In the present study, a relative elevation of the intracellular Na^+ (black vs. whites, males vs. females, black females vs. white females, black males vs. white males) was associated with a lower activity of erythrocyte Na^+-K^+-ATPase as demonstrated by either the Na^+ or K^+ kinetics of the enzyme activation curves. The analysis of the K^+ activation curves revealed K_a and n values comparable with those of previous investigations (28, 35). These values are similar in each group with the exception of erythrocytes of the white females which demonstrate a somewhat higher K_a value than the other groups. The large standard deviation, however, in this particular group renders this difference questionable. The V_{max} values are significantly lower for groups demonstrating higher intracellular Na^+ with the exception of black vs. white males, where the Na^+ activation isotherms do not appear to have different V_{max} levels. The finding of lower Na^+-K^+-ATPase in groups with higher intracellular Na^+ is further supported by the fact that when the Na^+-K^+-ATPase data from all groups were pooled at four selected medium concentrations of Na^+ and K^+, the enzyme activity displayed negative correlations with the intracellular Na^+.

The elevated RBC Na^+ concentration in the black group could result from a higher membrane permeability for this cation, a possibility that seems unlikely since other reports did not show such a phenomenon (16, 36, 37). Another cause for the racial differences in the intracellular Na^+ concentration could be an altered activity of Na^+,K^+-cotransport (16, 17). At present, a lack of agreement exists in regard to this possibility (38–40). Therefore, it seems reasonable to conclude that the difference in erythrocyte Na^+ concentration between blacks and whites is at least partially a result of a variation in the Na^+-K^+-ATPase activity, while a contribution of the Na^+,K^+-cotransport system to this difference may also exist.

To our knowledge, we are the first to report sex differences in RBC Na^+-K^+-ATPase activity. Regardless of race, females exhibited higher activity of the enzyme at varying medium concentrations of both Na^+ and K^+. This is due to sex-related differences in V_{max} values. From Table III, it appears that there is hardly an overlap between the V_{max} values of the 18 females and 19 males. It should be noted that these two groups are balanced with respect to racial extraction (i.e., 9 blacks and 9

whites in the female group and 9 blacks and 10 whites in the male group). Additional support for the higher Na^+-K^+-ATPase activity in the women emerges from the measurements of the intracellular Na^+ and K^+. Erythrocytes of the women showed a significantly lower intracellular Na^+ and a higher K^+ of borderline significance as compared with men. This latter finding concurs with several studies (2, 4, 5), but not others (6, 7, 33, 34).

Our conclusion that normotensive whites have a higher erythrocyte Na^+-K^+-ATPase activity than blacks is in agreement with findings by previous investigators (3, 15). Other studies, performed by one group (36, 41), showed no racial or sex-related differences in erythrocyte ouabain-sensitive Rb^+ uptake (the Na^+-pump). These studies are difficult to interpret because the transport medium contained only 3 μM Rb^+ and no K^+. This Rb^+ concentration is insufficient for a full activation of the Na^+-pump. Further support for our contention of racial and sex-related differences in erythrocyte Na^+-K^+-ATPase activity have been derived from our subsequent study (unpublished data). In this study, we have shown a lower number of Na^+-pump units (measured by [^{3}H]ouabain binding) in erythrocytes of blacks and males as compared with whites and females, and the number of Na^+-pump units was inversely related to the intracellular Na^+ concentration. Kinetic analysis of the [^{3}H]ouabain binding indicated that the lower number of Na^+-pump units was probably not related to the presence of an endogenous, digoxin-like factor competing with the exogenous ouabain.

The finding that erythrocyte membrane Na^+-K^+-ATPase activity measured in vitro is lower in blacks and males than whites and females does not imply that its physiological analog, the Na^+-pump, in the former two groups, operates in vivo at a lower level. To accomplish the same Na^+-pump activity, erythrocytes of blacks and males may function at a higher intracellular Na^+ concentration than whites and females. Thus, a "trade off" for a "normal" operation of the Na^+-pump at a lower number of Na^+-pump units is a higher intracellular Na^+ concentration.

A lower Na^+-K^+-ATPase activity may result from: (*a*) inherited differences in the structure of the enzyme, (*b*) intrinsically lower number of enzyme units, and (*c*) the sustained effect of extracellular modulators. A potential modulator is the putative digitalis-like Na^+ transport inhibitory factor (STIF) (42–44). According to an hypothesis, which is well-documented in animal studies, high BP may develop in susceptible subjects because their kidneys have blunted sodium excretory capacity, thus leading to Na^+ retention and expansion of the extracellular fluid volume. This results in enhanced release of STIF, which through inhibition of the renal tubular Na^+-K^+-ATPase diminishes Na^+ reabsorption, thereby leading to augmented Na^+ excretion. The consequence is a tendency for correction of the extracellular fluid volume expansion. However, high circulating levels of STIF cause inhibition of the Na^+-K^+-ATPase in various tissues including the vascular smooth muscle (44). Inhibition of the Na^+-K^+-ATPase in this tissue can lead to vasoconstriction and elevation of the BP (45–48).

Blacks, as a group, appear to exhibit several characteristics of this hypothetical model (49). Normotensive blacks demonstrate a blunted natriuresis following NaCl loading (50, 51) and a higher sensitivity in BP response to NaCl (51, 52). These findings coupled with demonstrations of lower PRA in blacks vs. whites (10, 51, 53, 54) suggest a relatively expanded extracellular fluid volume in blacks vs. whites (55). Furthermore, the decrease in urinary K^+ excretion in blacks noted by us and others (10, 56) could be due to a decrease in K^+ intake (10, 57), though a lower renal Na^+-K^+-ATPase activity (58, 59) can be an additional factor responsible for this finding. If K^+ intake is similar in blacks and whites, yet the urinary K^+ excretion in the former is lower, gastrointestinal or sweat losses in blacks must be higher for body balance to be maintained. There have been no published investigations addressing this matter.

In conclusion, we have demonstrated that RBC Na^+-K^+-ATPase activity in blacks and males is lower than in whites and females and that lower activity of the enzyme is associated with higher intracellular Na^+ concentration. These findings were obtained despite heterogeneity of our sample population with respect to geographic locality of origin in the blacks, ethnic variations in the whites, familial history of hypertension, and other environmental factors. Thus, the identification of sex and racial-related differences in erythrocyte Na^+-K^+-ATPase activity implies that they exist irrespective of potential effects of the aforementioned factors. The biological variabilities in Na^+-K^+-ATPase may be incidental and of no consequence to BP regulation. However, it is quite possible that a similar tendency in the Na^+-K^+-ATPase and intracellular Na^+ regulation in VSMC of blacks and males, as compared with whites and females, may contribute to the predisposition of the former two groups to vasoconstriction and hypertension. The results also indicate that balancing sample populations according to sex and race is essential in studying the RBC Na^+-K^+-ATPase.

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