# Intracellular Ionic Consequences of Dietary Salt Loading in Essential Hypertension

## Relation to Blood Pressure and Effects of Calcium Channel Blockade

**Lawrence M. Resnick, Raj K. Gupta, Beatrice DiFabio, Mario Barbagallo, Samuel Mann, Rosemarie Marion, and John H. Laragh**

*Division of Endocrinology/Hypertension, Wayne State University Medical Center, Detroit, Michigan 48201; Cardiovascular Center, New York Hospital–Cornell Medical Center, New York 10021; and Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York 10463*

## Abstract

**To study the ionic basis of salt sensitivity in hypertension, $^{19}F$-, $^{31}P$-, and $^{23}Na$-nuclear magnetic resonance techniques were used to measure cytosolic free calcium ($Ca_i$), pH ($pH_i$), free magnesium ($Mg_i$), and sodium ($Na_i$) in erythrocytes of essential hypertensive subjects ($n = 19$). Individuals were studied for 2 mo each on low- ($U_{Na}V < 50$ meq/d) and high- (UNaV > 200 meq/d) salt diets, with the concomitant administration of nifedipine (10 mg t.i.d.) or placebo tablets for 1 mo of each diet. Salt loading elevated $Ca_i$ and $Na_i$ while suppressing $Mg_i$ and $pH_i$; these changes occurred predominantly in salt-sensitive subjects ($n = 9$). Nifedipine blunted the pressor response to salt loading > 50% ($\Delta$ diastolic BP [high–low salt vs placebo] = 5±2 vs 14±2 mmHg, $P < 0.05$) and reversed salt-induced ionic changes, lowering $Ca_i$ and elevating $Mg_i$ and $pH_i$. Regardless of the definition of salt sensitivity, continuous relationships were observed between the pressure response to salt loading, the levels of $Ca_i$ ($r = 0.726$, $P < 0.001$), $Na_i$ ($r = 0.747$, $P < 0.001$), and $pH_i$ ($r = -0.754$, $P < 0.001$), and the salt-induced change in $Mg_i$ ($r = -0.757$, $P < 0.001$). Altogether, these results emphasize the reciprocal and coordinate nature of intracellular ionic changes in response to dietary salt loading and calcium channel blockade in essential hypertension. They suggest that salt sensitivity is mediated by cellular calcium accumulation from the extracellular space, in association with magnesium depletion and acidification. Lastly, interpretation of intracellular ion measurements in the future will require concurrent assessment of dietary salt intake. (*J. Clin. Invest.* 1994. 94:1269–1276.) Key Words: NMR spectroscopy • intracellular ions • pH • calcium • magnesium**

## Introduction

Epidemiological as well as direct intervention studies repeatedly have demonstrated that altered dietary intake of mineral salts such as sodium chloride can result, in at least some "sensitive" individuals, in clinically significant differences in blood pressure. Current theories differ, however, as to how increased availability of salt to the organism as a whole results in the vasoconstrictor and other organ system responses characteristic of clinical salt-sensitive hypertension. Common to many current formulations is the proposal that an initially sodium chloride–derived signal is somehow translated at the cellular level to a calcium signal, resulting in an increased cytosolic free calcium and, thus, in increased vascular smooth muscle tone and elevated blood pressure (1). However, the relations between pressor responses to salt and the concomitant alterations in the different intracellular ionic species remain unclear.

Our group has used nuclear magnetic resonance (NMR)[1] spectroscopic techniques to study intracellular cations in peripheral blood and has demonstrated abnormalities of intracellular free magnesium (2), intracellular pH (3), and cytosolic free calcium levels (4) in hypertension and allied metabolic disorders. These data complement earlier studies in essential hypertensive subjects where we observed salt-induced alterations of extracellular serum ionized calcium and of various calcium regulatory hormones that in turn were reversed and or prevented by calcium channel blockade (5, 6). These studies led us to hypothesize that salt-sensitive hypertensive disease is critically dependent on enhanced cellular calcium accumulation from the extracellular space.

To further test this hypothesis and to directly assess the blood pressure and intracellular ionic consequences of dietary salt loading in man, we studied essential hypertensive subjects at extremes of salt intake both in the presence and absence of concurrent therapy with the calcium channel blocking agent, nifedipine. Our results, reported previously in abstract form (7, 8), demonstrate (*a*) that coordinated, linked changes in intracellular sodium, free calcium, free magnesium, and intracellular pH occur in response to salt loading, (*b*) that these changes occur predominantly among salt-sensitive individuals, and (*c*) that they are closely linked to both the pressor effects of salt and the hypotensive effects of calcium channel blockade. Our data also suggest that the previously reported enhanced efficacy of calcium channel blockade in low renin and sodium-volume forms of hypertension may be explained by their ability to reverse specifically dietary salt–induced changes in cytosolic free calcium and intracellular pH.

This study was presented in part at the American Federation of Clinical Research in Washington D.C. on 7 May 1990 and at the International Society of Hypertension in Montreal, Canada on 22 June 1990.

Address correspondence to Lawrence M. Resnick, M.D., Division of Endocrinology/Hypertension, Wayne State University Medical School, University Health Center-4H, 4201 St. Antoine, Detroit, MI 48201.

*Received for publication 3 August 1993 and in revised form 7 April 1994.*

J. Clin. Invest.



0021-9738/94/09/1269/08 $2.00
Volume 94, September 1994, 1269–1276

1. *Abbreviations used in this paper:* DBP, diastolic BP; NMR, nuclear magnetic resonance; PRA, plasma renin activity; RBC, red blood cell; SS and SI, salt-sensitive and -insensitive, respectively.

## Methods

Essential hypertensive subjects were recruited and followed at the Hypertension Center of the New York Hospital. The protocol followed was approved by the Committee on Human Rights in Research, and informed consent was obtained from the subjects after the nature of the procedures had been explained. Essential hypertension was diagnosed in untreated subjects on the basis of outpatient blood pressures > 150/90 mmHg on at least three occasions and the absence of any history, physical examination, or laboratory evidence of secondary forms of hypertension. Subjects discontinued medications ≥ 7 wk before beginning any dietary maneuvers and had been off diuretics for ≥ 6 mo.

### *Dietary methods*

During the baseline evaluation period, participants discontinued all foods with potential pharmacological effects and were instructed in keeping food diaries, accurately weighing foods, measuring salt added to food, and estimating food portions of food eaten away from home. Two 24-h food diaries submitted at the end of the baseline period and a diet history questionnaire were used to individualize food intake plans while the low- and high-salt diets were administered.

The low salt diet was achieved by eliminating from the diet all salt-containing compounds of significance, often substituting foods of similar composition from the same food group. The high salt intake was achieved by adding measured amounts of high-salt bouillon and/or table salt and soy sauce to the baseline daily intake. Subjects were instructed to take salt-containing condiments throughout the day with meals.

### *Study methods*

Drug intervention with nifedipine used a randomized, placebo-controlled, single-blind crossover design in which 2-month periods of low (UNaV < 50 meq/d) and high (UNaV > 200 meq/d) dietary sodium chloride intake were ingested according to the methods described above. On each level of salt intake, subjects were assigned to 1 mo of nifedipine, 10 mg t.i.d., as well as 1 mo of treatment with similarly appearing placebo tablets, also taken t.i.d.

Patients were evaluated at monthly intervals after an overnight fast, 2–4 h after taking their morning pill (nifedipine or placebo) with water. The patient arrived on each occasion with a 24-h urine specimen collected the day before being seen and with two 24-h food diaries, one of which coincided with the day of the 24-h urine collection. An aliquot of the urine specimen was checked for urinary sodium content and, if the total sodium excretion was within the accepted limits for that assigned diet, blood tests were obtained for analysis of intracellular ion content. Urine was analyzed for total sodium, potassium, calcium, magnesium, and creatinine excretion. Blood pressure was obtained manually in the supine, seated, and standing positions by the research nursing staff. Patients in the seated position also had their blood pressure evaluated with an automatic device (Arteriosonde; Roche Diagnostics, Nutley, NJ) set to measure and record pressure every 2 min for 30 min. If urinary sodium excretion was not within the range specified for that diet period, additional time was spent with the dietitian to achieve the goal salt intake, and the patient was given another 7–10 d to comply. Persistent failure to achieve goal sodium chloride excretion levels resulted in the patient being dropped from the study.

At the time of each monthly visit, nifedipine or placebo pills were returned to the research nursing staff who estimated compliance on the basis of pill counts. A new consignment of pills, numerically coded, was allocated to the patient at the beginning of each monthly period.

Serum electrolytes, blood urea nitrogen, creatinine, and urinary electrolytes, calcium, magnesium, and creatinine excretion, were measured by automated techniques. Plasma renin activity and urinary aldosterone excretion were measured by standard radioimmunoassay techniques.

### *NMR intracellular ion analyses*

*$^{31}$P-NMR analysis of $Mg_i$ and $pH_i$.* 10 ml of heparinized blood was spun at 2,000 rpm for 10 min, and the plasma was saved to be used in the analysis of $Ca_i$ (see below). The remaining erythrocyte fraction was decanted into a 10-mm NMR tube, and $^{31}$P-NMR spectra were recorded at 81 MHz and 37°C for 30 min on a spectrometer (XL200; Varian Analytical Instruments, Sunnyvale, CA) in the Fourier transform mode and with wide-band proton noise decoupling.

The intracellular free magnesium concentration, $Mg_i$, was determined according to the formula:

$$Mg_i = K_d(MgATP) \times (\phi^{-1} - 1) \quad (9)$$

where $K_d$ (MgATP) is the apparent dissociation constant for the reaction MgATP = $Mg^{2+}$ + ATP, = $0.4 \times 10^{-5}$ M under physiological conditions at 37°C and at pH 7.2; and $\phi = ATP_{free}/ATP_{total}$ as determined from the chemical shift differences of the $\alpha$ and $\beta$ phosphoryl group resonances of ATP in the $^{31}$P-NMR spectrum.

*$pH_i$.* Measurement of the chemical shift difference of the 3- and 2-phosphoryl resonances of 2,3-diphosphoglycerate (2,3 DPG) on the $^{31}$P-NMR spectra at known different absolute pH levels, allows for a determination of $pH_i$ in experimental samples (10). Packed red blood cells (RBCs) were lysed by repeated freezing and thawing, and aliquots of the hemolysate were titrated to pH 7.0–7.4, using HCl or NaOH. $^{31}$P-NMR spectra were obtained at each pH value, and the chemical shift difference of the 3- and 2-phosphoryl resonances of 2,3 DPG were plotted against the pH value at which that spectrum was obtained. This titration curve was linear within the pH range tested and was used to determine the $pH_i$ of unknowns (3).

*$^{19}$F-NMR analysis of $Ca_i$.* $Ca_i$ was determined in RBCs by a modification of the method of Murphy et al. (11), using the fluorinated, membrane-permeant Ca chelator, QUIN-MF-AM.

10 ml of heparinized blood was spun at 10,000 rpm for 10 min, and the plasma was decanted. The packed cells were added to 100 ml Hanks' balanced salt solution buffer titrated to a pH of 7.4 with $NaHCO_3$. Cells were loaded with 20 $\mu$M QUIN-MF-AM for 25 min at 37°C in a shaking water bath. Loaded cells were then spun at 10,000 rpm for 10 min, the supernatant medium was aspirated, and the cells were resuspended and incubated for 90 min in fresh buffer together with the originally decanted plasma fraction. Cells were then centrifuged, washed again with fresh buffer together with plasma, and recentrifuged and decanted into 10-mm NMR tubes for analysis. $^{19}$F-NMR spectra were run on a spectrometer (Varian Analytical Instruments) operating at 470.4 MHz and 37°C. Free cytosolic calcium, $Ca_i$, was calculated according to the formula:

$$Ca_i = K_{d,app} \times (Ca\text{–QUIN-MF})/(\text{QUIN-MF}),$$

where the $K_{d,app}$ of Ca–QUIN-MF was calculated at 126 nM in RBC hemolysates at 37°C, and where Ca–QUIN-MF/QUIN-MF was determined from the ratio of areas of the QUIN-MF and Ca–QUIN-MF resonance peaks on the $^{19}$F-NMR spectrum.

To approximate more closely the in situ cellular environment, we equilibrated calcium-bound and -free QUIN-MF in red cell hemolysates to determine the apparent dissociation constant, $K_d$, rather than in buffer solutions. The $K_d$ thus determined, 126 nM, is lower than those for the fluorescent probes currently used to measure $Ca_i$ and thus allows a greater sensitivity in distinguishing differences among the low $Ca_i$ values we observed in red blood cells.

*$^{23}$Na-NMR analysis of $Na_i$.* The shift reagent, dysprosium trispolyphosphate $[Dy(PPPi)_2]^{7-}$ (5 mM) was mixed with 10 ml heparinized blood and then was centrifuged. $^{23}$Na-NMR spectra were then obtained in packed cells as well as in the decanted plasma fraction to assess intracellular sodium, $Na_i$, and to normalize for Na in the extracellular space, according to the formula:

$$Na_i = [A_i/A_o \times S_o/(1 - S_o)] \times Na_o \quad (12),$$

where $A_i$, $A_o$ are the integrated areas of NMR signals in the intracellular and extracellular space, respectively; $S_o$ is the fractional extracellular space in the NMR window; and $Na_o$ is the plasma Na concentration obtained by standard automated techniques.

When human RBCs are analyzed repeatedly, the interassay variability of the above NMR-based ionic measurements for $pH_i$ is ~ 0.0005 U and, for $Mg_i$, $Na_i$, and $Ca_i$, < 5%.

*Table I. Basal Clinical and Laboratory Characteristics of Study Patients*

| | Salt-sensitive ($n$ = 9) | Salt-insensitive ($n$ = 10) |
|---|---|---|
| Age (y) | 58±2 | 56±3 |
| Sex (male/female) | 7/2 | 5/5 |
| Race (black/white) | 2/7 | 0/10 |
| Weight (body mass index) | 27.9±2.1 | 25.5±1.9 |
| Diastolic BP (mmHg) | 153±8 | 156±8 |
| Diastolic BP (mmHg) | 101±3 | 102±2 |
| Blood urea nitrogen (mg %) | 15.9±1.1 | 17.5±1.6 |
| Cr (mg %) | 1.0±0.06 | 1.0±0.07 |
| $U_{Na}V$ (meq/d) | 83±6 | 75±7 |
| PRA (ng/ml per hour) | 1.9±0.3 | 3.0±0.5* |

* $P < 0.05$ vs. salt-sensitive.

Salt sensitivity was defined as a diastolic BP (DBP) on high salt > 5% greater versus the low salt diet. The effects of low and high salt diets, and placebo and nifedipine phases were compared for both salt-sensitive (SS) and -insensitive (SI) subjects by ANOVA in which salt sensitivity (SS vs SI) was a grouping factor, and the measurements on high and low salt, on placebo and nifedipine, were each considered separate treatments. Post-ANOVA $t$ tests (unpaired, Bonferroni) were performed, adjusting the degrees of freedom appropriately. Paired $t$ tests were performed to analyze the effects of salt and of nifedipine independently of how salt sensitivity was defined. Relationships between salt- and nifedipine-induced changes in pressure and intracellular ion levels were analyzed by linear regression analysis. All results are expressed as mean±SEM.

## Results

*Clinical and laboratory data.* Basal clinical and laboratory values for the 19 patients for whom complete data were obtained are described in Table I. On the basis of diastolic pressure responses to high and low salt diets while on placebo tablets (see Methods), 9 patients (52±2 y; 7 female, 2 male; 7 white, 2 black) were classified as SS, whereas 10 (56±3 y; 5 male, 5 female; 10 white) were classified as SI. Significant differences in the data reported below remained unchanged whether or not the two black subjects were included in the analysis. Except for elevated blood pressure, basal clinical and laboratory data were not outside normal limits and did not differ significantly among SS versus SI patient subgroups, with the exception of plasma renin activity, which was lower in subjects subsequently found to be SS versus SI (1.9±0.3 vs 3.0±0.5 ng/ml per hour, $P < 0.05$).

Urinary electrolyte excretion values are recorded in Table II. Steady state, 24-h average urinary sodium levels met the designated goal ranges in all the reported patients, although on the low salt diet, SS exhibited a lower UNaV than did SI individuals ($P < 0.05$). Urinary 24-h potassium excretion remained constant both on the two dietary salt periods and on placebo versus nifedipine treatment phases. Urinary calcium excretion was also higher in SI compared with SS subjects on the low-salt diet and was higher on high versus low salt intakes both in SS and SI subjects on placebo and nifedipine. Additionally, nifedipine significantly increased average calcium excretion values in both SS and SI subjects (Table II). Urinary magnesium excretion was only modestly affected by differing dietary salt intake and although all levels tended toward higher values on high versus low salt, these differences were not consistently statistically significant. To ascertain whether we could successfully achieve and maintain patients on high and low salt–containing diets without concomitantly altering dietary levels of other minerals such as calcium and magnesium, we have in the past computer analyzed 3-d food records for nutrient and mineral content in patients undergoing the identical dietary salt protocol using the same dietitian personnel and have compared these values to the 24-h urinary excretion values for all the mineral species. Under the above conditions, it was found that urinary excretion values closely paralleled the food record data, further supporting the present data indicating that calcium and magnesium intake on the high and low salt diet phases were not significantly different.

Plasma renin activity (PRA) values did not differ at either extreme of dietary salt intake among SS versus SI individuals. Similarly, both plasma renin activity and urinary aldosterone excretion values were significantly suppressed on high versus low salt intakes in both SS and SI subjects. Furthermore, chronic nifedipine treatment did not significantly change either PRA or urinary aldosterone excretion. These results and all subsequently reported data (see below) were unaffected by the order of the diets or drug treatments administered.

*Blood pressure and intracellular ionic effects of salt.* Table

*Table II. Urinary Electrolyte Excretion and Renin–Aldosterone System Responses to Dietary Salt*

| | Low salt | | | | High salt | | | |
|---|---|---|---|---|---|---|---|---|
| | Salt sensitive | | Salt insensitive | | Salt sensitive | | Salt insensitive | |
| | Placebo | Nifedipine | Placebo | Nifedipine | Placebo | Nifedipine | Placebo | Nifedipine |
| $U_{Na}V$ (meq/d) | 17±2 | 22±4 | 33±6* | 33±5 | 295±26‡ | 328±34‡ | 286±36‡ | 283±23‡ |
| $U_KV$ (meq/d) | 84±11 | 79±6 | 68±7 | 69±4 | 83±5 | 82±7 | 81±9 | 70±8 |
| $U_{Ca}V$ (mg/d) | 90±11 | 118±19‖ | 141±16* | 135±10 | 178±30‡ | 222±33‡‖ | 222±18‡ | 274±33‡‖ |
| $U_{Mg}V$ (meq/d) | 8.5±2.0 | 7.6±1.3 | 7.2±1.0 | 7.1±0.8 | 10.5±1.4 | 8.3±12.1 | 8.3±0.7 | 8.5±0.8 |
| $U_{Creat}V$ (g/d) | 1.7±0.1 | 1.8±0.2 | 1.7±0.1 | 1.5±0.1 | 1.7±0.1 | 1.8±0.2 | 1.8±0.1 | 1.5±0.1 |
| $U_{Aldo}V$ ($\mu$g/d) | 20±3 | 23±4 | 18±2 | 27±8 | 9±1‡ | 9±1‡ | 9±2‡ | 8±2‡ |
| PRA (ng/ml per hour) | 2.7±0.5 | 4.0±0.9 | 3.7±0.7 | 6.2±1.6 | 1.3±0.4‡ | 2.0±0.7 | 2.4±0.6§ | 3.2±0.9‡ |

* Significant at $P < 0.05$ vs. ss. ‡ Significant at $P < 0.01$ vs. low salt. § Significant at $P < 0.05$ vs. low salt. ‖ Significant at $P < 0.05$ placebo.

*Table III. Blood Pressure and Ionic Effects of Dietary Salt*

| | Low salt | | | | High salt | | | |
|---|---|---|---|---|---|---|---|---|
| | Salt sensitive | | Salt insensitive | | Salt sensitive | | Salt insensitive | |
| | Placebo | Nifedipine | Placebo | Nifedipine | Placebo | Nifedipine | Placebo | Nifedipine |
| Systolic BP (mmHg) | 142±6 | 125±4* | 155±6 | 137±5*‡ | 171±10§ | 143±8‖¶ | 164±6 | 138±6¶ |
| Diastolic BP (mmHg) | 89±3 | 82±2¶ | 101±2‡ | 92±3‡¶ | 103±3§¶ | 87±2* | 102±2 | 89±3* |
| Serum $Na^+$ (meq/liter) | 139±0.6 | 139±0.5 | 138±0.8 | 138±0.6 | 139±0.6 | 140±0.6 | 139±0.9 | 140±0.8 |
| Serum $K^+$ (meq/liter) | 4.2±0.05 | 4.3±0.09 | 4.2±0.1 | 4.2±0.08 | 3.9±0.07‖ | 4.2±0.1¶ | 4.0±0.08 | 4.1±0.1 |
| Serum $Ca_T$ (mg%) | 9.3±0.1 | 9.5±0.1 | 9.5±0.2 | 9.9±0.4 | 9.4±0.1 | 9.7±0.1 | 9.4±0.2 | 9.9±0.2 |
| Serum $Mg_T$ (meq/liter) | 1.90±0.04 | 2.0±0.06 | 1.87±0.1 | 1.89±0.09 | 1.83±0.04 | 1.95±0.05 | 1.81±0.07 | 1.80±0.10 |
| Serum $CO_2$ (mg %) | 23±2.0 | 25±1.2 | 22±0.8 | 26±1.1¶ | 25±0.5 | 25±0.8 | 24±1.0‖ | 26±0.9 |
| $Na_i$ (meq/liter) | 8.0±.15 | 9.4±1.6 | 5.4±1.0 | 6.6±0.1 | 10.8±2.1‖ | 10.4±0.7 | 6.1±0.3‡‖ | 6.9±0.7 |
| $Ca_i$ (nM) | 32.3±3.6 | 30.2±1.2 | 32.7±4.9 | 22.0±6.1* | 46.5±8.0‖ | 31.4±5.1* | 36.7±7.6 | 28.8±5.2 |
| $Mg_i$ ($\mu$M) | 207±21 | 199±8 | 196±12 | 206±17 | 143±16‖ | 203±25 | 199±9‡ | 205±15 |
| pHi | 7.15±0.02 | 7.32±0.02¶ | 7.23±0.01‡ | 7.27±0.02¶ | 7.10±0.01‖ | 7.27±0.03¶ | 7.21±0.01‡ | 7.25±0.01¶ |

* Significant at $P < 0.01$ vs. placebo. ‡ Significant at $P < 0.05$ vs. SS. § Significant at $P < 0.01$ vs. LS. ‖ Significant at $P < 0.05$ vs. LS. ¶ Significant at $P < 0.05$ vs. placebo.

III reports the blood pressures in SS and SI subjects on each dietary salt intake on nifedipine versus placebo treatment. By definition, in SS on placebo tablets, blood pressure rose significantly on the high- versus low-salt diet ($\Delta$ systolic BP = 29±6 mmHg, $P < 0.001$; $\Delta$DBP = 14±2 mmHg, $P < 0.001$). Conversely, in SI, no significant changes in diastolic pressures were observed (0±3 mmHg, $P$ = NS), and systolic pressures rose only minimally (9±6 mmHg, $P$ = NS) with salt loading.

Table III and Fig. 1 and 2 also demonstrate the intracellular ionic responses to salt loading. For the group as a whole, dietary salt loading elevated cytosolic free calcium, $Ca_i$ ($P < 0.05$), and intracellular sodium, $Na_i$ ($P < 0.001$), while it suppressed intracellular pH, $pH_i$ ($P < 0.01$), and free magnesium, $Mg_i$ ($P < 0.05$) (Fig. 1). These changes occurred predominantly among SS, not SI subjects (Fig. 2). Thus, $Mg_i$ levels were significantly lower ($P < 0.05$), and $Na_i$ levels significantly higher ($P < 0.05$) in SS versus SI subjects on high dietary salt intake. On both salt diets, $pH_i$ was lower ($P < 0.05$) in SS versus SI individuals. Intracellular sodium rose significantly in all the subjects, but the magnitude of the rise was greater in SS versus SI individuals ($P < 0.05$; Fig. 2). For the group as a whole, when salt-induced changes in the ions were themselves compared, it was found that the changes in $Ca_i$ were correlated with the changes in $Na_i$ ($r = 0.504$, $P < 0.05$), $Mg_i$ ($r = -0.513$, $P < 0.05$), and $pH_i$ ($r = -0.378$, $P$ = NS).

Furthermore, for all subjects, independent of the definition of salt sensitivity, significant relationships were observed between the various intracellular ionic species and blood pressure. Significant, positive relationships were observed between both intracellular sodium and intracellular free calcium and the salt-induced change in diastolic pressure ($Na_i$: $r = 0.747$, $P < 0.001$; $Ca_i$: $r = 0.726$, $P < 0.001$). Additionally, the salt-induced change in pressure was closely and inversely related to the salt-induced change in free magnesium ($r = -0.757$, $P < 0.001$). Lastly, salt-induced changes in both systolic ($r = -0.612$, $P < 0.001$) and diastolic ($r = -0.754$, $P < 0.001$) (Fig. 3) pressures were inversely related to $pH_i$ levels measured on the high salt diet and, for the systolic pressure change, to the basal $pH_i$ on low salt ($r = -0.857$, $P < 0.001$). No significant relationships were observed between basal blood pressures or the effect of salt loading and basal urinary excretion values or changes in these values.

*Blood pressure and intracellular ionic effects of nifedipine.* Nifedipine significantly lowered blood pressure on both high- and low-salt diets in all subjects and lowered pressure equally among SS and SI individuals on a given salt intake, as shown in Table III. For the group as a whole, however, diastolic pressure fell to a greater extent on the high salt diet ($\Delta$DBP = −14±2 vs −8±2 mmHg, $P < 0.05$), and this corresponded to greater nifedipine-induced changes in $Ca_i$ and $Mg_i$ on high versus low salt diets (see below). This effect was especially true among SS individuals ($\Delta$DBP = −16±2 mmHg on high salt vs −7±2 mmHg on low salt, $P < 0.05$).

When compared with the ability of salt to elevate blood pressure, nifedipine significantly blunted the pressor effect of salt in SS individuals (Fig. 4). Thus, the diastolic pressure response to salt loading among SS subjects taking nifedipine was no longer statistically significant and, quantitatively, was reduced > 50% ($\Delta$DBP on salt + nifedipine = 5±2 mmHg vs on salt + placebo = 14±2 mmHg; $P < 0.05$, nifedipine vs placebo) (Table IV). Hence, nifedipine decreased salt sensitivity in SS subjects.

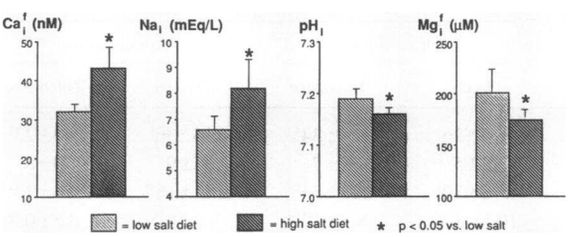


*Figure 1.* $^{19}F$-, $^{23}Na$-, and $^{31}P$-NMR spectroscopic measurement of intracellular RBC cytosolic free calcium ($Ca_i^f$), intracellular sodium ($Na_i$), intracellular pH ($pH_i$), and intracellular free magnesium ($Mg_i^f$) levels in unmedicated essential hypertensive subjects studied on low- (UNaV < 50 meq/d) and high- (UNaV > 200 meq/d) salt diets.

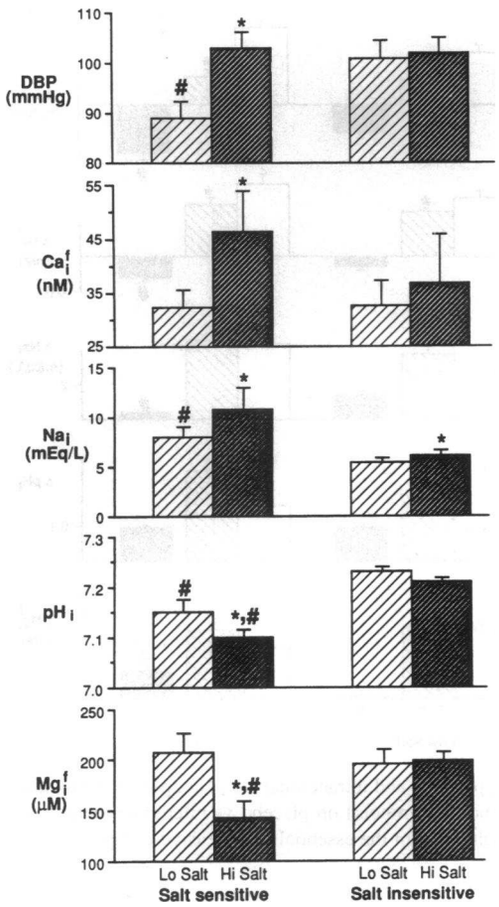


*Figure 2.* Blood pressure and intracellular ionic responses to low and high dietary salt intakes in salt-sensitive vs -insensitive essential hypertensive subjects. * $P < 0.05$ vs lo salt; # $P < 0.05$ vs salt insensitive.

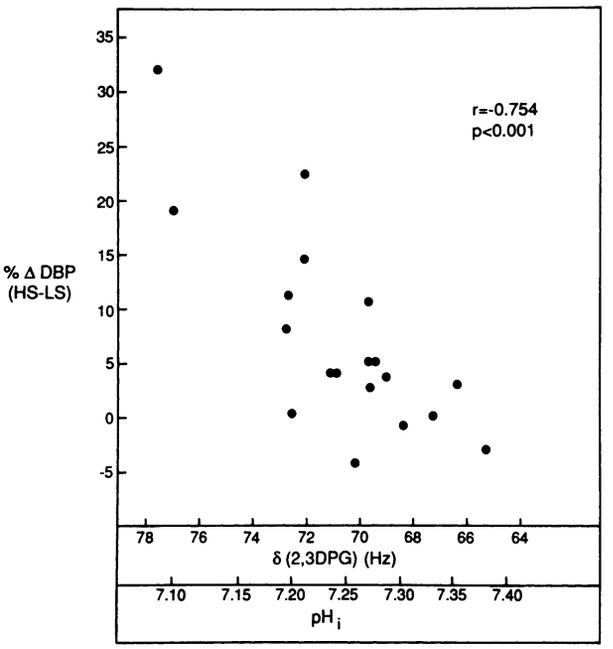


*Figure 3.* Inverse relation of salt-induced pressor responses to salt-induced intracellular pH levels in essential hypertensive subjects.

of the hypertensive state and induced by salt loading in SS hypertensive individuals.

## Discussion

The mechanism by which increased dietary salt intake can elevate blood pressure in a significant proportion of the hyperten-

Nifedipine-induced changes in blood pressure were also associated with parallel changes in the various intracellular ion species measured. These calcium-antagonist–induced ionic changes were observed in all subjects and on each dietary salt intake, as shown in Fig. 5. For the group as a whole compared with placebo, nifedipine significantly lowered $Ca_i$ ($P < 0.05$) on both low- and high-salt diets. This effect occurred to a greater extent on the high-salt diet ($\Delta Ca_i = -11.3 \pm 2.1$ vs $-6.6 \pm 1.7$ nM, $P < 0.05$), and is attributable to the fall in $Ca_i$ among SS patients (see Table III), who conversely, on the low-salt diet did not display any significant change in $Ca_i$. Reciprocally, nifedipine elevated $Mg_i$ levels, but only on the high-salt diet ($\Delta Mg_i = 32 \pm 9$ vs $2 \pm 6$ mM, $P < 0.05$). Conversely, nifedipine elevated $Na_i$ levels only on the low-salt diet ($\Delta Na_i = 1.3 \pm 0.2$ vs $0.3 \pm 0.2$ meq/liter, $P < 0.05$). Intracellular pH was consistently elevated by nifedipine on both salt diets (Fig. 5). Furthermore, just as the ability of salt to raise pressure was related to baseline $pH_i$: the lower the $pH_i$, the greater the salt-induced pressor response (see above), the antihypertensive efficacy of nifedipine ($\Delta$DBP) was also closely related to the baseline $pH_i$: the lower the $pH_i$, the greater the nifedipine-induced fall in blood pressure ($r = 0.645$, $P < 0.001$). Altogether then, with the exception of $Na_i$, nifedipine restored toward normal the deviations of blood pressure and of $Ca_i$, $Mg_i$, and $pH_i$ characteristic

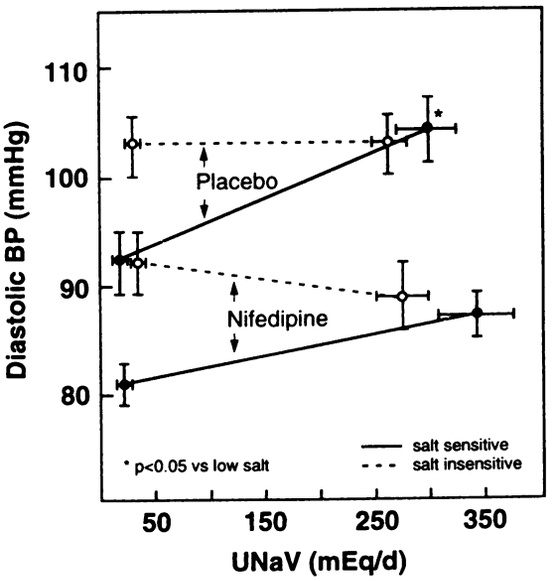


*Figure 4.* The effects of high and low dietary salt intakes on diastolic blood pressure in salt-sensitive hypertensive subjects studied on placebo vs nifedipine therapy. A significant salt-induced elevation in blood pressure ($P < 0.05$) occurred on placebo. On nifedipine, the difference in diastolic pressure on low vs high salt was no longer significant.

*Table IV. Nifedipine Reverses Blood Pressure Effects of Salt Loading: BP (High Salt) − BP (Low Salt)*

| | Placebo | Nifedipine | Δ |
|---|---|---|---|
| SS ($n$ = 9) | | | |
| Systolic BP | 29±6 | 18±4 | −11±4 |
| Diastolic BP | 14±2 | 5±2* | −9±2 |
| SI ($n$ = 10) | | | |
| Systolic BP | 9±6 | 1±5 | −8±5 |
| Diastolic BP | 0±3 | −3±3 | −3±3 |

All SS $P < 0.05$ vs SI. * $P < 0.05$ nifedipine vs placebo.

sive population remains undefined. Inadequate suppression of renin system or sympathetic nerve activity (13), and/or exaggerated stimulation of putative endogenous digoxin-like molecules (1, 14), of calcium-regulating hormones such as $1,25(OH)_2D$ (5, 15), PTH (16), and/or of the newly described parathyroid hypertensive factor (17), have all been invoked as possible etiologic factors. Ultimately, however, regardless of the extracellular mediators involved, a salt-induced alteration in cellular ion homeostasis has been presumed but not adequately demonstrated.

To better understand the clinical phenomenon of salt-sensitive hypertension directly at the cellular ionic level, we measured the blood pressure and intracellular ionic effects of dietary salt loading with and without co-administration of nifedipine in essential hypertensive subjects. We observed (*a*) a significant rise in intracellular sodium occurring in all subjects on high versus low dietary salt intakes, but exaggerated in salt-sensitive subjects; (*b*) salt loading in salt-sensitive, but not salt-insensitive individuals coordinately elevates cytosolic free calcium and suppresses intracellular pH and free magnesium levels. For all subjects, salt-induced elevations in blood pressure were closely related to the ability of salt loading to alter intracellular ion levels; and (*c*) that the ability of calcium channel blockade to reverse salt-induced changes in blood pressure is closely related to its ability to reverse the ionic effects of dietary salt loading, nifedipine reducing $Ca_i$ and elevating $Mg_i$ and $pH_i$ levels.

Given the differences between RBCs and other cell populations, appropriate caution should be exercised in extending the results of this study to other tissues. Nevertheless, the close reciprocal relationships observed between salt- and nifedipine-induced changes in blood pressure and in RBC ion levels suggest that these ionic events in RBCs reflect similar ionic events in other tissues as well. Furthermore, since red cells lack intracellular organelles, these results support the concept that salt-sensitive hypertension is both dependent on and mediated by cellular calcium accumulation from the extracellular space and by associated changes in intracellular $Mg_i$ and pH. Moreover, these data provide a cellular ionic basis for understanding the previously observed enhanced hypotensive efficacy of calcium channel blockade in sodium volume forms of hypertensive disease (18–20). Lastly, of practical importance for research in this field, proper interpretation of intracellular ion measurements among various patient populations in the future will require concurrent assessment and/or control of dietary salt intake.

The use of NMR spectroscopy to measure intracellular min-

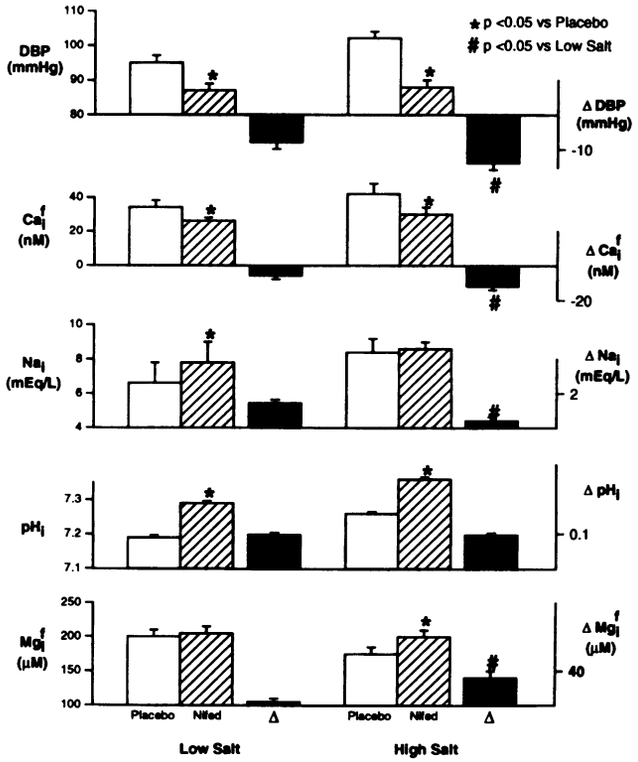


*Figure 5.* Blood pressure and intracellular $Ca_i$, $Na_i$, $pH_i$, and $Mg_i$ levels (see Fig. 1 legend for symbols) on placebo vs nifedipine therapy on low- and high-salt diets for the essential hypertensive group as a whole.

eral ions has received increasing attention (21). $^{31}$P-NMR spectra allow intracellular free magnesium and intracellular pH to be measured noninvasively and directly in packed cells and yields normal values for these ions in RBCs ($Mg_i$ = 220–250 mM; $pH_i$ = 7.28±0.02) and in other tissues comparable to or lower than other more invasive techniques (22, 23). Similarly, we measured intracellular free calcium in RBCs by using $^{19}$F-NMR spectroscopy, in which cells are loaded and then incubated with the flourinated EDTA-derived probe, QUIN-MF. Using this technique, basal levels of $Ca_i$ in RBCs of normotensive subjects, 20–30 nM, are in complete agreement with values obtained using other NMR (11, 24, 25) or fluorescent (26) probes. Lastly, the NMR measurement of $Na_i$ is based on the addition of a cellular nonpermeant chemical shift reagent, $[Dy(PPP_i)_3]^{7-}$, which remains in the extracellular space and thus allows both intracellular and extracellular sodium to be clearly distinguished and quantitated. However, ~ 15% of the total intracellular sodium may be "NMR-invisible" and is presumed to represent membrane-bound and otherwise immobilized sodium. This property of measuring only "free" sodium, in addition to being able to precisely distinguish extracellular vis a vis intracellular sodium, may inadvertently help to explain why we were able to observe consistent increases in NMR $Na_i$ in all subjects on high versus low dietary salt intakes when other careful studies have only been able to measure consistent increases in sodium transport pump activities (27) or have only seen changes when pressor responses were observed (28, 29).

This study complements and supports previous data suggesting that cellular calcium uptake from the extracellular space is critical to the pressor effects of salt loading. Thus, dietary

salt-induced increases in blood pressure were closely and quantitatively related to salt-induced decreases in serum ionized calcium and to stimulation of $1,25(OH)_2D$ (5). We suggested that the fall in extracellular ionized calcium represented a reciprocal calcium transfer intracellularly mediated by factors such as $1,25(OH)_2D$. Similarly, in normotensive dogs, while neither saline nor calcium infusions alone raised blood pressure, the combination of both saline and calcium was significantly pressor (30). Furthermore, pretreatment of these animals with a calcium channel antagonist prevented any significant pressor responses (30). These findings have recently been confirmed, salt-induced elevations of blood pressure being closely related to decreased serum ionized calcium and to a reciprocal increase in intracellular free calcium in both platelets and lymphocytes (31–33). We cannot distinguish in this study, however, the extent to which salt-induced changes in blood pressure and/or intracellular ions are due to intrinsic cellular defects of membrane transport, to the elaboration of circulating extracellular factors that alter cellular ion transport, or to a combination of both.

Indeed, each of the measured ion species may participate in the hypertensive process and in the pressor effects of salt. Mechanistically, calcium serves as part of the final common pathway mediating contraction in skeletal, cardiac, and vascular smooth muscle (34). The direct relation of free cytosolic calcium in platelets (35) and in RBCs (4) to the level of blood pressure suggests that this biochemical effect of calcium is both clinically relevant and reflected in a variety of tissues. Similarly, fasting RBC free magnesium levels are closely and inversely related to the blood pressure in normotensive and hypertensive subjects (2), and experimentally induced magnesium depletion increases smooth muscle contractility and can cause frank hypertension (36, 37). Furthermore, salt-induced hypertension has previously been associated with suppression of total RBC magnesium levels (30). Magnesium may exert its effects by virtue of its interactions with calcium and/or sodium. Thus, magnesium blunts the contractile effects of calcium in vascular smooth muscle (38), inhibits calcium binding to calmodulin (39), stimulates the rectifying calcium-dependent potassium channel (40–42), and stimulates $Na^+,K^+$ATPase activity (43). Suppressed intracellular free magnesium would therefore be expected to potentiate calcium-mediated contractions, exacerbate the resultant cellular depolarization, and cause cellular sodium accumulation, as has been demonstrated (44).

Intracellular pH is also a critical control element in normal cellular function (23), and we have previously demonstrated lower intracellular pH levels in essential hypertensive versus normotensive human subjects, in rats with renin-dependent, 2K-1C Goldblatt hypertension, and in DOC-saline hypertensive rats versus their normotensive controls (3). Furthermore, maneuvers that raised or lowered intracellular pH consistently changed blood pressure in the opposite direction: the higher the pH, the lower the blood pressure (3). These results have recently been confirmed, and increased total body acid production in hypertension, especially salt-sensitive forms of hypertension, has also been reported (45, 46). The mechanisms by which an altered $pH_i$ might increase blood pressure remain undefined, but increases in cytosolic free calcium appear associated with intracellular acidification (47, 48).

Since salt-loading in this study resulted in coordinate changes in each of these mineral ion species, we cannot ascertain from these results alone which, if any, of these ions might be primarily responsible for mediating the pressor effects of salt and which might be only secondarily involved. Indeed, the multiple correlations we observed in the present study between the various intracellular ion species, blood pressure, and salt sensitivity (see Results) may also reflect similar close relationships among these ions themselves. Thus, we have found basal $Ca_i$ and $Na_i$, as well as $Mg_i$ and $pH_i$, to be directly related, while $Ca_i$ and $Mg_i$ were linked in an inverse manner (4). However, our recent work suggests that while elevation of cytosolic free calcium and/or suppression of free magnesium may be necessary, they may not be sufficient conditions for the emergence of elevated blood pressure and that intracellular pH may play a pivotal role in and/or be a marker for the hypertensive process. Thus, even in the absence of hypertension, subjects with obesity and/or non–insulin-dependent diabetes mellitus exhibited elevated $Ca_i$ and suppressed $Mg_i$ levels compared with their equally normotensive thin and nondiabetic controls (49). Only in the hypertensive subjects, whether thin, obese, diabetic, or not, were $pH_i$ levels consistently lower (49). The importance of $pH_i$ is further emphasized by the results of this present study. Not only were the pressor effects of dietary salt loading closely related to the basal $pH_i$, the lower the $pH_i$, the greater the pressor response, but the antihypertensive effects of nifedipine were equally closely related to the $pH_i$.

Lastly, this study provides a cellular ionic basis for the enhanced antihypertensive efficacy of calcium channel blockade previously noted in volume-dependent hypertensive states such as DOC-saline and Dahl salt-sensitive rat models and in essential hypertensive subjects with low plasma renin activity and/or lower serum ionized calcium values (19, 20, 50). We have previously suggested that the lower average extracellular ionized calcium observed in the low renin state reflects a greater dependence of blood pressure on cytosolic calcium accumulation from the extracellular space vis a vis from internal stores (51), hence explaining their greater sensitivity to calcium channel blocking agents such as nifedipine (50). This present study provides additional evidence in favor of this hypothesis, by directly demonstrating in RBCs devoid of intracellular calcium stores, the linked blood pressure and cellular calcium elevating effects of salt loading and the reversal of these pressure and calcium effects by nifedipine. Furthermore, in the presence of nifedipine, dietary salt loading in salt-sensitive subjects no longer significantly elevated blood pressure; diastolic pressures did not rise out of the normal range (Table III). Hence, the enhanced clinical utility of calcium channel blocking agents in low renin and/or sodium volume-dependent forms of hypertension may derive from their ability to at least partially reverse salt-dependent cellular ionic changes. On the other hand, it is reasonable that nifedipine lowered pressure to at least some degree in all the subjects studied here, including salt-insensitive individuals, since a variety of mechanisms other than salt also contribute to cellular calcium uptake, the inhibition of which would lower average cytosolic free calcium levels and thus blood pressure.

Altogether, these results demonstrate physiologically similar cellular ionic mechanisms underlying the ability of dietary salt to exacerbate hypertension and of calcium channel blockade with nifedipine to ameliorate it, and emphasize the critical role of altered cellular ion metabolism in the hypertensive process. Regardless of mechanistic considerations, however, proper interpretation of intracellular mineral ion measurements in future studies may require a concurrent assessment of dietary salt intake. Using NMR spectroscopic techniques to measure intracel-

lular ion levels in peripheral blood samples may also be useful in distinguishing clinically salt-sensitive subjects and is the focus of current studies.

## References


1. Blaustein, M. P. 1977. Sodium ions, calcium ions, blood pressure regulation, and hypertension: a reassessment and a hypothesis. *Am. J. Physiol.* 232:C165–C173.

2. Resnick, L. M. 1984. Intracellular free magnesium in erythrocytes of essential hypertension: Relation to blood pressure and serum divalent cations. *Proc. Natl. Acad. Sci. USA.* 81:6511–6515.

3. Resnick, L., R. K. Gupta, R. E. Sosa, M. L. Corbett, and J. H. Laragh. 1987. Intracellular pH in human and experimental hypertension. *Proc. Natl. Acad. Sci. USA.* 84:7663–7667.

4. Resnick, L., R. K. Gupta, and J. H. Laragh. 1990. RBC cytosolic free calcium levels in hypertension: relation to blood pressure and other cations. *Am. J. Hypertens.* 3:59A.

5. Resnick, L., J. P. Nicholson, and J. H. Laragh. 1985. Alterations in calcium metabolism mediate dietary salt sensitivity in essential hypertension. *Trans. Assoc. Am. Phys.* 98:313–321.

6. Resnick, L., J. P. Nicholson, and J. H. Laragh. 1989. The effects of calcium channel blockade on blood pressure and calcium metabolism. *Am. J. Hypertens.* 2:927–930.

7. Resnick, L., R. Gupta, and J. H. Laragh. 1990. Calcium channel blockade reverses salt sensitivity: role of intracellular ions. *Clin. Res.* 38:259*a*. (Abstr.)

8. Resnick, L., R. Gupta, and J. H. Laragh. 1990. Role of intracellular cations in dietary salt sensitivity. *Clin. Res.* 38:476*a*. (Abstr.)

9. Gupta, R., J. Benovic, and J. Rose. 1978. The determination of the free magnesium level in the human red blood cell by $^{31}$P-NMR. *J. Biol. Chem.* 253:6172–6176.

10. Moon, R. B., and J. H. Richards. 1973. Determination of intracellular pH by $^{31}$P magnetic resonance. *J. Biol. Chem.* 248:7276–7278.

11. Murphy, E., L. Levy, L. R. Berkowitz, E. P. Orringer, S. A. Gabel, and R. E. London. 1986. NMR measurements of cytosolic free calcium in human red blood cells. *Am. J. Physiol.* 251:C496–C507.

12. Gupta, R. and P. Gupta. 1982. Direct observation of resolved resonances from intra- and extracellular sodium 23 ions in NMR studies of intact cells and tissue using dysprosium (III) tripolyphosphate as paramagnetic shift reagent. *J. Mag. Reson.* 47:344–350.

13. Sealey, J., J. Blumenfeld, M. Bell, M. S. Pecker, S. Sommers, and J. H. Laragh. 1988. Presidential Address. On the renal basis for essential hypertension: nephron heterogeneity with discordant renin secretion and sodium excretion causing a hypertensive vasoconstriction-volume relationship. *J. Hypertens.* 6:763–777.

14. DeWardener, H. E., G. A. MacGregor. 1980. Dahl's hypothesis that a saluretic substance may be responsible for a sustained rise in arterial pressure: its possible role in essential hypertension. *Kidney Int.* 18:1–9.

15. Resnick, L. M. 1987. Uniformity and diversity of calcium metabolism in hypertension: a conceptual framework. *Am. J. Med.* 82(Suppl. 1B):16–26.

16. Campese, V. 1986. Calcium, parathyroid hormone, and the sympathoadrenal system. *Am. J. Nephrol.* 6(Suppl. 1):29–32.

17. Lewanczuk, R., L. M. Resnick, J. Blumenfeld, J. H. Laragh, and P. Pang. 1990. A new circulating hypertensive factor in the plasma of essential hypertensive subjects. *J. Hypertens.* 8:105–108.

18. Zawada, E. T., and M. Johnson. 1984. Calcium chelation and calcium channel blockade in anesthetized acute renovascular hypertensive rats. *Miner. Electrolyte Metab.* 10:366–370.

19. Hall, C., and S. Hungerford. 1983. Prevention of DOCA-salt hypertension with the calcium blocker nitrendipine. *Clin. Exp. Hypertens. Part A.* 5:721–728.

20. Iriuchijima, J. 1980. Effect of calcium antagonist nifedipine on blood pressure of various hypertensive rats. *Hiroshima J. Med. Sci.* 29:15–19.

21. Gupta, R., and P. Gupta. 1984. NMR studies of intracellular metal ions in intact cells and tissues. *Annu. Rev. Biophys. Bioeng.* 13:221–246.

22. Alvarez-Leefmans, F., F. Giralde, and S. Garmino. 1987. Intracellular free magnesium in excitable cells: its measurement and its biologic significance. *Can. J. Physiol. Pharmacol.* 65:915–925.

23. Roos, A., and W. F. Boron. 1981. Intracellular pH. *Physiol. Rev.* 61:296–434.

24. Murphy, E., L. R. Berkowitz, E. Orringer, L. Levy, S. A. Gabel, R. E. London. 1987. Cytosolic free calcium levels in sickle red blood cells. *Blood.* 69:1469–1474.

25. Jelicks, L. A., J. Weaver, S. Pollack, R. K. Gupta. 1989. NMR studies of intracellular free calcium, free magnesium and sodium in the guinea pig reticulocyte and mature red blood cell. *Biochem. Biophys. Acta.* 1012:261–266.

26. Lew, V. L., R. Y. Tsien, C. Miner, R. M. Bookchin. 1982. Physiological ($Ca^{2+}$) level and pump-leak turnover in intact red cells measured using an incorporated Ca chelator. *Nature (Lond.).* 298:478–481.

27. Canessa, M., C. Lasky, B. Falkner. 1990. Red blood cell Na transport as a predictor of blood pressure response to a sodium load in young blacks and whites. *Hypertension (Dallas).* 16:508–514.

28. Pedersen, K. E., P. Jest, N. A. Klitgaard, J. Rokkedal-Nielsen, T. Johansen. 1986. Effect of oral salt loading on blood pressure and lymphocyte sodium metabolism in borderline hypertension. *Acta Med. Scand. Suppl.* 714:81–85.

29. Oshima, T., E. W. Young, K. Hermsmeyer, D. A. McCarron. 1992. Modification of platelet and lymphocyte calcium handling and blood pressure by dietary sodium and calcium in genetically hypertensive rats. *J. Lab. Clin. Med.* 119:151–158.

30. Nicholson, J., L. Resnick, J. Cigarroa, D. Marion, E. J. Vaughan, J. H. Laragh. 1991. The pressor effect of sodium-volume expansion is calcium mediated. *Am. J. Hypertens.* 4:904–908.

31. Oshima, T., H. Matsuura, K. Matsumoto, K. Kido, G. Kajiyama. 1988. Role of cellular calcium in salt sensitivity of patients with essential hypertension. *Hypertension (Dallas).* 11:703–707.

32. Shingu, T., H. Matsuura, M. Kusaka, M. 1991. Significance of intracellular free calcium and magnesium and calcium-regulating hormones with sodium chloride loading in patients with essential hypertension. *J. Hypertens.* 9:1021–1028.

33. Kainouchi, M., H. Matsuura, T. Shingu, I. Inoue, K. Ishida, R. Ozono, T. Fujii, A. Yuasa, T. Oshima, G. Kajiyama. 1991. Time-course of changes in intracellular cations and blood pressure with salt loading in patients with essential hypertension. *J. Hypertens.* 9(Suppl. 3):s302–s303.

34. Kuriyama, H., I. Uyshi, H. Sueuk, A. Kitamura, T. Itoh. 1982. Factors modifying contraction-relaxation cycle in vascular smooth muscles. *Am. J. Physiol.* 243:H641–H662.

35. Erne, P., P. Bolli, E. Burgissen, F. Buhler. 1984. Correlation of platelet calcium with blood pressure: effect of antihypertensive therapy. *N. Engl. J. Med.* 319:1084–1088.

36. Altura, B. M., B. T. Altura. 1981. Magnesium ions and contraction of vascular smooth muscles: relationship to some vascular diseases. *Fed. Proc.* 40:2672–2679.

37. Altura, B. M., B. T. Altura, A. Gebrewold. 1984. Magnesium deficiency and hypertension: correlation between magnesium-deficient diets and microcirculatory changes in situ. *Science (Wash. DC).* 223:1315–1317.

38. Altura, B. M. 1982. Magnesium and the regulation of contractility of vascular smooth muscle. *Adv. Microcirc.* 11:77–113.

39. Tasi, M., T. Drackenberg, E. Thulin, S. Forsen. 1987. Is the binding of magnesium (II) to calmodulin significant? An investigation by magnesium-25 nuclear magnetic resonance. *Biochemistry.* 26:3635–3643.

40. Treischmann, U., G. Isenberg. 1989. $Ca^{2+}$-activated $K^+$ channels contribute to the resting potential of vascular myocytes. $Ca^{2+}$-sensitivity is increased by intracellular $Mg^{2+}$-ions. *Pfluegers Arch. Eur. J. Physiol.* 414(Suppl 1):S183–S184.

41. Squire, L. G., O. H. Petersen. 1987. Modulation of $Ca^{2+}$- and voltage-activated $K^+$ channels by internal $Mg^{2+}$ in salivary acinar cells. *Biochem. Biophys. Acta.* 899:171–175.

42. Golowasch, R. S., A. Kirkwood, C. Miller. 1986. Allosteric effects of $Mg^{2+}$ on the gating of $Ca^{2+}$-activated $K^+$ channels from mammalian skeletal muscle. *J. Exp. Biol.* 124:5–13.

43. Zemel, M., J. Kraniak, P. Standley, J. Sowers. 1988. Erythrocyte cation metabolism in salt-sensitive hypertensive blacks as affected by dietary sodium and calcium. *Am. J. Hypertens.* 1:386–392.

44. Turlapaty, P. D. M. V., B. M. Altura. 1978. Extracellular magnesium ions control calcium exchange and content of vascular smooth muscle. *Eur. J. Pharmacol.* 52:421–423.

45. Batlle, D. C., A. Saleh, G. Rombola. 1990. Reduced intracellular pH in lymphocytes from spontaneously hypertensive rat. *Hypertension (Dallas).* 15:97–103.

46. Batlle, D. C., A. M. Sharma, M. W. Alsheikha, M. Sobrero, A. Saleh, C. Gutterman. 1993. Renal acid excretion and intracellular pH in salt-sensitive genetic hypertension. *J. Clin. Invest.* 91:2178–2184.

47. Vaughan-Jones, R. D., W. J. Lederer, D. A. Eisner. 1983. $Ca^{2+}$ ions can affect intracellular pH in mammalian cardiac muscle. *Nature (Lond.).* 301:522–524.

48. Tornquist, K., A. J. Tashjian. 1992. pH homeostasis in pituitary GH4C1 cells: basal intracellular pH is regulated by cytosolic free $Ca^{2+}$ concentration. *Endocrinology.* 130:717–725.

49. Resnick, L. M., R. Gupta, K. K. Bhargava, H. Gruenspan, M. Alderman, J. H. Laragh. 1991. Cellular ions in hypertension, diabetes, and obesity: a nuclear magnetic resonance spectroscopic study. *Hypertension (Dallas).* 17:951–957.

50. Resnick, L. M., J. P. Nicholson, J. H. Laragh. 1987. Calcium, the renin-angiotensin system, and the hypotensive response to nifedipine. *Hypertension (Dallas).* 10:254–258.

51. Resnick, L. M., J. Laragh, J. E. Sealey, M. Alderman. 1983. Divalent cations in essential hypertension. Relations between serum ionized calcium, magnesium, and plasma renin activity. *N. Engl. J. Med.* 309:888–891.