

Relationship between (Na + K)-ATPase activity, lipid peroxidation and fatty acid profile in erythrocytes of hypertensive and normotensive subjects

Ramón Rodrigo · Jean P. Bächler · Julia Araya ·
Hernán Prat · Walter Passalacqua

Received: 22 December 2006 / Accepted: 17 March 2007 / Published online: 5 April 2007
© Springer Science+Business Media B.V. 2007

Abstract Oxidative stress may play a role in the pathogenic mechanism of essential hypertension. Lipid peroxidation can alter the cellular structure of membrane-bound enzymes by changing the membrane phospholipids fatty acids composition. We investigated the relationship between (Na + K)-ATPase activity, lipid peroxidation, and erythrocyte fatty acid composition in essential hypertension. The study included 40 essential hypertensive and 49 healthy normotensive men (ages 35–60 years). Exclusion criteria were obesity, dyslipidemia, diabetes mellitus, smoking, and any current medication. Patients underwent 24-h ambulatory blood pressure monitoring and blood sampling. Lipid peroxidation was measured in the plasma and erythrocytes as 8-isoprostane or malondialdehyde (MDA), respectively. Antioxidant capacity was measured as ferric reducing ability of plasma (FRAP) in the plasma and as reduced/oxidized glutathione (GSH/GSSG ratio) in erythrocytes. (Na + K)-ATPase activity and fatty acids were determined in erythrocyte membranes. Hypertensives

had higher levels of plasma 8-isoprostane, erythrocyte MDA, and relative percentage of saturated membrane fatty acids, but lower plasma FRAP levels, erythrocyte GSH/GSSG ratio, (Na + K)-ATPase activity and relative percentage of unsaturated membrane fatty acids, compared with normotensives. Day-time systolic and diastolic blood pressures correlated positively with lipid peroxidation parameters, but negatively with (Na + K)-ATPase activity. These findings suggest that the modulation of (Na + K)-ATPase activity may be associated with changes in the fatty acid composition induced by oxidative stress and provide evidence of a role for this enzyme in the pathophysiology of essential hypertension.

Keywords Lipid peroxidation · Essential hypertension · (Na + K)-ATPase · Fatty acids

Introduction

Reactive oxygen species (ROS) have been shown to be involved in the pathogenesis of hypertension [1, 2]. Hypertension causes 7.1 million deaths annually [3] and is a major risk factor for cardiovascular disease mortality [4]. An imbalance between the ROS generation and antioxidant defense systems in the body resulting in oxidative stress has been reported in spontaneously hypertensive rats [5] and human essential hypertension [6]. ROS production could damage arterial walls including an impairment of the endothelium-dependent vasodilation or endothelial dysfunction [7].

The interaction of ROS with biological membranes produces a variety of functional modifications due to either direct interaction with the molecular cell machinery and/or oxidative modification of the environment of biological

R. Rodrigo (✉) · J. P. Bächler
Laboratory of Renal Pathophysiology, Molecular and Clinical
Pharmacology Program, Institute of Biomedical Sciences,
Faculty of Medicine, University of Chile, Independencia 1027,
Casilla, 70058 Santiago 7, Chile
e-mail: rrodrigo@med.uchile.cl

J. Araya
Department of Nutrition, Faculty Of Medicine, University
of Chile, Santiago, Chile

H. Prat
Cardiovascular Center, University of Chile Clinical Hospital,
Santiago, Chile

W. Passalacqua
Nephrology Unit, University of Chile Clinical Hospital,
Santiago, Chile

macromolecules [8]. Lipid peroxidation contributes to the loss of cellular functions through the inactivation of membrane enzymes and cytoplasmic proteins. In erythrocytes, increased lipid peroxidation generates changes in lipid composition, thus, altering the phospholipid fatty acid profile and membrane fluidity [9]. The validity of erythrocyte fatty acid composition as a reliable biomarker of that in other organs has been supported by recent studies showing a significant correlation of erythrocyte polyunsaturated fatty acids (PUFAs) composition with that found in the liver [10] and muscles [11].

Lipid composition of the erythrocytes has been implicated in the modulation of membrane-bound enzymes, such as (Na + K)-ATPase, Ca²⁺-ATPase, Mg²⁺-ATPase, and acetylcholinesterase [12]. (Na + K)-ATPase activity depends on its close interaction with phospholipids rich in PUFAs. Since PUFAs are the major targets of ROS renders the membrane phospholipids particularly sensitive to metabolic conditions associated with oxidative stress. Consequently, an increase in PUFAs enhances the vulnerability of membranes to undergo modifications in fatty acid composition [13], thus, impairing the (Na + K)-ATPase micro-environment needed for optimal enzyme activity [14]. (Na + K)-ATPase inhibition triggers Ca²⁺ entry and increases the myogenic tone and contractility in arterial smooth muscle cells [15]. This results in an increase in peripheral vascular resistance, the hemodynamic hallmark of hypertension [16]. The aim of the present study was to test the hypothesis that increased lipid peroxidation leads to changes in cell membrane fatty acid composition that are functionally related to blood pressure elevation through an impairment of (Na + K)-ATPase activity.

Materials and methods

Study design

A cross-sectional design applied to 40 hypertensive and 49 healthy normotensive subjects. The study protocol was approved by the local Ethics Committee of the University of Chile Clinical Hospital, and performed in accordance with the Helsinki Declaration II. All participants signed written consent and no complications were encountered during the study.

Patients

We recruited essential hypertensive patients (stage 1, Seventh Joint National Committee) [3] who were not under pharmacological treatment. Inclusion criteria were male sex and age between 35 and 60 years. Potential participants were recruited from outpatients consulting at the

Cardiovascular Center of the University of Chile Clinical Hospital. Hypertension was defined as the mean day-time blood pressure values ≥ 135 mm Hg systolic, or ≥ 85 mm Hg diastolic, by ambulatory blood pressure monitoring [17]. Exclusion criteria were smoking, obesity (BMI > 30 kg m⁻²), diabetes, hypercholesterolemia, heart, liver or kidney disease, and use of any current medication. Normotensive volunteer subjects participated as controls. Potential participants were subjected to clinical history evaluation, physical examination, and laboratory screening (serum glucose, creatinine, and aldosterone, and lipid profile) in order to ensure that both normotensive subjects and hypertensive patients met the inclusion and exclusion criteria.

Ambulatory blood pressure monitoring

Blood pressure levels were determined through ambulatory blood pressure monitoring on a regular workday (during 24 h from 8:30 AM) using an oscillometric monitor (SpaceLabs 90207, SpaceLabs Inc., Redmond, WA), previously checked for accuracy against simultaneous measurements by mercury sphygmomanometer. The mean day-time value of blood pressure was registered. This device fulfills the validation criteria's of the British Hypertension Society protocol [18] and the Association for the Advancement of Medical Instrumentation (AAMI) for studies in ambulatory condition [19]. The oscillometric accuracy, assessed by Spacelabs-intra-arterial average differences, was -0.6 ± 5.9 and 0.9 ± 6.4 mm Hg (means \pm standard deviation), for systolic and diastolic pressures, respectively, which are within the AAMI accuracy standard. The estimated oscillometric reproducibility was -0.3 ± 3.2 and 0.1 ± 3.5 mm Hg (means \pm standard deviation), for systolic and diastolic pressures, respectively [20, 21].

Blood samples

Venous blood samples (< 10 ml) were collected in chilled vacutainers containing disodium EDTA (1.5 mg ml⁻¹). Plasma and red blood cell lysates, were stored at -70°C . Samples for 8-isoprostane measurement were collected in plastic tubes previously treated with antioxidant butylated hydroxytoluene (final concentration 1 mmol l⁻¹). Erythrocyte membranes were isolated by ultra-centrifugation (Sorvall Inc. Newton, Connecticut, USA) at $100,000$ g for 45 min and stored in eppendorf tubes containing histidine-sucrose buffer (pH 6.8) at -80°C until used for (Na + K)-ATPase and Mg²⁺-ATPase activity measurements.

Ferric reducing ability of plasma (FRAP)

Plasma antioxidant status was assessed by measuring its ability to reduce ferric to ferrous iron (FRAP) with a detection limit 10 $\mu\text{mol l}^{-1}$ [22].

Glutathione

Reduced glutathione (GSH) and glutathione disulfide (GSSG) were assayed by fluorometry [23]. The GSH/GSSG ratio was also determined, as a parameter of intracellular redox status.

Malondialdehyde (MDA)

Lipid peroxides were assayed spectrophotometrically at 532 nm by the thiobarbituric acid reaction at pH 3.5, followed by solvent extraction with a mixture of *n*-butanol/pyridine (15/1, v/v) [24]. Tetramethoxy-propane was used as the external standard and the level of lipid peroxides was expressed as nmol malondialdehyde (MDA)/g Hb.

8-Isoprostane

8-Isoprostane concentration in plasma, recognized as a reliable biomarker of lipid peroxidation *in vivo* [25], was determined by using ELISA kit (Cayman, Ann Arbor, MI) and results expressed as pg/ml.

ATPases

Activities of (Na + K)-ATPase and Mg⁺²-ATPase were measured by the method of Katz and Epstein [26]. The assay mixture consisted in (mM) of 100 NaCl, 20 KCl, 6 ATP (vanadium free), 6 MgCl₂ and 10 imidazole buffer (pH 7.8), was pre-incubated at 37°C for 1 min, and the reaction was started by the addition of ATP. After 15 min reaction was stopped by the addition of 1 ml ice-cold 25% (w/v) trichloroacetic acid. Inorganic phosphate was measured in the supernatant by the method of Taussky and Shorr [27]. (Na + K)-ATPase activity was calculated from the difference between the amount of inorganic phosphate released in the presence and in the absence of K⁺ in the incubation medium. Mg⁺²-ATPase activity was calculated from the difference between the amounts of inorganic phosphate released in the absence of K⁺ at zero time. Protein concentration was measured by the method of Lowry et al. [28]. The specific activities were expressed as μmol inorganic phosphate released per milligram protein per hour.

Extraction and separation of phospholipids from erythrocyte membranes

Blood samples collected in syringes containing 5% (w/v) EDTA as anticoagulant were centrifuged at 2,500 rpm for 15 min at 4°C to separate erythrocytes. Erythrocyte membranes were isolated according to Huertas et al. [29]. Membrane lipids were extracted as described by Bligh and Dyer [30].

Preparation and analysis of fatty acid methyl esters (FAME)

Fatty acids from erythrocyte membranes were methylated. The phospholipids were eluted from silica gel with two 15 ml portions of chloroform/methanol/water (10:10:1, by vol.). Solvent was evaporated in a stream of nitrogen, and 10 mg tricosanoic acid (23:0, internal standard) was added prior to the esterification with 0.2 N sodium-methanol for 30 min at 40°C, and then, with H₂SO₄-methanol as described for alkaline methylation [31]. Samples were cooled and the fatty acid methyl esters (FAME) were extracted with 0.5 ml hexane and analyzed by gas-liquid chromatography.

A Hewlett-Packard gas chromatograph (model 6890)(Palo Alto, CA, USA) equipped with a capillary column (50 m × 0.22 mm BPX70; 0.25U QC 0.08 SGE) was employed to separate FAME. The temperature was programmed from 180°C to 230°C at 2°C min⁻¹ with a final hold, separating 12:0 to 22:6*n*-3. Both detector and injector temperatures were set at 240°C. Hydrogen was used as carrier gas, at a flow rate of 1.5 ml min⁻¹ and split ratio of 1:80. FAME was identified by comparing their retention times with individual purified standards and quantified using a Hewlett-Packard integrator (HP 3396 Series III).

Materials

All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), Merck (Darmstadt, Germany) and Riedel-de Hën (Germany), and were of the highest commercial grade available.

Statistical analysis

Descriptive statistics of variables used the means and standard error of the means (SEM). The source of variation between normotensive and hypertensive subjects was assessed by unpaired Students *t*-test, for normally distributed parameters, with a *P* value < 0.05 for statistical significance. The association of variables was studied by Pearson correlation test due to their Gaussian distribution.

Results

Clinical characteristics

Clinical characteristics of the 89 subjects in the hypertensive and normotensive groups are shown in Table 1. All parameters were within the normal range and were not different between the two groups except for significant

Table 1 Clinical characteristics of essential hypertensive patients ($n = 40$) and healthy normotensive subjects ($n = 49$)

Characteristic	Normotensive ($n = 49$)	Hypertensive ($n = 40$)	<i>P</i> Value
Age (year)	43.4 ± 1.1	44.6 ± 1.4	0.49
Body mass index (kg/m ²)	24.8 ± 1.2	25.9 ± 1.3	0.53
Blood glucose (mmol/l)	4.93 ± 0.07	5.09 ± 0.07	0.11
Creatinine (μmol/l)	79.6 ± 15.0	81.4 ± 9.7	0.93
Total cholesterol (mmol/l)	4.59 ± 0.16	4.81 ± 0.14	0.31
High-density lipoprotein (mmol/l)	1.26 ± 0.04	1.18 ± 0.05	0.18
Low-density lipoprotein (mmol/l)	2.71 ± 0.07	2.86 ± 0.09	0.21
Triglycerides (mmol/l)	1.34 ± 0.06	1.49 ± 0.08	0.12
Awake systolic BP (mm Hg)	118.5 ± 1.3	138.1 ± 2.1	<0.001*
Awake diastolic BP (mm Hg)	76.6 ± 1.7	92.1 ± 1.4	<0.001*
Heart rate (beats/min)	72.8 ± 1.2	74.1 ± 1.3	0.47

Data are expressed as means ± SEM. BP, blood pressure

* Significant difference by unpaired Student's *t*-test

higher mean day-time systolic (SBP) and diastolic blood pressure (DBP) in the hypertensive group ($P < 0.001$),

Antioxidant status and oxidative stress-related parameters

The antioxidant status measured in plasma (FRAP) and erythrocyte (GSH/GSSG ratio) as well as lipid peroxidation products (plasma 8-isoprostane and erythrocyte MDA) are shown in Table 2. FRAP levels and GSH/GSSG ratios were 27% and 31% lower ($P < 0.001$) in hypertensive compared to normotensives subjects, respectively. In addition, erythrocyte MDA and plasma 8-isoprostane levels were 23% and 43% higher in the hypertensives than

Table 2 Plasma and erythrocyte antioxidant status and lipid peroxidation-related parameters of the study participants

Parameter	Normotensive subjects ($n = 49$)	Hypertensive patients ($n = 40$)	<i>P</i> value
<i>Plasma</i>			
FRAP (μmol/l)	419.6 ± 9.7	307.1 ± 11.2	<0.001*
8-isoprostane (pmol/l)	78.7 ± 2.8	112.7 ± 3.2	<0.001*
<i>Erythrocytes</i>			
GSH/GSSG ratio	7.46 ± 0.08	5.17 ± 0.17	<0.001*
Malondialdehyde (nmol/g Hb)	301.9 ± 1.1	372.2 ± 1.4	<0.02*

Data are expressed as means ± SEM. FRAP, ferric reducing ability of plasma; GSH, reduced glutathione; GSSG, oxidized glutathione. * Significant difference by unpaired Student's *t*-test

normotensive subjects, respectively ($P < 0.01$). Furthermore, hypertensive and normotensive mean day-time SBP and DBP were positively correlated with MDA and 8-isoprostane levels (Fig. 1).

Activity of ATPases, lipid peroxidation and systolic blood pressure

Activities of (Na + K)-ATPase and Mg²⁺-ATPase in the erythrocytes of both groups are shown in Fig. 2A. Erythrocyte (Na + K)-ATPase activity was 14% lower in the hypertensives compared to normotensives ($P < 0.03$). In contrast, no significant differences were found in the Mg²⁺-ATPase activity ($P = 0.25$). The correlation between erythrocyte MDA and plasma 8-isoprostane levels with (Na + K)-ATPase activity is shown in Fig. 2B and C, respectively. Erythrocyte (Na + K)-ATPase activity correlated negatively with both MDA and 8-isoprostane in hypertensive and normotensive subjects. In addition, mean day-time SBP and DBP showed a negative correlation with (Na + K)-ATPase activity (Fig. 3) but not with Mg²⁺-ATPase in both groups.

Erythrocytes fatty acid composition

The fatty acid composition of erythrocytes phospholipids is shown in Fig. 4. The 16:00, 18:0 and 24:0 fatty acid levels were higher and 14:1, 16:1, 20:1, 22:1, 20:4 n -6, 22:2 n -9, and 22:6 n -3 levels were lower ($P < 0.05$) in hypertensive compared to normotensives. Whereas 14:00, 20:0, 22:0, 18:1, 24:1, 18:2 n -6, 18:3 n -6, 20:5 n -3 showed no significant differences between the two groups. The relative percentage of saturated fatty acids (SAFAs) were 86% higher but that of monounsaturated fatty acids (MUFAs) and PUFAs were 29% and 21% lower in hypertensives than the normotensives, respectively ($P < 0.001$)(Fig. 4A).

Discussion

The findings of the present study confirm previous data reporting association of blood pressure with both antioxidant status and oxidative stress-related parameters [2]. Furthermore, our data provides a new viewpoint related to fatty acid composition and (Na + K)-ATPase activity in patients with essential hypertension. This suggests an involvement of membrane lipid profile in the modulation of (Na + K)-ATPase activity and its role in the pathogenesis of blood pressure elevation.

The lower erythrocyte GSH/GSSG ratio and plasma FRAP levels (parameters indicative of the antioxidant defenses) in hypertensives, is consistent with the higher lipid peroxidation. Antioxidants normally contribute to the

Fig. 1 Pearson correlation of plasma and erythrocyte lipid peroxidation parameters to mean day-time systolic (A, C) and diastolic (B, D) blood pressure of normotensive ($n = 49$) (open circles and dotted line) and hypertensive ($n = 40$) (solid circles and line) participants, respectively. Hemoglobin (Hb)

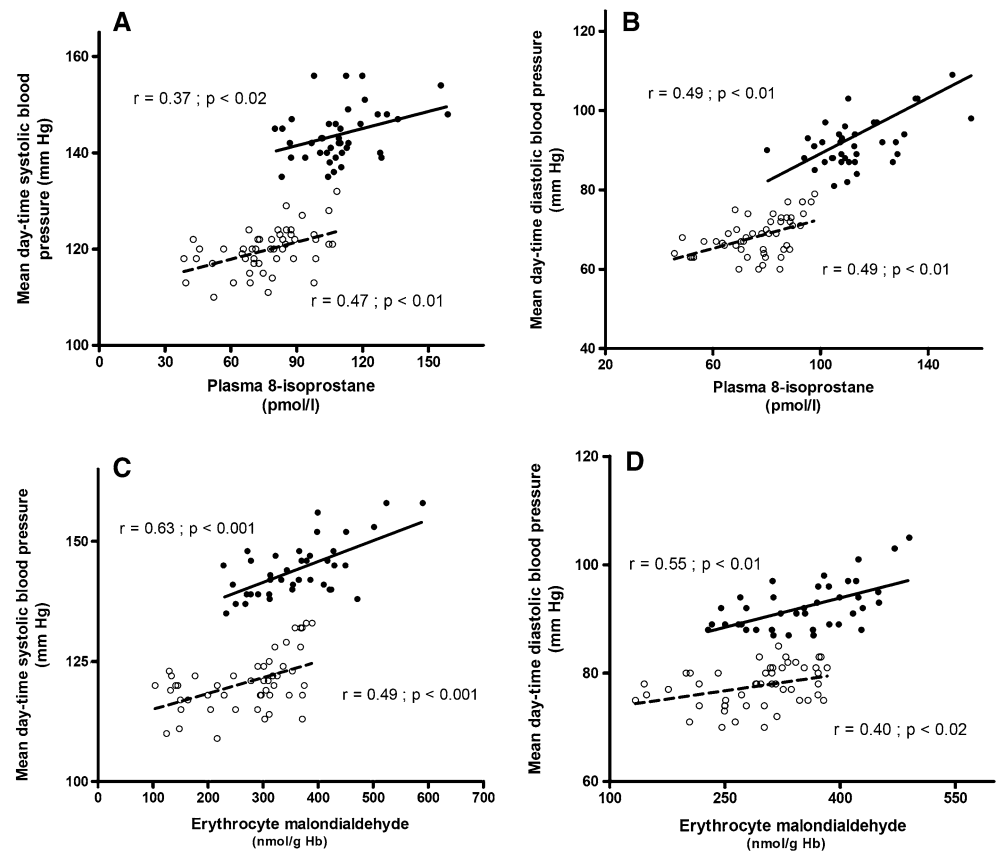
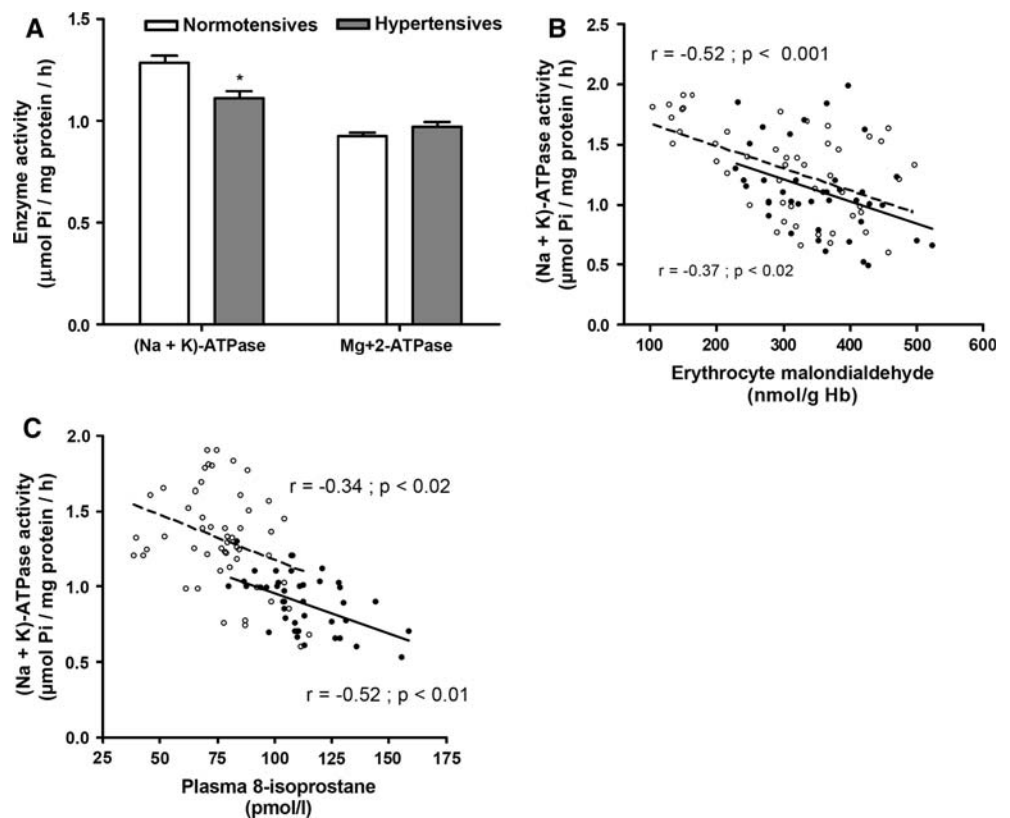


Fig. 2 Erythrocyte ATPase activity (mean \pm SEM) in both study groups. * $P < 0.03$ vs. normotensives (A). Pearson correlation of (Na + K)-ATPase activity to malondialdehyde (B), 8-isoprostane (C) of normotensives ($n = 49$) (open circles and dotted line) and hypertensives ($n = 40$) (solid circles and line) participants



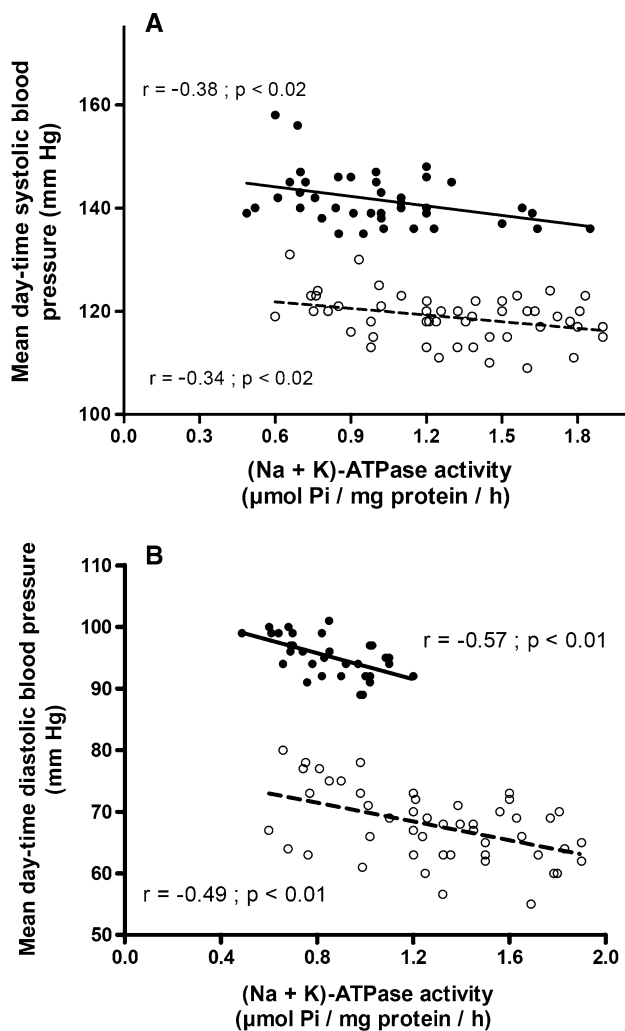


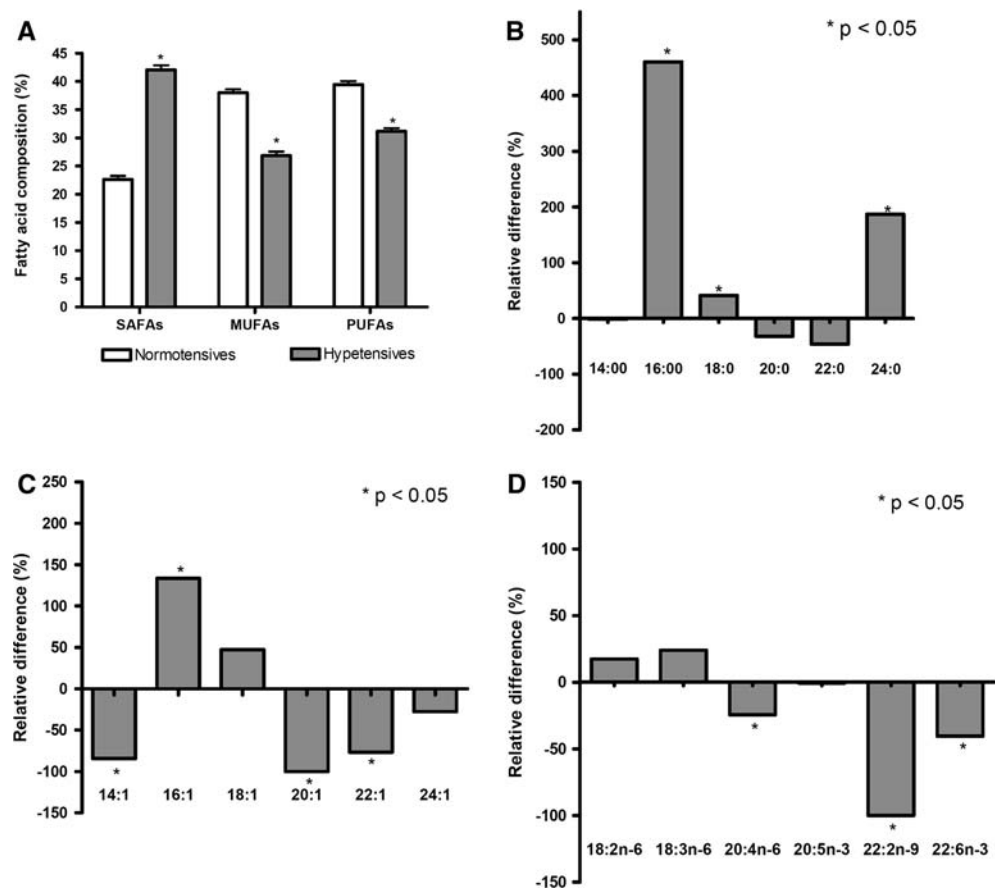
Fig. 3 Pearson correlation of (Na + K)-ATPase activity to mean day-time systolic (A) and diastolic (B) blood pressure of normotensive ($n = 49$) (open circles and dotted line) and hypertensive ($n = 40$) (solid circles and line) participants, respectively

prevention of peroxidation of polyunsaturated fatty acids. Erythrocytes MDA and plasma 8-isoprostane levels were elevated in hypertensives (Table 2) and show a positive correlation with both mean day-time SBP and DBP (Fig. 1).

ROS are increasingly implicated in human hypertension, since they exert important effects in vascular biology [32]. This suggests that ROS are contributors in mediating vasoconstriction occurring in hypertensive patients [1, 32–34]. However, to the best of our knowledge, differences in fatty acid pattern and its functional relationship with oxidative stress-related parameters in essential hypertension has not been reported. The present findings of increased lipid peroxidation in hypertensives are consistent with specific changes in membrane fatty acid profile, showing that the relative proportion of SAFAs was increased and

those of MUFAs and PUFAs were decreased (Fig. 4A), this could cause structural changes in plasma membrane [35]. The physicochemical properties of plasma membranes are partly determined by the degree of fatty acid unsaturation [36]. The incorporation of PUFAs into membrane phospholipids has been suggested to damage cellular membranes by virtue of the susceptibility of highly unsaturated acyl fatty acid to peroxidation [37, 38]. Our findings would support the idea that the unsaturation grade of the membrane lipids is related to lipid peroxidation susceptibility, although the method used for measurement of fatty acids gives only a relative proportion of saturated, mono- and polyunsaturated fatty acids. All of the above mentioned membrane effects could contribute to modulate the functional activity of membrane-bound enzymes. Lipid peroxidation, contributes to the loss of some cellular function through the inactivation of membrane-bound enzymes and native ion channels [8]. Accordingly, previous studies have shown that oxidative stress causes an inhibition of Ca^{+2} , Mg^{+2} -ATP-ase in the erythrocytes [39] and sodium channel dysfunction in cardiomyocytes [40]. In agreement with this view, our data demonstrate that the impairment of erythrocyte (Na + K)-ATPase activity associated positively with lipid peroxidation (Figs. 2B and C). Impairment of (Na + K)-ATPase activity could be due to the loss of its optimal interaction with the membrane components, as a consequence of increased lipid peroxidation. Although a direct inhibition by peroxynitrite, as occurs in liver plasma membranes, should not be discarded [41]. In contrast, the lack of significant differences in the activity of erythrocyte Mg^{+2} -ATP-ase between hypertensives and normotensives may indicate a lesser functional dependence of this enzyme activity, respect to (Na + K)-ATPase, upon the interaction with the membrane ROS targets. Therefore, it could be suggested that Mg^{+2} -ATP-ase is causally unrelated to oxidative stress-mediated hypertension. This view is supported by the fact that Mg^{+2} -ATP-ase in spontaneously hypertensive rats showed no relationship with blood pressure [42]. In contrast, (Na + K)-ATPase activity is expected to be influenced by the fatty acid unsaturation [13], in oxidative stress settings due to the vulnerability of PUFAs to ROS attack [43, 44]. Therefore, it is likely that the involvement of fatty acid composition in the modulation of membrane-bound (Na + K)-ATPase enzyme, thus, explaining the possible functional effect derived from alterations in fatty acid pattern. The above mentioned considerations could give an explanation to the finding of lower levels of (Na + K)-ATPase activity in hypertensive than normotensive participants (Fig 2A). Attempts to analyze the contribution of n-6/n-3 PUFA ratio in the modulation of both (Na + K)-ATPase activity and blood pressure were not carried out in the present study. Although it has been reported that n-6/n-3 PUFA ratio may be

Fig. 4 Plasma membrane fatty acid composition of erythrocytes (means \pm SEM) in both study groups. * $P < 0.001$ vs. normotensives, (A). Relative differences (%) in the proportions of SAFAs (B), MUFAs (C) PUFAs (D) of hypertensive ($n = 40$) compared to normotensive ($n = 49$) participants. The positive or negative values indicate higher or lower proportions, respectively. SAFAs, saturated fatty acids; MUFAs mono-unsaturated fatty acids; PUFAs poly-unsaturated fatty acids



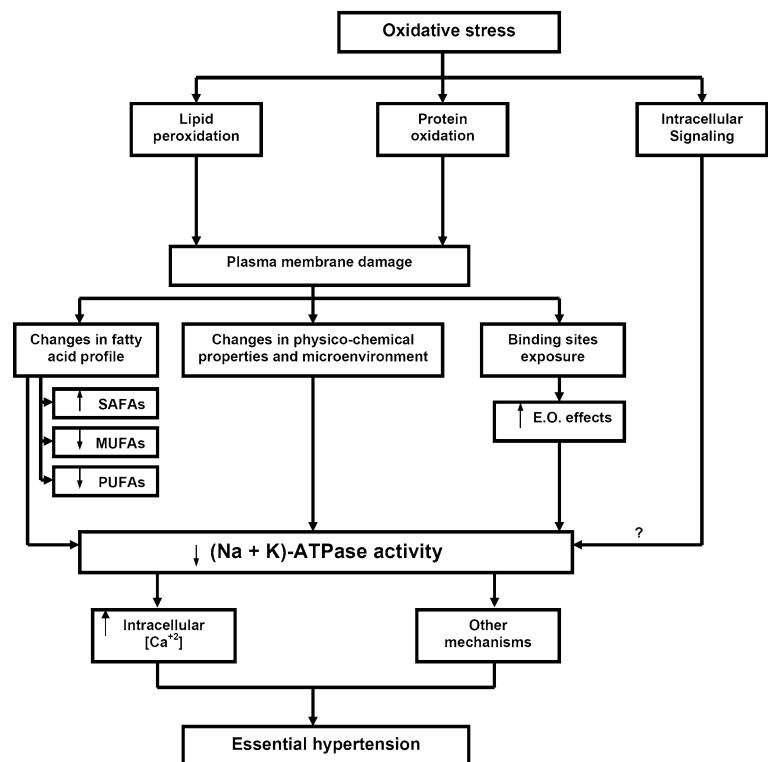
valuable in interpreting the biomarker data on fatty acid profile associated with prevention of coronary heart disease [45]. More recent studies based on large series of trials, concluded that focusing on the ratio distracts from the more important issue of the abundance of the $n-3$ PUFAs to be the single most important dietary change that can be made to improve cardiovascular health [46, 47].

The ubiquity of (Na + K)-ATPase in mammalian cells and its function in the vascular wall has suggested its role as a contributory factor in the pathogenesis of hypertension. Since the erythrocyte fatty acid profile changes reported in this study are expected to occur in other cell types also, it could be pointed out that the elevation of local Na⁺ on the submembrane area due to the diminution of (Na + K)-ATPase activity, in arterial smooth muscle cells, could facilitate Ca²⁺ entry through Na⁺/Ca²⁺ exchanger type 1 (NCX1). Consequently, a rise in cytosolic Ca²⁺ concentration would contribute in the elevation of blood pressure [48]. Nevertheless, other effects likely to influence the modulation of (Na + K)-ATPase, due to increased lipid peroxidation and/or protein oxidation, should not be discarded. Thus, it could be speculated that the above mentioned structural membrane alterations may result in an increased exposure of the endogenous cardiac glycoside binding site of the (Na + K)-ATPase. The cardiac glycoside

or endogenous ouabain (E.O.) is a ligand that behaves as a natural regulator of (Na + K)-ATPase in vivo [49, 50] and thus it may play a role in the mechanism of hypertension [51, 52]. The specific binding site of E.O. has a strong evolutionary conservation among all species [53]. This ligand has the only known receptor in (Na + K)-ATPase [54], whose α_2 isoform can mediate in the development of hypertension either through intracellular ion exchange or signaling cascades [55]. Functionally, E.O. can exert cardiotoxic and vasotonic actions dealing to the development of human hypertension [56]. Consequently, it seems reasonable to assume that structural changes induced by lipid peroxidation in erythrocyte membrane, as well as in other cell types, could result in increased exposure of E.O. binding sites, thus, contributing to the elevation of blood pressure by the alternative above mentioned mechanism (Fig 3). The lack of these specific E.O. binding sites in the Mg²⁺-ATPase molecule prevents E.O.-enzyme interaction, thereby accounting for the differential response of erythrocyte (Na + K)-ATPase and Mg²⁺-ATPase activities in causing essential hypertension. A schema to explain this hypothesis is shown in Fig. 5.

In summary, these findings suggest that the modulation of (Na + K)-ATPase activity may be associated with changes in the fatty acid composition induced by oxidative

Fig. 5 Schema of a proposed hypothesis to explain the effects of oxidative stress on the cellular plasma membrane fatty acid composition and membrane-bound elements to account for the elevation of blood pressure through the modulation of (Na + K)-ATPase activity in essential hypertension. E.O., endogenous ouabain



stress or with oxidative stress directly, or both, and provide evidence of a role for this enzyme in the pathophysiology of essential hypertension.

Acknowledgments The authors wish to thank the Fondo Nacional de Investigación Científica y Tecnológica (FONDECYT, grant number 1040429, Chile Government), Procaps Laboratory (Colombia) and Gynopharm CFR Laboratory (Chile) for their financial support of this study. The technical assistance of Mr. Diego Soto is also acknowledged.

References

- Lassègue B, Griendling K (2004) Reactive oxygen species in hypertension. An update. *Am J Hypertens* 17:852–860
- Touyz RM, Schiffrin EL (2004) Reactive oxygen species in vascular biology: implications in hypertension. *Histochem Cell Biol* 122:339–352
- Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr et al (2003) Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. *Hypertension* 289:2560–2572
- Stidley CA, Hunt WC, Tentori F, Schmidt D, Rohrscheib M, Paine S et al (2006) Changing relationship of blood pressure with mortality over time among hemodialysis patients. *J Am Soc Nephrol* 17:513–520
- Chen X, Touyz RM, Bae Park J, Schiffrin EL (2001) Antioxidant effects of vitamins C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke-prone SHR. *Hypertension* 38:606–611
- Pedro-Botet J, Covas MI, Martin S, Rubies-Prat J (2000) Decreased endogenous antioxidant enzymatic status in essential hypertension. *J Hum Hypertens* 14:343–345
- John S, Schmieder RE (2003) Potential mechanisms of impaired endothelial function in arterial hypertension and hypercholesterolemia. *Curr Hypertens Rep* 5:199–207
- Stark G (2005) Functional consequences of oxidative membrane damage. *Membr Biol* 205:1–16
- Cazzola R, Rondanelli M, Russo-Volpe S, Ferrari E, Cestaro B (2004) Decreased membrane fluidity and altered susceptibility to peroxidation and lipid composition in overweight and obese female erythrocytes. *J Lipid Res* 45:1846–1851
- Elizondo A, Araya J, Rodrigo R, Poniachik J, Signorini C, Sgherri C, Comporti M, Videla LA (2007) Docosahexaenoic acid and docosapentaenoic acid levels in liver and erythrocyte phospholipids from obese non-alcoholic fatty liver disease patients. *Obesity* 15:24–31
- Felton CV, Stevenson JC, Godsland IF (2004) Erythrocyte-derived measures of membrane lipid composition in healthy men: associations with arachidonic acid at low to moderate not high insulin sensitivity. *Metabolism* 53:571–577
- Vajreswari A, Rupalatha M, Rao PS (2002) Effect of altered dietary n-6-to-n-3 fatty acid ratio on erythrocyte lipid composition and membrane-bound enzymes. *J Nutr Sci Vitaminol (Tokyo)* 48:365–370
- Bartoli GM, Palozza P, Luberto C, Franceschelli P, Piccioni E (1995) Dietary fish oil inhibits human erythrocyte Mg,NaK-ATPase. *Biochem Biophys Res Commun* 213:881–887
- Rodrigo R, Castillo R (2006) Pathophysiological mechanisms of renal damage by alcohol consumption: involvement of oxidative stress. In: Yoshida R (ed) *Trends in Alcohol Abuse and Alcoholism Research*. Nova Publishers
- Grover AK, Samson SE, Robinson S, Kwan CY (2003) Effects of peroxynitrite on sarcoplasmic reticulum Ca²⁺ pump in pig coronary artery smooth muscle. *Am J Physiol Cell Physiol* 284:C294–C301
- Hamilton BP, Blaustein MP (2006) Molecular mechanisms linking sodium to hypertension: report of a symposium. *J Investig Med* 54:86–94

17. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr et al (2003). Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. *Hypertension* 289:2560–2572
18. Myers MG, Tobe SW, McKay DW, Bolli P (2005) New algorithm for the diagnosis of hypertension. *Am J Hypertens* 18:1369–1374
19. O'Brien E, Mee F, Atkins N, O'Malley K (1991) Short report: accuracy of the Spacelabs 90207 determined by to the British Hypertension Society Protocol. *J Hypertens* 9:573–574
20. Groppelli A, Omboni S, Parati G, Mancia G (1992) Evaluation of noninvasive blood pressure monitoring devices Spacelabs 90202 and 90207 versus resting and ambulatory 24-h intra-arterial blood pressure. *Hypertension* 20:227–232
21. Sun M, Tien J, Jones R, Ward R (1996) A new approach to reproducibility assessment: clinical evaluation of SpaceLabs Medical oscillometric blood pressure monitor. *Biomed Instrum Technol* 30:439–448
22. Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem* 239:70–76
23. Hissin PJ, Hilf R (1976) A fluorometric method for determination of oxidized and reduced glutathione in tissues. *Anal Biochem* 74:214–226
24. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95:351–358
25. Pradelles P, Grassi J, Maclouf J (1985) Enzyme immunoassays of eicosanoids using AchE as label: an alternative to radioimmunoassay. *Anal Chem* 57:1170–1173
26. Katz AI, Epstein FH (1967) The role of sodium–potassium-activated adenosine triphosphatase in the reabsorption of sodium by the kidney. *J Clin Invest* 46:1999–2011
27. Taussky HH, Shorr E (1953) A microcolorimetric method for the determination of inorganic phosphorus. *J Biol Chem* 202:675–685
28. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275
29. Huertas JR, Palomino N, Ochoa JJ, Quiles JL, Ramirez-Tortosa MC, Battino M, Robles R, Mataix J (1998) Lipid peroxidation and antioxidants in erythrocyte membranes of full-term and preterm newborns. *Biofactors* 8:133–137
30. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction. *Can J Biochem Physiol* 37:911–917
31. Araya J, Rodrigo R, Orellana M, Rivera G (2001) Red wine raises HDL and preserves long-chain polyunsaturated fatty acids in rat kidney and erythrocytes. *Br J Nutr* 86:189–195
32. Redon J, Oliva MR, Tormos C, Giner V, Chaves J, Iradi A, Saez GT (2003) Antioxidant activities and oxidative stress byproducts in human hypertension. *Hypertension* 41:1096–1101
33. Kashyap MK, Yadav V, Sherawat BS, Jain S, Kumari S, Khullar M et al (2005) Different antioxidants status, total antioxidant power and free radicals in essential hypertension. *Mol Cell Biochem* 277:89–99
34. Hozawa A, Ebihara S, Ohmori K, Kuriyama S, Ugajin T, Yayoi Koizumi Y et al (2004) Increased plasma 8-isoprostane levels in hypertensive subjects: the Tsurugaya project. *Hypertens Res* 27:557–561
35. Gibson RA, Neumann MA, Burnard SL, Rinaldi JA, Patten GS, McMurchie EJ (1992) The effect of dietary supplementation with eicosapentaenoic acid on the phospholipid and fatty acid composition of erythrocytes of marmoset. *Lipids* 27:169–176
36. Knapp HR, Hullin F, Salem N Jr (1994) Asymmetric incorporation of dietary *n*-3 fatty acids into membrane aminophospholipids of human erythrocytes. *J Lipid Res* 35:1283–1291
37. Alexander-North LS, North JA, Kiminyo KP, Buettner GR, Spector AA (1994) Polyunsaturated fatty acids increase lipid radical formation induced by oxidant stress in endothelial cells. *J Lipid Res* 35:1773–1785
38. Van Ginkel G, Sevanian A (1994) Lipid peroxidation-induced membrane structural alterations. *Methods Enzymol* 233:273–288
39. Leclerc L, Marden M, Poyart C (1991) Inhibition of the erythrocyte (Ca²⁺+Mg²⁺)-ATPase by nonheme iron. *Biochim Biophys Acta* 1062:35–38
40. Fukuda K, Davies SS, Nakajima T, Ong BH et al (2005) Oxidative mediated lipid peroxidation recapitulates proarrhythmic effects on cardiac sodium channels. *Circ Res* 97:1262–1269
41. Kocak-Toker N, Giris M, Tulubas F, Uysal M, Aykac-Toker G (2005) Peroxynitrite induced decrease in Na⁺, K⁺-ATPase activity is restored by taurine. *World J Gastroenterol* 11:3554–3557
42. Norman RI, Achall N. (1993) Cell membrane microviscosity and Ca(2+)-Mg(2+)-ATPase activity do not contribute to hypertension in the spontaneously hypertensive rat model. *Clin Sci (Lond)* 85:585–591
43. Liu Y, Longmore RB (1997) Dietary sandalwood seed oil modifies fatty acid composition of mouse adipose tissue, brain, and liver. *Lipids* 32:965–969
44. Begin ME (1990) Fatty acids, lipid peroxidation and diseases. *Proc Nutr Soc* 49:261–267
45. De Lorgeril M, Salen P (2003) Dietary prevention of coronary heart disease: focus on omega-6/omega-3 essential fatty acid balance. *World Rev Nutr Diet* 92:57–73
46. Harris WS (2006) The omega-6/omega-3 ratio and cardiovascular disease risk: uses and abuses. *Curr Atheroscler Rep* 8:453–459
47. Wijendran V, Hayes KC (2004) Dietary *n*-6 and *n*-3 fatty acid balance and cardiovascular health. *Annu Rev Nutr* 24:597–615
48. Iwamoto T, Kita S (2006) Topics on the Na⁺/Ca²⁺ exchanger: role of vascular NCX1 in salt-dependent hypertension. *J Pharmacol Sci* 102:32–36
49. Hallaq HA, Hauptert GT Jr (1989) Positive inotropic effects of the endogenous Na⁺/K⁺-transporting ATPase inhibitor from the hypothalamus. *Proc Natl Acad Sci USA* 86:10080–10084
50. Shaikh IM, Lau BW, Siegfried BA, Valdes R Jr (1991) Isolation of digoxin-like immunoreactive factors from mammalian adrenal cortex. *J Biol Chem* 266:13672–13678
51. Manunta P, Stella P, Rivera R et al (1999) Left ventricular mass, stroke volume, and ouabain-like factor in essential hypertension. *Hypertension*. 34:450–456
52. Zhang W, Huang BS, Leenen FH (1999) Brain renin-angiotensin system and sympathetic hyperactivity in rats after myocardial infarction. *Am J Physiol* 276:H1608–H1615
53. Dostanic-Larson I, Van Huysse JW, Lorenz JN, Lingrel JB (2005) The highly conserved cardiac glycoside binding site of Na,K-ATPase plays a role in blood pressure regulation. *Proc Natl Acad Sci USA* 102:15845–15850
54. Dostanic I, Paul RJ, Lorenz JN, Theriault S, Van Huysse JW, Lingrel JB (2005) The alpha2-isoform of Na-K-ATPase mediates ouabain-induced hypertension in mice and increased vascular contractility in vitro. *Am J Physiol Heart Circ Physiol* 288:H477–H485
55. Kaplan JH (2005) The sodium pump and hypertension: a physiological role for the cardiac glycoside binding site of the Na, K-ATPase. *Proc Natl Acad Sci USA* 102:15723–15724
56. Bova S, Blaustein MP, Ludens JH, Harris DW, DuCharme DW, Hamlyn JM (1991) Effects of an endogenous ouabain-like compound on heart and aorta. *Hypertension* 17:944–950