Increased Sodium-Calcium Exchange in Arterial Smooth Muscle of Spontaneously Hypertensive Rats

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We compared sodium-calcium (Na-Ca) exchange in vascular smooth muscle between spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. Aortic rings of 11 SHR and 11 WKY rats aged 11–12 weeks were superfused with physiological saline, and isometric tension was measured. Systolic blood pressure was higher in SHR (174±12 mm Hg) than in WKY rats (132±4 mm Hg): 1) In the presence of 10 μM phentolamine, 10 μM verapamil, and 5 mM caffeine, reduction of ionized extracellular sodium concentration ([Na +]0) from normal (139.2 mM) to 1.2 mM (replaced by N-methyl-D-glucamine) caused an external Ca 2+-dependent increase in tonic tension (calcium entry by Na-Ca exchange). The rate of increase was higher in SHR (35.4±3.9 mg/min) than in WKY rats (17.9±1.3 mg/min) (p<0.01). 2) In the presence of phentolamine, verapamil, and caffeine, relaxation from low-Na+ contraction was promoted by external calcium removal. The rate of relaxation was directly related to [Na +]. The rates of relaxation at normal (139.2 mM) [Na +] were higher in SHR than in WKY rats (p<0.05). The rates of relaxation at 1.2 mM [Na +], (calcium extrusion by adenosine triphosphate–driven calcium pump) were not different between SHR (11.6±2.8 mg/min) and WKY rats (8.9±2.5 mg/min). The increase in the rates of relaxation from 1.2 mM to normal (139.2 mM) [Na +] (calcium extrusion by Na-Ca exchange) was greater in SHR (34.9±6.6 mg/min) than in WKY rats (17.1±4.5 mg/min) (p<0.05). These observations suggest that Na-Ca exchange in vascular smooth muscle is increased in SHR and might be involved in the mechanisms for hypertension in SHR. (Hypertension 1989;13:890–895)

Contraction of vascular smooth muscle (VSM) is normally triggered by a rise in the cytoplasmic free calcium concentration ([Ca2+]c) as a result of calcium entry from the extracellular fluid or the release of calcium from the sarcoplasmic reticulum (SR). Relaxation of VSM is promoted by extrusion of calcium across the plasma membrane or resequestration of calcium in the SR. Two types of mechanisms may be involved in calcium entry: calcium-selective channels (both agonist receptor–operated and voltage-gated) and sodium-calcium (Na-Ca) exchange (reverse mode). Likewise, two types of mechanisms may be involved in calcium extrusion: an adenosine triphosphate (ATP)–driven calcium pump (mediated by a calmodulin-modulated, calcium-dependent ATPase) and Na-Ca exchange (forward mode). The Na-Ca exchange can mediate either net calcium entry or exit, depending on the prevailing sodium electrochemical gradient across the plasmalemma ∆μNa.

Two reports showed the abnormality in Na-Ca exchange in VSM of hypertensive animals. A study in sarcolemmal vesicles suggested a slight increase in Na+-Ca2+ exchange, and a preliminary report in VSM cells showed an increase in Na+-Ca2+ exchange. But these are not contraction experiments. By using caffeine to minimize SR calcium sequestration we examined aortic rings of spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats to determine whether the Na-Ca exchange in arterial smooth muscle was abnormal or not in hypertension. In this study, we used the rate of tension development as a measure of the rate of Ca2+ delivery to the contractile elements.

Materials and Methods

Tissues

Small rings of thoracic aorta, 2–2.5 mm long by 1–1.5 mm diameter, were used for these experiments. Eleven male SHR (aged 11–12 weeks) and 11 age-matched male WKY rats were killed by decap-
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1.2mM-Na⁺ (NMG)

FIGURE 1. Plots of contraction induced in aortic rings of Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) by reduction of ionized extracellular sodium concentration ([Na⁺]) from normal (139.2 mM) to 1.2 mM (replaced by N-methyl-D-glucamine [NMG]) in presence of 5 mM caffeine, 10 μM verapamil, and 10 μM phentolamine. Periods of exposure to 1.2 mM [Na⁺], are indicated by bars in the figure. Rate of contraction was higher in SHR than in WKY rats.

itation; the thoracic aorta was rapidly excised and placed into Krebs' solution at 37°C. The arteries were cleaned of surrounding connective tissue and equilibrated in the warm Krebs' solution until resting tension was stabilized at 500 mg (for at least 1 hour) before data collection was initiated.

Solutions

The tissues were incubated in a modified Krebs' solution that contained (mM): NaCl 138, KCl 4.7, NaH₂PO₄ 1.2, CaCl₂ 1.8, MgSO₄ 1.2, glucose 10, and HEPES 10, adjusted to pH 7.4 with Tris, and gassed with 100% O₂. In calcium-free solutions, calcium was replaced with equimolar MgCl₂. In low-sodium solutions, N-methyl-D-glucamine (NMG) was used as the iso-osmotic sodium replacement, and pH was adjusted to 7.4 with hydrogen chloride.

Special Reagents and Drugs

The drugs used were verapamil HCl (Eisai Co., Tokyo, Japan), caffeine (Sigma Chemical Co., St. Louis, Missouri), and phentolamine HCl (CIBA GEIGY Co., Basel, Switzerland).

Contraction Measurements

Two thin (0.4 mm diameter) stainless steel hooks were inserted through the lumen of the artery rings. One hook was fixed to the floor of a small jacketed tissue chamber (1 ml); the other hook was connected to a force transducer (model ME-4021, MEC Ltd., Tokyo, Japan) that was mounted immediately above the tissue chamber. Isometric tension was continuously monitored and recorded on a strip chart recorder. The tissue was steadily superfused by a peristaltic pump (Minipuls 2, Gilson, Villiers Le Bel, France) at a rate of 2 ml/min with well-oxygenated incubation fluid at 37°C. To minimize the influence of SR calcium sequestration in VSM, tissues were routinely treated with caffeine; that is, caffeine was added to the superfusion fluids (final concentration 5 mM) and was allowed to reach a steady concentration in the incubation fluid within the tissue chamber (40-50 minutes). The rate of tension development was calculated from the rising slope that was almost constant 3-10 minutes after the solution exchange.

Blood Pressure Measurements

Systolic blood pressure was measured by the tail-cuff method before decapitation.

Statistical Analysis

Values are expressed as mean±SEM. The difference was assessed by the unpaired t test or two-way analysis of variance.

Results

Blood Pressure, Body Weight, and Heart Rate

Systolic blood pressure was higher in SHR (174±12 mm Hg) than in WKY rats (132±4 mm Hg) (p<0.001). Heart rate tended to be higher in SHR (452±24 beats/min) than in WKY rats (393±21 beats/min). Body weight was not different between SHR (297±16 g) and WKY rats (292±14 g).

Contraction Experiments

Figure 1 shows examples of the contraction induced by the reduction of ionized extracellular sodium concentration ([Na⁺]) from normal (139.2 mM) to 1.2 mM in aortic rings of SHR and WKY rats. This increase in tonic tension was entirely external Ca²⁺-dependent and appeared to be due to calcium entry by Na-Ca exchange. As shown in
**Figure 2.** Graph of relation between ionized extracellular sodium concentration and rate of tension development in Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). Data were all obtained in presence of 5 mM caffeine, 10 μM verapamil, and 10 μM phentolamine. Rate of tension development was calculated from the rising slope as illustrated in Figure 1. \( n = 5 \) (WKY rats); \( n = 6 \) (SHR). Standard error bars are shown on the graph. *p<0.05; **p<0.01.

Figure 1, the rate of contraction was higher in SHR than in WKY rats. Figure 2 shows the relation between \( [Na^+]_o \) and the rate of tension development in SHR and WKY rats. As shown in the figure, the rate of tension development was inversely related to \( [Na^+]_o \). The rates of tension development were significantly higher in SHR than in WKY rats at 1.2 mM \( [Na^+]_o \) (p<0.01), 7.5 mM \( [Na^+]_o \) (p<0.01), and 15 mM \( [Na^+]_o \) (p<0.05) \( [Na^+]_o \). Also the rates of tension development at 30 mM, 45 mM, and 60 mM \( [Na^+]_o \) tended to be higher in SHR than in WKY rats.

The absolute magnitude of the peak of the low sodium–induced tension development was significantly higher in SHR (200.4±21.8 mg tension) than in WKY rats (121.8±8.9 mg tension) at 1.2 mM \( [Na^+]_o \) (p<0.02).

**Relaxation Experiments**

Figure 3 shows examples of the relaxation from low Na⁺ (1.2 mM)–induced contraction after the removal of external Ca²⁺ in aortic rings of SHR and WKY rats. The Ca²⁺-free (relaxation) solution contained normal (139.2 mM) Na⁺. As shown in the figure, the rate of relaxation was higher in SHR than in WKY rats. Figure 4 also shows examples of the relaxation from low Na⁺–induced contraction. In this case, the Ca²⁺-free (relaxation) solution contained only 1.2 mM Na⁺. As shown in the figure, the rates of relaxation were much slower than that in Figure 3 and were not different between SHR and WKY rats.

Figure 5 shows the relation between \( [Na^+]_o \) and the rate of relaxation of aortic rings in SHR and WKY rats. The rate of relaxation was directly related to \( [Na^+]_o \). The rates of relaxation at normal (139.2 mM) \( [Na^+]_o \) were significantly higher in SHR (46.5±6.3 mg/min) than in WKY rats (26.0±5.2 mg/min) (p<0.05). The rates of relaxation at 60 mM and 30 mM \( [Na^+]_o \) tended to be higher in SHR than in WKY rats. The rates of relaxation at only 1.2 mM \( [Na^+]_o \) (calcium extrusion by ATP-driven calcium pump) were not different between SHR (11.6±2.8 mg/min) and WKY rats (8.9±2.5 mg/min).

Figure 6 shows the increase in the rates of relaxation from 1.2 mM to normal (139.2 mM) \( [Na^+]_o \) (calcium extrusion by Na-Ca exchange) in aortic rings of SHR and WKY rats. The increase was significantly greater in SHR (34.9±6.6 mg/min) than in WKY rats (17.1±4.5 mg/min) (p<0.05).

We calculated the ratio of Na-Ca exchange to ATP-driven calcium pump in calcium extrusion. The ratio was 2.5±0.8:1 in VSM of WKY rats and increased to 3.8±1.1:1 in VSM of SHR.

**Discussion**

The elevated blood pressure of most forms of hypertension is characterized by increased peripheral vascular resistance with normal cardiac output. The increased peripheral resistance reflects a functional alteration of VSM that may be manifest by increased reactivity to vasopressor agents that include norepinephrine, angiotensin II, and vasopressin. The generalized increase in vascular reactivity makes it likely that the mechanisms that link receptor activation to contraction are altered in hypertension; the availability of calcium for contraction is modified. There is a known link between sodium metabolism and calcium regulation in VSM cells, namely, Na-Ca exchange. Therefore, we compared the Na-Ca exchange in VSM of SHR and WKY rats.

**Contraction Experiments**

Small rings of rat thoracic aorta were used for these experiments. The rate of contractile tension development in these rings was used as a measure of the rate of Ca²⁺ delivered to the contractile apparatus. In this study, we did not measure the maximal tension in low sodium–induced contraction. The long time superfusion of the tissues in the very low sodium solution would damage the tissues. Therefore, we superfused the tissues in the low sodium solution for about 10 minutes. Contractions were induced by lowering the external sodium con-
centration [Na$^+_{\text{in}}$], which is one means of reducing $\Delta\mu_{\text{Na}}$. As shown in Figure 1, reduction of [Na$^+$]$_{\text{in}}$ resulted in increased tonic tension.

The calcium that activates arterial smooth muscle contraction, activator Ca$^{2+}$, comes from two sources, the extracellular fluid and the SR. Ca$^{2+}$ sequestration in SR may complicate estimates of Ca$^{2+}$ entry that are based on contraction measurements in tissues that have a substantial SR. So caffeine was used to minimize SR calcium sequestration and, thus, to emphasize the plasma membrane systems that transport calcium and help to regulate [Ca$^{2+}$]. Calcium channel blocker was used to inhibit calcium entry through the calcium channel. The $\alpha$-blocker was used to inhibit the effect of endogenous $\alpha$-adrenergic receptor agonists.

As a result, calcium entry through Na-Ca exchange was significantly higher in SHR than in WKY rats. This is in accord with tracer flux data in the aorta and mesenteric artery of SHR; that is, Matlib et al$^6$ reported that Ca$^{2+}$ uptake in Na$^{+}$-loaded cell membrane vesicles isolated from mesenteric arteries of SHR (calcium entry through Na-Ca exchange) was slightly increased compared with WKY rats. Sperti and Colucci,$^7$ by using $^{45}$Ca$^{2+}$ uptake in VSM cells cultured from aortas of SHR and WKY rats, reported a significant increase of Na$^{+}$-Ca$^{2+}$ exchange in VSM of SHR.

**Relaxation Experiments**

We evaluated calcium extrusion through Na-Ca exchange by examination of the effect of external sodium on the rate of relaxation after the induction of contractions of modest amplitude (Figures 3 and 4). Such relaxation is usually dependent on removal of calcium from the cytosol either by sequestration in the SR or by extrusion across the sarcolemma via the ATP-driven calcium pump or Na-Ca exchange. The influence of SR calcium sequestration in VSM was minimized by performing the experiments in the presence of caffeine. All the experiments were performed in the presence of phentolamine and verapamil to minimize the effects of endogenous catecholamines and calcium movements through calcium-selective channels.

**FIGURE 3.** Plots of relaxation in aortic rings of Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) from low Na$^+$ (1.2 mM)–induced contraction after removal of external Ca$^{2+}$ in presence of 5 mM caffeine, 10 $\mu$M verapamil, and 10 $\mu$M phentolamine. Ca$^{2+}$-free (relaxation) solution contained 139.2 mM Na$^+$. Rate of relaxation was higher in SHR than in WKY rats. NMG, N-methyl-D-glucamine.

**FIGURE 4.** Plots of relaxation in aortic rings of Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) from low Na$^+$ (1.2 mM)–induced contraction after removal of external Ca$^{2+}$ in presence of 5 mM caffeine, 10 $\mu$M verapamil, and 10 $\mu$M phentolamine. Ca$^{2+}$-free (relaxation) solution contained only 1.2 mM Na$^+$. Rates of relaxation were much slower than that in Figure 3 and were not different between SHR and WKY rats. NMG, N-methyl-D-glucamine.
Since the Na-Ca exchange operates in parallel with the ATP-driven calcium pump in calcium extrusion, we tried to compare the relative roles of the calcium transport mechanisms between SHR and WKY rats. That is, to determine the effect of calcium extrusion via the ATP-driven calcium pump, we measured the rate of relaxation in sodium-depleted media after removal of external calcium (to avoid the Na-Ca exchange-mediated calcium entry that occurs when $[Na^+]_o$ is reduced). The increment in the rate of relaxation, on restoring external sodium, could then be attributed to Na-Ca exchange.

As a result, though ATP-driven calcium pump was not different between SHR and WKY rats, Na-Ca exchange was significantly greater in SHR than in WKY rats. This suggests that in SHR, the relative role of Na-Ca exchange in calcium extrusion compared with calcium pump is greater than in WKY rats. In fact, our relaxation experiments showed that the mean ratio of Na-Ca exchange to ATP-dependent calcium pump in calcium extrusion was 3.8:1 in VSM of SHR and was 2.5:1 in VSM of WKY rats. This suggests that in calcium extrusion, Na-Ca exchange might be more important than ATP-driven calcium pump in VSM of SHR.

It is known that internal calcium is required at a site not involved in translocation to activate Na-Ca exchange-mediated calcium entry. That is, the turnover of the exchanger and the exchanger-mediated transport of calcium in both directions across the sarcolemma are activated by internal calcium. This implies that the higher the $[Ca^{2+}]_i$, the more the $[Ca^{2+}]_j$ (and thus, tone) will be biased by the Na-Ca exchange. It has been reported that intracellular calcium concentration might be increased more in SHR than in WKY rats. Therefore, there is a possibility that Na-Ca exchange in SHR is greater than in WKY rats due to, in part, the increased intracellular calcium concentration.
The complete blockade of calcium channels by calcium channel blocker and the complete blockade of the uptake mechanism by caffeine were not examined directly. However, calcium channel blockers are known to block some receptor-operated channels as well as voltage-gated calcium channels in VSM. We reported that the calcium channel blocker verapamil (10 μM) almost completely abolished contractions evoked by potassium (100 mM) depolarization. Moreover, we reported that the reduction in [Na+]o to 30 mM did not affect tension in the absence of caffeine, but it did induce a progressive rise in tonic tension in the presence of caffeine (5 mM). This observation suggests that net Ca2+ influx was increased when [Na+]o was reduced; a substantial rise in [Ca2+]i was then prevented by Ca2+ sequestration in the SR in the absence but not in the presence of caffeine.

The aforementioned tension measurements provide only indirect information about the influence of Δ[Na+]o on [Ca2+]i. By using fura-2 with digital imaging microspectrofluorometry, we observed that lowering [Na+]o, induced contractions and a rise in [Ca2+]i in single, dissociated bovine tail artery smooth muscle cells. In this study, membrane potential, internal pH, and internal sodium were not measured. There is a possibility that these variables are changed when extracellular sodium or calcium is changed. Further investigation is required to clarify the role of these variables.

In conclusion, Na-Ca exchange was increased in VSM of SHR compared with VSM of WKY rats; therefore, an intrinsic abnormality of Ca2+ homeostasis in VSM of SHR is implicated.

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