Sodium Depletion Increases Calcium-Activated Left Ventricular Pressure in the Rat

GianPaolo Rossi and Fetnat M. Fouad-Tarazi

Left ventricular dP/dt has been reported to be enhanced in the perfused isovolumic paced heart (Langendorff preparation) isolated from sodium-depleted rats. In this study, we tested the hypothesis that this positive inotropic effect is caused by increased responsiveness of the contractile myofilaments to calcium. To address this question, the calcium–left ventricular pressure relation during tetanization of the whole heart at different extracellular calcium concentrations (2.5, 5.0, 7.5, 12.5, and 15.0 mM) was studied in 19 sodium-depleted (low sodium diet plus diuretics) and 18 control (regular sodium intake) Sprague-Dawley normotensive rats. Results showed that the maximum calcium-activated left ventricular pressure (induced by tetanization) was significantly higher in sodium-depleted than in control rats at 12.5 mM calcium concentration (170±27 [SD] mm Hg in sodium-depleted rats vs. 112±14 mm Hg in control rats, \( p = 0.0005 \)). The calcium concentration at which half-maximal left ventricular pressure response was attained, however, was not different between the two groups. We conclude that long-term in vivo sodium depletion increased the maximum calcium-activated left ventricular pressure without altering the sensitivity of the contractile machinery to calcium. (Hypertension 1990;15:894–899)

Dietary sodium restriction and diuretics are widely used in the treatment of hypertension and heart failure\(^{1,2}\) because of their well-known beneficial hemodynamic effects secondary to sodium depletion.\(^{3}\) Surprisingly little information is available regarding the direct effects of long-term sodium depletion on the heart.\(^{4}\)

We have reported previously that, under controlled preload, afterload, heart rate, and coronary perfusion, the left ventricular (LV) contractility index (positive dP/dt) was enhanced in hearts isolated from sodium-deprived normotensive rats in comparison with control hearts obtained from age-matched rats maintained on regular sodium intake.\(^{5}\) We have also found that total ventricular calcium content is significantly increased in sodium-depleted rats.\(^{6}\) The physiological relevance of this increased calcium in activating cardiac contraction in sodium depletion was not established, however, in our previous reports. In this study, we investigated an alternative hypothesis that long-term sodium depletion increases contractility by increasing the responsiveness of the contractile myofilaments to calcium. We studied this possibility by inducing tetanization of the whole isolated heart, a novel approach for studying the contractile properties of the intact heart.\(^{7}\) In this method, the maximal calcium-activated pressure generated by the heart constitutes a well-defined end point on the subsigmoidal calcium concentration–LV pressure relation and can be used for unambiguous comparison between hearts of different experimental groups.\(^{7}\)

Methods

Animal Model and Number

Normotensive Sprague-Dawley rats were studied under conditions of either sodium depletion or regular sodium intake. The number of animals varied in the individual protocols; the total number was 19 sodium-depleted rats and 18 control rats on regular sodium intake (Table 1).

Study Design

Six-week-old Sprague-Dawley normotensive rats (Hilltop Lab., Scottsdale, New Jersey) received a regular sodium diet for 6 weeks until they were accustomed to our animal facilities. At 12 weeks of
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895

mmHg

300

200

100

0

I SECOND

12.5 mM [Ca^{2+}]

mmHg

300

200

100

0

I SECOND

12.5 mM [Ca^{2+}]

FIGURE 1. Recordings showing example of tetanization of a control rat heart performed at extracellular calcium concentration ([Ca^{2+}]) of 12.5 mM. Tetanies were found to be very reproducible.

Last tetanus is smoother and longer because rapid stimulation was maintained to deliberately stop experiment. (Each division corresponds to 1 second on time scale.)

age, they were weight-matched and randomly assigned to either a regular or a low sodium intake. To limit differences between groups apart from sodium intake, the same commercial low sodium diet (sodium 0.06 mg/g, potassium 9.6 mg/g, calcium 6.2 mg/g, gross energy 4.84 kcal/g, ICN Pharmaceuticals, Costa Mesa, California) was given to all rats. A regular sodium intake (3.2 meq/day) was achieved by supplementing 0.5 g % NaCl to drinking distilled water (RS group). Sodium depletion was attained in the other group by drinking distilled water and injection of intraperitoneal furosemide (5 mg/kg body wt) for 4 consecutive days at the beginning of the study and at the 16th week of age (low sodium plus diuretic [LSD] group); furosemide was used to override the renal sodium-sparing mechanisms likely to be activated during dietary sodium restriction. The dietary sodium manipulation was maintained for 6 weeks. During this period, body weight and systolic blood pressure (tail-cuff indirect method) were measured weekly in all rats. At weeks 2–4 of the dietary period, five rats were randomly selected from each of the two dietary groups and put individually into metabolic cages to measure individual water and food intake, 24-hour electrolyte urinary excretion, and blood chemistry.

Langendorff Study

At the age of 18 weeks, rats were killed after pentobarbital anesthesia (50 mg/kg body wt i.p.) and during positive pressure ventilation to avoid hypoxic damage to the myocardium. Briefly, the excised heart was securely attached by its aortic stump to the Langendorff apparatus, and retrograde perfusion was immediately started by using an oxygenated (100% O_2) buffer containing (mM) NaCl 108, KCl 5, MgCl_2 1, NaHCO_3 20, glucose 10, CaCl_2 2.5, and HEPES 5 (pH 7.40 at 37°C). Details of the preparation have been previously published. LV pressure was obtained (Millar Instrs., Houston, Texas) during cardiac pacing (Grass SD9, Grass Instr. Co., Quincy, Massachusetts) at a rate of 260 beats/min (5-msec stimulus at 2 V). The LV balloon secured around the tip of the Millar catheter was then filled with water until end-diastolic pressure reached 0 mm Hg. After 30 minutes was allowed for equilibration, LV pressure was recorded on a Brush recorder (Gould Inc., Cleveland, Ohio). Then, the balloon inside the left ventricle was distended stepwise until either LV developed pressure reached a plateau or end-diastolic pressure reached 10 mm Hg; our previous observations showed that this value is usually associated with the maximum developed pressure. Then hearts were exposed to a 4-minute infusion of 1 μM ryanodine to block calcium release from the sarcoplasmic reticulum. Tetanization of the heart was achieved by pacing at 10 Hz with rectangular pulses of 50–70-msec duration and 2.0 V; tetanies from the same heart were found to be reproducible when the heart was allowed to rest for 60–90 seconds after each tetanus (Figure 1). We could not continue ryanodine infusion after 4 minutes because LV pressure declined even after stopping the infusion at the risk of complete cardiac standstill. For the same reason, we could not infuse ryanodine throughout the experiment.

In initial experiments (group 1), tetanization was performed at extracellular calcium concentration ([Ca^{2+}]) of 12.5 mM (group 1, n=6 RS and n=6 LSD). To better evaluate the relation between different [Ca^{2+}], and maximum calcium-activated pres-

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LSD, low sodium plus diuretic; RS, regular sodium.
sure elicited by tetanization, a second series of hearts (group 2) was exposed sequentially to 2.5, 5.0, 7.5, and 15.0 mM [Ca\^2+]\textsubscript{0} after pretreatment with ryanodine (group 2, n=7 RS and n=8 LSD). Tetanization was produced twice at each level of [Ca\^2+]\textsubscript{0} by the same pacing pattern. The average pressure attained during the two tetanies was calculated and used for data analysis. At the end of each experiment, right ventricular and LV weights were measured with a Mettler PC 440 precision balance (Mettler Instr. Corp., Hightstown, New Jersey).

**Statistics**

Results are expressed as mean±SD. Two-sided Student's t test for unpaired data, one-way and repeated-measures two-way analysis of variance (ANOVA), and regression analysis were used when appropriate. Differences were considered significant at p values of less than 0.05. Statistical analysis was performed by using both PROPHET Software, sponsored by National Institutes of Health, and program P2V of Biomedical Data Package (BMDP Statistical Software, Inc., Aston, Pennsylvania). The sigmoid function fitted to data points in Figure 4 was applied by using the PROPHET'S curve-fitting routine (Fit Function command).

**Results**

**Effect of Sodium Depletion on Intact Rats**

Daily water, food, calcium, potassium, and magnesium intake was similar in the two groups. The lower sodium intake in the LSD group of rats versus the RS group was reflected in lower urinary sodium excretion (Table 2); however, serum sodium concentration was not significantly different between the two dietary groups. Average body weight was almost identical in the two groups before dietary manipulation (428±19 g in the LSD group vs. 423±16 g in the RS group); however, body weight was significantly lower in the LSD group of rats in comparison with the RS group as early as the end of the first week of the diet. At the end of the sixth dietary week, body weight was 10% lower in the LSD group than in the RS group of rats (483±62 vs. 529±34 g, \(p<0.05\)).

No difference in systolic blood pressure was observed at any time between the two groups. At the end of the study, LV weight was significantly lower in the LSD group than in the RS group of rats (965±140 vs. 1,068±187 mg, \(p<0.05\)); however, no significant difference was detected in LV weight/body weight ratio between the two groups.

**Effect of Sodium Depletion on Myocardial Contractility**

*Results in group 1: Baseline data. LV end-diastolic pressure (preload) required to attain the plateau of LV developed pressure was not significantly different between the two dietary groups (16.7±4.45 mm Hg in the LSD group vs. 15.8±3.75 mm Hg in the RS group). This maximum LV developed pressure (at \(L_{\text{max}}\)) was higher in sodium-depleted rats than in control rats (92.6±17.3 mm Hg in the LSD group and 74.8±9.3 mm Hg in the RS control rats, \(p=0.05\)).

**Effect of ryanodine.** The negative inotropic response was similar in the hearts of the rats in the LSD group and in the hearts of the rats in the control group (Figure 2) and persisted after the withdrawal of ryanodine; it was only minimally reversible by exposure to 12.5 mM [Ca\^2+]\textsubscript{0} (Figure 2).
Tetanization of the heart. Figure 3 illustrates tetanies in a heart from a control rat and a heart from a sodium-depleted rat. In experiments performed in group 1 at a calcium concentration of 12.5 mM, the maximum calcium-activated LV pressure was significantly higher in the LSD group of rats in comparison with the RS group of rats (170.3±26.5 and 112.4±13.4 mm Hg, respectively, p=0.0005).

Results in group 2. The aim of this protocol was to define the \([Ca^{2+}]_o\)-LV pressure relation during tetanization. Hearts of rats in both the LSD and the RS groups showed that absolute nonnormalized maximum calcium-activated LV pressure was significantly lower at 2.5 mM as compared with 5.0 mM calcium concentrations. On the other hand, maximum calcium-activated LV pressure was not significantly different in rats in the RS group between 5, 7.5, and 15 mM calcium concentrations; also, it was not significantly different in rats in the LSD group between 5 mM and 7.5 mM \([Ca^{2+}]_o\). The difference in the nonnormalized maximum calcium-activated pressure was not significant at any of these calcium concentrations between rats in the LSD group and rats in the control group. Irreversible tetanization leading to a stone heart, however, occurred in four of six hearts of rats in the LSD group that stopped after recording tetanies at 7.5 mM \([Ca^{2+}]_o\), whereas it was possible to continue obtaining tetanies in five of the seven hearts of rats in the control group up to \([Ca^{2+}]_o\) of 15 mM. Of the two hearts of rats in the RS group that stopped before application of 15 mM \([Ca^{2+}]_o\), only one showed evidence of stone heart. To assess differences in the calcium concentration required to produce half-maximum LV tetanized pressure response, a sigmoid function was fitted to all data points belonging to each diet within group 2 (Figure 4); this calculated calcium concentration was unchanged by dietary sodium manipulation (2.23 mM in the LSD group vs. 2.18 mM in the RS group).

Discussion

Sodium depletion in our rats was documented by the metabolic studies showing almost negligible urinary sodium excretion. Impaired body growth in these rats was in agreement with previously published data\(^{11-13}\); the time-related increase in body
weight was blunted in sodium-depleted rats partly because of a decrease in body water, but also reflected an overall reduction in organ growth. This interpretation might also explain the lower LV weight in sodium-depleted rats.

In this study, a novel approach was used to assess in vitro myocardial contractility after in vivo sodium depletion. Although tetanization of the heart has been reported in different species, the mammalian heart was believed to be non tetanizable. In this study, however, we report for the first time successful tetanization of whole perfused heart in the rat. Tetanization was achieved by stimulating the heart at high frequency at various calcium concentrations in the medium after exposure of the heart to ryanodine to block calcium handling in the sarcoplasmic reticulum. The high frequency of stimulation opens the slow calcium channels and, therefore, produces sustained calcium entry. Under such conditions, an excellent correlation between intracellular and extracellular calcium concentration was demonstrated during tetanization of the ferret papillary muscle. Moreover, tetanies were shown to be reproducible without causing irreversible calcium overload and cell necrosis. Accordingly, this strategy of tetanization is considered useful for comparison of different experimental groups of hearts. Additionally, it was shown that beyond a certain level, tetanization force reaches a plateau despite concomitant increase in intracellular calcium concentration, indicating saturation of force with respect to calcium.

In our study, maximum calcium-activated LV pressure was significantly higher in the rats in the LSD group versus the control group at a calcium concentration of 12.5 mM. At other levels of calcium concentrations, however, differences between rats in the LSD group and rats in the control group were nonsignificant. The overall lower nonnormalized LV pressure in group 1 during tetanization at 12.5 mM Ca\(^{2+}\) as compared with data obtained in group 2 at [Ca\(^{2+}\)]\(_{o}\) of 5, 7.5, and 15 mM might be related to differences in the experimental conditions.

The increased pressure elicited by tetanization of the heart of sodium-depleted rats (at a [Ca\(^{2+}\)], of 12.5 mM) suggests that the number of force-generating cross bridges, the average force generated by each cross bridge, or both are increased. This finding, together with the observation of a higher incidence of stone heart at high calcium concentrations in the sodium-deprived hearts, also suggests an increased vulnerability to calcium entry and overload. Our finding of increased maximal calcium-activated force after in vivo sodium depletion might explain our previously observed increased inotropic effect of sodium depletion during twitch contraction. Increased responsiveness of the contractile machinery to calcium after in vivo sodium depletion might be related to the previously reported increased total calcium content (normalized per gram of tissue) in the ventricles of sodium-deprived rats. Unfortu...
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