Sodium Induces Hypertrophy of Cultured Myocardial Myoblasts and Vascular Smooth Muscle Cells

Abstract—The mechanisms of sodium-induced myocardial hypertrophy and vascular hypertrophy are poorly understood. We tested the hypothesis that a high sodium concentration can directly induce cellular hypertrophy. Neonatal rat myocardial myoblasts (MMbs) and vascular smooth muscle cells (VSMCs) were cultured in a 50:50 mixture of DMEM and M199 supplemented with 10% fetal bovine serum. When the monolayers reached ~80% confluence, normal sodium medium (146 mmol/L) was replaced with high sodium media (152 mmol/L, 160 mmol/L, and 182 mmol/L) for up to 5 days. Increasing sodium from a baseline concentration of 146 mmol/L to the higher concentrations for 5 days caused dose-related increases in cell mean diameter, cell volume, and cellular protein content in both MMbs and VSMCs. Increasing the sodium concentration by only 4% (from 146 mmol/L to 152 mmol/L) caused the following respective changes in MMbs and VSMCs: 8.5% and 8.7% increase in cell mean diameter, 27.6% and 27.0% increase in cell volume, and 55.7% and 46.7% increase in cellular protein content. The rate of protein synthesis, expressed as [3 H]leucine incorporation, increased by 87% and 99% in MMbs after exposure to 152 mmol/L and 160 mmol/L sodium, respectively, compared with the 146-mmol/L sodium control group. Exposure of MMbs to medium with a sodium concentration of 10% above normal, ie, 160 mmol/L, caused a significant decrease (range, 26% to 44%) in the rate of protein degradation at multiple time points over a 48-hour period compared with normal sodium control cells. The increase in cellular protein content caused by 160 mmol/L sodium returned to normal within 3 days after MMbs were returned to a normal sodium medium. These findings support the hypothesis that sodium has a direct effect to induce cellular hypertrophy and may therefore be an important determinant in causing myocardial and/or vascular hypertrophy in subjects with increased sodium concentration in the extracellular fluid. (Hypertension. 1998;31:1083-1087.)

Key Words: sodium ■ hypertrophy ■ myocardial myoblasts ■ muscle, smooth, vascular

Sodium homeostasis profoundly influences the cardiovascular system in normotensive and hypertensive subjects and is a major risk factor for cardiovascular morbidity and mortality independent of other cardiovascular risk factors (for review, see Reference 1). Mounting evidence from animal, epidemiological, and clinical studies suggests that a high dietary salt intake is associated with myocardial hypertrophy.2–6 Although the mechanism of salt-induced myocardial hypertrophy is poorly understood, dietary salt intake is thought to modify the process of myocardial hypertrophy by hemodynamic and/or nonhemodynamic mechanisms.7

The development of cardiac hypertrophy in response to pressure and/or volume overload is generally considered to be an adaptive mechanism to normalize ventricular wall stress. High blood pressure is one of the most powerful determinants of LVH,8 and several studies5,9–11 have shown that a high dietary salt intake is associated with increased blood pressure. Therefore, many investigators consider an increased pressure load on the myocardium to be a major cause of LVH in subjects with salt-induced hypertension.

More recent evidence points to a close relationship between the development and persistence of LVH and sodium intake, which may be independent of blood pressure.2,4,6,12–14 Frohlich and associates12 reported that a high sodium diet not only caused further cardiac enlargement in spontaneously hypertensive rats but also increased cardiac mass in normotensive Wistar-Kyoto rats in the absence of increased blood pressure. Studies in humans have shown that the intracellular sodium concentration of red blood cells is positively correlated with the degree of LVH.15 Together these results suggest that sodium-induced cardiac hypertrophy may be partially mediated by a direct action of sodium on the myocardium, independent of hemodynamic factors.

The present study sought to determine whether sodium can directly induce hypertrophy of individual cells that are not exposed to many of the in vivo factors, such as high blood pressure or increased cardiac output, commonly associated with a high salt diet. The results indicate that increasing the concentration of sodium in cell culture medium can induce hypertrophy of neonatal rat MMbs and VSMCs. The hypertrophy...
seems to result from an increase in the rate of cellular protein synthesis and a decrease in the rate of cellular protein degradation and can be totally reversed within a few days by returning the sodium concentration of the medium to normal levels.

**Methods**

**Cell Culture**

Rat MMbs and rat VSMCs were used (American Type Culture Collection, Rockville, Md). MMbs were derived from embryonic BDIX rat tissue, and VSMCs were derived from the thoracic aorta of DBIX embryonic rats. Both cell lines were grown in 50% DMEM (GIBCO) plus 50% M199 (GIBCO) supplemented with 10% FBS (HyClone), 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B. This medium is referred to as “standard medium.” The cells were seeded into sterile culture flasks at ~5×10^5 cells/cm² and incubated at 37°C in a humidified 5% CO₂/95% O₂ atmosphere. Eighteen hours after subculture, the medium was replaced with fresh medium. The medium was changed every 72 hours. Confluent monolayers were established between 4 and 6 days after seeding. Both MMbs and VSMCs were used between passages 16 and 24 in these experiments.

**Exposure to High Sodium and Reinstitution of Normal Sodium**

Both MMbs and VSMCs were plated in T-25 flasks or 24-well plates at ~5×10^5 cells/cm² using standard medium. When the monolayers reached ~80% confluence, the standard medium, which had a mean±SD sodium concentration of 146±2.0 mmol/L, was replaced with media having the following concentrations of sodium: 152±2.3 mmol/L (4% above normal), 160±2.7 mmol/L (10% above normal), and 182±3.1 mmol/L (25% above normal). The high sodium medium was made by simply adding sodium chloride (Sigma Chemical Co) to the standard medium. The medium pH ranged from 7.3 to 7.4 and was similar between normal sodium medium and high sodium medium. After 5 days of exposure to high sodium medium, the cells still excluded trypan blue dye (>95%), and the levels of lactate dehydrogenase (LD-L 20 assay kit, Sigma) were not increased in the medium. Other experiments were performed in which cells were exposed to high sodium medium (160 mmol/L) for 48 hours and then incubated in normal sodium medium (146 mmol/L) for an additional 3 days.

**Cell Number, Cell Diameter, and Cell Volume**

MMbs or VSMCs cultured in normal or high sodium media were harvested using 0.25% trypsin–0.2% EDTA (Sigma) to obtain a single cell suspension. Cell number was determined using a hemocytometer. Mean cell diameter was determined on 50 randomly chosen cells in each sample. The cells were magnified using a 40× Leitz objective (NA 0.70) and focused on a high-resolution video monitor (PVM-1343MD, Sony). Mean cell diameter was determined using image analysis software (Optimas Co). Because the suspended cells were virtually spherical, it was possible to calculate cell volume from mean cell diameter.

**Cellular Protein Content**

Cell suspensions obtained by detachment from T-25 flasks were centrifuged for 5 minutes at 500g. The lysates were prepared using a modification of a method described previously. The cell pellets were washed three times in the following solution: PBS, 2 mmol/L PMSF, 1 mmol/L AEBSF, 2 mmol/L benzamidine, 10 μg/mL aprotinin, and 10 μg/mL leupeptin (all from Sigma), pH 7.4. The cells were then resuspended in a solution containing Tris-HCL (pH 7.4), 0.5% NP-40, 100 mmol/L NaCl, and 5 mmol/L MgCl₂, with protease inhibitors (at the same concentration as the PBS wash). Cells were ruptured by pushing the cell suspension through a 30-gauge needle. The homogenate was then clarified at 14 000 rpm for 5 minutes. Cell protein content was determined in duplicate using BSA as the standard (Bio-Rad Protein Assay Kit, Bio-Rad Laboratories).

**DNA Synthesis**

The uptake of [³H]thymidine by MMbs and VSMCs was used as an indicator of DNA synthesis, as described previously. Briefly, the cells were seeded into 24-well plates at 10^5 cells/cm² in standard medium. The following day, the medium was removed and replaced with fresh standard medium for 24 hours. The cells were then cultured in 4% FBS DMEM-M199 medium containing various concentrations of sodium for 72 hours: 146 mmol/L (control), 152 mmol/L, 160 mmol/L, or 182 mmol/L. During the last 6 hours of incubation, the cells were pulsed with [³H]thymidine (Amersham) by adding 1 μCi per well. The cells were then washed, harvested, and processed for counting in a scintillation counter.

**Protein Synthesis**

The rate of protein synthesis was estimated by incorporation of [³H]leucine (Amersham) into the cells, as described previously. Briefly, the cells were seeded into 24-well plates at 10^5 cells/cm² in standard medium. The following day, the medium was removed and replaced with fresh standard medium for 24 hours. The cells then cultured in 4% FBS DMEM-M199 medium containing various concentrations of sodium (146, 152, 160, and 182 mmol/L) for another 72 hours. [³H]Leucine (1 μCi/well) was added to the cells during the last 6 hours of incubation. At the end of the 6-hour labeling period, the cells were rapidly rinsed three times with ice-cold PBS. Radioactivity in the cells was determined using a scintillation counter.

**Protein Degradation**

The ratio of the radioactivity released from the cells to the radioactivity remaining in the cells.

**Statistical Analyses**

All determinations were performed in six samples for each group, and each experiment was repeated at least two times. Where indicated, data are presented as mean±SD or SE. Differences were considered to be statistically significant if a value of P<0.05 by way of a paired r test. All statistical calculations were performed using StatView software (BrainPower).

**Results**

**Effects of Sodium on Cell Size and Protein Content**

Cell size and cellular protein content were determined after MMbs and VSMCs were exposed to media having various
Effects of Sodium on Cell Protein Turnover

To elucidate the underlying mechanism of sodium-induced hypertrophy in vitro, the rate of cellular protein synthesis and degradation was investigated in MMbs cultured in medium having a normal sodium concentration (146 mmol/L) and in medium having a high sodium concentration. Fig 2 shows that the rate of protein synthesis, estimated as [3H]leucine incorporation into the cells, increased by 87% in MMbs and 80% in VSMCs when the cells were exposed to medium having a sodium concentration of 182 mmol/L (25% above control). Fig 3 shows that exposing MMbs to medium having a sodium concentration of 10% above normal, ie, 160 mmol/L, caused a significant decrease in the rate of protein degradation at multiple time points over a 48-hour period compared with normal sodium control cells.

Reversibility of Hypertrophy by Reinstition of Normal Sodium

Experiments were conducted to determine whether sodium-induced hypertrophy of cells can be reversed by returning the hypertrophied cells to normal sodium medium. Fig 5 shows that 2 days of exposure to medium having a sodium concentration of 160 mmol/L caused the cellular protein content to increase by 27%. A slight decrease in cellular protein content occurred 1 day after the reinstition of normal sodium medium; protein content.

Table

<table>
<thead>
<tr>
<th>Sodium Group</th>
<th>Cell Diameter, μm</th>
<th>Cell Volume, μm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMbs</td>
<td></td>
<td></td>
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<tr>
<td>146 mmol/L</td>
<td>10.0±0.2</td>
<td>527.3±63.2</td>
</tr>
<tr>
<td>152 mmol/L</td>
<td>10.8±0.2*</td>
<td>673.1±47.9*</td>
</tr>
<tr>
<td>160 mmol/L</td>
<td>11.1±0.2*</td>
<td>704.3±70.7*</td>
</tr>
<tr>
<td>182 mmol/L</td>
<td>11.7±0.1*</td>
<td>841.4±68.2*</td>
</tr>
<tr>
<td>VSMCs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>146 mmol/L</td>
<td>9.8±0.5</td>
<td>494.2±82.4</td>
</tr>
<tr>
<td>152 mmol/L</td>
<td>10.6±0.5*</td>
<td>627.7±99.2*</td>
</tr>
<tr>
<td>160 mmol/L</td>
<td>11.8±0.3*</td>
<td>843.6±79.8*</td>
</tr>
<tr>
<td>182 mmol/L</td>
<td>12.0±0.5*</td>
<td>868.3±93.5*</td>
</tr>
</tbody>
</table>

Data are given as mean±SD of 100 randomly selected cells from two independent experiments. Control group is 146 mmol/L sodium for both MMbs and VSMCs. *P<.05.

Concentrations of sodium for 5 days: 146 mmol/L (control), 152 mmol/L (4% above control), 160 mmol/L (10% above control), and 182 mmol/L (25% above control). As shown in the Table and Fig 1, increasing the concentration of sodium in the medium caused a dose-related increase in cell mean diameter, cell volume, and cellular protein content in both MMbs and VSMCs. Increasing the sodium concentration in the medium by as little as 4% above normal, ie, from 146 to 152 mmol/L, caused the cellular protein content to increase by 55.7% in MMbs and 46.7% in VSMCs, as shown in Fig 1. This lowest level of sodium also increased cell mean diameter by 8.5% in MMbs and 8.7% in VSMCs and increased cell volume by 27.6% in MMbs and 27.0% in VSMCs, as shown in the Table.

Figure 1. Effects of sodium on cellular protein content. After MMbs and VSMCs had been exposed to the various concentrations of sodium for 5 days, the cellular protein content was determined using Bio-Rad protein assay. The control sodium concentration in the medium was 146 mmol/L. Data are expressed as mean±SD from two independent series of experiments (n=6). *P<.05.

Figure 2. Effect of sodium on protein synthesis in MMbs. The rate of protein synthesis was determined over a 6-hour period after the cells had been exposed to the various concentrations of sodium for 18 hours. The control sodium concentration in the medium was 146 mmol/L. Data are expressed as mean±SD from two independent series of experiments (n=6). *P<.05.

Figure 3. Effects of sodium on protein degradation in MMbs. Relative protein degradation rate is expressed as the ratio of released [14C]phenylalanine over incorporated [14C]phenylalanine. Data are expressed as mean±SD from two independent series of experiments (n=6). *P<.05.
Sodium has a direct action to induce cellular hypertrophy in both MMbs and VSMCs. Cellular hypertrophy is estimated as cellular protein content. The hypertrophy appears to result from an increase in the rate of protein synthesis and a decrease in the rate of protein degradation. These findings are consistent with clinical studies showing that increasing the sodium concentration used in the present study to induce cellular hypertrophy can be achieved in the intact animal under multiple physiological and pathophysiological conditions. Further studies are needed to investigate the relationship between cardiovascular hypertrophy and intracellular/extracellular sodium concentrations in subjects with high dietary salt intake, especially long-term high dietary salt intake.

In the present study, the high sodium medium was made by simply adding sodium chloride to normal medium. It is unlikely, however, that increased chloride concentration played an important role in the induction of cellular hypertrophy. In a previous study, Pasquie and associates concluded that the sodium ion, regardless of whether it is given as sodium chloride or sodium citrate, is
associated with cardiac hypertrophy. A more difficult question is whether increases in osmolarity in the media caused by the addition of sodium may have played a role in the induction of cellular hypertrophy. Although we have not addressed this question systematically, our preliminary data suggest that incubating neonatal rat MMbs in medium containing 20 mMol/L mannitol had no effect on cellular protein content (0.24±0.04 ng/cell), compared with control cells incubated in mannitol-free medium in which the cellular protein content was 0.25±0.05 ng/cell. On the basis of this initial study, it is unlikely that increased osmolarity plays a significant role in sodium-induced cellular hypertrophy; however, further studies are needed to confirm or refute this initial assessment.

Cellular hypertrophy is characterized by an enlargement of cell size, as well as an increase in cellular protein content. Our data indicate that sodium-induced cellular hypertrophy was not accompanied by cellular hyperplasia and that the hypertrophy was caused by an increase in the rate of cellular protein synthesis and a decrease in the rate of cellular protein degradation. Other studies have shown a positive correlation between LVH, intracellular sodium concentration, and the activity of the Na-\(H\) exchanger.28 Book and associates29 reported that the expression of Na-\(K\)-ATPase isofrom was altered in the hypertrophied left ventricles of rats. Interestingly, in a rat model, long-term salt loading induced an increase in the activity of Na-\(K\)-ATPase in kidney, heart, liver, muscle, and aorta.30 We can speculate that sodium-induced hypertrophy involves alteration of the Na-\(H\) exchanger activity or Na-\(K\) exchanger activity. Another possibility is that sodium alters the expression of growth factors such as transforming growth factor-\(\beta\), basic fibroblast growth factor, and platelet-derived growth factor. Further studies are needed to elucidate the cellular and molecular mechanisms of sodium-induced cellular hypertrophy.

In conclusion, we have demonstrated that sodium can directly induce cellular hypertrophy in cultured neonatal rat MMbs and VSMCs, that the hypertrophy can be reversed by the reinstitution of normal sodium concentration medium, and that the hypertrophy is due to an increase in the rate of protein synthesis and a decrease in the rate of protein degradation. These findings support the hypothesis that the direct cellular effect of sodium may be an important determinant in causing myocardial and/or vascular hypertrophy in subjects with high dietary salt intake.

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