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INTRACELLULAR CATION CONCENTRATIONS IN ESSENTIAL HYPERTENSION AND CHRONIC RENAL FAILURE

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ABSTRACT

The aim of this study was to test basal and after treatment erythrocyte sodium and calcium concentrations, and calcium-ATPase activity and platelet cytosolic free calcium and pH in 20 normotensive controls, 20 hemodialysis-dependent chronic renal failure patients and in 18 essential hypertensives.

Prior to treatment, essential hypertensive and uremic patients presented similar higher platelet calcium concentrations and lower pH than the normotensive control group. The erythrocyte sodium, calcium, and magnesium concentrations were only significantly elevated in chronic renal failure, with a significant decrease in the calcium-ATPase activity in the latter population.

Hemodialysis partially reversed these intracellular ionic abnormalities with normalization of platelet pH . Significant correlations have been noted between weight loss and decreases in platelet calcium concentration ($r=0.60$, $p<0.01$) or in erythrocyte sodium ($r=0.50$, $p<0.05$). The systolic blood pressure decrease was only correlated to the increase in calcium-ATPase activity ($r=0.57$, $p<0.05$).

Antihypertensive treatment (captopril and nifedipine) only tended to normalize the intracellular calcium concentration with correlation between the decrease of the latter and blood pressure decrease ($r=0.64$ for the systolic blood pressure and 0.68 for the diastolic blood pressure, $p<0.01$).

Thus, in essential hypertension and in uremia, some cellular ionic abnormalities exist in platelets in baseline condition. Moreover, in uremia, erythrocyte presents abnormal ionic pattern. Some, but not all of these abnormalities could be corrected by treatment affecting blood pressure (cellular calcium) in essential hypertension or by hemodialysis (cellular sodium, calcium, and pH). In the latter treatment, the changes are linked to extracellular fluid modification. In essential hypertension, the intracellular calcium reduction was linked to blood pressure decrease.

INTRODUCTION

The relationship between sodium (Na) and high blood pressure (BP) is still disputed. Among theories proposed, one supposed the existence of abnormality of the Na transport across the cell membrane leading to an increase in intracellular Na concentration followed by an increase in intracellular calcium (Ca) concentration, the well-known trigger for stimulation of contractile proteins at the vascular smooth muscle level. One hypothesis for these ionic modifications proposes the existence of a plasma Na-K-ATPase inhibitor responsible for this sodium increase. Another proposition would be a hyperactivity of the sodium-proton (Na-H) exchange with alkalinisation of the cells (1).

For testing these theories, human erythrocytes and platelets were used as they are more easily available than smooth muscle cells, postulating the existence of a diffuse membrane abnormality. Cells were obtained from individuals suffering from primary hypertension and also from chronically hemodialysed patients. The latter model has been frequently characterized by hypertension and the presence of large amount of plasma Na-K-ATPase inhibitor activity (2,3).

Our aim was as follows :

1. What are the effective baseline blood cell cation abnormalities in essential hypertension and in chronic renal failure (by

measuring Na, Ca, and H cations)?

2. Could these alterations be reversed during BP decrease (by antihypertensive or hemodialysis treatment)?

3. Could these reversible abnormalities have some relationship with the pathogenesis of BP changes, postulating that the reversibility would be the same at the vascular smooth muscle level?

MATERIAL

The study was approved by the hospital's committee on Ethics and approval was also obtained from each patient before starting. The arterial BP was taken after 10 minutes in supine position using a Hawksley Random Zero Sphygmomanometer. Two measurements of BP (Korotkoff phase V for diastolic BP) were taken 5 minutes apart and the BP reported was the mean of these values.

The normotensive controls (NC) (n=20) were recruited from hospital staff (n=10) and people attending our hospital for routine examination (n=10). All of these NC were healthy and none was taking any medication at the time of the study.

The essential hypertensive patients (EHT) (n=18) were recruited from hypertensive patients attending the out-patient clinic of our University hospital. These patients were untreated, presented BP level above 140 and/or 90 mmHg and no evident secondary cause of high BP. They were free of aspirin for at least 10 days and without cardiovascular complications.

The uremic patients tested (U) (n=20), with end-stage renal disease of various causes, were anuric, and none was taking loop diuretics. They were only slightly hypertensive before hemodialysis, and the elevated BP was reduced during the hemodialysis treatment.

TABLE 1

Descriptive Characteristics of NC, U, and EHT Populations

VARIABLES	NC (n=20)	U (n=20)	EHT (n=18)
AGE (years)	41±16*	53±14	50±15
SEX RATIO (M/F)	12/8	8/12	10/8
BMI (Kg/m²)	25±4	26±4	27±4
SYSTOLIC BP (mmHg)	124±10*	149±20*	164±14*
DIASTOLIC BP (mmHg)	74±8	77±10	100±7*
HEART RATE (B/min)	74±12	76±11	72±12

(ANOVA with BONFERRONI test): * = $p<0.05$ compared to other groups

BMI = body mass index

The table 1 describes the characteristics of our populations. All of our patients were caucasian.

All blood drawings were performed in the morning, in fasting conditions. For uncomplicated EHT, measurements were first done prior to and following 1 month of treatment with either a calcium entry blocker (nifedipine, n=9) or an angiotensin converting enzyme inhibitor (captopril, n=9). For end-stage renal disease patients, erythrocyte and platelet measurements were performed at the beginning and at the end of a bicarbonate buffer hemodialysis session using a cuprophan hollow fiber membrane (dialysate composition in mM : Na 140; K 1; Ca 1.5; Bicarbonate 35). During the session heparin perfusion was used to maintain the whole blood activated clotting time between 200 and 250 sec, ultrafiltration was performed to target the "dry"

weight. The latter was calculated according to regular post-dialysis echocardiographic assesment of cardiac volume normalization, clinical absence of oedema, and the lowest weight tolerated without muscular cramps. In these patients, plasma renin activity (PRA), blood pH and calcium concentration, and parathyroid hormone 1-84 (PTH) have been measured at the beginning and the end of the hemodialysis session.

METHODS

ERYTHROCYTE ION MEASUREMENTS.

Five ml freshly drawn heparinized venous blood were centrifuged at 4°C, 1500 g for 10 minutes. Plasma and buffycoat were discarded. One half of the remaining erythrocytes was washed 3 times with ice-cold choline chloride 140 mM, and hemolyzed in bidistilled water for Na determinations. The other half of erythrocytes was used to determine Ca concentration according to GAFTER et al (4) : in brief, 4 washings were performed with cold sodium chloride (150 mM) to yield 5 ml packed washed cells and addition of lanthanum chloride 10% in trichloracetic acid 10%. All these determinations have been performed by an atomic Perkin Elmer 130 spectrophotometer. Results are expressed in mmol/l of cells for the sodium and in μmol/l of cells for Ca concentrations.

Erythrocyte calcium-ATPase activity was also measured according to the method described by CHA et al (5), and expressed in μmol Pi liberated/h per mg protein.

PLATELET FREE CYTOSOLIC CALCIUM AND pH MEASUREMENTS.

Ten ml venous blood were collected in Becton dickinson C.T.A.D. vacutainer tubes containing: citrate, adenosine,

theophylline, and dipyridamole, which allow excellent reproducible results in each patient.

Intracellular free calcium was estimated using the fluorescent probe fura 2-acetoxymethylester (FURA 2-AM). Blood was centrifuged at 150 g for 10 minutes at room temperature (20°C). The platelet rich plasma was then diluted with 1 ml Hepes buffer (in mM : NaCl 140; KCl 5; MgSO4 1; glucose 6; HEPES sodium 10; EGTA 4; pH 7.4) and centrifuged at 200 g for 10 minutes. The pellet was gently resuspended into 1 ml HEPES buffer without EGTA. Thereafter, the Fura 2-AM loaded cells were left at room temperature (20°C) for 30 minutes to be sure the fluorescent probe was completely cleaved by cytoplasmic esterases into Fura 2. In order to remove non internalized probe, the specimens were gel filtered through a 15 ml Sepharose 2-B column equilibrated with HEPES buffer pH 7.4 just before the fluorescent measurement. The columns were equilibrated and eluted with Hepes buffer. The very small amount of extracellular dye noted after such gel filtration was checked according to the procedure detailed by OSHIMA et al (6) using EGTA in the extracellular medium to complex extracellular Ca and measuring the fluorescent signal decrease, and this amount was substracted from the total fluorescent signal in the presence of extracellular $CaCl_2$.

Platelet counts of each sample were adjusted to $1x10^8$ cells/ml. External CaCl2 was added to the cell suspension at a concentration of 1 mM. Fluorescent measurements were performed using glass cuvettes at 20°C with a Perkin Elmer LS-5 Luminescence Spectrometer. The excitation wave-length was 340 nm with 2.5 nm slit and the emission wave-length was 500 nm with 5 nm slit.

Intracellular free calcium levels were determined according to POLLOCK et al (7) from the equation :

$$(Ca)_{IC}\ (nmol/l) = Kd\ (F - F\ min)/(Fmax - F)$$

where F was in arbitrary units of fluorescence, Kd (the dissociation constant for calcium binding to the indicator)= 224 nmol/l. Fmax was determined by lysing the cells with digitonin 50 mM and thus exposing Fura 2 to 1 mM Ca; Fmin was determined by adjusting the pH of lysed cells to 8.5 with TRIS 20 mM and chelating the calcium with addition of EGTA 10 mM. A correction of Fmin was always done if necessary after lysing the platelets, by adding manganese chloride 2 mM (which totally quenched Fura-2 and gave by this way the autofluorescence of the cells). The equation for calculating Fmin from F_{Mn} was : $Fmin = F_{Mn} + (F\ max - F_{Mn})/3.7$ (7). The average intracellular calcium concentration was calculated from the values obtained from 2 different aliquots of platelets from each patient. The intra-essay and inter-essay variations were less than 5 and 7 %, respectively. The delay between drawing blood and the end of the estimation was approximately 2 hours.

The platelet intracellular pH (final concentration of 1×10^8 cells/ml) has been determined according to RINK et al (8) with the fluorescent probe 2, 7 bis carboxyethyl-5, 6 carboxyfluorescein acetoxymethylester (BCECF-AM) 5 μM with an excitation wavelength of 500 nm and a 5 nm slit, the emission wavelength was 530 nm with 10 nm slit, at room temperature (20°C). BCECF fluorescences were calibrated by lysing the cells with digitonin 50 μM to release the probe into the extracellular medium and the fluorescent signal was recorded at known values of pH measured with a combination glass electrode reference (radiometer) directly inserted in the cuvette. A calibration

curve for BCECF fluorescence with NIGERICIN 2 μg/ml in a high KCl buffer at different external pH were performed in every sample and the pH reported was the corrected value as detailed by ASTARIE (9). Moreover, dye leakage did not exceed 13 % per hour at 20°C. The intra- and inter- essay variations were less than 3 and 1.5 %, respectively. All measurements were performed within 2 hours after blood sampling.

STATISTICAL ANALYSIS

All data are represented as means ± SD. The data were found to be normally distributed with no significant degree of skewness. Analysis of variance with BONFERRONI correction was used for comparing the 3 groups of patients and Multiple analysis of variance (MANOVA) test allowed to measure the influence of age and gender in the different populations. Where only 2 independent groups of means were to be compared, a simple t-test was performed. A paired t-test was used to evaluate the difference between initial and final values. Correlations have also been measured between the different quantitative parameters. Only p values less than 0.05 were regarded as statistically significant.

RESULTS

Table 2 shows the baseline results for the 3 populations. The red blood cell Na concentration was higher in uremic patients than in the normotensive control population, but not different from essential hypertensive patients. Red blood cell Ca concentration was significantly higher and Ca-ATPase activity lower in uremia than in the 2 other populations. The intracellular free calcium in platelets was increased and pH decreased in both uremic and essential hypertensive patients.

TABLE 2
Erythrocyte Na, Ca Concentrations, Ca-ATPase Activity, Platelet Ca and pH in NC, U, and EHT Patients.

VARIABLES	NC (n=20)	U (n=20)	EHT (n=18)
ERYTHROCYTE		<0.05	
Na (mmol/l)	8.8 ± 2.2	---- 10.6 ± 2.6	10.2 ± 1.8
Ca (μmol/l)	7.05± 4	13.6 ± 6*	9 ± 3
Ca-ATPase (μmol P/h per mg prot)	1.1 ± 0.3	0.7 ± 0.2*	1.07± 0.2
PLATELET			
Ca (nmol/l)	91 ±19*	105 ±16	106 ±12
pH (units)	7.10± 0.04*	7.05± 0.03	7.05± 0.04

ANOVA with BONFERRONI test : * $p<0.05$ with the 2 other groups

In the untreated essential hypertensive patients, systolic and diastolic BP correlated with erythrocyte Na concentration ($r=0.72$ and $=0.66$, respectively; $p<0.01$) and with platelet Ca ($r=0.72$ and $=0.68$, respectively; $p<0.01$). In uremic patients, systolic and diastolic BP only correlated with platelet Ca ($r=0.80$ and 0.52; $p<0.001$ and <0.05, respectively). Platelet Ca moreover correlated with erythrocyte Na concentration in essential hypertensive patients ($r=0.65$; $p<0.01$) and in uremia ($r=0.50$; $p<0.02$).

No significant influence of age or gender has been found, by the MANOVA test, on different parameters : body mass index, erythrocyte Na, Ca concentrations, Ca-ATPase activity, platelet pH and Ca concentration. However, age influenced systolic BP ($p<0.03$).

Antihypertensive treatment in essential hypertension decreased systolic and diastolic BP but was not associated with changes in the erythrocyte ionic concentrations except the Ca concentration which was decreased. Platelet pH did not change;

TABLE 3

Comparison between Captopril and Nifedipine Treatment for 1 Month in EHT Patients.

VARIABLES	CAPTOPRIL (n=9)		NIFEDIPINE (n=9)	
	BEFORE	AFTER	BEFORE	AFTER
SBP (mmHg)	165 ± 10	151 ± 12*	162 ± 17	148 ± 15*
DBP (mmHg)	99 ± 5	92 ± 15*	101 ± 8	90 ± 8*
ERYTHROCYTE				
Na (mmol/l)	10.2± 1.3	10.2± 2.9	10.2± 2.3	10 ± 2.8
Ca (μmol/l)	9 ± 3	7.4± 3*	9.3± 3	7.1 ± 2.7*
Ca-ATPase (μmol P/h per mg prot)	1.1± 0.2	1.1± 0.2	1 ± 0.3	1 ± 0.1
PLATELET				
Ca (nmol/l)	107 ± 10	94 ± 13*	104 ± 13	90 ± 17*
pH (units)	7.05± 0.03	7.05±0.03	7.05±0.04	7.05±0.02

* : $p<0.05$ between before and after treatment

however treatment reduced the cytosolic free calcium (table 3). No significantly difference was found in the response between nifedipine and captopril groups for any of the parameters tested. The variation in the platelet Ca concentration induced by lowering BP has been correlated to systolic and diastolic BP changes ($r=0.64$ for the systolic and $r=0.58$ for the diastolic BP; $p<0.01$).

Table 4 represents the influence of hemodialysis (with a weight loss of 2.2 ± 1.3 Kg) on BP, and blood cell parameters. During treatment, systolic BP, plasma PTH, erythrocyte Na and Ca concentrations significantly decreased, but Ca-ATPase activity and platelet pH increased . In these uremic patients, the plasma PTH was not correlated to any clinical or blood cell ionic changes during this treatment. The decrease in erythrocyte Na or platelet Ca concentration has been correlated to the weight loss during hemodialysis ($r=0.50$; $p<0.05$ and 0.60;

TABLE 4

Influence of Hemodialysis on Blood Pressure and Blood Cell Ions.

VARIABLES	HEMODIALYSIS (n=20)	
	BEFORE	AFTER
SBP (mmHg)	149 ± 20	129 ± 24**
DBP (mmHg)	77 ± 10	75 ± 12
PRA (ng/ml x h)	1.6 ± 0.4	3.2 ± 1.5**
Blood pH(units)	7.23± 0.05	7.35± 0.33**
Blood Ca(mM)	2.34± 0.18	2.83± 0.15***
PTH(pg/ml)	174 ± 58	110 ± 68*
ERYTHROCYTE		
Na (mmol/l)	10.6 ± 2.6	10 ± 2.4*
Ca (μmol/l)	13.8 ± 6	10.8 ± 3.8***
Ca-ATPase (μmol P/h per mg prot)	0.7 ± 0.2	0.9 ± 0.1***
PLATELET		
Ca (nmol/l)	106 ± 16	87 ± 14**
pH (units)	7.06± 0.03	7.10± 0.03**

* $p<0.05$; ** $p<0.01$; *** $p<0.001$ weight loss 2.2 ± 1.3 Kg

$p<0.01$,respectively). These ionic modifications have however not been correlated to BP change. On the other hand, the increase in Ca-ATPase activity during this treatment significantly correlated to weight loss ($r=0.53$; $p<0.05$) and to decrease in systolic BP ($r=0.57$; $p<0.05$).

DISCUSSION

The aims of this study has been to evaluate first the cation abnormalities in blood cells from essential hypertensive and uremic patients (the latter represent a well-known model of secondary hypertension) as compared to normotensive controls. Secondly, we intended to appreciate the reversibility of these abnormalities when lowering BP either by antihypertensive drugs

in essential hypertension or by hemodialysis with ultrafiltration in chronic renal failure. The potential relationships between these blood cell abnormalities and BP level have also been tested.

In the essential hypertensive group, a higher platelet free calcium concentration was observed with a decrease in platelet pH as compared to the normotensive control group. No significant difference was noted between these groups for the erythrocyte ionic composition or basal Ca-ATPase activity.

The findings in the literature with respect to the erythrocyte Na concentration in essential hypertensive patients are somewhat varied, with reports of increased, decreased, or unaltered values comparing to normotensive controls (10). The controversy in these results could be explained by different selection criteria of this population. As a matter of fact, BRAMLEY (11) proposed that age and obesity could be responsible for the higher Na concentration noted in essential hypertension. In this study, however, we have not been able to confirm this point, perhaps due to the rather small size of our hypertensive sample. Another explanation for the discrepant results in the literature could be provided by some methodological differences in the procedure for measurement of Na concentration : i.e. storage temperature, delay before measurements (12, 13).

In primary hypertension, however, we have found significant correlations between erythrocyte Na concentration and BP, between platelet Ca and BP in agreement with ERNE et al (14), and between these blood cell ionic parameters themselves, suggesting some pathophysiological role played by these ions in the high BP levels noted in this disease. The increase in platelet Ca could at least in part be the result of plasma influence as proposed by LINDNER et al (15) and PANG et al (16) but also it could be due to a change in the sodium gradient across the membrane by opening of voltage-dependent calcium chanels or decreasing the Na-Ca exchange activity. WEBER et al (17) found, in the plasma of hypertensives, a sodium transport inhibitor which produced a dose-contraction response of isolated rabbit forearm artery which was abolished in calcium free medium, suggesting a role of sodium in this increase of intracellular calcium coming from outside the cells.

The plasma membrane Ca-ATPase plays a critical role in maintaining calcium homeostasis via the stimulation of calcium efflux (18). In our study, the basal erythrocyte Ca-ATPase was not activated in spite of the increase in calcium concentration, expressing some relative defect in its activity. Many controversial results have been published concerning the activity of this enzyme : either increased (19), unaltered (20) or decreased (21).

However, we have also noted a rather provocative decrease in platelet pH activity in essential hypertension. Many works in the literature report alkalinisation of these cells and leukocytes in this population (22, 23). Again a controversy exists about this parameter as measured in essential hypertension since WEDER et al (24) or TOKUDOME et al (25) did not find any difference between the intracellular pH in platelets of normotensive and essential hypertensive patients. Moreover, BATTLE (26) in lymphocytes and RESNIK (27) in erythrocytes showed a decrease in cellular pH in hypertensives. This controversy may be linked to the type of cell used or the methodology of cell storage and warrants further studies.

Yet, an elevation in calcium would lead to a normal compensatory increase in Ca pump activity (functioning as an obligatory Ca^{2+}- H^{+} exchange). The latter could play a role in lowering the pH by driving proton into the cell while extruding calcium from the cell. However, in our study, the Ca-ATPase activity is not increased. Thus, the cytosolic acidification must be explained by other mechanisms than activation of Ca-ATPase enzyme activity. Conversely, this cell acidification could attenuate the Ca^{2+}-H^{+} exchange, thereby increasing intracellular calcium and also stimulating, in parallel, the Na^{+}-H^{+} antiport. The increase in calcium concentration and stimulation of Na^{+}-H^{+} exchange have been noted in vascular smooth muscle cells and proposed by AVIV (1, 28) as the mechanism responsible for essential hypertension (either salt-sensitive or salt-resistant form).

On the other hand, antihypertensive therapy decreases BP and erythrocyte and platelet Ca concentrations. These changes were intercorrelated. The effect on erythrocyte Ca concentration was neither due to a stimulation of Ca-ATPase, nor to a modification of intracellular Na. The effect of antihypertensive treatment

on the platelet Ca has already been noted (14, 15), without any difference in the type of antihypertensive drugs used. Yet, in acute oral administration of nifedipine or captopril, LENZ et al (29) have noted a similar BP decrease in both treated groups, but only a decrease in platelet Ca in the nifedipine treated group. Moreover, in their study, no correlation was observed between BP and platelet Ca changes.

In uremia, we have confirmed previous observations of an increase in erythrocyte Na concentration before hemodialysis (30, 31, 32, 33) which may be due to a plasma Na-K-ATPase inhibitor activity (33, 36). In agreement with GAFTER et al (4), we have noted an increase of erythrocyte total Ca concentration, accounting for the high incidence of erythrocyte morphologic abnormalities (i.e. ecchynocytosis) noted in this population. Our results for the Ca concentration are somewhat lower than those reported by these authors. The erythrocyte Ca-ATPase activity, as previously shown (4, 37) is largely decreased (by approximately 50 %) in chronic renal failure and could be one explanation for the increase in erythrocyte Ca concentration. For GAFTER's group (4), the inhibition of the Ca-ATPase activity is due to a dialysable factor present in uremic serum. The improvement of this pump activity noted in our patients after hemodialysis would agree with this proposition.

As already noted by some (34, 35), but not all authors (38), the platelet cytosolic Ca is increased in the uremic population before hemodialysis treatment. Moreover, we have found in uremia an excellent correlation between BP and platelet free Ca level in agreement with the literature (35). The latter biological parameter is also significantly correlated to erythrocyte Na concentration and appears to be of pathophysiological importance in the genesis of high BP.

As no positive relationship has been noted between intact PTH and platelet cytosolic Ca, the PTH seems not to be the main part of this potential link.

The platelet pH activity in this kind of secondary hypertension, is decreased, perhaps as a consequence of the chronic plasma acidosis seen in these patients. No influence of gender and/or age has been observed in uremic people on all the parameters tested.

During hemodialysis, most of the ionic abnormalities described tended to be corrected, in spite of only a slight effect on BP (the decrease in systolic BP only reaches a level of significance). However, good correlations exist between weight loss, a marker of extracellular volume reduction and erythrocyte Na or platelet Ca changes. This effect may be explained by a modification of the Na-K pump activity (39). Our work gives original information on platelet Ca which decreases during hemodialysis. Correlations exist however between BP changes and changes in erythrocyte Ca-ATPase activity but not with the other blood cell ionic determinations. This is probably due to the existence of other more powerful mechanisms for BP regulation in these uremic subjects during hemodialysis such as sympathetic nervous system activation and plasma renin-angiotensin stimulation (noted in our study) in response to plasma volume contraction. The platelet pH also tends to be normalized with the blood pH correction during the session, in agreement with others (40, 41), whatever hemodialysis technic is used.

We conclude that some similarities exist in the intracellular platelet but not erythrocyte ionic abnormalities observed in primary and secondary hypertension, some of which appear reversible. In essential hypertension, significant correlation has been noted between platelet Ca change and BP decrease during antihypertensive treatment. This has not been found in chronic renal failure, probably due to other influences and mechanisms on the regulation of the blood cell ionic concentrations and BP.

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