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# Altered Vascular Calcium Regulation in Hypertension

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The present study was aimed at investigating whether the vascular calcium regulation is altered in hypertension. Two-kidney, one clip (2K1C) and deoxycorticosterone acetate (DOCA)-salt hypertension were made in rats, and their thoracic aortae were taken 4 weeks later. The isometric contractile response and calcium uptake of the endothelium-denuded aortic preparations were determined. Caffeine (0.1～35 mmol/L) induced a greater contraction in 2K1C and DOCA-salt hypertension than in normotensive control. When the vascular calcium store was functionally-depleted by a repeated exposure to caffeine, it took longer to reload the store and to resume the initial contraction force in response to caffeine in both 2K1C and DOCA-salt hypertension. The vascular $^{45}Ca$ uptake following the functional depletion of the cellular store was also greater in both models of hypertension than in control. Ryanodine, calcium channel activator of the sarcoplasmic reticulum, attenuated the restoration of caffeine-induced vascular contraction, which was not affected by either 2K1C or DOCA-salt hypertension. Nifedipine, an L-type $Ca^{2+}$ channel blocker, attenuated the restoration of caffeine-induced contraction, which was not affected by DOCA-salt hypertension, but was more pronounced in 2K1C hypertension. Nifedipine also diminished the vascular $^{45}Ca$ uptake, which was not affected by DOCA-salt hypertension, but was more pronounced in 2K1C hypertension. Ouabain, a $Na^+$, $K^+$-ATPase inhibitor, increased the caffeine-induced contraction by a similar magnitude in control and 2K1C hypertension, which was, however, markedly attenuated in DOCA-salt hypertension. Ouabain enhanced the vascular $^{45}Ca$ uptake, the degree of which was not affected by 2K1C hypertension, but was markedly attenuated in DOCA-salt hypertension compared with that in control. Cyclopiazonic acid, a selective inhibitor of $Ca^{2+}$-ATPase of the sarcoplasmic reticulum, attenuated the restoration of caffeine-induced contraction, which was not affected by 2K1C hypertension, but was more marked in DOCA-salt hypertension. These results suggest that the increased vascular calcium storage may be attributed to an enhanced calcium influx in 2K1C hypertension, and to an impaired $Na^+$-$K^+$ pump activity of the cell membrane and subsequently increased calcium pump activity of the cellular store in DOCA-salt hypertension.

Key Words: Vascular calcium store, Caffeine-sensitive, 2K1C hypertension, DOCA-salt hypertension, $^{45}Ca$ uptake

## INTRODUCTION

The concentration of calcium ions in the vascular smooth muscle cell is the single most important factor initiating and maintaining the vascular contractility. Chronic hypertension has been associated with increased calcium levels in the vascular smooth muscle cell, which is in turn causally related with an attenuated response to vasodilators as well as an augmented response to vasoconstrictors (Cheng & Shibata, 1981).

There are two physiological sources for the delivery of calcium in the vascular smooth muscle. One supplies calcium through voltage- and ligand-

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dependent membrane channels, opened by electrical stimuli and vasoactive agents, respectively. The resulting contractile responses are of tonic character and exhibit a great susceptibility to the inhibition of calcium antagonists. The other pathway consists of calcium delivery through the release from cellular storage sites. The resultant contraction responses are of phasic nature and are less sensitive to changes in transmembrane calcium supply.

It has been reported that calcium storage of the vascular sarcoplasmic reticulum is increased in genetic hypertension, resulted from an increased calcium permeability through the sarcolemma (Bukowski, 1990; Kanagy et al, 1994). However, the vascular calcium regulation in secondary hypertension such as renal hypertension largely remains unexplored.

The present study was aimed at investigating if and how the vascular calcium regulation are altered in hypertension. Two-kidney, one clip (2K1C) and deoxycorticosterone acetate (DOCA)-salt hypertension were made in rats. The calcium capacity, calcium release and calcium loading characteristics of the cellular store were determined by examining vasocontraction response to caffeine and assessing $^{45}Ca$ uptake of the thoracic aorta isolated from these two models of hypertension.

## METHODS

*Development of 2K1C and DOCA-salt hypertension*

2K1C hypertension was made using male Sprague-Dawley rats (160~200 g) by constricting the left renal artery with a silver clip having an internal gap of 0.2 mm under ketamine anesthesia. The contralateral kidney was left untouched. They were kept for 4 weeks until used. DOCA-salt hypertension was made using rats of the same species. One week after left unilateral nephrectomy, the animals were subcutaneously implanted with silastic DOCA (200 mg/kg) strip under ketamine anesthesia. They were then supplied with 0.9% NaCl drinking solution until used 4 weeks later. Arterial blood pressure and heart rate were directly measured under thiopental (50 mg/kg, ip) anesthesia. Age-matched and non-treated rats were used as control.

*Recording isometric tension*

Thoracic aorta was taken and prepared into rings, 4~5 mm long each. The endothelium was destructed by gently rubbing the lumen of the ring with a cotton swab. The ring was suspended in a tissue bath (5 mL) containing physiological salt solution (PSS) at 37°C, while being continuously bubbled with 95% $O_2$-5% $CO_2$ (pH 7.4). The composition (in mmol/L) of the PSS used was: NaCl 130, KCl 4.7, $NaHCO_3$ 14.9, $KH_2PO_4$ 1.18, $MgSO_4$ 1.17, EDTA 0.026, dextrose 5.5, and $CaCl_2$ 1.6. For a $Ca^{2+}$-free PSS, $CaCl_2$ was omitted and EGTA ($5\times10^{-5}$ mol/L) was added.

One end of the ring was fixed to the bottom of the bath and the other attached to a force-displacement transducer (Grass Model FT03, Quincy, MA, USA). The ring was stretched using a passive force (2.0 g), and allowed to stand for 1~2 h before starting the experimental protocol.

Caffeine has been known to cause contraction by directly releasing calcium from the sarcoplasmic reticulum, being independent of second messengers (Kanaide et al, 1987). Therefore, to deplete the vascular calcium store, the aortic preparations were exposed to repeated challenges with caffeine (35 mmol/L, for 5 min each) in the $Ca^{2+}$-free media. When no further contraction was eventually obtained by the caffeine, calcium was reintroduced in the media to reload the intracellular calcium stores. The media were then again replaced with $Ca^{2+}$-free PSS, and contractile responses to caffeine were recorded after 1~3 min.

*$^{45}Ca$ uptake*

$^{45}Ca$ uptake of the aortic preparation was measured as described by previous investigators (van Breemen et al, 1995). After 1-h equilibration period elapsed in unlabelled PSS, aortic segments without functional endothelium were incubated for 5 min in a medium containing $^{45}Ca$ (0.5 μCi/mL). The tissues were then washed for 50 min in ice-cold $Ca^{2+}$-free PSS containing EGTA (2 mmol/L) to remove extracellular $^{45}Ca$ of the vasculature. Aortic segments were dissolved by incubating at 55°C overnight in 0.5 N NaOH-5% SDS solution. Scintillation cocktail (2 mL) was added to each vial, and the radioactivity of $^{45}Ca$ was counted in a liquid scintillation counter. The amount of uptake was presented as $^{45}Ca$ pmole/g

tissue.

*Drugs*

Drugs used were DOCA, caffeine, nifedipine, ouabain, phenylephrine, ryanodine, and cyclopiazonic acid (CPA), all purchased from Sigma Chemical Company (St. Louis, MO, USA). CPA was dissolved in DMSO (<0.01%). Ryanodine, nifedipine and ouabain were prepared in absolute ethanol (<0.001%). Other reagents were dissolved in distilled water. $^{45}Ca$ was obtained from Dupont Company (Boston, MA, USA).

*Statistical analysis*

Values are expressed as means±SEM. The statistical significance of the difference between groups was evaluated by one-way ANOVA followed by Bonferroni's test for multiple comparisons. The concentrations producing a half-maximal response ($EC_{50}$) were determined after logic transformation of normalized dose-response curve.

## RESULTS

*Arterial pressure and heart rate*

Table 1 shows mean arterial pressure, heart rate, and body weight in control, 2K1C, and DOCA-salt rats. The arterial pressure was significantly higher in 2K1C and DOCA-salt rats than in control. The heart rate in 2K1C rats was lower than that in control. Body weight did not significantly differ among the groups.

**Table 1.** Mean arterial pressure (MAP), heart rate (HR), and body weight (BW) in control, 2K1C, and DOCA-salt rats

| | Control (n=12) | 2KlC (n=10) | DOCA (n=10) |
|---|---|---|---|
| MAP (mmHg) | 129±10 | 179±15** | 172±12** |
| HR (beats/min) | 383±13 | 335±18* | 368±15 |
| BW (g) | 320±10 | 300±11 | 340±14 |

Values are means±SEM. n=number of rats. *p<0.05, **p<0.01; compared with control.

*Calcium capacity of the caffeine-sensitive store*

Aortic rings were repeatedly challenged with caffeine (35 mmol/L) in $Ca^{2+}$-free PSS. This procedure culminated in a functional depletion of calcium of caffeine-sensitive cellular stores, as evidenced by a failure of the caffeine to induce a further contraction.

When no contraction was eventually obtained by the caffeine, calcium was reintroduced in the media to reload the store with calcium. Fig. 1 shows con-

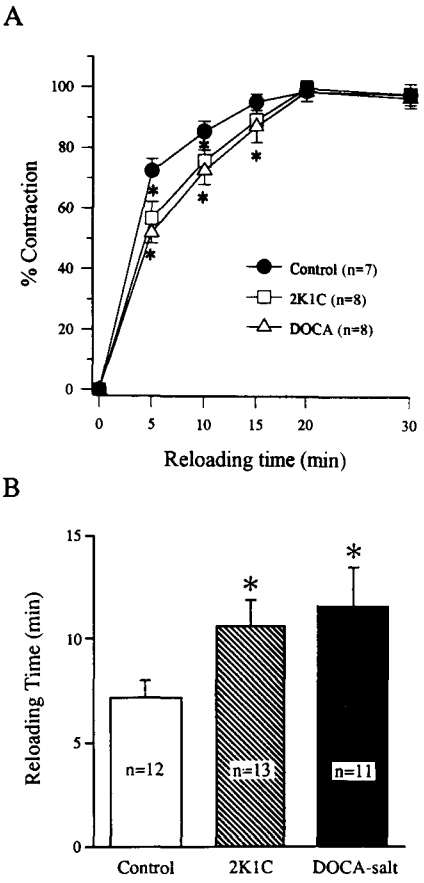


**Fig. 1.** A: Contraction responses to caffeine (35 mmol/L) as a function of reloading time. Aortic rings were repeatedly exposed to caffeine (35 mmol/L) in $Ca^{2+}$-free PSS containing EGTA (50 μmol/L), and then were reintroduced with calcium (1.6 mmol/L). B: Reloading time to reach 75% of the initial maximal contraction to caffeine. n=number of experiments. *p<0.05, compared with control.

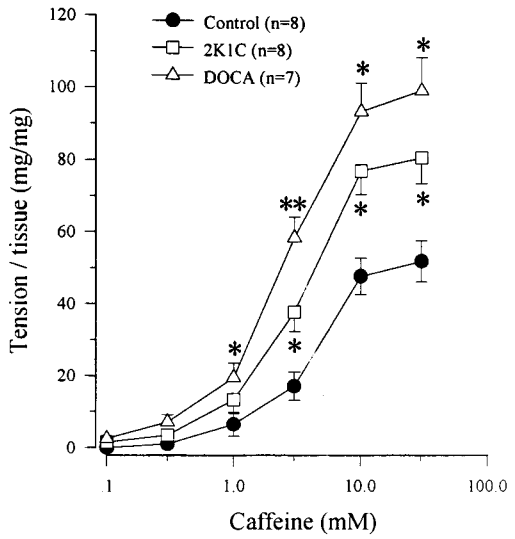


**Fig. 2.** Contractile responses to caffeine (35 mmol/L) of the aortic rings which had been depleted of and reloaded with calcium for 20 min. *p<0.05, **p<0.01; compared with control.

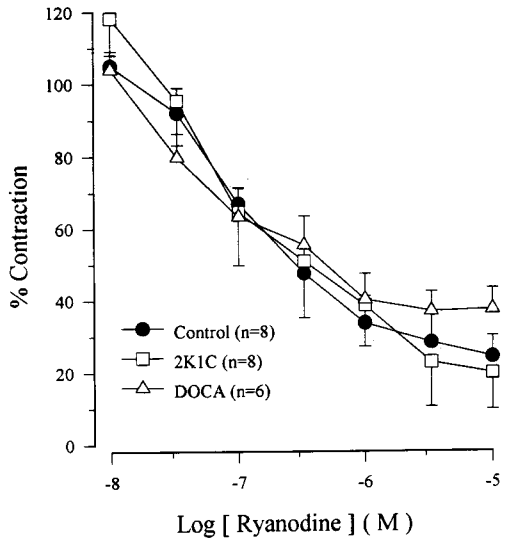


**Fig. 4.** Effects of ryanodine on the caffeine (35 mmol/L)-induced contraction. Ryanodine was added 5 min before reloading the calcium store for 20 min after its functional depletion.

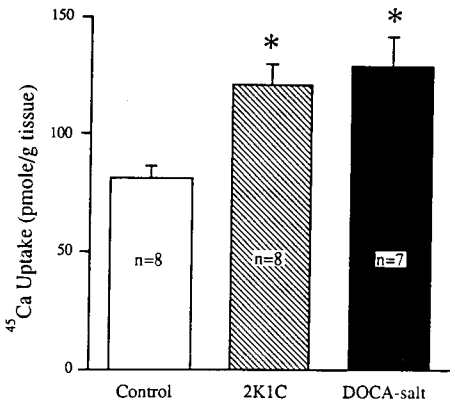


**Fig. 3.** Amounts of $^{45}$Ca uptake after a functional depletion of caffeine-sensitive $Ca^{2+}$ stores. The calcium was depleted by the repeated exposure to caffeine, and then $^{45}$Ca uptake was allowed for 7 min. *p<0.05, compared with control.

traction responses to caffeine as a function of loading time. The contractile force developed in response to caffeine (35 mmol/L) was increased in a reloading time-dependent manner. It took longer in hypertension than in control to reload the store and to resume the initial contraction force to caffeine.

Fig. 2 shows responses to caffeine of aortic rings reloaded with calcium for 20 min. Although the maximal contraction induced by caffeine was two-to three-fold greater in hypertension than in control, $EC_{50}$ did not significantly differ among them (5.1±0.8 mmol/L in control, 4.7±0.7 mmol/L in 2K1C hypertension, 4.0±0.6 mmol/L in DOCA-salt hypertension; n=6 each).

The amount of $^{45}$Ca uptake following the functional depletion of calcium stores was significantly higher in 2K1C and DOCA-salt hypertension than in control (Fig. 3).

### *Releasing property of the $Ca^{2+}$ store*

Fig. 4 illustrates the effect of ryanodine, applied in between the functional depletion of the calcium store and reintroduction of extracellular calcium, on contractile responses to caffeine. Ryanodine inhibited the caffeine-induced contraction in a dose-dependent manner, which was not affected by either 2K1C or DOCA-salt hypertension.

### *Refilling characteristics of the depleted $Ca^{2+}$ store*

Fig. 5 shows the effect of nifedipine on the caffeine-induced contraction and $^{45}$Ca uptake. Nifedipine-treatment inhibited the caffeine-induced contraction in a

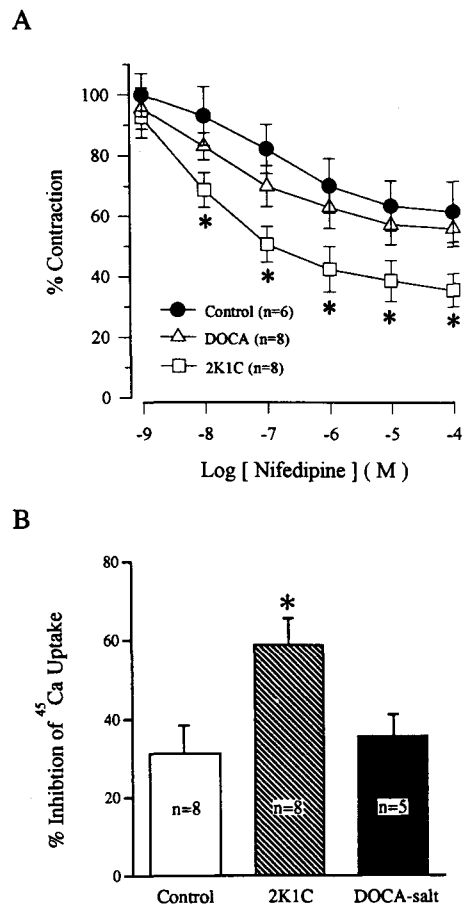


**Fig. 5.** Effects of nifedipine on the caffeine (35 mmol/L)-induced contraction and $^{45}Ca$ uptake. Nifedipine of each concentration was added 5 min before reloading the calcium store for 20 min (A). Data represent those obtained in the presence of $10^{-4}$ mol/L nifedipine (B). *p $<0.05$, compared with control.

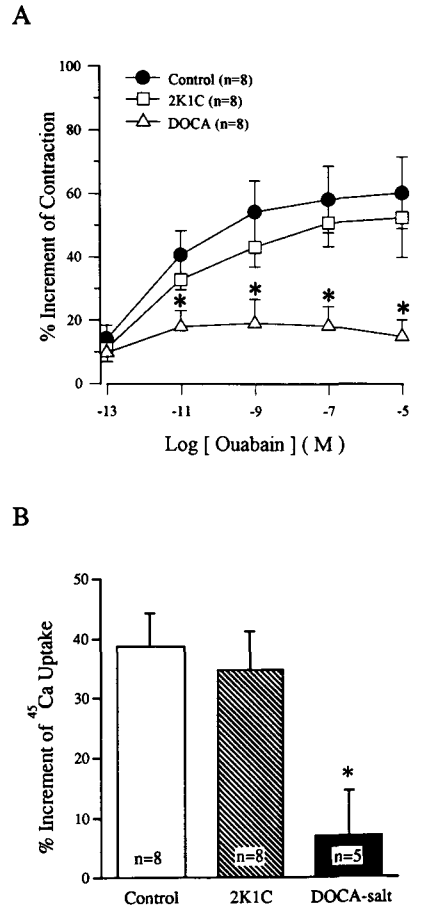


**Fig. 6.** Effects of ouabain on the caffeine (35 mmol/L)-induced contraction and $^{45}Ca$ uptake. Ouabain of each concentration was applied 5 min before reloading the calcium store for 20 min (A). Data represent those obtained in the presence of $10^{-4}$ mol/L ouabain (B). *p< 0.05, compared with control.

dose-dependent manner, which was not affected by DOCA-salt hypertension, but was more pronounced in 2K1C hypertension than in control. $^{45}Ca$ uptake of the depleted calcium store was markedly inhibited by nifedipine in 2K1C hypertension, while not affected in DOCA-salt hypertension.

Fig. 6 shows the effect of ouabain on the caffeine-induced contraction and $^{45}Ca$ uptake. Ouabain treatment enhanced the caffeine-induced contraction in a dose-dependent manner in 2K1C hypertension by a similar magnitude to that in control. On the contrary, ouabain did not affect the caffeine-induced contraction in DOCA-salt hypertension. $^{45}Ca$ uptake was enhanced by ouabain in 2K1C hypertension as in control, but was not affected by DOCA-salt hypertension.

Fig. 7 depicts the effect of CPA on the caffeine-induced contraction. CPA inhibited the caffeine-induced contraction, which was not affected by 2K1C hypertension, but was more pronounced in DOCA-salt hypertension compared with that in control.

## DISCUSSION

It has been widely shown that the intracellular calcium concentration is increased in hypertension (Cheng & Shibata, 1981; Suzuki et al, 1979). Although

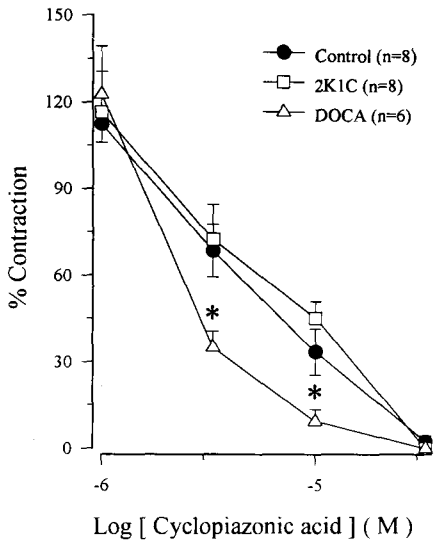


**Fig. 7.** Effects of cyclopiazonic acid on the caffeine (35 mmol/L)-induced contraction. Cyclopiazonic acid of each concentration was added 5 min before reloading the calcium stores for 20 min. *$p<0.05$; compared with control.

the activity of the plasmalemmal voltage-dependent $Ca^{2+}$ channel has been found to be increased in hypertension (Kruse et al, 1994), the function of the cellular calcium stores has not been well studied.

The present study showed a two-to-three-fold greater contractile force development in response to caffeine in both 2K1C and DOCA-salt hypertensive aortae than in normotensive control. In addition, the force developed was dependent on the time elapsed to reload the cellular store following its functional depletion, and it took longer in hypertension than in control to reload the cellular calcium store to resume the initial contraction force. The caffeine-induced contraction after depletion-reloading of the cellular calcium store as well as the amount of calcium uptake assessed using $^{45}Ca$ was enhanced in 2K1C and DOCA-salt hypertension, as has been shown in other types of hypertension (Rusch & Hermsmeyer, 1988; Storm et al, 1992; Ohya et al, 1993). However, $EC_{50}$ for caffeine did not significantly differ between either 2K1C or DOCA-salt hypertension and normotension. Therefore, the greater force development in these two models of hypertension cannot be attributed to an altered caffeine-sensitivity of the calcium store, but to an increased capacity of the store, being in accord with other types of hypertension (Bukowski, 1990; Sugiyama et al, 1990).

Kanagy et al (1994) suggested that the increased vascular calcium store in spontaneously hypertensive rats counteracts the elevated cytosolic calcium resulted from an increased calcium influx through the plasmalemma, while the calcium releasing-property of the sarcoplasmic reticulum is normal. In the present study, ryanodine, an opener of the sarcoplasmic reticular calcium channel, was found to attenuate the caffeine-induced contraction in hypertension by a similar magnitude to that in normotension, also suggesting that the channel property of the calcium store was not altered.

Three different pathways refilling the depleted calcium store have been sofar known: (1) the voltage-dependent $Ca^{2+}$ channel of the plasmalemma (Low et al, 1992; Xuan et al, 1992), (2) calcium pump of the store, and (3) $Na^{+}$-$Ca^{2+}$ exchange of the plasmalemma (Weiss et al, 1993; Borin et al, 1994). In the present study, nifedipine, a calcium channel blocker, caused an attenuation of the caffeine-induced contraction in 2K1C hypertension. Furthermore, $^{45}Ca$ uptake after a functional depletion of the cellular calcium stores was significantly decreased by nifedipine in 2K1C hypertension, but not in DOCA-salt hypertension. Taken together, the increased calcium storage in 2K1C hypertension may be attributed to an increase in the plasmalemmal calcium influx through the voltage-dependent channel. However, the mechanism sequestrating calcium in the intracellular storage beyond the step involving the plasmalemmal $Ca^{2+}$ channel is still unknown.

A reduced $Na^{+}$-$K^{+}$ pump activity has been demonstrated in DOCA-salt hypertension (Hamlyn et al, 1988; Pamnani et al, 1978; Pamnani et al, 1989). The impaired activity of the $Na^{+}$-$K^{+}$ pump may enhance the reverse $Na^{+}$-$Ca^{2+}$ exchange, resulting in an increased $Ca^{2+}$ influx (Weiss et al, 1993). In the present study, ouabain, a $Na^{+}$-$K^{+}$ ATPase inhibitor, enhanced the caffeine-induced contraction in 2K1C hypertension and normotension. However, it did not affect the contraction in DOCA-salt hypertension, reflecting an already-reduced $Na^{+}$-$K^{+}$ pump activity. In addition, ouabain was of little effect in enhancing $^{45}Ca$ uptake in DOCA-salt hypertension. On the contrary, the inhibited caffeine-induced contraction due to the treatment with CPA, a $Ca^{2+}$-ATPase inhibitor, before reloading calcium, was more prono-

unced in DOCA-salt hypertension, representing an increased calcium pump activity. It is likely that the activity of $Na^+$-$K^+$ pump is attenuated, and calcium pump activity of the cellular store is increased in DOCA-salt hypertension.

In conclusion, our results are consistent with the hypothesis that the vascular calcium storage is increased in 2K1C and DOCA-salt hypertension. The increased storage may be attributed to an enhanced calcium influx through the voltage-dependent calcium channel in 2K1C hypertension, and to an impaired plasmalemmal $Na^+$-$K^+$ pump activity and subsequently increased calcium pump activity of the cellular store in DOCA-salt hypertension.

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