Abnormal Platelet Function and Calcium Handling in Dahl Salt-Hypertensive Rats

Yun Li, Takeshi Adachi, Victoria M. Bolotina, Cathy Knowles, Kenneth A. Ault, Richard A. Cohen

Abstract—The effect of dietary salt on platelet function and Ca\(^{2+}\) homeostasis was studied in Dahl (DS) rats, a genetic model of salt-sensitive hypertension. DS rats were fed a high-salt (DSHS) or a low-salt diet (DSLS) for up to 4 weeks, and the effects of salt loading on systolic blood pressure, platelet P-selectin expression, and platelet Ca\(^{2+}\) homeostasis were measured. The high-salt diet increased blood pressure and markedly increased the amount of ionomycin (IM)-releasable Ca\(^{2+}\) in platelet intracellular stores (Ca\(^{2+}\)/IM). The alteration in Ca\(^{2+}\) stores was not prevented when the hypertension was prevented by treatment with hydralazine and reserpine. The Ca\(^{2+}\) store filling during platelet exposure to 1 mmol/L Ca\(^{2+}\) for 5 minutes and the rate of sarcoplasmic/endoplasmic Ca\(^{2+}\) ATPase–dependent Ca\(^{45}\) uptake were higher in DSHS compared with that in DSLS. There was a decrease in thrombin-induced Ca\(^{2+}\) influx in platelets from DSHS; consistent with this, agonist-induced P-selectin expression was decreased. In DSLS, nitric oxide accelerated reloading of platelet Ca\(^{2+}\) stores after their emptying by thrombin but failed to do so in DSHS. These results indicate that in DS rats, a high-salt diet increases sarcoplasmic/endoplasmic Ca\(^{2+}\) ATPase activity and the Ca\(^{2+}\)/IM but decreases the reuptake of Ca\(^{2+}\) caused by nitric oxide. Decreases in Ca\(^{2+}\) influx and platelet P-selectin expression might be explained by changes in intracellular Ca\(^{2+}\) stores in DSHS rats, which apparently is a heritable response to a high-salt diet. (Hypertension. 2001;37:1129-1135.)

Key Words: salt • calcium • diet • nitric oxide • rats, Dahl • platelets

Epidemiological studies show that there is an association between a dietary high-salt diet and arterial hypertension in salt-sensitive individuals.\(^1,2\) The Dahl-Rapp salt-sensitive (DS) rat has been used as a genetic model of human salt-sensitive hypertension.\(^3\) When challenged with a high-salt diet, DS rats have an attenuated pressure natriuresis, renal medullary vasoconstriction, renal injury, and hypertension.\(^4\) However, the mechanism by which salt sensitivity alters vascular function and mediates hypertension is not clear.

Platelets play a key role in the development of vascular disease and related thrombotic events. Cytosolic calcium concentration ([Ca\(^{2+}\)]\(_i\)) is the main determinant of platelet activation and aggregation. Results from studies in hypertensive rats\(^5,7\) and individuals with essential hypertension\(^6,7\) indicate that hypertension is associated with altered platelet Ca\(^{2+}\) homeostasis. Platelets also have been used as a model reflecting smooth muscle Ca\(^{2+}\) homeostasis.\(^5,7\) In DS rats, the effect of hypertension on platelet Ca\(^{2+}\) homeostasis is controversial, with both increases and decreases in [Ca\(^{2+}\)]\(_i\) having been reported.\(^6,9\)

Similar to other types of cells, the levels of [Ca\(^{2+}\)]\(_i\) in platelets are controlled by its influx and efflux as well as the content and release from intracellular Ca\(^{2+}\) storage sites (dense tubular system). In platelets, there are no voltage-regulated Ca\(^{2+}\) channels,\(^10\) and the mechanisms controlling receptor-regulated Ca\(^{2+}\) influx are not clear. Intracellular Ca\(^{2+}\) stores play an important role in Ca\(^{2+}\) homeostasis and calcium influx. In the resting state, most of the Ca\(^{2+}\) is in the intracellular Ca\(^{2+}\) stores, whose content is controlled by sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) and inositol trisphosphate receptors. A number of studies\(^11\) including those on platelets\(^12\) indicate that intracellular stores regulate Ca\(^{2+}\) entry. We recently showed that nitric oxide (NO), a known inhibitor of platelet activation, inhibits thrombin-induced Ca\(^{2+}\) influx indirectly by accelerating SERCA-dependent refillling of the Ca\(^{2+}\) stores.\(^13\)

This study determined the effect of long-term dietary salt loading on the amount of Ca\(^{2+}\) in intracellular stores, agonist-stimulated Ca\(^{2+}\) entry, SERCA activity, and P-selectin expression in platelets from DS and salt-resistant (DR) rats. The effect of a high-salt diet on the changes in platelet intracellular Ca\(^{2+}\) stores caused by NO was also investigated.

Methods

Animals and Isolation of Rat Platelets

Male DS and DR rats (8 to 9 weeks of age, from Harlan Sprague-Dawley) were divided into 2 groups. The low-sodium diet group was fed a 0.12% NaCl diet. The high-sodium diet group was fed a diet...
supplemented with 8% NaCl. Some DS rats receiving 8% NaCl also received antihypertensive drugs in the drinking water (hydralazine, 80 mg/L; reserpine, 1.4 mg/L).14 Systolic arterial blood pressure and body weight were measured before and 3 to 4 days, 2 weeks, or 4 weeks after initiation of salt diets. Blood pressure was measured by tail-cuff method. Blood (8 to 10 mL) was drawn from the abdominal aorta of pairs of rats that were fed low-salt or high-salt diets on the same day and was put into tubes containing 1/10 volume of 3.8% trisodium citrate. Platelet-rich plasma was obtained by centrifuging the blood for 10 minutes at 350g at room temperature. Platelet pellets were obtained by centrifugation at 750g for 10 minutes and then resuspended and washed in HEPES buffer consisting of (mmol/L): 137 NaCl; 2.7 KCl; 1 MgCl2 ·6H2O; 3.3 NaH2PO4; 5.5 glucose; and 3.8 HEPES (pH 7.4).

Measurement of Platelet Cytosolic Ca2+ Concentrations

Before each individual experimental run, isolated platelets (107/mL) were loaded with the Ca2+ indicator Fura-2 AM (2.5 µmol/L) at 37°C for 10 minutes. The extracellular dye was removed by centrifugation, and platelets were resuspended in 2 mL HEPES buffer immediately before Ca2+ measurements. Fluorescence was measured at 37°C under constant stirring in a Hitachi F-4500 spectrofluorometer, with excitation wavelength alternating between 340 and 380 nm every 0.5 second and emission wavelength at 510 nm. Recordings were corrected for autofluorescence that was determined in unloaded platelets. Platelet cytosolic Ca2+ concentration was estimated by the ratio of fluorescence detected at the two excitation wave lengths (R510/R340).

As shown in Figures 1 and 2, the amount of Ca2+ in platelet intracellular stores was estimated by 2 protocols. In the first protocol, Fura-2–loaded platelets were suspended in Ca2+-free medium and a maximum dose of ionomycin (IM, 5 µmol/L) was applied to release all releasable Ca2+ from intracellular stores (Ca2+IM). In the second protocol, platelets were resuspended in 1 mmol/L Ca2+ buffer for 5 minutes to augment platelet intracellular stores that may have depleted during isolation in Ca2+ chelating buffers. After removing the extracellular Ca2+ with EGTA, ionomycin was added to measure Ca2+IM. In both protocols, Ca2+IM was estimated from the amount of Ca2+ released by ionomycin in two different ways: (1) the peak [Ca2+]i increase after IM (expressed as the change in R340/380 between the baseline and peak) and (2) the integral of changes in the fluorescence ratio during the first 5 minutes of the IM release transient.

Platelet P-Selectin Expression

Platelet P-selectin expression was used as a marker of platelet activation and was measured in whole blood by flow cytometry as described.15 P-selectin expression was measured with no added stimulus (spontaneous) and after activation by agonists. Briefly, 5 µL of whole blood was placed immediately into 4 tubes containing 50 µL PBS as negative control, 50 µL PBS plus 10 µL rabbit anti–P-selectin to determine spontaneous activation, and either 5 µmol/L ADP plus 500 µmol/L epinephrine or thrombin (Thr, 0.5 U/mL) and anti–P-selectin antibody for agonist-induced activation. Each sample was mixed and washed with PBS and centrifuged at 1700g for 5 minutes. Then, 20 µL of a solution containing secondary antibody was added and incubated in the dark. Finally, the samples were resuspended in 500 µL of PBS and analyzed by flow cytometry as described.15 Results are expressed as percentage of platelets staining for P-selectin compared with unstained samples.

Measurements of 45Ca2+ Uptake

SERCA activity was measured by 45Ca2+ uptake in platelet lysates. Washed platelets were disrupted by sonication, and the total lysates were used for 45Ca2+ uptake assay. Paired samples from DS rats receiving a high-salt diet (DSHS) and DS rats receiving a low-salt diet (DSLS) were studied on the same day. Uptake was assayed in samples from both groups of rats without or with added thapsigargin (TG, 10 µmol/L), a specific inhibitor of SERCA, which was added, mixed, and incubated at 37°C for 15 minutes before assay. Ca2+ uptake buffer (30 mmol/L Tris-HCl, pH 7.0, 100 mmol/L KCl, 5 mmol/L sodium azide, 6 mmol/L MgCl2, 0.15 mmol/L EGTA, 0.12 mmol/L CaCl2, 10 mmol/L oxalate) was mixed with 1 µCi/mL 45CaCl2 and 2 mmol/L ATP at 37°C. The reaction was started by adding 300-µg aliquots of protein into the uptake mixture (500 µL) and was stopped at 10, 30, and 60 minutes by filtration of 0.1 mL of the mixture through Whatman GF/C glass filters. The filters were washed twice with 2.5 mL of wash buffer consisting of 30 mmol/L imidazole, 250 mmol/L sucrose, and 0.5 mmol/L EGTA and counted by liquid scintillation. 45Ca2+ uptake was calculated by counting the radioactivity standardized by protein concentration, which was determined by the Bradford method. The rate of TG-sensitive 45Ca2+ uptake was considered as SERCA activity and was compared in DSHS and DHLS.

Materials

NO gas was obtained from Matheson. Saturated NO solution was prepared as described16 and stored at 4°C. A flexible, plastic 1-L intravenous bag was filled with 750 mL distilled water and bubbled with nitrogen gas to remove oxygen. Then, 30 mEq Bio-Rad analytical grade anion exchange resin was mixed in the water before

Figure 1. Amount of IM-releasable Ca2+ in platelet intracellular stores (Ca2+/IM) in DSLS or DSHS. Responses were obtained in platelets obtained in Ca2+-free solution without Ca2+ refilling. Top: Representative trace of IM-induced Ca2+ release in DSLS superimposed on that from DSHS. Fura-2–loaded platelets were resuspended in Ca2+-free medium and 5 µmol/L IM was applied. Ca2+/IM was evaluated in two ways: first by the IM-induced peak Fura-2 ratio and second by integral obtained during initial 5 minutes. Middle: Summarized data from 8 rats per group showing that without Ca2+ refilling Ca2+/IM as assessed by either IM-induced Fura-2 peak (left panel) or integral (right panel) was increased significantly in DSHS (black bar) compared with that in DSLS (white bar). Bottom: Summarized data showing that Ca2+/IM assessed by IM-induced Fura-2 peak (without Ca2+ refilling) was increased in DSHS for 3 to 4 days, 2 weeks, and 4 weeks. Two weeks of antihypertensive treatment did not prevent change in platelet Ca2+/IM.

Material

NO gas was obtained from Matheson. Saturated NO solution was prepared as described16 and stored at 4°C. A flexible, plastic 1-L intravenous bag was filled with 750 mL distilled water and bubbled with nitrogen gas to remove oxygen. Then, 30 mEq Bio-Rad analytical grade anion exchange resin was mixed in the water before
the bag was purged with nitrogen gas for 30 minutes. (Any nitrite or nitrate that is formed by the reaction of NO with residual oxygen is retained by the resin.) The bag was then purged with NO gas to give a dissolved gas concentration at 4°C of 3.1±0.6 mmol/L (n=5) and was stable for 1 week as measured by a chemiluminescent method (Siever NOA model 270 NO analyzer). Subsequent NO dilutions were made from the saturated NO solution by drawing off the solution from the bag with a gas-tight syringe and dissolving in sealed tubes filled with deoxygenated solution (bubbled with nitrogen for 1 hour).

4CaCl₂ was obtained from New England Nuclear. Fura-2/AM was from Molecular Probes. Thr, ADP, epinephrine, and IM were from Sigma Chemical.

Calculations and Statistical Analysis
The Hitachi software was used for data collection, calculation of Fura-2 emission ratio, and data analysis. An unpaired t test was used to determine the significance of differences in the means of data between the rats with low-salt and high-salt diets. A repeated-measures ANOVA was performed to compare 4Ca²⁺ uptake in DSHS and DSLS platelets over time. Data were expressed as mean±SEM; n indicates the number of animals. A value of P<0.05 was considered significant.

Results
Effect of High Salt on Systemic Blood Pressure
Before initiating salt diets, there was no significant difference in systemic blood pressure or body weight between DS and DR rats. After 4 weeks of the high-salt (8% NaCl) diet, the blood pressure in DS rats increased from 120±2 to 200±6 mm Hg (n=8, P<0.05). There was no significant change in systemic blood pressure in DR rats after the same period of the high-salt diet (from 122±2 to 123±2 mm Hg, n=5). Also, in DS rats after 4 weeks of the low-salt (0.12% NaCl) diet, the blood pressure did not increase significantly (from 122±2 to 125±2 mm Hg, n=8). In DS rats, 2 weeks of the high-salt diet also caused a marked increase in blood pressure (200±8 mm Hg, n=5). Antihypertensive treatment normalized the blood pressure in DS rats fed the 8% NaCl diet for 2 weeks (110±6 mm Hg, n=3, P<0.05 compared with those not treated with antihypertensive agents).

Amount of Ca²⁺ in IM-Releasable Stores
The amount of Ca²⁺ in Ca²⁺/IM is shown for platelets from DS rats given the low-salt and high-salt diets in Ca²⁺-free conditions (Figure 1). In addition, the Ca²⁺/IM was also assessed after Ca²⁺ store refilling, which also changed the kinetics of Ca²⁺ release from the stores (Figure 2). With both protocols and regardless of whether the stores were quantified by the integral of the Fura-2 [Ca²⁺]ₜ transient or its peak, platelets from the DS rats fed the high-salt diet showed significantly greater IM-evoked [Ca²⁺]ₜ release than those of DS rats fed with low-salt diet. These data indicate that in DS rats, a high-salt diet increased the amount of releasable Ca²⁺ in intracellular Ca²⁺ stores.

Figure 1 also shows the time course of the change in IM-releasable stores in DS rats fed a high-salt diet as well as the effect of antihypertensive treatment. Compared with the IM-releasable stores in DS rats given the low-salt diet, the Ca²⁺/IM was increased in DS rats after the high-salt diet for 3 to 4 days, 2 weeks, or 4 weeks. Despite the fact that antihypertensive treatment prevented the rise in blood pressure, the antihypertensive drugs did not affect the change in platelet calcium stores caused by the high-salt diet.

The same protocol was used to measure the effect of dietary salt on platelets from DR rats. The Table indicates that in DR rats, regardless of salt diet fed for 4 weeks, there was no significant difference in Ca²⁺/IM either before or after Ca²⁺ refilling.

Thr-Induced Ca²⁺ Influx and Platelet Activation
Thr-induced Ca²⁺ response was compared in DS rats given low-salt and high-salt diets. Figure 3 shows the effect of dietary salt on the Thr-induced [Ca²⁺]ₜ response in the presence of 1 mmol/L extracellular Ca²⁺. In platelets from DS
rats given a high-salt diet, thrombin-evoked \([\text{Ca}^{2+}]_i\) response was decreased and the time to reach the peak was increased compared with that in DS rats given the low-salt diet (Figure 3, \(P<0.05\)). In the presence of extracellular \(\text{Ca}^{2+}\), the Thr-induced \([\text{Ca}^{2+}]_i\) response is a reflection of net \(\text{Ca}^{2+}\) release, influx, influx, and reuptake. To experimentally separate \(\text{Ca}^{2+}\) influx from the \(\text{Ca}^{2+}\) release from intracellular \(\text{Ca}^{2+}\) stores, Thr (0.5 U/mL) also was applied in \(\text{Ca}^{2+}\)-free buffer to platelets whose stores had not been reloaded with \(\text{Ca}^{2+}\) (Figure 4). Thr caused a small increase in \([\text{Ca}^{2+}]_i\), representing only \(\text{Ca}^{2+}\) release from intracellular stores, which then declined to a steady-state level. Addition of 1 mmol/L extracellular \(\text{Ca}^{2+}\) 5 minutes after thrombin further increased \([\text{Ca}^{2+}]_i\), as a result of \(\text{Ca}^{2+}\) influx. There was no significant difference in thrombin-induced \(\text{Ca}^{2+}\) release in \(\text{Ca}^{2+}\)-free buffer in DS rats fed a low-salt or a high-salt diet. However, during \(\text{Ca}^{2+}\) influx, \([\text{Ca}^{2+}]_i\) increased to significantly lower levels in DS rats fed the high-salt diet compared with those fed the low-salt diet.

Figure 5 shows the effect of a high-salt diet on platelet \(\text{P-selectin}\) expression in DS rats. There was no significant difference in \(\text{P-selectin}\) expression under basal conditions, but \(\text{P-selectin}\) expression both by the combination of ADP (5 \(\mu\text{mol/L}\)) and epinephrine (500 \(\mu\text{mol/L}\)) as well as Thr (0.5 U/mL) decreased significantly in DS rats fed the high-salt diet compared with those fed the low-salt diet.

\(\text{Ca}^{2+}\) Store–Refilling Capacity

The above results indicate that in DS rats, a high-salt diet increased the amount of IM-releasable \(\text{Ca}^{2+}\) in platelet intracellular stores and diminished Thr-induced \(\text{Ca}^{2+}\) influx and agonist-stimulated \(\text{P-selectin}\) expression. One possible cause for such changes could be a change in reuptake into the intracellular stores. To test this hypothesis, two different approaches were used.

As shown in Figures 1 and 2, when compared in platelets from the same rats, the amount of releasable \(\text{Ca}^{2+}\) in intracellular stores was significantly larger when measured after incubating 5 minutes in 1 mmol/L extracellular \(\text{Ca}^{2+}\). This indicates that during the exposure of platelets to extracellular \(\text{Ca}^{2+}\), \(\text{Ca}^{2+}\) entered the cell and was taken up into intracellular stores. The increase in the integral of IM-induced \(\text{Ca}^{2+}\) transient between that determined in \(\text{Ca}^{2+}\)-free buffer (Figure 1) and after exposure to 1 mmol/L \(\text{Ca}^{2+}\) (Figure 2) was analyzed as an indicator of \(\text{Ca}^{2+}\) store filling. In DS rats fed the high-salt diet, the increase in IM-releasable \(\text{Ca}^{2+}\) stores
expressed as the integral before and after Ca\(^{2+}\) refilling was significantly higher than that in DS rats fed the low-salt diet (200±26 versus 60±42, \(P<0.05\)).

Because SERCA is mainly responsible for refilling intracellular Ca\(^{2+}\) stores, we hypothesized that increased activity of this enzyme is responsible for the larger amount of Ca\(^{2+}\) in intracellular stores and greater refilling of intracellular Ca\(^{2+}\) stores in platelets from DS rats fed high-salt diet compared with those fed the low-salt diet. Platelet SERCA activity was estimated by measuring the TG-sensitive Ca\(^{2+}\) uptake in crude platelet lysates. As shown in Figure 6, in DS rats fed the high-salt diet, TG-sensitive \(^{45}\)Ca\(^{2+}\) uptake was significantly greater compared with that in DS rats fed the low-salt diet (ANOVA, \(P<0.05\), \(n=5\)). The rate of \(^{45}\)Ca\(^{2+}\) uptake over the first 10 minutes was also significantly greater in lysates from platelets of DS rats fed the high-salt diet when compared with that of DS rats fed the low-salt diet (Figure 6).

**Effect of NO on IM-Releasable Stores**

In human platelets, NO inhibits store-operated Ca\(^{2+}\) influx indirectly by accelerating the SERCA-dependent refilling of intracellular stores because its effect is blocked by BHQ, 2,5-di-(tert-butyl)-1,4-benzohydroquinone, another specific inhibitor of SERCA.\(^{13}\) The same protocol was used in rat platelets, and the effect of NO on Ca\(^{2+}\) stores in platelets from DS rats fed high-salt and low-salt diets was evaluated. In the absence of extracellular Ca\(^{2+}\), Thr (2.5 U/mL) released intracellular Ca\(^{2+}\), and, 5 minutes later, a maximal dose of IM was added to release the remaining portion of Ca\(^{2+}\) from the stores. When NO (10\(^{-6}\) μmol/L) was applied 30 seconds before Thr, the amplitude of the Thr response was decreased and the amplitude of the IM-induced Ca\(^{2+}\) release was enhanced compared with control, as shown in Figure 7. This indicates that after Ca\(^{2+}\) is released from the stores by Thr,

Figure 6. \(^{45}\)Ca\(^{2+}\) uptake in lysates of platelets from DSLS and DSHS. Top, TG-sensitive \(^{45}\)Ca\(^{2+}\) uptake (nmol/mg protein) in DSLS and DSHS (\(n=5\)). \(^{45}\)Ca\(^{2+}\) uptake was significantly greater in platelets from DSHS by ANOVA. Bottom, Rate of TG-sensitive uptake over initial 10 minutes (nmol/mg protein per minute) was significantly higher in platelets from DSHS compared with DSLS (unpaired \(t\) test).

Figure 7. Effect of NO on refilling of IM-releasable platelet Ca\(^{2+}\) stores. Upper trace shows experiments performed in absence of extracellular Ca\(^{2+}\). NO (1 μmol/L) was added 30 seconds before Thr (2.5 U/mL). IM (5 μmol/L) was applied 5 minutes after Thr to release all stored Ca\(^{2+}\). Example shown is that of DSLS platelets in which Ca\(^{2+}\)/IM is larger in platelets treated with NO. Bottom, Peak ionomycin-induced Fura-2 ratio was analyzed; peak ratio responses are shown in bar graph. NO significantly increased IM peak response in DSLS but not in DSHS (\(n=4\)).

NO can promote Ca\(^{2+}\) reuptake into the stores. To examine the effect of dietary salt on the ability of NO to promote Ca\(^{2+}\) reuptake into intracellular stores, the effect of NO on IM-releasable Ca\(^{2+}\) store in platelets from DS rats fed low-salt and high-salt diets are compared in Figure 7. In DS rats fed the low-salt diet, NO significantly increased the amount of Ca\(^{2+}\) in IM-releasable stores by 38%, whereas the initially larger Ca\(^{2+}\) store in platelets of DS rats fed the high-salt diet was not significantly affected by NO.

**Discussion**

This study demonstrates that a high-salt diet increases blood pressure and the amount of Ca\(^{2+}\) in intracellular stores in platelets from DS rats. We also found that the increase in Ca\(^{2+}\) in the stores could be explained by an increase of their refilling by higher SERCA activity. The increase in Ca\(^{2+}\) stores was associated with a decrease in agonist-induced Ca\(^{2+}\) entry as well as an apparent decrease in the ability of NO to further refill intracellular Ca\(^{2+}\) stores. Antihypertensive treatment prevented the rise in blood pressure that occurred with the high-salt diet, but the changes in platelet Ca\(^{2+}\) stores still occurred. This indicates that the change in platelet Ca\(^{2+}\) stores is unrelated to the increased blood pressure itself. Furthermore, because this alteration in Ca\(^{2+}\) homeostasis did not occur in salt-resistant rats given a high-salt diet, this result suggests that the change in platelet Ca\(^{2+}\) stores is linked to the genetic susceptibility to salt-induced hypertension in the DS rat.

Because of the similarities in Ca\(^{2+}\) homeostasis between platelets and smooth muscle and their easy accessibility from patients, platelets have been used as an attractive model to explore the relationship between Ca\(^{2+}\) homeostasis and vascular diseases. Earlier studies found that resting platelet
[Ca\textsuperscript{2+}], is increased in both human essential hypertension\textsuperscript{6} and spontaneously hypertensive rats and that there is a positive relationship between resting platelet Ca\textsuperscript{2+} concentration and blood pressure.\textsuperscript{3} However, the changes in platelet resting [Ca\textsuperscript{2+}], in DS rats fed the high-salt diet are controversial. Vasdev et al.\textsuperscript{8,17} found that in DS rats fed a high-salt diet, platelet resting [Ca\textsuperscript{2+}] increased. On the other hand, Ishida et al\textsuperscript{9} found that high dietary salt decreases resting [Ca\textsuperscript{2+}], in platelets from DS rats.

Recently, it was suggested that in human platelets, the resting [Ca\textsuperscript{2+}], is neither an indicator of blood pressure level nor of platelet Ca\textsuperscript{2+} homeostasis.\textsuperscript{18} These investigators showed that in a heterogeneous population of patients, the blood pressure positively correlated with the Ca\textsuperscript{2+} in the dense tubular system, \(V_{\text{max}}\) of SERCA, and SERCA protein expression. They also showed that platelets from black Americans have a greater amount of Ca\textsuperscript{2+} in intracellular stores than those from white Americans.\textsuperscript{19} Our results provide evidence in rat platelets that the amount of Ca\textsuperscript{2+} in intracellular stores increases in salt-sensitive hypertension, which is also a characteristic type of hypertension in blacks. Thus, it may be that intracellular Ca\textsuperscript{2+} stores are a better indication of platelet Ca\textsuperscript{2+} homeostasis both in human and rat platelets in pathological conditions.

Apart from the increase in the amount of Ca\textsuperscript{2+} in platelet intracellular Ca\textsuperscript{2+} stores, we found a decrease in agonist-stimulated Ca\textsuperscript{2+} influx in platelets of salt-sensitive rats fed a high-salt diet. This is consistent with a store-operated mechanism governing Ca\textsuperscript{2+} influx in rat platelets. In a variety of cell types, Ca\textsuperscript{2+} entry is thought to be dependent on agonist-induced Ca\textsuperscript{2+} release and depletion of Ca\textsuperscript{2+} stores.\textsuperscript{11} Although the mechanism that relays the extent of the filling of Ca\textsuperscript{2+} stores in the dense tubules to the plasma membrane to regulate external Ca\textsuperscript{2+} influx is not fully understood, Kimura et al\textsuperscript{20} showed that in normal human platelets, overloading intracellular Ca\textsuperscript{2+} stores decreases agonist-evoked external Ca\textsuperscript{2+} entry. Our study indicates that pathological overloading of platelet intracellular Ca\textsuperscript{2+} stores, at least in those of hypertensive DS rats, may decrease agonist-induced Ca\textsuperscript{2+} entry.

Unlike reports indicating that Thr-evoked Ca\textsuperscript{2+} entry is increased in rats with spontaneous hypertension\textsuperscript{21} or patients with diabetes,\textsuperscript{22} Dicha et al\textsuperscript{22} showed that in both salt-dependent hypertensive Sabra or DS rats, there was neither a marked elevation of basal [Ca\textsuperscript{2+}], nor an augmented response to Thr. Ishida et al\textsuperscript{23} described significantly lower [Ca\textsuperscript{2+}]. In resting platelets of DOCA salt–treated rats and unchanged responses to thrombin. It appears that alterations in platelet Ca\textsuperscript{2+} handling are different in salt-dependent and spontaneous forms of genetic hypertension. The mechanism for this difference is not clear, although our study suggests that increased Ca\textsuperscript{2+} in the intracellular stores may explain decreased agonist-induced Ca\textsuperscript{2+} influx.

An increased amount of Ca\textsuperscript{2+} in the intracellular stores may be due to decreased Ca\textsuperscript{2+} release or increased Ca\textsuperscript{2+} reuptake. This study provides two lines of evidence to suggest that augmented Ca\textsuperscript{2+} reuptake is responsible for the increase in the amount of releasable Ca\textsuperscript{2+} in the intracellular stores. The first is that in DS rats fed a high-salt diet, the capacity to directly refill Ca\textsuperscript{2+} stores is increased. When platelets from DS rats were suspended in Ca\textsuperscript{2+}-free medium and then in the presence of extracellular Ca\textsuperscript{2+}, the increase in IM-releasable Ca\textsuperscript{2+} from the store was greater than that in DS rats fed a low-salt diet. Because SERCA is the main mechanism responsible for Ca\textsuperscript{2+} reuptake, it is logical to propose that sequestering mechanisms of Ca\textsuperscript{2+} into intracellular Ca\textsuperscript{2+} stores are more active in DS rats fed a high-salt diet. Second, increased SERCA activity was directly demonstrated by measuring \(^{45}\text{Ca}^{2+}\) uptake in platelet lysates. Several studies have reported that platelet SERCA activity is related to blood pressure and is increased in spontaneously hypertensive rats and hypertensive patients. Resink et al\textsuperscript{24} have documented a substantial increase in platelet SERCA activity in patients with essential hypertension. Papp et al\textsuperscript{25} showed that the total Ca\textsuperscript{2+} ATPase activity in mixed platelet membranes isolated from spontaneously hypertensive rats was higher than that in Wistar-Kyoto rats. Our finding that SERCA activity is increased in platelets of DS rats fed a high-salt diet is consistent with an increased expression of SERCA protein. The fact that antihypertensive treatments did not prevent the changes in platelet Ca\textsuperscript{2+} stores suggests that the changes observed in SERCA activity may be due to the genetic response to a high-salt diet rather than to hypertension itself.

Higher SERCA activity might also explain the observation that the effect of NO on intracellular Ca\textsuperscript{2+} stores is decreased in platelets from DS rats fed a high-salt diet. Previous studies in our laboratory showed that NO inhibits store-operated Ca\textsuperscript{2+} influx in human platelets by promoting SERCA-dependent refilling of Ca\textsuperscript{2+} stores.\textsuperscript{13} This effect of NO could explain the entirety of its ability to reduce [Ca\textsuperscript{2+}], because its effects were blocked by inhibitors of SERCA. In rat platelets, we also found that after Ca\textsuperscript{2+} is released from the stores by Thr, NO increases the amount of Ca\textsuperscript{2+} in the IM-releasable stores. The change caused by NO was less in DS rats fed the high-salt diet. This might be interpreted to indicate that in DS rats fed a high-salt diet, SERCA activity is upregulated and the amount of Ca\textsuperscript{2+} in intracellular stores is already greater, so when NO was applied, the augmentation in store by NO was relatively smaller. This could mean that the amount of Ca\textsuperscript{2+} in intracellular stores is near maximal in hypertensive DS rats.

Summary
This study demonstrates that a high-salt diet, associated with the development of hypertension in DS rats, increased platelet SERCA activity and the amount of Ca\textsuperscript{2+} in intracellular stores and decreased agonist-induced Ca\textsuperscript{2+} entry and the response to NO. The modifications in Ca\textsuperscript{2+} stores may also occur in other cell types. The increased amount of Ca\textsuperscript{2+} in platelet intracellular stores and decreased response to NO in platelets may be involved in the pathogenesis of salt-induced hypertension and in the development of cardiovascular complications.

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References


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