Control of the Erythrocyte Free Ca\(^{2+}\) Concentration in Essential Hypertension

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Since Ca\(^{2+}\) ions seem to directly participate in the control of erythrocyte membrane structure and deformability, and because cell Ca\(^{2+}\) metabolism has been repeatedly proposed to be modified in hypertension, the intracellular calcium ion concentration ([Ca\(^{2+}\)]\(_i\)) was investigated in red blood cells from hypertensive and normotensive subjects. [Ca\(^{2+}\)]\(_i\), was measured by using the fluorescent Ca\(^{2+}\) chelator fura-2. Red blood cell [Ca\(^{2+}\)]\(_i\), was increased in hypertensive compared with normotensive subjects in the whole population and further increased when hypertensive were compared with age-matched normotensive subjects. An inverse relation between age and [Ca\(^{2+}\)]\(_i\), was observed when calculated with blood pressure adjusted. In hypertensive patients, high [Ca\(^{2+}\)]\(_i\), values were associated with a reduced erythrocyte deformability. The initial rate of \(\text{d}[\text{Ca}\(^{2+}\)]/\text{d}t\) did not differ between the two blood pressure groups. Similarly, when the extracellular Ca\(^{2+}\) concentration was elevated from 1 to 2 mmol/l, [Ca\(^{2+}\)]\(_i\), increased by 16±4% (\(p<0.03\)) in red blood cells from both groups, thus maintaining a significant difference between hypertensive and normotensive subjects. Under these conditions, the addition of 10\(^{-8}\) mol/l nicardipine, a dihydropyridine Ca\(^{2+}\) antagonist, decreased [Ca\(^{2+}\)]\(_i\), by 15±4% (\(p<0.05\)) and 7±5% in erythrocytes from hypertensive and normotensive subjects, respectively, thereby reducing the difference in [Ca\(^{2+}\)]\(_i\), observed between these two groups. This nicardipine effect was positively correlated to the initial [Ca\(^{2+}\)]\(_i\),. In the presence of 5 \(\mu\)mol/l W7, a calmodulin antagonist, [Ca\(^{2+}\)]\(_i\), increased significantly only in erythrocytes from hypertensive patients (26±6%, \(p<0.01\)). These results demonstrate that essential hypertension is associated, in the erythrocyte, with an increased cytosolic Ca\(^{2+}\) concentration, higher reduction of [Ca\(^{2+}\)]\(_i\), by nicardipine, and an enhanced activity of the calmodulin-dependent regulatory mechanisms. (Hypertension 1992; 19:167-174)

The intracellular free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) in erythrocytes is the direct balance of both Ca\(^{2+}\) influx and Ca\(^{2+}\) pump activity with the participation of membrane and cytosolic Ca\(^{2+}\) binding proteins as an intracellular Ca\(^{2+}\) buffer. The calmodulin-stimulated Ca\(^{2+}\), Mg\(^{2+}\)-ATPase, the membrane outwardly directed Ca\(^{2+}\) pump, is the only mechanism by which these cells maintain a low intracellular Ca\(^{2+}\) concentration.\(^1\) Ca\(^{2+}\) ions participate in the control of cell membrane structure and deformability.\(^2\)\(^-\)\(^4\) In particular, Ca\(^{2+}\) and calmodulin directly regulate skeletal protein interactions in the erythrocyte membrane and Ca\(^{2+}\) loading induces changes in the activities of calpain and acid endopeptidases, which are responsible for the selective degradation of cytoskeletal proteins.\(^5\)

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At the erythrocyte level, essential hypertension is associated with abnormalities such as increases in volume, number, and aggregability; a decrease in deformability;\(^6\)\(^-\)\(^11\) and an altered Ca\(^{2+}\) handling.\(^12\)\(^-\)\(^14\) The activity of the Ca\(^{2+}\) pump has been investigated by several groups. Vincenzi et al\(^13\)\(^,\)\(^15\) reported that, according to the anti-calmodulin agent used, trifluoperazine or the 48/80 compound, its basal activity was decreased or unchanged in hypertensive subjects. The basal activity was also found unchanged in EGTA-treated membranes, but its stimulation by calmodulin was reduced when compared with that of normotensive subjects.\(^12\)\(^,\)\(^16\) Essential hypertension has been also reported to be associated with decreased Ca\(^{2+}\) uptake\(^17\) and membrane Ca\(^{2+}\) binding\(^14\)\(^,\)\(^18\)\(^,\)\(^19\) and increased or decreased total intracellular calcium content, measured by flame spectrometry or flameless atomic absorption.\(^20\)\(^,\)\(^21\) Recently, the erythrocyte [Ca\(^{2+}\)]\(_i\), evaluated by nuclear magnetic resonance of a fluorinated derivative of quin-2 (2-[(2-bis(carboxymethyl)-amino-5-methylphenoxy)-methyl]-6-methoxy-8-bis[carboxymethyl]aminoquin-
direct index of the erythrocyte internal viscosity, a
the internal
to be modified by the medium viscosity,

Rheological Parameters

Values of the laboratory were 5.00±0.45 arbitrary
units (AU) for healthy subjects 30-60 years old
(n=6).

Patients and Subjects

Eighty-seven Caucasian subjects were included in
this study after they gave informed consent. Fifty were
patients with mild-to-moderate hypertension (supine
blood pressure greater than 140/90 mm Hg, mean age,
48±1 years; 30 men and 20 women with mean body
mass index [BMI] of 27±0.5 and 22.3±0.3 kg/m²,
respectively). Thirty-seven were normotensive healthy
volunteers (mean age, 31±2 years; 29 men with BMI
23.8±0.4 kg/m² and eight women with BMI 22.8±0.5
kg/m²); their mean arterial blood pressure averaged
117±2 and 85±2 mm Hg, respectively. All the patients
and subjects were untreated for at least 4 weeks before
blood sampling. None had cardiac, neurological, or
renal complications or peripheral vascular disease.

Preparation of Red Blood Cells

Venous blood (5 ml) was collected into tubes
containing 1/10 volume of the anticoagulant: 2.73% citric acid, 4.48% trisodium citrate, and 2% glucose. Experiments were performed within 4 hours after
blood sampling. Blood was kept at room temperature
and centrifuged at 20°C for 5 minutes at 530g maxi-
mum just before the experiments. Plasma and buffy
coat were removed by suction, and packed cells were
immediately diluted to the required hematocrit.

Rheological Parameters

The rigidity index of the erythrocytes was evalu-
ated in hypertensive patients only. It was measured in
duplicate within half an hour of venipuncture by the
method of Hanss24 with a hemorheometer SPO1
(IMH, Paris, France). The initial filtration flow
(through a nucleopore filter with pores 5 μm in
diameter) of 0.4 ml packed cells diluted in 5 ml (7%
hematocrit) of Hanks’ buffer solution (Pasteur Diag-
nostic, Paris, France) was determined at 37°C at a
constant pressure. The intra-assay variability was
5.00±0.32% (six measures of each sample, n=6).
Values of the laboratory were 5.00±0.45 arbitrary
units (AU) for healthy subjects 30–60 years old
(mean age 43±4 years).

As the spectral characteristics of the fluorescent
Ca²⁺ chelator fura-2 have been previously observed
to be modified by the medium viscosity,25 the internal
viscosities of red blood cells from hypertensive and
normotensive subjects were compared. The mean
corpuscular hemoglobin concentration (MCHC), a
direct index of the erythrocyte internal viscosity,26,27
was calculated from hematocrit and erythrocyte hem-
oglobin content, determined with a Coulter
counter, of 5 ml of blood sampled in heparin-coated
tubes. It did not differ between the two blood pressure
groups (34.1±0.1 versus 33.7±0.2 g/100 ml), which allowed the use of the same calibration param-
ters to calculate [Ca²⁺].

Measurements of Cytosolic Free Ca²⁺ Concentra-
tion in Intact Red Blood Cells

Measurements of [Ca²⁺], in intact erythrocytes
were performed with fura-2.23 This method allows
valid determination of [Ca²⁺], in intact erythrocytes in
spite of hemoglobin. Fura-2-tetrakis(acetoxymethyl-
ester) (fura 2-AM) was dissolved in dry dimethylsul-
foxide at a concentration of 1 mmol/l. Aliquots were
stored dessicated at −20°C and were diluted with
buffer A [in mmol/l: NaCl 123, KCl 5, MgCl₂ 1, CaCl₂
1, glucose 10, HEPES 25 (N’-[2-hydroxyethyl]pi-
erazine-N’-[2-ethanesulfonic acid], pH 7.4 at 37°C)
immediately before incubation. Erythrocytes sus-
pended at 1% hematocrit in buffer A were incubated
for 45 minutes at 37°C with 0.5 μmol/l fura 2-AM.
They were then diluted to 0.1% hematocrit in buffer A
containing 1 mmol/l MnCl₂ to quench the extra-
cellular fura-2 signal and excitation spectra immedi-
ately recorded on a Spex fluorolog CM111 equipped
with a 450 W xenon lamp (Instrument SA; Jobin
Yvon, Longjumeau, France). Excitation measure-
ments corrected with rhodamine 6G quantum
counter were performed with an excitation band-
width of 1.8 nm at 510 nm emission wavelength and
9.0 nm bandwidth. All the experiments were per-
formed at 37°C in quartz suprasil microcuvettes with
optical pathways of 1 and 5 mm. Free Ca²⁺ concen-
trations were calculated according to the equation
established by Poenie et al28:

$$[\text{Ca}^{2+}] = K \times (R - R_0)/(R_s - R)$$

with

$$K = K_0 \times (F_0/F_s)^{385}$$

where R is the ratio of the fluorescence intensities
measured at 335 and 385 nm, Ro is the same ratio in
the absence of Ca²⁺, Rs is the same ratio when
saturated with Ca²⁺, K is the dissociation constant of
fura-2–calcium complex, and Fo and Fs are the
fluorescence intensities with zero and saturating
Ca²⁺, respectively, measured at a 385 nm excitation
wavelength.

The parameters (Ro, Rs, K) were determined from
fura-2–calcium fluorescence calibration experiments
performed at 37°C with EGTA-calcium buffers, simi-
lar to the intracellular medium in terms of pH (7.2),
ionic strength (in mmol/l: NaCl 10, KCl 120, free
Mg²⁺ 0.4, glucose 10, HEPES 25, and variable Ca²⁺
concentrations ranging from 1 mmol/l to 20 μmol/l),
and viscosity (14 mPa×sec). Under these conditions,
Ro=0.88±0.04, Rs=13.2±3.1, and K=1.0±0.2 μM
(n=6). Intracellular viscosity was mimicked by add-
ing 21 g/l polyvinylpyrrolidone to the incubation medium to produce a viscosity of 14 mPa·s at 37°C, similar to that described in erythrocytes. The intracellular fura-2 concentration was similar in the two blood pressure groups (2.8±0.3 and 2.5±0.2 10⁻⁵ mol/l in the normotensive and hypertensive groups, respectively). As observed previously in normotensive subjects, [Ca⁺²], values were observed to be stable between 15 and 90 minutes. The kinetics of fura 2-AM uptake and de-esterification were not markedly different in hypertensive patients since [Ca⁺²], values were stable between 30 and 90 minutes (92.2±6.6 versus 86.2±6.7 nmol/l, respectively). An incubation time of 45 minutes was used for [Ca⁺²], determinations. The de-esterification of the dye was completed under these conditions since the spectral characteristics of lysed cells (50 µl fura-2-loaded cells in 450 µl bidistilled water) were similar to those of fura-2 in the presence of saturating Ca⁺² concentration (maximal intensity at 336 nm, Rs=34±8, n=5).

The interassay and intra-assay variabilities were 6.3±2.1% and 5.8±2.0% (n=6–8), respectively. All determinations were performed at least in duplicate.

Determination of ⁴⁵Ca⁺² Influx in Intact Red Blood Cells

Ca⁺² influx experiments were performed according to a method derived from that described by Waller et al. Packed erythrocytes isolated as described above were washed twice by centrifugation for 5 minutes at 1,500g and 4°C in nine volumes of physiological saline, twice in nine volumes of buffer A without Ca⁺² (buffer B), and then suspended in the same buffer at 20–30% hematocrit. After incubation for 30 minutes at 37°C, the experiment was initiated by addition of CaCl₂ and ⁴⁵Ca⁺² (Amersham, Les Ulis, France) at final concentrations of 1 mmol/l and 5 µCi/ml, respectively. Aliquots (0.1 ml) of cell suspension were withdrawn as a function of time, diluted with 0.9 ml buffer B at 0°C, and centrifuged for 5 seconds at 12,000g in an Eppendorf microcentrifuge. The supernatants were discarded, and the pellets were suspended in 1 ml buffer B and recentrifuged as above. This washing procedure was repeated twice. The whole procedure took less than 5 minutes. Erythrocyte pellets were then lysed by successive additions of 0.5 ml bidistilled water and 0.5 ml of 5% (wt/vol) trichloroacetic acid under vigorous agitation. After centrifugation for 2 minutes at 12,000g, 0.9 ml supernatant was diluted in 5 ml picofluor (Packard, Downers Grove, Ill.) and ⁴⁵Ca was counted.

To check that the accumulated [Ca⁺²], indeed represented an intracellular uptake and not a binding, erythrocytes were first Ca⁺² loaded in the presence of 1 mmol/l CaCl₂ and 25 µCi/ml ⁴⁵Ca⁺², washed twice in medium B, and resuspended in this medium at the same hematocrit as initially. Then 2 µmol/l ionomycin (Calbiochem, San Diego, Calif.) was added, and 0.1-ml aliquots withdrawn as a function of time were treated as described above. For each donor, uptake experiments were performed at least in duplicate.

Statistical Analysis

Results are expressed as mean±SEM. The number of independent experiments is indicated by n. The significance of the differences between the groups was assessed by two-tailed unpaired or paired Student's t tests as indicated. Correlation coefficients between parameters were calculated by linear regression. Ca⁺² uptake initial rates were calculated by computerized polynomial analysis.

Results

Intracellular Free Ca⁺² Concentration in Red Blood Cells From Hypertensive Patients and Normotensive Subjects

[Ca⁺²⁺], was determined in fura-2–loaded red blood cells from 50 untreated hypertensive patients and 37 normotensive subjects. The mean [Ca⁺²⁺], of hypertensive patients was significantly higher than that of the normotensive subjects (85±2 versus 77±2 nmol/l,
In the whole population, $[Ca^{2+}]_i$ values did not correlate with age ($r = -0.062$), with blood pressure ($r = 0.124$), or with BMI ($r = 0.015$) (Figure 1). However, when the correlation between $[Ca^{2+}]_i$ and age was calculated with blood pressure adjusted, it became significant ($r = -0.202$, $p < 0.05$). Similarly, when the correlation between $[Ca^{2+}]_i$ and blood pressure was calculated with age adjusted, it reached significance ($r = 0.239$, $p < 0.05$). In contrast, $[Ca^{2+}]_i$ did not correlate with BMI when calculated with blood pressure or age adjusted ($-0.035$ or $0.035$, respectively).

When data were analyzed separately for each sex, $[Ca^{2+}]_i$ remained similarly correlated with age ($n = 59$, $r = 0.300$, $p < 0.05$ with blood pressure adjusted) and mean blood pressure ($r = 0.441$, $p < 0.01$ with age adjusted) in men but not in women. When only male hypertensive patients were considered, a clear inverse relation between age and erythrocyte $[Ca^{2+}]_i$ was observed ($n = 30$, $r = -0.584$, $p < 0.001$). It remained valid when calculated with blood pressure adjusted ($r = 0.634$, $p < 0.001$).

Since erythrocyte $[Ca^{2+}]_i$ seemed to be influenced by two factors, age and blood pressure, it was also compared in age-matched normotensive and hypertensive subgroups. As indicated in Table 1, a clear difference in $[Ca^{2+}]_i$ between hypertensive patients and normotensive subjects was obtained ($p < 0.001$). It was more marked in men than in women. In male subjects only, erythrocyte $[Ca^{2+}]_i$ was positively correlated to mean blood pressure ($n = 29$, $r = 0.441$, $p < 0.01$) (Figure 2).

**Rheological Parameters**

Since $Ca^{2+}$ ions participate in the control of membrane structure and deformability, the rigidity index of red blood cells was measured in 30 hypertensive patients (17 men and 13 women). Its values ranged between 4.7 and 12.8 with a mean value of $8.6 \pm 0.6$ AU. The individual values were positively correlated with those of $[Ca^{2+}]$, measured in parallel ($r = 0.373$, $n = 30$, $p = 0.042$).

**Variations in $Ca^{2+}$ Influx and Calmodulin Activity in Erythrocytes From Hypertensive and Normotensive Subjects**

To investigate the respective roles of $Ca^{2+}$ influx and calmodulin in the high $[Ca^{2+}]_i$ observed in erythrocytes from hypertensive and normotensive subjects, $Ca^{2+}$ influx and calmodulin activity were assayed in erythrocytes from 30 hypertensive patients (17 men and 13 women) and 30 normotensive subjects (17 men and 13 women). The results are shown in Figure 3. $Ca^{2+}$ influx was significantly increased in hypertensive patients compared to normotensive subjects ($p < 0.01$). Calmodulin activity was also increased in hypertensive patients ($p < 0.01$) compared to normotensive subjects. These results suggest that the high $[Ca^{2+}]_i$ observed in erythrocytes from hypertensive patients is due to an increased $Ca^{2+}$ influx and an increased calmodulin activity. Further studies are needed to determine the exact mechanisms involved.
Erythrocytes from hypertensive patients, [Ca\textsuperscript{2+}], was measured under various experimental conditions. These experiments were performed with 12 hypertensive patients (eight men and four women; mean age, 50\pm3 years) and nine normotensive subjects (five men and four women; mean age, 46\pm2 years. These two age-matched subgroups were representative of the age-matched groups studied above in terms of mean blood pressure and [Ca\textsuperscript{2+}], (123\pm4 versus 91\pm2 mmHg, p<0.001 and 84\pm4 versus 71\pm2 nmol/l, p<0.01, respectively).

**Influence of Ca\textsuperscript{2+} influx.** To evaluate the participation of Ca\textsuperscript{2+} influx in the increase of [Ca\textsuperscript{2+}], observed in erythrocytes from hypertensive patients, the initial rate of 45\textsuperscript{Ca}\textsuperscript{2+} uptake was measured in intact red blood cells (Figure 3). The addition of ionomycin, a Ca\textsuperscript{2+} ionophore, induced a rapid and total release of the accumulated 45\textsuperscript{Ca}\textsuperscript{2+}, thereby confirming that we were dealing with intracellular uptake and not with membrane binding. As illustrated in Figure 4, the initial rate of 45\textsuperscript{Ca}\textsuperscript{2+} uptake did not differ between hypertensive and normotensive subjects (45\pm6 and 47\pm5 \textmu mol/l cells/hr, respectively). The influence of a rise in extracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{o}) on [Ca\textsuperscript{2+}] was also evaluated in hypertensive and normotensive subjects. When [Ca\textsuperscript{2+}]\textsubscript{o} was increased from 1 to 2 mmol/l, [Ca\textsuperscript{2+}] increased significantly by 17\pm7% (from 84\pm4 to 97\pm4 mmol/l, p=0.025) and by 15\pm5% (from 71\pm2 to 81\pm4 mmol/l, p=0.028 by paired t tests) in erythrocytes from hypertensive and normotensive subjects, respectively (Figure 5). In the presence of 2 mmol/l extracellular Ca\textsuperscript{2+}, the difference in [Ca\textsuperscript{2+}], values between hypertensive and normotensive subjects was maintained, (97\pm4 versus 81\pm4 mmol/l, respectively, p<0.01 by unpaired t test). The addition of 10\textsuperscript{-7} mol/l nicardipine, a dihydropyridine Ca\textsuperscript{2+} antagonist, decreased [Ca\textsuperscript{2+}], by 15\pm4% (from 97\pm4 to 83\pm3 mmol/l, p=0.013) in erythrocytes from hypertensive patients and by 7\pm5% (from 81\pm4 to 74\pm3 mmol/l) in those from normotensive subjects (Figure 5). The nicardipine-dependent decrease in [Ca\textsuperscript{2+}], was positively correlated to the initial [Ca\textsuperscript{2+}]\textsubscript{i} value (r=0.65, p<0.01, n=21) (Figure 6) indicating that the higher the [Ca\textsuperscript{2+}], the larger the nicardipine effect. In spite of the low number of subjects, this correlation was also significant when results from each blood pressure group were analyzed separately (r=0.62 and 0.65, respectively, p<0.05 for both groups). This suggests that the nonsignificant effect obtained in normotensive subjects was related to their low initial [Ca\textsuperscript{2+}]\textsubscript{i} values.

**Effect of the calmodulin antagonist W7 on [Ca\textsuperscript{2+}].** Since the activity of the Ca\textsuperscript{2+} pump is regulated by the Ca\textsuperscript{2+}-calmodulin complex,\textsuperscript{1} the role of calmodulin in the high [Ca\textsuperscript{2+}], observed in erythrocytes from hypertensive subjects was evaluated by measuring [Ca\textsuperscript{2+}], in the presence of W7 [N-(6-aminohexyl)-1-naphthalenesulfonamide hydrochloride], a calmodulin antagonist. W7 is reported to affect the Ca\textsuperscript{2+}, induced conformational change of calmodulin, thus preventing its interaction with the target enzyme.\textsuperscript{31}

In the presence of 5 \textmu mol/l W7, [Ca\textsuperscript{2+}], was enhanced significantly by 26\pm6% (from 84\pm4 to 101\pm5 nmol/l, p<0.01) in erythrocytes from hypertensive patients, whereas it increased slightly but not signif—

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**Figure 3.** Line graphs show time course of 45\textsuperscript{Ca}\textsuperscript{2+} uptake into intact red blood cells and the effect of the Ca\textsuperscript{2+} ionophore ionomycin. Red blood cells prepared as described in "Methods" were diluted to 20–30% hematocrit. Uptake was initiated by addition of a 1 mmol/l CaCl\textsubscript{2} solution containing 10 \muCi/ml 45\textsuperscript{Ca}, then stopped at various times by addition of 100 \mu l cell (○) suspension to 900 \mu l buffer B kept at 0°C. Efflux of accumulated 45\textsuperscript{Ca} was initiated by addition of 2 mmol/l ionomycin (●). Results are expressed as mean of six independent experiments.

**Figure 4.** Graph shows initial rate of 45\textsuperscript{Ca}\textsuperscript{2+} uptake into red blood cells from hypertensive (HT) (●) and normotensive (NT) (○) subjects. Initial rates of 45\textsuperscript{Ca}\textsuperscript{2+} influx were calculated by computarized analysis of time-course experiments.
the presence of hemoglobin has hampered until recently the investigation of \([\text{Ca}^{2+}]_i\) in erythrocytes from hypertensive patients.** The absence of correlation between \([\text{Ca}^{2+}]_i\) when calculated with blood pressure adjusted. This was also observed when only data from male subjects were considered. The absence of correlation between \([\text{Ca}^{2+}]_i\) and blood pressure or age in women may be due to the small number of subjects studied or to the influence of additional factors such as hormonal variations. The effect of age seemed to be more marked in male hypertensive patients than in normotensive subjects. Previous results have demonstrated that, in spite of the decreased erythrocyte fluidity and the increased aggregation tendency observed in hypertensive patients, there was only in these patients an inverse relation between age and aggregation tendency.**

Erythrocyte \(\text{Ca}^{2+}\) content has been reported to participate in the control of cell deformability, which is reduced by calcium load and improved by \(\text{Ca}^{2+}\) antagonists.** The erythrocyte rigidity indexes measured in this study in patients with moderate hypertension were similar to those previously determined using the same methodology.*** In the patients of the hypertensives, the presence of hemoglobin has hampered until recently the investigation of \([\text{Ca}^{2+}]_i\) in hypertensive (●) and normotensive (○) subjects. Cell preparation and \([\text{Ca}^{2+}]_i\) determinations were performed as described in "Methods." Erythrocytes were first incubated 10 minutes at 37 °C with or without \(10^{-7}\) mol/l nicardipine (Ni), then 10 minutes with 1 or 2 mM CaCl\(_2\). \(+p<0.05\) by two-tailed paired Student's t test for values measured in the presence of 2 mM extracellular \(\text{Ca}^{2+}\) compared with those obtained with 1 mmol/l extracellular \(\text{Ca}^{2+}\). \(+p<0.05\) by two-tailed paired Student's t test for values measured in the presence of nicardipine compared with those obtained in its absence. \(+p<0.01\) by two-tailed unpaired Student's t test for values from hypertensive patients compared with those from normotensive subjects. **

Discussion

Numerous abnormalities of \(\text{Ca}^{2+}\) metabolism have been reported to be associated with essential hypertension.** In particular, altered \(\text{Ca}^{2+}\) handling has been described in various blood cells.** Upon hypertensive patients compared with normotensive subjects.

Figure 5. Histogram shows influence of extracellular \(\text{Ca}^{2+}\) concentration \(([\text{Ca}^{2+}]_o\)) and effect of the \(\text{Ca}^{2+}\) antagonist nicardipine on erythrocyte free \(\text{Ca}^{2+}\) concentrations \(([\text{Ca}^{2+}]_i)\) in hypertensive (●) and normotensive (○) subjects. Cell preparation and \([\text{Ca}^{2+}]_i\) determinations were performed as described in "Methods." Erythrocytes were first incubated 10 minutes at 37 °C with or without \(10^{-7}\) mol/l nicardipine (Ni), then 10 minutes with 1 or 2 mM CaCl\(_2\). \(p<0.05\) by two-tailed paired Student's t test for values measured in the presence of 2 mM extracellular \(\text{Ca}^{2+}\) compared with those obtained with 1 mmol/l extracellular \(\text{Ca}^{2+}\). \(p<0.05\) by two-tailed paired Student's t test for values measured in the presence of nicardipine compared with those obtained in its absence. \(p<0.01\) by two-tailed unpaired Student's t test for values from hypertensive patients compared with those from normotensive subjects.

Erythrocyte \(\text{Ca}^{2+}\) content has been reported to participate in the control of cell deformability, which is reduced by calcium load and improved by \(\text{Ca}^{2+}\) antagonists.** The erythrocyte rigidity indexes measured in this study in patients with moderate hypertension were similar to those previously determined using the same methodology.*** In the patients of the...
The initial rates of Ca\(^{2+}\) influx measured by our method were in the same range as those determined in blood platelets.\(^{40-42}\) Its presence in erythrocytes argues for a major role of the plasma membrane in the altered Ca\(^{2+}\) handling observed in essential hypertension. In this cell, [Ca\(^{2+}\)]\(\text{in}\), is only regulated by two plasma membrane mechanisms, Ca\(^{2+}\) influx and extruding pump.

The initial rates of Ca\(^{2+}\) influx measured by our method were in the same range as those determined previously in quin-2–loaded erythrocytes by others,\(^{43,44}\) thus indicating a real Ca\(^{2+}\) influx without significant participation of Ca\(^{2+}\) efflux. The initial rate of \(\text{Ca}^{2+}\) uptake did not differ between erythrocytes from hypertensive and normotensive subjects, in accordance with the previous study of Ronquith and Frithz,\(^{17}\) who observed that the fast component of \(\text{Ca}^{2+}\) uptake was similar in the two groups. A rise of the extracellular Ca\(^{2+}\) concentration from 1 to 2 mmol/l, which has previously been described to nearly double the initial rate of Ca\(^{2+}\) uptake and to increase [Ca\(^{2+}\)], by 15%,\(^{45}\) did not modify the difference in [Ca\(^{2+}\)] observed between hypertensive and normotensive subjects. Since Ca\(^{2+}\) influx in erythrocytes is partially inhibited by dihydropyridine Ca\(^{2+}\) antagonists,\(^{43-46}\) we also studied the influence of nicardipine on [Ca\(^{2+}\)] and found it to be higher in hypertensive than in normotensive subjects, the high [Ca\(^{2+}\)] values being more reduced by nicardipine than the low values. This suggests that the dihydropyridine-sensitive component participates for a larger part in the total Ca\(^{2+}\) influx in essential hypertensive than in normotensive subjects.

A modified Ca\(^{2+}\)-extruding pump activity, as assessed by a decreased degree of calmodulin stimulation, has been reported in both erythrocytes and platelets from essential hypertensive patients.\(^{12,16,35}\) In this study, we observed that the presence of a calmodulin antagonist increased further the level of [Ca\(^{2+}\)], in erythrocytes from hypertensive patients. This might reflect either a higher sensitivity of the calmodulin–calcium pump complex to this antagonist or a higher stimulation of the Ca\(^{2+}\) pump by the calcium–calmodulin complex in hypertensive patients. This last hypothesis is more likely since this may reflect a higher activity of the Ca\(^{2+}\) pump in hypertensive patients than in normotensive subjects in response to the increase in [Ca\(^{2+}\)]. This may suggest that the Ca\(^{2+}\) pump cannot reestablish normal [Ca\(^{2+}\)] levels in hypertensive patients.

In conclusion, we demonstrate with the fluorescent calcium chelator fura-2 that the cytosolic Ca\(^{2+}\) concentration is increased in erythrocytes from essential hypertensive patients. This increase in [Ca\(^{2+}\)], is associated with an increased nicardipine-sensitive effect and an altered function of calmodulin in the erythrocyte Ca\(^{2+}\) handling.

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![Figure 7. Plots show effect of the calmodulin antagonist W7 on erythrocyte free Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]) in hypertensive (●) and normotensive (○) subjects. Cell preparation and [Ca\(^{2+}\)] measurements were performed as described in "Methods." Before fura-2 loading, cells were incubated 10 minutes at 37°C with (5×10\(^{-6}\) mol/l) or without W7. **p<0.01 by two-tailed paired Student's t test for values measured in the presence of W7 compared with those measured in its absence. *p<0.05, **p<0.01 by two-tailed unpaired Student's t test for values from hypertensive patients compared with those from normotensive subjects.](https://hyper.ahajournals.org/doi/fig/10.1161/01.HYP.7.6.1404?ua=1)
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