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Decreased NKCC1 Activity in Erythrocytes From African Americans With Hypertension and Dyslipidemia

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Abstract

Background—Recent studies demonstrated a key role of ubiquitous isoform of Na⁺,K⁺,2Cl⁻ co-transport (NKCC 1) in regulation of myogenic tone and peripheral resistance. We examined the impact of race, gender, and plasma lipid on NKCC 1 activity in French Canadians and African Americans with hypertension and dyslipidemia.

Methods—NKCC and passive erythrocyte membrane permeability to K⁺, measured as ouabain-resistant, bumetanide-sensitive, and (ouabain+bumetanide)-resistant ⁸⁶Rb influx, respectively, were compared in 111 French-Canadian men, 107 French-Canadian women, 26 African-American men, and 45 African-American women with essential hypertension and dyslipidemia.

Results—The African-American men and women were 7 years younger and presented twofold decreased plasma triglycerides compared to their French-Canadian counterparts ($P < 0.01$) whereas body mass index (BMI), total cholesterol, low-density lipoprotein, and high-density lipoprotein (HDL) were not different. NKCC was respectively 50 and 38% lower in the African-American men and women than in the French Canadians ($P < 0.005$) without any differences in passive erythrocyte membrane permeability for K⁺. We did not observe any impact of age on NKCC in all groups under investigation, whereas plasma triglycerides correlated positively with the activity of this carrier in the French-Canadian men only.

Conclusions—NKCC 1 activity is lower in erythrocytes of African Americans with essential hypertension and dyslipidemia than in Caucasian counterparts. We suggest that decreased NKCC 1 may contribute to the feature of the pathogenesis of salt-sensitive hypertension seen in African Americans.

Keywords

African Americans; blood pressure; hypertension; Na⁺,K⁺,Cl⁻ co-transport; plasma lipids; renal complications; salt retention

Since the initial observations of increased monovalent ion exchange across erythrocytes from spontaneously hypertensive rats,^{1,2} such cells lacking a nucleus and intracellular organelles have been widely studied for the functional characterization of ion transport pathways in human hypertension. These investigations revealed that in erythrocytes from Caucasians with primary (essential) hypertension, ion transport is altered by augmented Na^+/Na^+ exchange, measured as the rate $^{23}\text{Na}^+_{\text{o}}/^{22}\text{Na}^+_{\text{i}}$ exchange or Na^+_{o} -dependent Li^+ efflux (Na^+/Li^+ countertransport), and the abnormal kinetic properties of $\text{Na}^+,\text{K}^+,\text{2Cl}$ co-transport (NKCC) indicated by increased maximal rate of the carrier activity and decreased its affinity for intracellular Na^+ (for review, see refs. 3–5).

Two NKCC isoforms have been cloned from vertebrate complementary DNA libraries. An ubiquitous NKCC1 has been detected in all types of cells studied so far, whereas renal-specific NKCC2 expression is limited to the apical membrane of epithelial cells from the thick ascending limb of Henle's loop and the macula densa. Apart from NKCC, the superfamily of Cl^- -coupled carriers, providing bidirectional electroneutral symport of monovalent ions, consists of Na^+,Cl^- (NCC) and K^+,Cl^- (KCC) co-transporters. Both NKCC1 and NKCC2 are inhibited by high-ceiling diuretics (HCD), such as bumetanide and furosemide, whereas NCC is resistant to HCD and inhibited by hydrochlorothiazide and other thiazide-derived diuretics (for review, see ref. 6). In contrast to NKCC, Na^+/Li^+ countertransporter has not yet been cloned, and its function remains poorly understood.⁴

It has been reported that hypertensive blacks are more frequently sodium-sensitive than hypertensive whites, and diuretics targeting NCC and NKCC2 remain the most reliable therapy either in combination or alone for hypertensive African Americans.^{7,8} These clinical data suggest that abnormal solute and fluid movement in absorptive and/or secretory epithelia contribute to enhanced salt sensitivity and sodium retention in African Americans with essential hypertension. This hypothesis is also consistent with the augmented activity of epithelial Na^+ channels, i.e., major regulators of salt reabsorption in distal tubules, seen in hypertensive African Americans⁹ and decreased NKCC1-mediated sweat gland function in blacks compared to whites.^{10,11}

It has been reported that NKCC is reduced in erythrocytes from blacks compared to whites (for review, see refs. 5,12). However, the limitations listed below complicate the comparative analysis of these data. First, hypertension was not controlled in several comparative black vs. white studies.^{13,14} Second, NKCC was shown to be higher in erythrocytes from Caucasian men compared to women.¹⁵ Mixed populations of men and women were investigated to analyze impact of race on erythrocyte ion transport in certain studies.^{13,14,16,17} Third, plasma lipids have been well documented to affect erythrocyte ion transport in Caucasian populations.¹⁸ The possible impact of distinct plasma lipid profiles has not been considered in white vs. black comparisons.

Keeping these observations in mind, we compared ion transport in erythrocytes from Caucasian and African-American men and women with essential hypertension and dyslipidemia. In contrast to nystatin- and 2,5-chloromercuribenzenesulfonic acid-treated erythrocytes employed in overwhelming number of previous investigations,^{13,14,16,17,19} we applied methods that allowed us to measure NKCC in intact cells with physiologically low intracellular Na^+/K^+ ratios. The possible influence of age, body mass index (BMI), and plasma lipids on the activity of ion transporters in erythrocytes from whites and blacks was examined by correlation analysis.

Methods

Patients

African Americans were examined at the Medical College of Wisconsin in Milwaukee, and French Canadians were investigated at Chicoutimi Hospital in the Saguenay–Lac-St-Jean region of Quebec, Canada, with identical protocols employed in both clinics. Hypertensive families were selected on the basis of having at least two siblings in the range from 18 to 55 years with hypertension and dyslipidemia, defined, respectively, as diastolic blood pressure >90 mm Hg, total cholesterol concentration >5.2 mmol/l, and high-density lipoprotein (HDL)-cholesterol <0.9 mmol/l. The exclusion criteria were secondary hypertension, diabetes mellitus, diastolic blood pressure >110 mm Hg on drug therapy, serum creatinine concentration >180 μ mol/l, BMI >35 kg/m², pregnancy, malignancy, substance abuse (including alcohol), and myocardial infarction or stroke in the last 6 months. Once at least two siblings in a family satisfied all the above-listed criteria, other siblings, not necessarily hypertensive, were also enrolled. Before the study, lipid-lowering medication was withdrawn for 1 month, and antihypertensive drugs for at least 1 week. For more details, see refs. (20,21). The protocol was approved by the Medical College of Wisconsin/Froedtert Memorial Lutheran Hospital institutional review board and the Human Investigation Committee of Chicoutimi Hospital.

Blood samples and ion transport measurement

Fasting blood samples were collected on an empty stomach in the morning into tubes containing 20–30 U/ml heparin as an anticoagulant, placed on ice and shipped for further analysis from both clinics to the Research Centre of the Centre hospitalier de l'Université de Montréal (CRCHUM). Previously, it was shown that blood storage with heparin at 2–4 °C for up to 4 days does not affect the erythrocyte ion transporters analyzed in the present study.¹⁵ Considering these data, ion transport was measured 24–48 h after blood withdrawal. NKCC and passive permeability for K⁺, mainly comprising KCC and lipid bilayer permeability, were quantified as ouabain-resistant, bumetanide-sensitive, and (ouabain +bumetanide)-resistant rates of ⁸⁶Rb influx, respectively, as described in detail elsewhere.¹⁵

Western blot was performed in accordance with a previously described protocol²² using anti-NKCC1 and anti-NKCC2 antibodies from Santa Cruz Biotechnology, Santa Cruz, CA. Human erythrocyte ghosts and protein extracts from rat kidney, aorta, and brain were obtained as described elsewhere.^{23,24}

Data analysis

Differences between African-American and French-Canadian subjects were examined by *t*-test (age, anthropometric parameters, lipids, ion transport, and blood pressure) and χ^2 analysis (gender). To analyze the predictors of ion transport activity, we carried out stepwise, block, multiple regression. This analysis tests a predictive linear model between a dependent variable and its predictors (independent variables) in multiple steps. At each step, a block of independent variables is included into the model on the basis of its partial correlation with the dependent variable. In the present study, four blocks were used: (i) age, sex (forced into the equation for control purposes); (ii) BMI, waist-to-hip ratio; (iii) total cholesterol, triglycerides, HDL, and low-density lipoprotein; (iv) awake and asleep systolic and diastolic blood pressures. Prior to this analysis, the dependent variables were tested for normality, outliers were excluded, and, if necessary, the data were transformed.

Results

Clinical characteristics of hypertensive families

Fifty-five French-Canadian families comprising 218 hypertensive siblings (111 men and 107 women) and 23 African-American families comprising 71 hypertensive siblings (26 men and 45 women) were included in this study (Table 1). Neither BMI nor total plasma cholesterol, low-density lipoprotein, and HDL was different between African Americans and French Canadians. Plasma triglycerides were decreased by 40 and 50% in African-American men and women ($P < 0.01$ and 0.001 , respectively). The African Americans were 7 years younger than the French Canadians.

Impact of race

NKCC activity was 45% lower in the mixed population of African-American men and women with essential hypertension than in their French-Canadian counterparts ($P < 0.005$, Figure 1). Importantly, the values of NKCC activity in French Canadians were consistent with activity of this carrier measured in other Caucasian populations.^{16,19} We did not observe any difference between the African Americans and French Canadians in passive erythrocyte permeability for K^+ (Figure 1).

Impact of gender

Previously, we reported that NKCC is increased in Caucasian men compared to women and sibling resemblance of NKCC in French-Canadian families with hypertension and dyslipidemia is higher in men than in women ($r = 0.39$ and 0.03 , respectively).¹⁵ Considering this, we analyzed the data for Caucasian and African-American men and women separately. Table 2 shows that NKCC was elevated by ~34% in the French-Canadian men compared to the women ($P > 0.05$). In contrast to whites, we did not find any difference in the activity of this carrier between the African-American men and women. Figure 2 illustrates the distribution pattern of NKCC activity among the French-Canadian and African-American men and women. In 40% of the African-American women and in 31% of the African-American men, the carrier's activity was $< 100 \mu\text{mol/liter}$ of cells/h. In contrast, only 2% of the French-Canadian men and 6% of the French-Canadian women showed such low NKCC. In both races, passive permeability of erythrocytes for K^+ was not affected by gender (Table 2).

Impact of age, BMI, and plasma lipids

The African-American men and women were 7 years younger and exhibited approximately twofold decreased plasma triglycerides compared to their French-Canadian counterparts (Table 1). To analyze the implication of these differences in altered NKCC, we undertook intraindividual correlation analyses separately for men and women. The possible impact of BMI, total cholesterol, low-density lipoprotein, and HDL was also subjected to correlation analysis, which led to several conclusions.

First, the correlation between passive erythrocyte permeability for K^+ was found to be race and gender dependent. Thus, this parameter correlated negatively with age in the French-Canadian men but positively in the African-American women (Table 3). We observed a highly significant correlation of plasma triglycerides with passive permeability to K^+ ($P = 0.00099$, Table 4) in the African-American women but not in the other groups subjected to this analysis.

Second, NKCC correlated positively with plasma triglycerides in the French-Canadian men ($P = 0.01$, Table 4). However, neither the French-Canadian nor the African-American women showed any significant correlation between these two parameters. Moreover, in the

African-American men, this correlation tended to be negative ($r = -0.235$, $P = 0.27$) rather than positive (Table 4).

Third, in the French-Canadian men, NKCC activity correlated positively with BMI ($P = 0.05$, Table 3) as well as with total cholesterol ($P = 0.03$) and HDL ($P = 0.04$, Table 4). A positive correlation between HDL and NKCC was also observed in African-American women ($P = 0.03$, Table 4). It should be underlined, however, that neither BMI nor total cholesterol and HDL were significantly different between the African Americans and French Canadians (Table 1).

Viewed collectively, these results argue against the impact of age, BMI, and plasma lipids on the decreased NKCC activity seen in African Americans with essential hypertension.

Western blot analysis

Human erythrocytes as well as rat brain, kidney, and aorta expressed ~ 116 kDa protein interacting with anti-NKCC1 antibodies (Figure 3). In contrast to NKCC1, the presence of NKCC2-immunoreactive protein was limited to the rat kidney.

Discussion

Western blot analysis showed that NKCC1 is the only isoform of NKCC expressed in human erythrocytes (Figure 3). This conclusion is consistent with the lack of bumetanide-sensitive component of ^{86}Rb uptake in erythrocytes isolated from *NKCC1*^{-/-} mice.^{25,26} Our results show that NKCC1 activity is sharply reduced in African Americans with essential hypertension compared to French-Canadian Caucasians (Figure 1). In French Canadians, the activity of this carrier is affected by gender (Table 2, Figure 2). In contrast, we did not observe any significant difference in NKCC activity between the African-American men and women (Table 2). These results strongly suggest that racial differences in the activity of this carrier, seen in our study as well as in previous investigations of mixed groups of men and women,^{13,14,16,17,19} are gender independent.

Our French-Canadian and African-American groups were well matched for BMI but were slightly different for age (Table 1). However, the lack of NKCC correlation with age in all groups under investigation (Table 3) and with BMI in French-Canadian women and in African-American women and men (Table 3) goes against the impact of age and BMI in racial differences of this carrier's activity.

The striking gender-, age-, and BMI-independent differences in NKCC observed between the African Americans and French Canadians with essential hypertension could be due to altered plasma lipids affecting ion fluxes across erythrocyte membranes.¹⁸ Indeed, plasma triglycerides were almost twofold lower in the African-American men and women compared to their French-Canadian counterparts (Table 1). However, the absence of correlation between plasma triglycerides and NKCC in the French-Canadian women and the African-American women and men (Table 4) argues against this hypothesis. It should be acknowledged, however, that the absence of statistical significance in some of the African-American correlations may reflect the smaller sample size (71 African Americans vs. 218 French Canadians).

Several working hypotheses might be proposed after analysis of the possible implication of decreased NKCC1 activity in features of the pathogenesis of essential hypertension and its cardiovascular and renal complications documented in African Americans.

First, NKCC1 plays a key role in transport of salt and osmotically obliged water across the basolateral membrane of secretory epithelium in distal tubule²⁷ and sweat glands.¹² Thus, decreased NKCC1 activity may lead to salt retention and the prevalence of salt-sensitive hypertension documented in African Americans.^{7,8} It should be stressed, however, that salt retention in African Americans with essential hypertension is accompanied by decreased plasma renin activity compared to their Caucasian counterparts, including French Canadians from the Saguenay–Lac-St-Jean region of Quebec.²⁸ In contrast, plasma renin activity is elevated by approximately threefold in *NKCC1* null mice^{27,29} that might be caused by direct involvement of NKCC1 in renin secretion demonstrated in comparative analysis of cultured juxtaglomerular granular cells from *NKCC1*^{+/+} and *NKCC1*^{-/-} mice.²⁹ Increased plasma renin activity as well as decreased plasma concentration of atrial natriuretic peptide and augmented expression of major renal Na⁺ transporters such as NKCC2, NCC, Na⁺/H⁺ exchanger (NHE3), and α 1 subunit of Na⁺, K⁺-ATPase in kidney lysates from *NKCC1*^{-/-} mice²⁷ should increase vascular volume and blood pressure, thus minimizing hypotension demonstrated in these genetically engineering animals by Flagella *et al.* and Wall *et al.*^{25,27} Importantly, augmented salt sensitivity^{7,8} and NKCC2-mediated reabsorption of salt and osmotically obliged water³⁰ detected in blacks possessing decreased NKCC1 activity are consistent with increased expression of NKCC2 (ref. 27) and salt-induced hypertension³¹ seen in NKCC1-deficient mice.

Second, it is well documented that HCD decrease [Cl⁻]; and hyperpolarize vascular smooth muscle cells. They also attenuate Ca²⁺ influx via voltage-gated L-type channels and reduce smooth muscle contractions triggered by diverse stimuli, including modest depolarization, α -adrenoceptor agonists, histamine, angiotensin II, thromboxane A₂, and oxytocin (for complete list of references, see refs. 32–35). More recently, we reported that these actions of HCD are absent in *NKCC1* null mice.²⁶ Thus, decreased NKCC1 seen in African Americans may contribute to attenuation rather than elevation of blood pressure via its manifestation in vascular smooth muscle cells involved in regulation of peripheral resistance.

Third, because of its key role in the maintenance of capillary blood flow and hydrostatic pressure,³⁶ the involvement of abnormal myogenic tone responses in protection against hypertension-induced organ damage is widely disputed.³⁷ Recently, it has been reported that HCD sharply lower myogenic tone in mouse mesenteric arteries²⁶ and completely abolish the myogenic responses of renal afferent arterioles of *in vitro* perfused hydronephrotic rat kidneys.³⁵ Importantly, this action of HCD was absent in mesenteric arteries isolated from *NKCC1*^{-/-} mice.²⁶ Viewed collectively, these data allowed us to propose that decreased NKCC1 contributes to attenuated myogenic tone that, in turn, leads to high blood pressure–induced kidney damage and approximately fourfold greater prevalence of end-stage renal disease documented in hypertensive African Americans compared to their Caucasian counterpart.^{21,38,39}

In conclusion, our study results show that NKCC1 activity in erythrocytes of African Americans with essential hypertension is twofold lower than in their French-Canadian counterparts. They also demonstrate that these differences are not caused by altered plasma lipid profile. Further experiments should be performed to uncover the mechanism of this phenomenon and its relationship to features of the pathogenesis of hypertension and its renal complications seen in African Americans.

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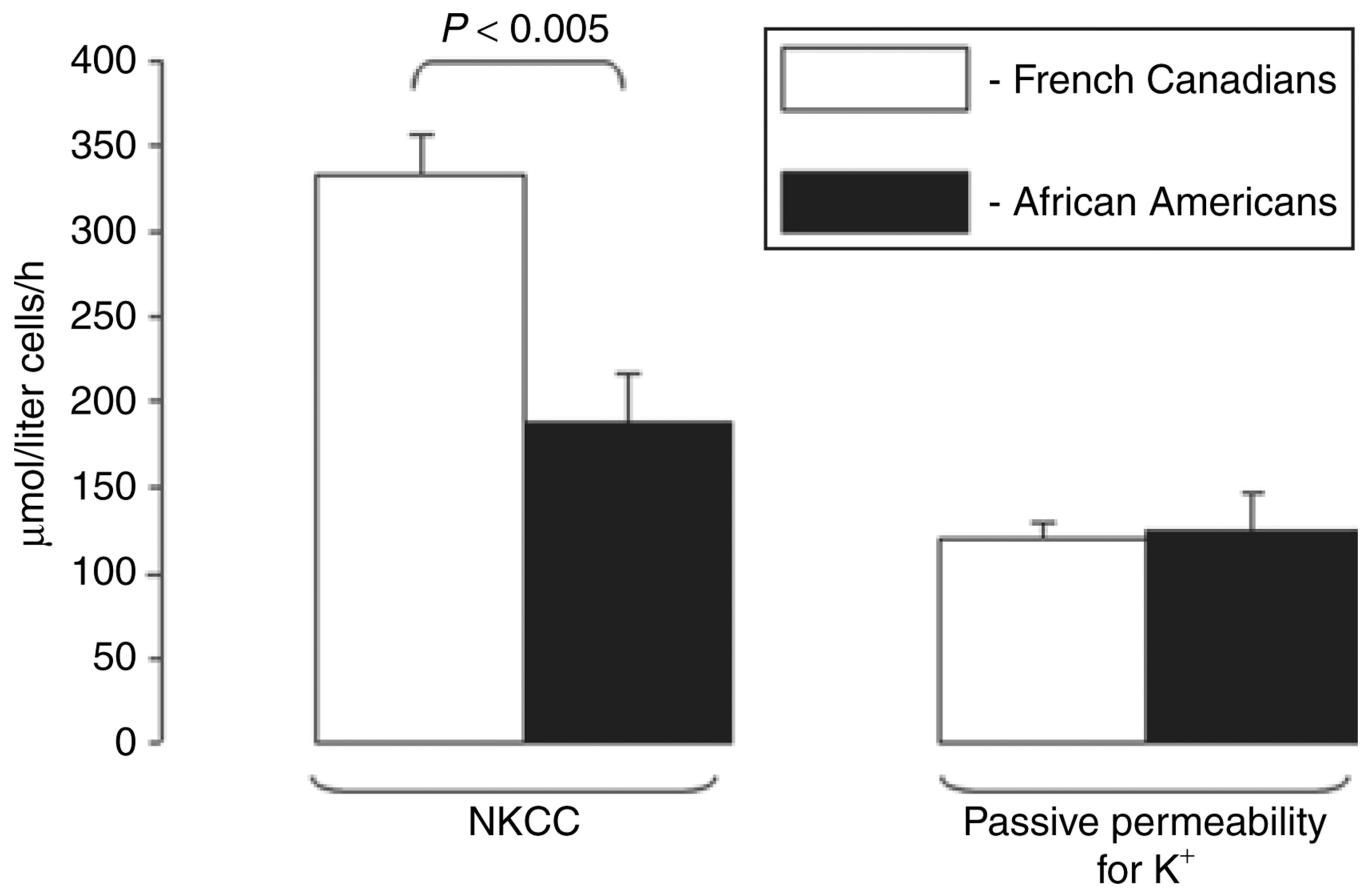


Figure 1. NKCC and passive permeability of erythrocyte membranes for K⁺ in French Canadians and African Americans with essential hypertension. Means ± s.e. are shown. NKCC, Na⁺,K⁺, 2Cl⁻ co-transport.

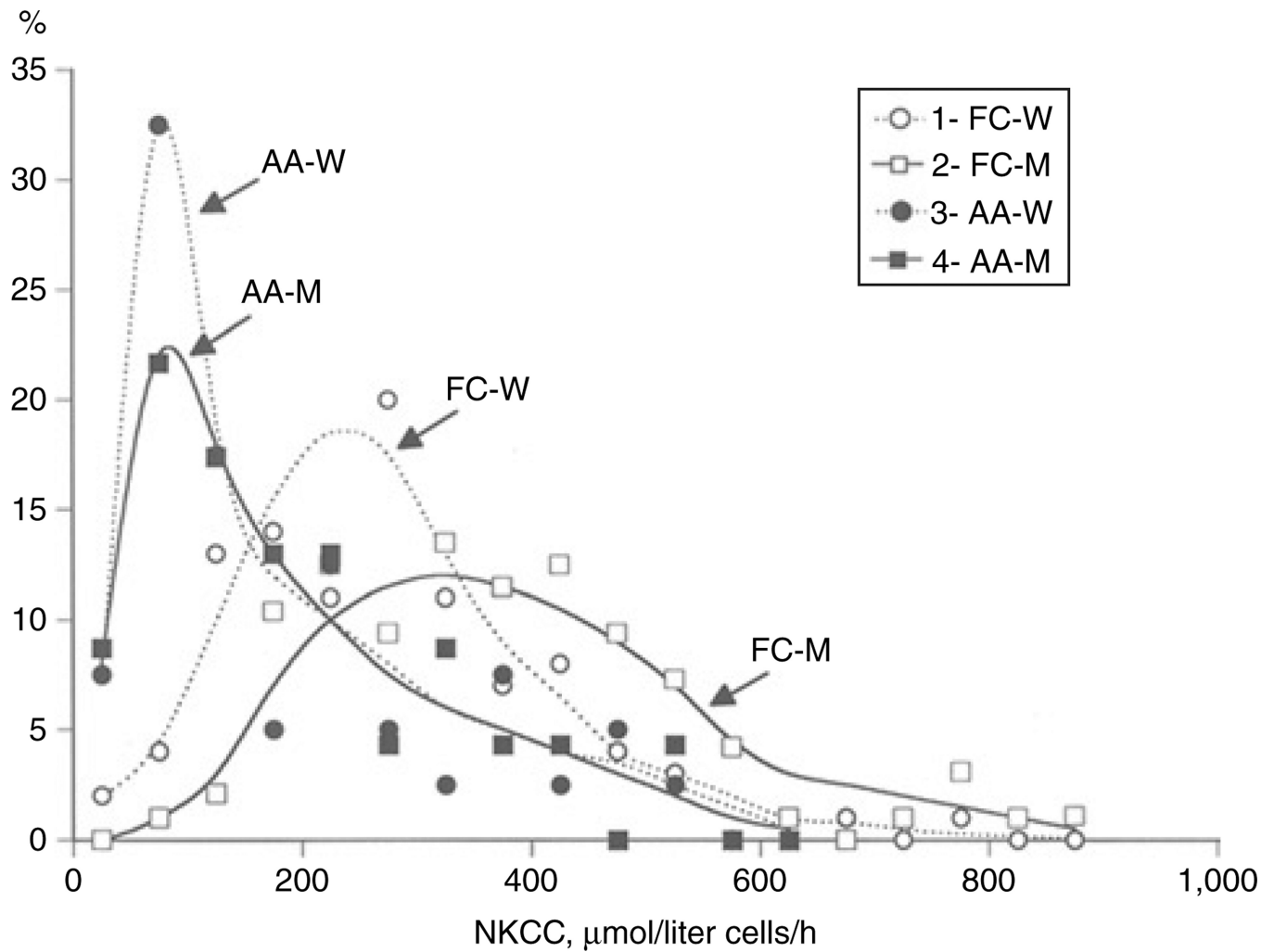


Figure 2. Distribution of African-American and French-Canadian men and women according to NKCC activity. The 50 $\mu\text{mol/liter cells/h}$ increment of NKCC was used to calculate the percentage of patients with the carrier's activity, indicated by white and black circles (French-Canadian and African-American women, respectively) and white and black squares (French-Canadian and African-American men, respectively). AA-M, African-American men; AA-W, African-American women; FC-M, French-Canadian men; FC-W, French-Canadian women; NKCC, $\text{Na}^+, \text{K}^+, 2\text{Cl}^-$ co-transport.

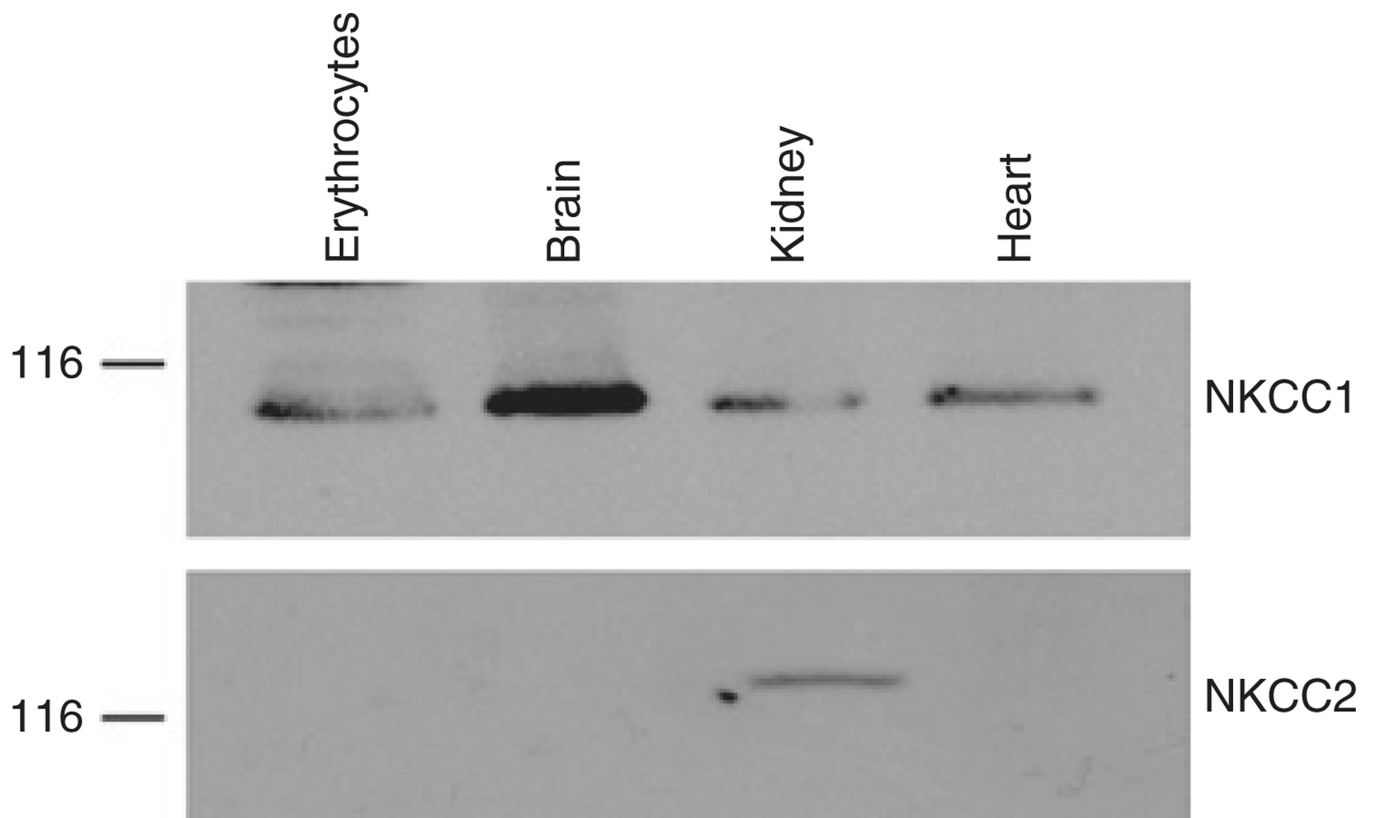


Figure 3. Representative western blots demonstrating the presence of NKCC1 and NKCC2 immunoreactive proteins in human erythrocytes and rat heart, kidney, and brain. NKCC, Na^+ , K^+ , 2Cl^- co-transport.

Table 1

Age, body mass index (BMI), and plasma lipids in French Canadians and African Americans with essential hypertension

	French Canadians		African Americans	
	Men (<i>n</i> = 111)	Women (<i>n</i> = 107)	Men (<i>n</i> = 26)	Women (<i>n</i> = 45)
Age	48.6 ± 4.6 (22–86)	52.9 ± 5.2 (23–88)	41.8 ± 8.4 (31–53)	45.6 ± 6.9 (28–56)
BMI, kg/m ²	28.64 ± 2.76 (21.90–38.76)	28.65 ± 2.80 (17.03–43.74)	29.55 ± 5.91 (18.92–43.67)	29.55 ± 5.91 (20.60–40.81)
Triglycerides, mmol/l	2.41 ± 0.23 (0.22–6.58)	2.23 ± 0.23 (0.24–7.03)	1.43 ± 0.29* (0.41–2.16)	1.10 ± 0.17** (0.52–2.34)
Cholesterol, mmol/l	5.21 ± 0.51 (0.62–7.81)	5.82 ± 0.59 (0.68–11.36)	4.99 ± 1.02 (2.34–8.22)	4.89 ± 0.75 (2.73–7.12)
LDL, mmol/l	3.22 ± 0.33 (0.38–5.50)	3.41 ± 0.36 (0.32–9.38)	3.30 ± 0.67 (1.24–5.77)	3.36 ± 0.51 (1.36–5.38)
HDL, mmol/l	1.05 ± 0.10 (0.18–2.17)	1.39 ± 0.14 (0.21–4.37)	1.02 ± 0.21 (0.51–2.22)	1.04 ± 0.16 (0.73–1.77)

Means ± s.e. values are given; extreme values are shown in parentheses.

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

* *P* < 0.01 and

** *P* < 0.001 compared to French-Canadian men and women, respectively.

Table 2

Ion transport in erythrocytes of French Canadians and African Americans with essential hypertension

Subjects	Ion transport, $\mu\text{mol/liter of cells/h}$	
	NKCC	Passive permeability for K^+
French-Canadian men	381 ± 37 (86–891)	102 ± 10 (58–157)
French-Canadian women	$285 \pm 28^*$ (28–823)	128 ± 12 (63–417)
African-American men	191 ± 39 (21–507)	125 ± 26 (50–248)
African-American women	176 ± 27 (2–559)	125 ± 19 (73–281)
$P_{1,3}$	<0.001	NS
$P_{2,4}$	<0.01	NS

Means \pm s.e. values are given; extreme values are shown in parentheses.

NKCC, Na^+ , K^+ , 2Cl^- co-transport; NS, not significant.

* $P < 0.05$ compared to French-Canadian men.

Table 3

Age, BMI, and erythrocyte ion transport in French-Canadian and African-American men and women with essential hypertension: correlation analysis

	NKCC	Passive permeability for K ⁺	NKCC	Passive permeability for K ⁺
	French-Canadian men		African-American men	
Age	<i>r</i> = 0.066	<i>r</i> = -0.222	<i>r</i> = 0.139	<i>r</i> = -0.215
	<i>P</i> = 0.49	<i>P</i> = 0.02	<i>P</i> = 0.51	<i>P</i> = 0.30
BMI	<i>r</i> = 0.186	<i>r</i> = -0.073	<i>r</i> = -0.315	<i>r</i> = 0.14
	<i>P</i> = 0.05	<i>P</i> = 0.45	<i>P</i> = 0.12	<i>P</i> = 0.51
	French-Canadian women		African-American women	
Age	<i>r</i> = -0.057	<i>r</i> = -0.026	<i>r</i> = 0.067	<i>r</i> = 0.145
	<i>P</i> = 0.56	<i>P</i> = 0.78	<i>P</i> = 0.67	<i>P</i> = 0.35
BMI	<i>r</i> = 0.165	<i>r</i> = -0.154	<i>r</i> = -0.059	<i>r</i> = 0.438
	<i>P</i> = 0.09	<i>P</i> = 0.12	<i>P</i> = 0.71	<i>P</i> = 0.03

Correlations with *P* < 0.05 appear in boldface.

BMI, body mass index; NKCC, Na⁺,K⁺,2Cl⁻ co-transport.

Table 4

Ion transport and plasma lipids in French-Canadian and African-American men and women with essential hypertension: correlation analysis

	NKCC	Passive permeability for K ⁺	NKCC	Passive permeability for K ⁺
	French-Canadian men		African-American men	
Triglycerides	<i>r</i> = 0.245	<i>r</i> = 0.150	<i>r</i> = -0.235	<i>r</i> = 0.267
	<i>P</i> = 0.01	<i>P</i> = 0.12	<i>P</i> = 0.27	<i>P</i> = 0.21
Cholesterol	<i>r</i> = 0.205	<i>r</i> = 0.099	<i>r</i> = -0.120	<i>r</i> = 0.14
	<i>P</i> = 0.03	<i>P</i> = 0.31	<i>P</i> = 0.22	<i>P</i> = 0.51
LDL	<i>r</i> = 0.06	<i>r</i> = 0.051	<i>r</i> = -0.103	<i>r</i> = 0.201
	<i>P</i> = 0.56	<i>P</i> = 0.61	<i>P</i> = 0.63	<i>P</i> = 0.35
HDL	<i>r</i> = 0.197	<i>r</i> = -0.062	<i>r</i> = 0.051	<i>r</i> = -0.273
	<i>P</i> = 0.04	<i>P</i> = 0.52	<i>P</i> = 0.81	<i>P</i> = 0.20
	French-Canadian women		African-American women	
Triglycerides	<i>r</i> = 0.139	<i>r</i> = 0.066	<i>r</i> = 0.093	<i>r</i> = 0.490
	<i>P</i> = 0.17	<i>P</i> = 0.51	<i>P</i> = 0.56	<i>P</i> = 0.00099
Cholesterol	<i>r</i> = 0.101	<i>r</i> = 0.08	<i>r</i> = -0.194	<i>r</i> = 0.175
	<i>P</i> = 0.31	<i>P</i> = 0.43	<i>P</i> = 0.22	<i>P</i> = 0.27
LDL	<i>r</i> = 0.06	<i>r</i> = 0.049	<i>r</i> = -0.152	<i>r</i> = 0.100
	<i>P</i> = 0.56	<i>P</i> = 0.64	<i>P</i> = 0.34	<i>P</i> = 0.53
HDL	<i>r</i> = 0.026	<i>r</i> = 0.039	<i>r</i> = 0.326	<i>r</i> = -0.068
	<i>P</i> = 0.79	<i>P</i> = 0.70	<i>P</i> = 0.03	<i>P</i> = 0.67

Correlations with $P < 0.05$ appear in boldface.

HDL, high-density lipoprotein; LDL, low-density lipoprotein; NKCC, Na⁺,K⁺,2Cl⁻ co-transport.