Increased calcium uptake in vascular tissue, leading to elevated cytosolic free calcium, has been implicated in the pathophysiology of hypertension. This study examined the dose-dependent effect of deuterium oxide (5%, 10%, or 20% in drinking water) on systolic blood pressure, aortic calcium uptake, and platelet cytosolic free calcium in spontaneously hypertensive rats. Starting at age 8 weeks, spontaneously hypertensive rats were divided into four groups of six animals each. The drinking water of groups 1, 2, 3, and 4 was replaced by 100% water and 5%, 10%, and 20% deuterium oxide in water, respectively, for another 7 weeks. Ten Wistar-Kyoto rats, age 8 weeks, were given 100% water for the next 7 weeks. The usual increase in systolic blood pressure and the associated increase in aortic calcium uptake and platelet cytosolic free calcium in spontaneously hypertensive rats at age 15 weeks was lowered in a dose-dependent manner by deuterium oxide. Deuterium oxide also prevented renal vascular changes in spontaneously hypertensive rats. A minimum dose of 10% deuterium oxide was needed to completely prevent the development of hypertension, elevated aortic calcium uptake, platelet cytosolic free calcium, and renal vascular changes in spontaneously hypertensive rats. (Hypertension 1991;18:550–557)

In the last decade, much attention has been given to the hypothesis that cellular calcium metabolism is abnormal in various forms of hypertension.1–6 Among the postulated defects are enhanced calcium influx across the cell membrane or reduced extrusion, or both, or sequestration of cytoplasmic calcium in association with elevated intracellular free calcium ([Ca^{2+}]_i). Because of the relative availability of platelets and similarity of calcium-dependent contractile processes shared by platelets and vascular smooth muscle cells,7 platelets have been used for analysis of the intracellular calcium.8 Although the resting levels of cytosolic [Ca^{2+}] have not been measured in vascular smooth muscle cells from hypertensive patients or hypertensive animal models, it is increased in platelets of patients with essential hypertension,9–13 spontaneously hypertensive rats (SHR), and Dahl salt-sensitive hypertensive rats11,14,15 and has a positive correlation with systolic blood pressure.9,12,13

Calcium antagonists inhibit both the calcium influx through Ca^{2+} channels and Ca^{2+} release from the Ca^{2+}-regulating sarcoplasmic reticulum and Ca^{2+} binding sites on the membrane of vascular smooth muscle.16,17 Oral administration of these calcium channel blockers decrease blood pressure in hypertensive human subjects,18–20 as well as SHR18,21,22 and Dahl salt-sensitive hypertensive rats23 but not in normotensive humans, normotensive Wistar-Kyoto (WKY) rats, and Dahl normotensive rats.

Deuterium oxide (D_2O), a stable nonradioactive isotope of water has been shown to inhibit calcium release and isometric tension in single muscle fibres of the barnacle.24 It has also been shown to reduce the L-type calcium channel conductance in isolated guinea pig myocytes.25 D_2O also depressed the contractile response of phenylephrine and KCl in a dose-dependent manner in rat aortic rings.26 We have shown recently that 25% D_2O in drinking water normalizes blood pressure in Dahl salt-sensitive hypertensive rats and when given to prehypertensive SHR prevents the development of hypertension. Twenty-five percent D_2O in drinking water also normalized elevated aortic calcium uptake in both SHR and Dahl salt-sensitive rats. However, it has no effect on blood pressure and vascular calcium uptake in normotensive rats.27,28 The objectives of the present study were to investigate the dose-dependent effect of 5%, 10%, and 20% D_2O in drinking water on

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systolic blood pressure, aortic calcium uptake, and platelet cytosolic $[\text{Ca}^{2+}]$, in SHR and to determine the minimum effective dose.

Methods

Animals, Diet, and Administration of Deuterium Oxide

Twenty-four male SHR of Okamoto-Aoki strain and 10 age-matched normotensive WKY rats (Charles River, Quebec, P.Q., Canada) were used in this study. All rats consumed standard rat chow and had free access to tap water. Starting at 8 weeks of age, SHR rats were divided into four groups of six animals each. The drinking water of groups 1, 2, 3, and 4 was replaced by 100% H$_2$O, 5% D$_2$O, 10% D$_2$O, and 20% D$_2$O in H$_2$O, respectively, for another 7 weeks. WKY rats (age 8 weeks) were given 100% H$_2$O for the next 7 weeks. Body weight and blood pressure of all rats were recorded weekly. Food and water intakes were recorded every second day. At the end of the experiment (age 15 weeks), rats were anesthetized with intraperitoneal sodium pentobarbital (10 mg/100 g body wt). After thoracic cage resection, blood was drawn into a vacutainer tube by intracardiac puncture for serum biochemistry and platelet preparation. Thoracic aorta was excised immediately for calcium-45 ($^{45}\text{Ca}^{2+}$) uptake measurements.

Laboratory Analysis

Sodium, potassium, calcium, and creatinine in serum were assayed on autoanalyzers, using ion-specific electrodes for sodium and potassium, compleximetric method for calcium, and reaction rate Jaffe Method for creatinine.

Measurements of $[\text{Ca}^{2+}]$ in Platelets

Platelet cytosolic $[\text{Ca}^{2+}]$ was measured essentially as described previously. Blood was collected into a vacutainer tube containing acid-citrate-dextrose (ACD) by cardiac puncture and platelet rich plasma was prepared by centrifugation at 120g for 20 minutes at room temperature. Platelet rich plasma was incubated for 30–45 minutes with 5 \text{M} \text{Fura-2 AM (Molecular Probes Inc., Eugene, Ore.) in a shaking water bath at 37°C. After incubation, extracellular Fura-2 AM was removed by passage through the Sepharose CL-2B column (8×200 mm) and platelets eluted with platelet buffer elution medium containing 10 \text{mM} \text{N}-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 145 \text{mM} \text{NaCl}, 4.5 \text{mM} \text{KCl}, 10 \text{mM} \text{D-glucose}, 5 \text{mM} \text{potassium chloride}, 1 \text{mM} \text{magnesium chloride}, 0.5 \text{mM} \text{magnesium chloride}, 1 \text{mM} \text{calcium chloride}, 6 \text{mM} \text{glucose}, and 0.1% \text{bovine serum albumin at pH 7.4 and 37°C. Platelets were incubated with the same buffer to a concentration of approximately 10^6 to 10^7 cells/ml (Coulter Counter, model STKS, Burlington, Canada) and incubated at 37°C for at least 30 minutes. The fluorescence excitation spectrum was scanned from 300 to 420 nm with emission wavelength fixed at 505 nm, using a Perkin-Elmer 512 double beam fluorescence spectrophotometer (Perkin-Elmer Corp., Analytical Instruments, Norwalk, Conn.). All measurements were done in duplicate, and the mean value was used for statistical calculation.}

The standard procedure for calculating $[\text{Ca}^{2+}]$ from dual wavelength measurements of Fura-2 was used that involves the following equation:

$$[\text{Ca}^{2+}] = K_d \frac{R - R_{\text{min}}}{(R_{\text{max}} - R)} \beta$$

where $R$ is the ratio of fluorescence of the sample at 340 and 380 nm; $R_{\text{max}}$ and $R_{\text{min}}$ the ratios for Fura-2 free acid at the same wavelengths in the presence of saturating Ca$^{2+}$, and in the normally zero Ca$^{2+}$, respectively; $\beta$ is the ratio of fluorescence of Fura-2 at 380 nm in zero and saturating Ca$^{2+}$; and $K_d$ is the dissociation constant of Fura-2 for Ca$^{2+}$, assumed to be 224 nM at 37°C. $R_{\text{max}}$ was determined by rupturing the cells with Triton X-100 (0.5%) and adding a saturating amount of calcium. $R_{\text{min}}$ was determined by adding Triton X-100 (0.5%) and ethylene-bis (oxethylendinitrieto) tetracetic acid (10 mmol/l) and increasing the pH to 8.3. Correction for autofluorescence was made by subtracting the fluorescence of unloaded cells from an equal density of cells loaded with Fura-2 to obtain a fluorescent signal that was solely representative of intracellular Fura-2. The results of platelet $[\text{Ca}^{2+}]$ are given in nanomoles per liter.

Measurements of Calcium Uptake by Thoracic Aortas

Calcium uptake by thoracic aortic tissues was measured as described previously. Briefly, rats were anesthetized and killed, and thoracic aortas immediately were excised and dissected free of connective tissue in a constantly oxygenated HEPES buffer (pH 7.4) solution containing: 150 mM NaCl, 4.5 mM KCl, 10 mM D-glucose, 5 mM HEPES, 1.5 mM CaCl$_2$, and 1 mM MgCl$_2$. Aortas were then cut into 2–3-mm-long segments. After an equilibration period of 2 hours at 37°C subsequent to tissue excision, aortic rings (in tissue holders) were incubated in 5 ml constantly oxygenated buffer containing $^{45}\text{Ca}^{2+}$ (5 \text{Ci/ml}) for 20 minutes at 37°C in a constantly shaking water bath. Subsequently, tissues were washed in cold (2°C) buffer for 2 minutes, followed by a second cold (2°C) buffer wash for 45 minutes to remove free $^{45}\text{Ca}^{2+}$. Under these conditions, efflux of intracellular calcium is prevented and only the intracellular calcium is effectively washed away. The tissues were then blotted; wet weights were measured and transferred to counting vials containing 100 \text{ul H}_{2}\text{O and 1 ml Protosol (New England Nuclear, Boston, Mass.) and placed in a water bath for 2 hours at 60–70°C. After digestion, 100 \text{ul glacial acetic acid was added to each vial followed by 10 ml liquid scintillation fluid (BDH Chemicals, Toronto, Canada), and the solution was counted in a Beckman liquid scintillation counter (Beckman Instruments, Fullerton, Cal-}
The uptake of Ca$^{2+}$ was expressed as micro-
moles Ca$^{2+}$ per kilogram wet weight of tissue per 20
minutes. This net uptake represents total Ca$^{2+}$
influx in the aortas.

**Systolic Blood Pressure Measurements**

Systolic blood pressure was recorded weekly us-
ing a tail-cuff method (model 5A Amplifier, ITTC
Life Science Instruments, Woodland Hills, Calif.).
Each pressure value was obtained by averaging four
individual readings.

**Tissue Histopathological Study**

Because 10% D$_2$O treatment in drinking water of
SHR prevented the development of hypertension, only
animals in this group and SHR and WKY rats given
100% H$_2$O were examined for tissue morphology. The
animals were killed and autopsy was performed. The
thoracic cavity was opened, and after splitting the
pericardium, the heart was examined in situ. Then the
heart was removed by dismembering it from great
vessels at the base, and the weight was recorded. The
right and left ventricles were split open, and the inter-
nal surface was examined. The abdominal cavity was
explored for any gross pathological change, and the
liver and kidney were removed and the weight re-
corded. Tissues from liver, heart, kidney, and adrenal
gland were fixed in 10% buffered formalin. Microscopic
slides of 5-μm-thick sections were prepared and stained with hematoxylin and eosin. The histopatholog-
ical analysis was done blindly.

**Statistical Analysis**

All data are expressed as mean±SD. Statistical anal-
yses of results were performed using Student’s t
test (unpaired) and Pearson correlation coefficient.

**Results**

**Effect of Oral Intake of Deuterium Oxide on
Serum Biochemistry, Body Weight, and Food
and Water Intake**

The initial mean body weight of SHR and WKY rats
was not significantly different (age 8 weeks) (Table 1).
Final body weight, serum sodium, potassium, calcium,
and creatinine in SHR after 7 weeks of D$_2$O treatment
(age 15 weeks) were not significantly different from
those of control (SHR and WKY rats given H$_2$O, age 15
weeks). Mean±SD values for food intake at the sev-
enth week of the experimental period were 37±4,
38±3, 36±4, 37±3, and 39±5 g/day/rat in SHR given
H$_2$O, 5% D$_2$O, 10% D$_2$O, 20% D$_2$O, and in WKY rats
given H$_2$O, respectively. Mean±SD values for water
intake at the seventh week of the experimental period
were 48±7, 50±4, 47±4, 50±6, and 52±4 ml/day/rat in
SHR given H$_2$O, 5% D$_2$O, 10% D$_2$O, 20% D$_2$O, and in
WKY rats given H$_2$O, respectively. There was no
significant difference in either food or water intake
among the groups.

**Effect of Oral Intake of Deuterium Oxide on Systolic
Blood Pressure of Spontaneously Hypertensive Rats**

SHR drinking H$_2$O displayed a continuous in-
crease in systolic blood pressure from 8 weeks on-
ward to 15 weeks of age when the study was com-
pleted (Table 2, Figure 1). Systolic blood pressure of
WKY normotensive rats was (mean±SD) 123±4 and
128±6 at week 0 (age 8 weeks) and week 7 (age 15
weeks), respectively. Eight-week-old prehypertensive
SHR placed on drinking water containing 10% or

### Table 1. Effect of Oral Deuterium Oxide Treatment on Body Weight and Biochemical Parameters of Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>100% H$_2$O</th>
<th>5% D$_2$O</th>
<th>10% D$_2$O</th>
<th>20% D$_2$O</th>
<th>100% H$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>339±15</td>
<td>343±18</td>
<td>337±21</td>
<td>342±16</td>
<td>355±20</td>
</tr>
<tr>
<td>Serum sodium (mmol/l)</td>
<td>146±0.94</td>
<td>147±1</td>
<td>145±1.4</td>
<td>145±2</td>
<td>146±1</td>
</tr>
<tr>
<td>Serum potassium (mmol/l)</td>
<td>5.3±0.29</td>
<td>5.5±0.45</td>
<td>5.4±0.54</td>
<td>5.6±0.52</td>
<td>5.3±0.32</td>
</tr>
<tr>
<td>Serum calcium (mmol/l)</td>
<td>2.67±0.07</td>
<td>2.68±0.06</td>
<td>2.70±0.07</td>
<td>2.71±0.06</td>
<td>2.64±0.05</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>37±3</td>
<td>38±4</td>
<td>38±2</td>
<td>37±3</td>
<td>41±5</td>
</tr>
</tbody>
</table>

Values are mean±SD at 15 weeks of age. Starting at 8 weeks of age, spontaneously hypertensive rats (SHR) were
given either 100% H$_2$O or deuterium oxide (D$_2$O) (5%, 10%, or 20%) as their drinking water for a period of 7 weeks
(n= six animals in each group). Ten Wistar-Kyoto (WKY) rats were given 100% H$_2$O.
20% D$_2$O showed no significant increase in systolic blood pressure over the 7-week period except during the first week. Blood pressure of these animals at 1–7 weeks of the experiment was not significantly different from WKY normotensive rats given H$_2$O. SHR given drinking water containing 5% D$_2$O showed significantly less ($p<0.01$) increase in blood pressure from week 1 to week 4 compared with SHR given water and showed no further increase in blood pressure after 4 weeks. Although blood pressure after 4 weeks was significantly lower than SHR given water, it was still significantly higher ($p<0.01$) as compared with normotensive WKY rats on H$_2$O. D$_2$O affects the increase in systolic blood pressure in SHR in a concentration-dependent manner. A minimum concentration of 10% D$_2$O in drinking water of SHR is required to prevent hypertension.

**Effect of Oral Intake of Deuterium Oxide on Platelet Cytosolic [Ca$^{2+}$]$i$ in Spontaneously Hypertensive Rats**

Cytosolic [Ca$^{2+}$]$i$ in platelets of untreated hypertensive rats at 15 weeks of age was significantly higher than that in normotensive WKY control rats; mean±SD values 155±14 and 68±12 nmol/l, respectively ($p<0.001$, Table 3). SHR given 5% D$_2$O for a period of 7 weeks had significantly lower platelet cytosolic [Ca$^{2+}$]$i$; mean±SD 93±15 nmol/l than in untreated SHR ($p<0.01$) but still higher than normotensive WKY control rats. SHR treated with 10% or 20% D$_2$O for a period of 7 weeks had normal platelet [Ca$^{2+}$]$i$; mean±SD 74±7 and 71±10 nmol/l, respectively, as compared with normotensive WKY rats drinking H$_2$O. There was a significant correlation $r=0.921$, $p<0.001$ between platelet [Ca$^{2+}$]$i$ and systolic blood pressure (Figure 2) for all groups together.

**Effect of Oral Intake of Deuterium Oxide on Calcium Uptake by Aortas of Spontaneously Hypertensive Rats**

Mean±SD values of calcium uptake by aortas of SHR given H$_2$O at age 15 weeks were significantly higher than those found in normotensive WKY rats given H$_2$O (Table 4). Aortas of SHR given 5% D$_2$O had significantly lower mean±SD values of calcium uptake than those found in SHR given H$_2$O but still significantly higher than those found in normotensive WKY rats given H$_2$O. SHR treated with 10% or 20% D$_2$O for a period of 7 weeks had normal values of aortic calcium uptake as compared with age-matched, normotensive WKY rats drinking H$_2$O (15 weeks).

**Effect of Oral 10% Deuterium Oxide on Organ Weight and Tissue Morphology**

Organ weights for SHR given H$_2$O, SHR given 10% D$_2$O, and WKY rats given H$_2$O were for liver (mean±SD) 13.2±0.77, 13.3±0.98, and 12.2±9.6; for heart 1.23±0.068, 1.28±0.080, and 1.10±0.052; for kidney 2.75±0.317, 2.50±0.137, and 2.72±0.358 g, respectively. There was no significant difference in liver, heart, and kidney weight and organ weight/body weight ratios in SHR given 10% D$_2$O compared with SHR on H$_2$O. Mean values of liver and heart weight and liver weight/body weight and heart weight/body weight ratios were significantly higher ($p<0.05$) in SHR given H$_2$O and SHR given 10% D$_2$O compared with WKY rats given H$_2$O. Mean values of kidney weight and kidney weight/body weight ratios were significantly different ($p<0.01$) from SHR given H$_2$O starting from week 1.

**Table 3. Effect of Oral Deuterium Oxide Treatment on Platelet Cytosolic Free Calcium of Spontaneously Hypertensive Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Platelet cytosolic free calcium (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR given H$_2$O</td>
<td>155±14*</td>
</tr>
<tr>
<td>SHR given 5% D$_2$O</td>
<td>93±15†</td>
</tr>
<tr>
<td>SHR given 10% D$_2$O</td>
<td>74±7</td>
</tr>
<tr>
<td>SHR given 20% D$_2$O</td>
<td>71±10</td>
</tr>
<tr>
<td>WKY given H$_2$O</td>
<td>68±12</td>
</tr>
</tbody>
</table>

Values for platelet cytosolic free calcium are mean±SD. $n=6$ animals in each group. Starting at 8 weeks of age, spontaneously hypertensive rats (SHR) were given either H$_2$O (100%) or D$_2$O (5%, 10%, or 20% in H$_2$O) as their drinking water for a period of 7 weeks. Wistar-Kyoto (WKY) rats were given 100% H$_2$O. Platelet cytosolic free calcium was measured in duplicate from each animal at 15 weeks of age.

*Values are significantly different ($p<0.01$) from other groups.
†Values are significantly different ($p<0.02$) from other groups.
weight and kidney weight/body weight ratio were not significantly different among various groups.

Histological changes were found in vascular tissue of kidneys of SHR given H2O. These changes were mild smooth muscle cell hyperplasia with some narrowing of the lumen in small arteries and arterioles (Figure 3A). In rats treated with 10% D2O, the arteriolar smooth muscle cell hyperplasia was minimal and arteriolar lumina were normal (Figure 3B). There was no apparent difference in tissue morphology of liver and heart in SHR given H2O and 10% D2O as compared with WKY normotensive rats given H2O.

Discussion

Abnormal contractile activity of vascular smooth muscle is considered to be one cause of hypertension. The contractile activity of vascular smooth muscle is regulated by the level of intracellular free calcium ions. Early work has shown that D2O depresses contraction of frog and barnacle muscle by uncoupling excitation-contraction without affecting the membrane potential. Recent work has shown that D2O reduces vascular smooth muscle cell contraction induced through agonist and depolarization activation of calcium channels in rats in a dose-dependent manner.

The results of the present study confirm previous reports of altered calcium metabolism in spontaneously hypertensive rats. Intracellular [Ca2+]i levels in platelets were found to be higher in hypertensive SHR than in normotensive WKY rats and were found to correlate directly with blood pressure. This association was supported by the observed direct relation between the concurrent change in intracellular [Ca2+]i and the antihypertensive response to treatment with D2O. This elevation in platelet [Ca2+]i, and its normalization with D2O in SHR may reflect changes in other tissues, including vascular smooth muscle, where increased vascular smooth muscle reactivity with elevated cytosolic [Ca2+] increases vascular resistance and elevated blood pressure. It has been suggested that cytosolic free calcium concentration in platelets reflects tone and structural changes of resistance vessels.

One of the possible mechanisms for increase in intracellular [Ca2+]i, in the vascular smooth muscle cells of patients with essential hypertension and hypertensive rats is suggested to be due to enhanced calcium influx. In the present study, D2O treatment lowered the elevated aortic calcium uptake in SHR. D2O treatment lowered both platelet intracellular [Ca2+]i and aortic calcium uptake in a concentration-dependent manner. Although 5% D2O in drinking water of SHR lowered blood pressure and elevated platelet intracellular [Ca2+]i, and aortic calcium uptake significantly, 10% D2O was needed to normalize these changes. We have shown earlier that 25% D2O in drinking water prevented hypertension in SHR and normalized elevated blood pressure in Dahl salt-sensitive hypertensive rats. In both SHR and Dahl salt-sensitive rats, elevated aortic calcium uptake was also normalized. However, D2O had no effect on systolic blood pressure and aortic calcium uptake in normotensive rats. Our previous experiments have demonstrated that D2O normalized the

![Graph showing relation between systolic blood pressure and cytosolic free calcium concentration in platelets of spontaneously hypertensive rats (SHR) given H2O (100%) or deuterium oxide (D2O) (5%, 10%, or 20% in H2O) as their drinking water for a period of 7 weeks starting from age 8 weeks (n=6 animals in each group). Rats were killed at 15 weeks of age. WKY, Wistar-Kyoto rats.](http://hyper.ahajournals.org/)

**TABLE 4. Effect of Oral Deuterium Oxide Treatment on Calcium Uptake by Aortas of Spontaneously Hypertensive Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>aCa2+ uptake (μmol/kg tissue/20 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR given H2O</td>
<td>747 ± 97*</td>
</tr>
<tr>
<td>SHR given 5% D2O</td>
<td>591 ± 41†</td>
</tr>
<tr>
<td>SHR given 10% D2O</td>
<td>438 ± 36</td>
</tr>
<tr>
<td>SHR given 20% D2O</td>
<td>453 ± 29</td>
</tr>
<tr>
<td>WKY given H2O</td>
<td>425 ± 41</td>
</tr>
</tbody>
</table>

Values for aCa2+ uptake are mean±SD. n=30 aortic rings from six rats in each group except Wistar-Kyoto (WKY) rats where n=50 aortic rings from 10 rats. Starting at 8 weeks of age, spontaneously hypertensive rats (SHR) were given either H2O (100%) or deuterium oxide (D2O) (5%, 10%, or 20% in H2O) as their drinking water for a period of 7 weeks. WKY rats were given 100% H2O. All calcium uptake measurements were done in normal HEPES (N-2-hydroxyethylpiperazine-N-2'-ethanesulfonic acid) buffer (without D2O). Rats were killed at 15 weeks of age. *Values are significantly different (p<0.01) from other groups. †Values are significantly different (p<0.01) from other groups.
KCl-induced high Ca\(^{2+}\) uptake in Sprague-Dawley rat aortas via voltage-operated Ca\(^{2+}\) channels. Similarly, the phenylephrine-induced high Ca\(^{2+}\) uptake via receptor-operated calcium channels was also reduced to the level of that in tissues of untreated controls. These in vitro effects of D\(_2\)O were concentration dependent.\(^{27,28}\) In experiments using guinea pig ventricular myocytes, others have reported that isotopic substitution of deuterium for hydrogen may affect cell surface Ca\(^{2+}\) channels.\(^{25}\) The antihypertensive effects of D\(_2\)O may be the result of increased blockage of calcium channels by bound deuterium ions.

Another possible mechanism of increase in cytosolic free calcium in hypertension has been suggested to be increased release of calcium intracellularly from sarcoplasmic reticulum.\(^{6}\) D\(_2\)O treatment may normalize elevated intracellular cytosolic free calcium by this mechanism. This concept is supported by findings that D\(_2\)O inhibits calcium release from the sarcoplasmic reticulum of barnacle muscle,\(^{24}\) presumably by altering the sarcoplasmic binding characteristics for calcium.\(^{37}\) It has been suggested that D\(_2\)O depresses mobilization of calcium ions by 1) lowering rate of release of calcium ions, 2) decreasing amount of calcium release, and 3) reducing diffusion of calcium ion.\(^{32,33}\)

We suggest above that during hypertension, the elevation in platelet cytosolic free calcium and its normalization with D\(_2\)O in SHR may reflect changes in vascular smooth muscle; however, the platelets themselves may also be implicated in the pathophysiology of hypertension. In hypertensive human subjects and hypertensive rats, platelet activation and increased formation of thromboxane A\(_2\), a potent...
platelet aggregant and vasoconstrictor, has been observed. Increased intracellular free calcium in platelets may influence several calcium-dependent processes leading to altered functional properties of the cells. Enhanced platelet aggregation can lead to vasoconstriction in vessels with damaged or functionally altered endothelium, and the phenomenon could play a role in the increased total peripheral resistance in essential hypertension. Furthermore, increased aggregation and secretion of vasoactive hormones and growth factors by platelets could be important factors in the development of atherosclerosis in hypertensive patients. D2O has been shown to enhance the polymerization of protein units of the microtubules-microfibres of the contractile system in human platelets, thus delaying one of the initial steps in platelet aggregation. In vitro D2O treatment inhibited shape change and aggregation in response to ADP in human platelets. D2O treatment may prevent hypertension by normalizing platelet cytosolic free calcium and increased platelet activation.

The present study shows no significant effect of D2O consumption on body weight; food and water intakes, and serum Na+, K+, Ca2+, and creatinine levels compared with age-matched SHR and WKY rat controls confirming the results of our earlier study using 25% D2O. In this study, 10% D2O treatment to SHR prevented the smooth muscle cell hyperplasia and narrowing of lumen in small arteries and arterioles in kidney. However, liver and heart weights, which were elevated in SHR given H2O, remained elevated in SHR given D2O. D2O treatment in SHR did not cause any adverse changes in liver, heart, and kidney morphology. These results suggest that 10% D2O treatment in drinking water of SHR for 7 weeks is not detrimental to their health and prevents renal vascular changes in these animals.

Whatever the fundamental mechanism of hypertension may be, increased cytosolic [Ca2+]i appears to be one of the mediating events. In view of the similarities between platelets and vascular smooth muscle cells, the observed relation between intracellular calcium in platelets, calcium uptake in aortas, and blood pressure provides further evidence for the important role of intracellular calcium in hypertension. It remains possible, however, that an increased intracellular calcium concentration is a consequence rather than a cause of elevated blood pressure.

In conclusion, we have shown that the in vivo antihypertensive effect of D2O in drinking water is dose dependent with a minimum dose of 10% needed to prevent the development of hypertension, elevated aortic calcium uptake, platelet cytosolic free calcium, and renal vascular changes in SHR.

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