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ARGUMENTS FOR THE PRESENCE OF A Na-K ATPase PUMP INHIBITOR IN THE PLASMA OF UREMIC AND ESSENTIAL HYPERTENSIVE PATIENTS

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ABSTRACT

The effect of salt and/or volume depletion has been tested in 6 end-stage renal disease and 11 essential hypertensive patients (HTA) on red blood cell (RBC) ionic fluxes. Volume depletion promotes an increase in the RBC Na-K ATPase activity with, as a result, a significant decrease in intracellular sodium concentration ((Na)ic). Moreover, a factor has been found in the plasma of uremic subjects which causes natriuresis when injected in rat renal arteries. The concentration of this factor decreases during dialysis in relation to the weight loss and the increase in the RBC Na-K pump activity. In essential hypertension, the effect of a low salt diet on the blood pressure is correlated with the improvement of RBC Na-K ATPase activity. These experiments illustrate the presence of a Na-K ATPase inhibitor in the plasma of these subjects, dependent on sodium and water balance.

INTRODUCTION

From the publication of Ambard and Beaujard (1) in the beginning of this century, sodium chloride intake has been proposed as one of the factors leading to chronic arterial hypertension although its importance and its exact mechanism of action still remain uncertain.

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One recent hypothesis postulates that the action of salt consumption on the blood pressure level could be explained by a plasma inhibitor of the Na-K ATPase, secreted when the plasma volume is expanded, for instance when sodium intake is high in the presence of a renal defect in salt excretion ability (2,6). It has been postulated that this factor causes natriuresis at the kidney level, leading in this way to the correction of the expanded plasma voluem. By inhibition of the Na-K ATPase in the vascular smooth muscle cells, this so-called "natriuretic factor" (NaF) may also increase the vascular resistances, and thereby the blood pressure level.

To confirm this theory, it would be necessary to study the Na and K fluxes directly in the target cells, the kidney tubular cells and the arteriolar smooth muscle cells. Unfortunately, such an approach is impossible in the clinical situation, and even, in experimental models, it remains difficult.

However, recent observations of abnormalities in RBC ionic fluxes suggest that the effect of the NaF is a general one, and that these RBC can be used as a convenient tool. Therefore, in this work, the transmembrane ionic fluxes were measured in RBC before and after modifications of plasma volume and total sodium content in patients with essential hypertension and in patients treated by hemodialysis for chronic renal failure. Hypertension is frequent in this disease and often related to sodium and volume overloading, a condition easily modified by hemodialysis.

METHODS

Ten to fifteen ml of freshly drawn venous blood are collected into heparinized tubes and centrifuged 10 minutes at 1750 g and 4°C. The plasma and buffy coat are carefully removed.

INTRACELLULAR SODIUM CONCENTRATION.

After 3 washings of 1 ml RBC in cold isotonic choline chloride (140mM), the intracellular sodium, potassium and lithium concentrations in these cells are determined after hemolysis in ion free distilled water using an atomic absorption spectrophotometer (Perkin Elmer 103).

RBC OUABAIN SENSITIVE SODIUM EFFLUX AND Na-K COTRANSPORT have been measured in duplicate in all the patients tested according to the PCMBS method proposed by Dagher et al., but slightly modified (4). Briefly, the remaining cells are loaded for 20 hours with sodium and choline and depleted of potassium at 4°C after PCMBS exposure to increase the membrane permeability (loading medium : NaCl 40 mM, KCl 3 mM, choline chloride 200 mM, sodium phosphate buffer 2.5 mM (pH 7.2 at 4°C), $MgCl_2$ 1 mM, ethylene glycoltetraacetate (EGTA) 1 mM, and parachloromercury-benzene sulfonate (PCMBS) 0.02 mM). After recovery of normal

membrane function (recovering medium for 1 hour at 37°C : NaCl 150 mM, $MgCl_2$ 1 mM, sodium phosphate buffer 5.4 mM (pH 7.2 at 37°C), adenine 2 mM, cysteine 4 mM, inosine 3 mM and glucose 10 mM), the cells are divided into 3 fractions put in sucrose magnesium media ($MgCl_2$ 75 mM, sucrose 85 mM, glucose 5 mM, MOPS Tris 10 mM (morpholinopropane sulfonic acid with Tris)), all Na and K free, except the first medium which contains KCl 2 mM. The second fraction is incubated in a solution with ouabain 0.1 mM and the third one with ouabain 0.1 mM and furosemide 1 mM.

The net Na and K efflux are measured at 37°C after 30, 60 and 90 minutes, except for the first medium where only the Na efflux can be measured after 15, 30, and 45 minutes. The ouabain sensitive Na efflux is calculated by the difference of fluxes between the first and the second media. The cotransport activity is obtained by the differences of fluxes between the second and third media.

Rb^{86} UPTAKE AND ^{3}H OUABAIN BINDING are measured in triplicate strictly according to the procedure described by De Luise et al (5).

Na-Li COUNTERTRANSPORT is studied in duplicate according to exactly the same technics proposed by Canessa et al (3).

PLASMA AND ULTRAFILTRATE FLUID NATRIURETIC ACTIVITIES are tested in vivo on rat kidneys according to the procedure used by Codon (7). Briefly, plasma or ultrafiltrate fluid samples are successively filtered at 4°C on different Amico filters (UM 50, XM 10 and UM 05). The fractons of molecular weight above 50000, which contain albumin and globulins, and below 500 with small molecules such as urea, creatinine, and ions are discarded. The so prepared materials from 10000 to 50000 (high molecular weight : HMW) and from 500 to 10000 (low molecular weight : LMW) are separately injected in duplicate into rat renal arteries with measurement of fractional sodium excretion (FRE Na) before and after injections. A natriuretic activity is considered as positive when FRE Na after injection at least doubles, and negative when FRE Na does not rise. It must be remembered that such fractions do not modify the glomerular filtration rate in test animals.

MATERIAL

RBC's of 6 male patients treated by hemodialysis (HD) for 12 to 60 months (average 20) were studied just before and just after a treatment session. All these patients, mean age 55 (ranging from 31 to 68), were free of congestive heart failure, infection, systemic or malignant disease. They did not receive digitalis or antihypertensive drugs. They were submitted first

to acetate HD (cuprophan capillary membrane 15-11 Travenol) and the next session 2 days later to hemofiltration (HF, polyacrylonitril membrane, Filtral 7 + 8, Hospal). This last technic allows more exact measurement of the amount of fluid removed during the session with the ability to collect this fluid easily.

RBC's of 8 other uremic subjects (6 males, 2 females, mean age 41), also chronically treated by HD, were collected at the end of such a treatment session and cross-incubated into their own plasma obtained at the beginning of that session and stored at 4°C.

RBC's of 16 essential hypertensive patients were studied (12 males, 4 females, mean age 36, 10 with a family history of high blood pressure, diastolic blood pressure between 90 to 110 mmHg). The subjects had been hypertensive from 3 months to ten years, were free of antihypertensive medications for at least 3 months and were submitted to a moderate salt restriction (about 5 gr NaCl/day) after one month of a placebo period. At each visit, supine blood pressure (BP) (mean of 3 determinations after 10, 20 and 30 minutes) and upright BP (after 1 minute in the standing position) were measured with a sphygmomanometer. Weight and supine and upright heart rate were also determined. After 30 minutes in the supine position, a blood sample was collected for plasma renin activity and RBC ionic flux analyses. On the day before each visit, the patient collected a 24 hour urine sample for ion and aldosterone excretion recordings.

STATISTICAL METHODS

All data are expressed as mean ± SD.

To compare the effect of one parameter on 2 small populations ($n<30$), the Student's t-test has been used. The paired t-test has permitted analysis of the effect of one parameter on one population.

RESULTS

IN THE UREMIC SUBJECTS, the effect of HD or hemofiltration on the RBC Na content and Na fluxes are quite similar (table 1).

When the RBC are compared before and after a HD session, one observes a clear decrease in the intracellular Na concentration. In parallel, there is a significant increase of the Na-K ouabain sensitive pump activity both during HD and HF, measured by the PCMBS ouabain sensitive Na efflux or the ouabain sensitive rubidium uptake. Both methods for the Na-K ATPase determination are well correlated ($r = 0.86$; $p<0.01$). Moreover, no change is observed in the number of active Na-K pump units as estimated by the ouabain binding. However, the cotransport activity remains unchanged during HD or HF.

TABLE 1

RBC Ionic Modifications During HD and HF (n = 6)

	HD		HF	
	Before	After	Before	After
$(Na)_{ic}$ (mm/l cell)	10.6 ± 2.1*	7.4 ± 1.6*	10 ± 1.8*	7.8 ± 1.7*
PCMBS ouab.S.Na efflux (µM/l cell x h)	2760 ± 1180**	4820 ± 1080**	2890 ± 1205**	4505 ± 1200**
Ouab.S.Rb upake (µM/l cell x h)	0.64 ± 0.2***	1.3 ± 0.5***	0.96 ± 0.5**	1.6 ± 0.8**
Ouabain binding (pM/l cell x h)	145 ± 60****	138 ± 48****	114 ± 54****	103 ± 44****
Furosemide S.Na cotransport (µM/l cell x h)	194 ± 45****	202 ± 74****	205 ± 115****	207 ± 98****

* $p < 0.02$
** $p < 0.05$
*** $p < 0.01$
**** NS

These modifications are identical whatever the technics used to treat the patient, either HD or HF, in spite of the fact that the sieving coefficients of the dialysis and hemofiltration membranes are different and the weight losses during the sessions are greater during HD (3.2 ± 0.9) than during HF (2.6 ± 1 Kg) ($p<0.01$).

When RBC's obtained at the end of a HD session are incubated for 1 hour in the plasma of the same subject, but collected at the beginning of the session, the ouabain sensitive Na efflux is inhibited (4993 ± 1378 after the session versus 3728 ± 740 µM/l cell x h when cross-incubated, $p<0.05$), with no change in the cotransport values (294 ± 107 versus 302 ± 90 µM/l cell x h, NS).

There is also a significant correlation between the Rb uptake improvement (in % of the initial value) and the weight loss during the sessions ($r = 0.79$; $p<0.01$). Only one patient shows a very slight improvement of the Na-K pump activity in spite of a moderate weight loss (2.5 Kg and 1.6 Kg during HD and HF, respectively). Indeed, the RBC Na-K ATPase activity of this patient before the treatment sessions is very low but remains low after the treatment (0.45 versus 0.63 µM/l cell x h for the

Rb uptake before and after HD and 0.69 versus 0.73 µM/l cell x h before and after HF, respectively).

A natriuretic activity (expressed by the increase of FRE Na compared with the control value) has been found in the low and the high molecular weight fractions of the plasma collected before the HD sessions in 4 of the 6 patients tested and disappears at the end of this treatment (table 2).
In these 4 patients this decrease in natriuretic activity during the session parallels the weight loss and is related to the increase in the RBC Na-K ATPase activity as estimated by the ouabain sensitive rubidium uptake. Patients number 3 and 5 do not show such a natriuretic activity in their plasma before the session. However, patient 3 also shows only a slight improvement of the RBC Na-K pump activity (40%), but patient number 5 who experienced a very large weight loss during HD (4.7 Kg) shows a very large increase in the RBC Na-K pump activity (185%). The same observation can be made during HF (table 3); but in this condition, a natriuretic activity can also be found in the ultrafiltrate fluid collected just at the start of the

TABLE 2

Measure of a Natriuretic Activity in Uremic Plasma (collected at the start (1) and the end (2) of an Hemodialysis session).

Patients		Natriuretic Activity (↑FRE Na)		Increase in Ouab. S.Rb uptake (%)	Weight Loss (Kg)
		(1)	(2)		
1.	HMW	2.9	0	83	3.8
2.	HWM	3.75	0	99	3.1
3.	LMW	0	0	40	2.5
	HMW	0	0		
4.	LMW	4.1	3	114	2.4
	HMW	2	0		
5.	LMW	0.5	0	187	4.7
	HMW	0	0		
6.	LMW	2	0	114	2.8
	HMW	0	0		

LWM from patients 1 and 2 have not been tested.

session. Again, as during HD, no natriuretic activity has been observed in the plasma of patients 3 and 5 before the sessions.

IN THE ESSENTIAL HYPERTENSIVE PATIENTS, from the 16 initially recruited, 2 had to be discarded after the placebo period, because of a diastolic BP above 110 mmHg and 3 after the salt restriction period because of nonobservance of the diet according to their 24 hour urinary sodium excretion. Thus 11 untreated essential hypertensive subjects (2 females, 9 males, mean age 37, 8 with a family history of high blood pressure) followed well the salt diet proposed according to the decrease in 24 H urinary sodium excretion and constitute our material for statistical analysis.

Table 4 summarizes the clinical and biological parameters for the first visit, after one month with a placebo, 1 pill per

TABLE 3

Measure of a Natriuretic Activity in Uremic Plasma and Ultrafiltrate Fluid (collected at the start (1) and the end (2) of an Hemofiltration session).

		Natriuretic Activity (↑ FRE Na)				Increase in Ouab. S. Rb uptake (%)	Weight (Loss Kg)
		Plasma		UF			
		(1)	(2)	(1)	(2)		
1.	LMW	(4.45)	--	--	--	34	2.2
	HMW	3	0	4.4	2.3		
2.	LMW	(4.45)	--	--	--		
	HMW	5.7	0	0	0	150	4
3.	LMW	0	0	0	0	4	1.6
	HMW	0	0	0	0		
4.	LMW	4.1	3	--	2.6	80	2.1
	HMW	2	0	3.7	0		
5.	LMW	0	2.3	3	2.4		
	HMW	0	1.7	3	2.1	95	3.8
6.	LMW	3.8	2.2	1.5	0		
	HMW	0	0	0	0	60	1.6

LMW of patients 1 and 2 have been pooled to be simultaneously tested together on Natriuresis, RBC ^{86}Rb uptake and Na efflux

TABLE 4

Effect of Salt Restriction on Clinical and Biological Parameters in Untreated Essential Hypertension (n = 11).

	First Visit	Placebo Period	Salt Restriction
UPRIGHT (mmHg)			
systolic BP	184 ± 16*	180 ± 19****	170 ± 2
diastolic BP	106 ± 6*	104 ± 6***	100 ± 6
mean BP	134 ± 7*	131 ± 10**	122 ± 8
SUPINE (mmHg)			
systolic BP	172 ± 12*	169 ± 14****	160 ± 10
diastolic BP	102 ± 6*	101 ± 6***	95 ± 4
mean BP	126 ± 6*	124 ± 7***	117 ± 5
HEART RATE (beats/min)			
upright	78 ± 7*	79 ± 10*	80 ± 8
supine	72 ± 7*	71 ± 6*	71 ± 8
WEIGHT (Kg)	78 ± 13*	79 ± 14*	77 ± 13
PLASMA RENIN ACTIVITY (ng/ml/h)	1.5 ± 0.8*	1.6 ± 1.1*	2.1 ± 1.9
NATRIURESIS			
mEq/24H	162 ± 64*	164 ± 24**	81 ± 18
mEq/gr creatinine	95 ± 27*	98 ± 29**	46 ± 19
KALIURESIS			
mEq/gr creatinine	56.3 ± 14*	53 ± 8*	56 ± 17
URINARY ALDOSTERONE			
µg/gr creatinine	8.4 ± 3.2*	8.8 ± 3.1*	9.4 ± 5.2

*	NS
**	$p<0.01$
***	$p<0.05$
****	$p<0.02$

day, and after 1 month with a salt restriction. Although a slight but insignificant decrease in BP is noted during placebo administration, there is no statistical difference for any parameters between the first visit and the placebo period. On the contrary, after the low salt diet, the upright and supine systolic, diastolic, and mean BP significantly decrease although heart rate, weight, plasma renin activity, 24 hours urinary aldosterone, potassium or calcium excretion do not change. At the RBC level, the (Na)ic significantly decreases after the diet (from 11.6 ± 3 to 9.7 ± 2 mM/l cell x h, $p<0.02$) with an increase in the Na pump activity (3160 ± 1220 during the placebo period to 4666 ± 637 µM/ l cell x h after the diet, $p<0.01$). Correlations exist between decreases in (Na)ic and mean BP ($r = 0.84$; $p<0.01$), between improvement in the Na-K pump activity and decrease in (Na)ic ($r = 0.81$; $p<0.01$) and between the improvement of the Na-K pump activity and decrease in mean BP ($r = 0.59$; $p<0.05$). No change is observed after the salt restriction for the cotransport (353 ± 170 versus 347 ± 171 µM/l cell x h after the diet, NS) and for the Na-Li countertransport (0.503 ± 0.24 versus 0.443 ± 0.23 mM/l cell x h after salt restriction, NS). For the 3 patients excluded because of non-compliance to the salt restriction, no change is noted for any clinical or biological parameters, so that the different correlations remain valid.

DISCUSSION

To test De Wardener's hypothesis that a plasma factor, secreted in response to a tendency of plasma volume to be expanded, causes generalized inhibition of Na-K ATPase in essential hypertensive subjects we have used 2 clinical models allowing modification of the sodium pool : chronic renal failure treated by HD and essential hypertension treated by salt restriction. Moreover, in end-stage renal disease, the presence of NaF in plasma has been investigated according to the technics developed by one of us (7). Both approaches are in good agreement with De Wardener's theory.

Some authors (8,13) have postulated that the abnormalities observed in RBC ionic fluxes of end-stage renal disease subjects are the consequence of some toxins accumulated due to decreased renal excretion. However, we already noted that some of these ionic pumps, for instance, the Na-K ATPase, are impaired by a volume dependent plasma inhibitor (9). In the present study, we confirm an inhibition of the ouabain sensitive Na-K pump activity in CRF before HD, and additionally, that this inhibition is modified by HD or HF. Moreover, the changes in the Na-K pump activity during HD and HF are strongly correlated with the weight loss, which corresponds to the amount of fluid removed during the dialysis sessions. This modification does

not appear to be due to exposure of the RBC to the dialysis membrane, because similar results for ionic fluxes have been obtained with 2 very different types of membrane (cuprophan and polyacrylonitril). Furthermore, cross-incubation experiments with plasma collected before and after the HD sessions reproduce the same inhibition of the Na-K ATPase suggesting the role of a plasma factor. This increase of fluxes during dialysis results from a stimulating effect due to modification of the rate of the Na-K pump activity, not due to an increase in the number of active pump units, as demonstrated by the absence of change in the ouabain binding before and after dialysis.

The increase in RBC Na-K pump activity has been measured with 2 different methods (PCMBS method and rubidium uptake) which are strongly correlated. It thus suggests that the PCMBS method does not impair the structure and function of at least the Na-K ATPase enzyme and that this methodology allows a quite correct measure of pump activity.

A natriuretic activity has been found in the plasma from 4 of the 6 patients at the start of both HD and HF. Its concentration falls at the end of the treatment in relation with the weight loss. In parallel, the RBC Na-K pump activity rises. The decrease in that plasma natriuretic activity during the session could for instance be the result of the removal of a NaF across the membrane. But some previous data (10), with infusion of isotonic saline solution during dialysis to prevent any weight loss while toxins are removed, also prevented the improvement of the RBC ouabain sensitive pump activity. These experiments and the relation between the decrease in natriuretic activity, the weight loss and the improvement in the RBC pump activity tend to show that during the HD sessions there is a decrease in the production of this natriuretic activity sensitive to the decrease in extracellular volume, rather than an elimination through the membrane. The absence of such a natriuretic activity at the start of the HD and HF in the plasma of 2 patients could be explained by the fact that this NaF is not always produced by every uremic subject in response to volume expansion. Another hypothetical explanation could arise from the fact that this factor is noted in the ultrafiltrate fluid from the 5th patient at the start of the HF session, whereas it is absent in his plasma drawn at the same time. It is possible that NaF could be polymerized to varying degrees in uremic patients. Patient 5 could have a natriuretic activity in his plasma with a molecular weight above 50,000 which could have been eliminated during our rough preparation of the natriuretic material, but which could be again found in the ultrafiltrate fluid after fragmentation into a lower molecular weight fraction which passed through the hemofilter membrane.

On the other hand, the third patient (tables 2 and 3) does not seem to have such a material either in HD or HF. This could

be explained by the fact that this patient could be hypovolemic before the dialysis session. Another explanation could be that this patient is the only one tested who is not of European origin. That suggests a racial influence in the production of this natriuretic factor. It should also be noted that he is the only one who does not follow very well the correlation between weight loss and the increase in RBC pump activity.

A final explanation for the absence of NaF in the plasma of some of our uremic patients despite weight loss could be that this factor so prepared does not act on the Na-K ATPase and thus is not the "natriuretic hormone" described by De Wardener et al. (6). Against this possibility, we have observed (unpublished data) that LMW fractions of plasma collected before HF inhibit the ouabain sensitive pump of RBC's taken from normal subjects. Thus, these fractions which promote natriuresis when injected into rat renal arteries could cause natriuresis by inhibition of renal Na-K ATPase.

Thus in end-stage chronic renal failure there is an inhibition of the RBC Na-K pump by a plasma factor, the secretion of which is sensitive to volume expansion. This factor which causes natriuresis in the rat kidney could be the natriuretic hormone proposed by Blaustein (2) and De Wardener (6) as playing a role in the pathogenesis of essential hypertension.

For its part, moderate dietary salt restriction significantly decreases upright and supine BP in essential hypertension after one month. The aim of this study, more than to show the favorable effect of salt restriction on BP, already demonstrated by some authors (11,12), was to note whether a volume dependent humoral factor is present in both essential hypertension and uremia. To test this possibility, we have measured the Na-K pump activity before and after modification of the sodium pool as realized in uremia by HD and HF. Moreover in this study we have tested 2 other RBC transport systems (the contransport and countertransport) which have also been described as abnormal in essential hypertension (3,4). In our hands, these last 2 systems are not affected by reduction of salt intake, whereas the Na-K pump activity improves in parallel with a decrease in (Na)ic during such a treatment, whatever this activity may be at the start of the study (again a very large distribution of the RBC Na-K ATPase activities is noted in essential hypertension as previously observed (10)). Yet those who had the lower pump activities before the diet have shown the greater hypotensive effect and improvement in their RBC Na-K pump activity. This biochemical determination of RBC Na-K ATPase activity could thus be a useful tool in identifying people who would respond the best to a low salt diet and in whom such a treatment must be first recommended. In essential hypertension, all the subjects are not affected to the same

extent regarding inhibition of the Na-K ATPase. This suggests (genetic or acquired) presence in only some patients of a renal defect in salt excretion with consequent fluid retention and production of the natriuretic factor, inhibiting the Na-K ATPase.

In conclusion, these experiments confirm the presence in plasma of a natriuretic inhibitor of the Na-K pump in essential hypertension, although affecting these subjects to various extent, and in volume overloaded uremic patients. However, the nature and the site of production of this factor still remain to be determined.

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