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Altered Calcium Current of the Vascular Smooth Muscle in Renal Hypertension

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The present study was aimed at investigating whether the calcium current in the vascular smooth muscle (VSM) cells is altered in renal hypertension. Two-kidney, one clip (2K1C) and deoxycorticosterone acetate (DOCA)-salt hypertension were made in Sprague-Dawley rats. Rats without clipping the renal artery or implanting DOCA were used as control for 2K1C and DOCA-salt hypertension, respectively. Four weeks after clipping, systolic blood pressure was significantly higher in 2K1C rats than in control (192±24 and 119±4 mmHg, respectively, n=16 each). DOCA-salt rats also showed a higher blood pressure (180±15 mmHg, n=18) compared with control (121±6 mmHg, n=14). VSM cells were enzymatically and mechanically isolated from basilar arteries. Single relaxed VSM cells measured 5~10 μm in width and 70~150 μm in length were obtained. VSM cells could not be differentiated in size and shape between hypertensive and normotensive rats under light microscopy. High-threshold (L-type) calcium currents were recorded using whole-cell patch clamp technique. The amplitude of the current recorded from VSM cells was larger in 2K1C hypertension than in control. Neither the voltage-dependence of the calcium current nor the cell capacitance was significantly affected by 2K1C hypertension. By contrast, the amplitude of the calcium current was not altered in DOCA-salt hypertension. These results suggest that high-threshold calcium current of the VSM cells is altered in 2K1C hypertension, and that calcium channel may not be involved in calcium recruitment of VSM in DOCA-salt hypertension.

Key Words: Two-kidney, One clip hypertension, Deoxycorticosterone acetate-salt hypertension, Vascular smooth muscle, Calcium current

INTRODUCTION

Functional characteristics, sensitivity to vasodilator, calcium metabolism and calcium channel properties, of the vascular smooth muscle (VSM) may be altered of hypertension (Cheng & Shibata, 1981; Webb & Bohr, 1981; Mulvany, 1984). Previous studies have demonstrated that VSM calcium channel properties were altered in spontaneously hypertensive rats (Hermsmeyer & Rush, 1989; Storm et al, 1992; Ohya et al, 1993; Wilde et al, 1994; Lozinskaya & Cox, 1997). However, little has been known on the electrophysiological characteristics of the VSM cell in other models of hypertension, such as two-kidney, one clip (2K1C) and deoxycorticosterone acetate (DOCA)-salt hypertension.

Our recent study has demonstrated that the calcium uptake of VSM after functional depletion of calcium stores was inhibited by a calcium channel blocker and a Na^+, K^+-ATPase inhibitor in 2K1C and DOCA-salt hypertensive rats, respectively (Kim et al, 1997). This finding suggests different routes of calcium recruitment in these models of hypertension. 2K1C hypertension may be associated with an altered role of the membrane calcium channel, while DOCA-salt hypertension may not be.

The objective of the present study was to investigate whether calcium influx in VSM is altered in renal hypertension. After inducing 2K1C and DOCA-salt hypertension in rats, electrophysiological charac-

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teristics of calcium current in the VSM cells were determined using whole-cell patch clamp technique.

METHODS

Development of hypertension and vascular myocyte preparation

Adult male Sprague-Dawley rats were used. 2K1C hypertension was induced by clipping of a 0.2 mm silver clip at the left renal artery under ketamine (50 mg/kg, IP) anesthesia. To develop DOCA-salt hypertension, rats were subcutaneously implanted DOCA strip one week after the unilateral nephrectomy, and supplied 1.0% saline as drinking water thereafter. Sham-operated rats without clipping or implanting served as control. They were used 4 weeks after the surgery.

On the day of experiment, systolic blood pressure was indirectly measured by tail-cuff method. It was significantly higher in 2K1C rats than in the control (192 ± 24 and 119 ± 4 mmHg, respectively; n=16 each, $p < 0.01$). DOCA-salt rats also showed significant increased blood pressure (180 ± 15 mmHg, n=18; $p < 0.01$) compared with control (121 ± 6 mmHg, n=14).

Rats were then decapitated using a guillotine. The brain was removed and placed in chilled solution containing (mM) NaCl 145, KCl 6, HEPES 10, and glucose 10. The pH was adjusted to 7.3 with NaOH. The basilar artery was removed for dispersion using iridectomy scissors.

Vessels were cleared of dura and glial tissues and minced for better accessing to the enzymes. Vessel fragments in Ca^{2+}- and Mg^{2+}-free solution were incubated at 37°C for 30 min. They were then placed in 1 ml of 0.1 mM Ca^{2+} salt solution containing 2 mg collagenase, 0.5 mg papain (both from Worthington Biochemical Co, Freehold, NJ, USA), and 1 mg dithiothreitol (Sigma Chemical Co, St. Louis, MO, USA) at 37°C for 20 min. The fragments were then washed three times to remove enzymes in Kraft-Brühe (KB) solution containing (mM) glutamic acid 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, HEPES 10, and EGTA 0.5. The pH was adjusted to 7.3 with KOH. They were mechanically triturated with two or three fire-polished Pasteur pipettes, having a tip diameter of 1~3 mm. Single, relaxed VSM cells were plated on the poly-d-lysine coated cover-slips in a multiwell tissue culture plate for 5 min. The plate was kept in refrigerator at 4°C.

Electrophysiological procedures

Calcium currents were recorded from isolated VSM cells using conventional whole-cell patch clamp techniques. Currents were recorded using an Axopatch 200 A patch clamp amplifier (Axon Instruments; Burlingame, CA, USA), sampled at 2 to 5 kHz by a computer interfaced with Digidata 1,200 (Axon Instruments). The computer generated the voltage protocols and recorded the resulting currents using pClamp software (version 6.0.2, Axon Instruments). Cell capacitance was estimated from the dial settings for capacitative compensation on the voltage clamp amplifier. All recordings were performed at room temperature (18~22°C).

Recording electrodes with 3 to 5 MΩ of resistance were filled with internal saline containing (mM) CsCl 100, TEACl 20, EGTA 10, HEPES 10, $CaCl_2$ 0.01, and MgATP 2 at pH 7.3 adjusted with CsOH. The external solution contained (mM) choline-Cl 110, HEPES 10, $BaCl_2$ 10, and $MgCl_2$ 2 at pH 7.3 adjusted with CsOH. Ba^{2+} instead of Ca^{2+} was used as the charge carrier for the calcium currents to improve the signal-to-noise ratio and to obviate calcium induced inactivation of calcium channels (Aaronson, et al, 1988). When the solution was transferred to the electrode, plastic cannula was used to avoid the possible release of Cu^{2+} and Zn^{2+} from metallic needles (Nam & Hockberger, 1992). All chemicals except for enzymes were obtained from Sigma Chemical Company (St. Louis, MO).

Statistics

Data were expressed as mean values$\pm$SD and compared by unpaired Student's *t* test. A value of $p < 0.01$ was considered as significant.

RESULTS

VSM cells varied in size and shape upon enzymatic isolation. The relaxed VSM cells were 70 to 150 μm in length, and 5 to 10 μm in width. They exhibited mostly either straight spindle or curved shape. No differentiation could be made by the shape and size between the control and hypertensive groups.

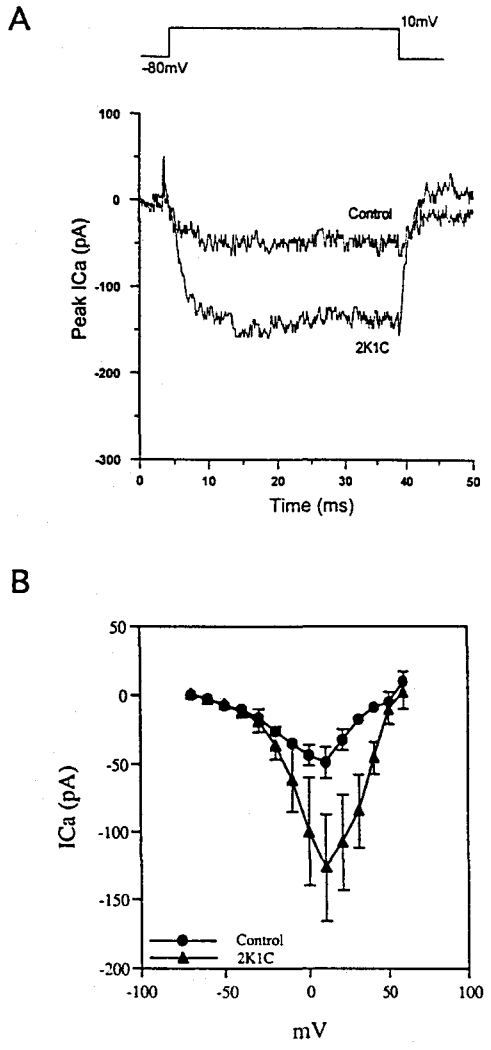

Fig. 1. High-threshold calcium currents in 2K1C hypertension and control. A. Peak currents in normotension (Control) and hypertension (2K1C) were generated by a single step with 35 ms duration to 10 mV from −80 mV. B. Current-voltage plots of the peak currents evoked by voltage steps from −70 mV to 60 mV. Circles represent control (n=11), and triangles denote 2K1C hypertension (n=16).

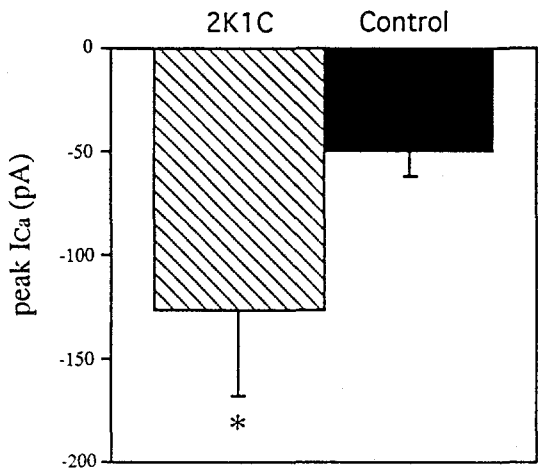

Fig. 2. Amplitude of peak calcium currents in 2K1C hypertension and control. Peak currents were elicited by a single step to 10 mV from −80 mV of holding potential in control (n=15) and 2K1C hypertension (n=26). *$p<0.01$; compared with control.

High-threshold calcium currents were obtained during 35 msec depolarizing steps to either 10 mV or −70 mV with 10 mV increments from a holding potential of −80 mV. The calcium currents were evoked by single step to 10 mV from −80 mV. The currents with fast activation and slow inactivation were recorded from VSM cells in both 2K1C and control groups (Fig. 1). Current-voltage (I-V) relationships of the current at steady-state were plotted to compare voltage dependence and threshold between two groups. The electrophysiological characteristics of the calcium current did not differ between 2K1C hypertension and control. However, the calcium current was significantly larger in 2K1C hypertension than in control (Fig. 2).

The calcium currents were also recorded from the DOCA-salt hypertensive and normotensive VSM cells under the same conditions as in 2K1C hypertensive cells (Fig. 3). The calcium current generated in DOCA-salt hypertension did not differ in voltage dependence, threshold and even magnitude from its respective control (Fig. 4).

I-V relationships obtained from steady-state calcium currents normalized with each cell capacitance is plotted in Fig. 5. Increased magnitude of calcium currents in 2K1C hypertension was not affected by cell surface area. Nor was the normalized calcium current different between DOCA-salt hypertension and control.

DISCUSSION

We observed that the high-threshold calcium current was increased in 2K1C hypertension. This finding is in line with the increased magnitude of the calcium current noted in other models of hypertension. In spontaneously hypertensive rats, for example, the calcium current was increased in the isolated cerebral artery (Wild et al, 1994), mesenteric artery (Cox & Lozinskaya, 1995; Lozinskaya & Cox, 1997),

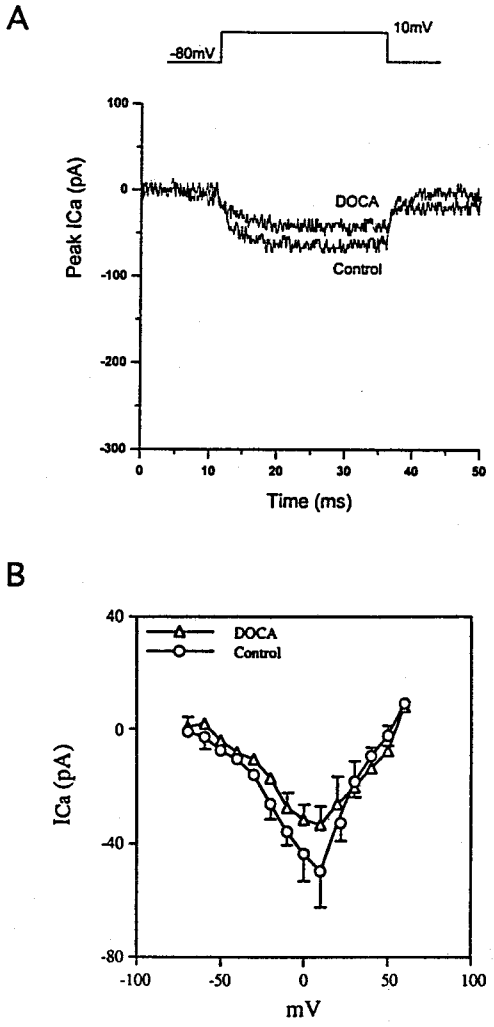

Fig. 3. Peak values and I-V relationships of calcium currents in the control and DOCA-salt hypertension. A. Peak calcium currents were generated by a single step with 25 ms duration to 10 mV from −80 mV in normotensive (Control) and hypertensive (DOCA) rats. B. I-V relationships of peak currents elicited by voltage steps from −70 mV to 60 mV in control (n=10) and hypertension (DOCA, n=12).

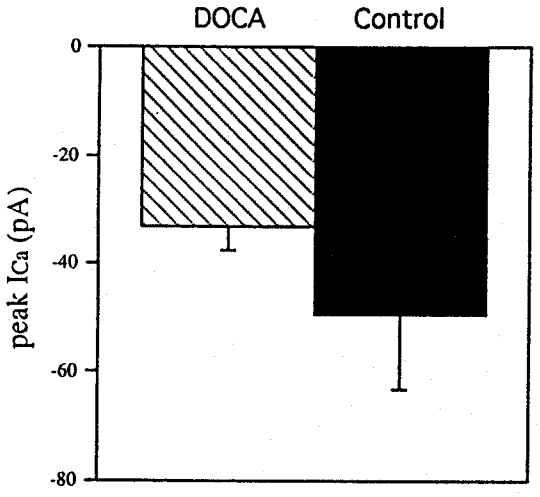

Fig. 4. Amplitudes of peak calcium currents in DOCA-salt hypertension and control. Peak currents were elicited by a single step to 10 mV from −80 mV of holding potential in normotensive (Control, n=12) and hypertensive (DOCA, n=16) rats.

and in primary cultured cells of azygos vein (Hermsmeyer & Rush, 1989). The high-threshold current may be more important in contributing to the calcium flux than the low-threshold calcium current, since the former has more slow inactivation and larger amplitude (Fox et al, 1987). Furthermore, the magnitude of low-threshold current remained unchanged in VSM cells of spontaneously hypertensive rat (Hermsmeyer & Rush, 1989). Therefore, the increased calcium current through the high-threshold channel may be functionally relevant in contributing to an increased vascular tone in 2K1C hypertension.

The changes in calcium current may result from altered properties, availability, or expression of the channel. Recently, single channel study in VSM demonstrated no changes in calcium channel properties in renal hypertensive rats (Simard et al, 1998). The increased calcium current in the whole cell may then be a reflection of an increased availability of the functional calcium channel in renal hypertension. However, the larger calcium current cannot be attributed to differences in voltage dependence. When the peak calcium current was recorded at the same step potential to 10 mV in both 2K1C and control rats, voltage dependence of the current was comparable between them. Our observation was in agreement with previous investigations showing little or no shift in voltage dependence in isolated cerebral artery (Wild et al, 1994) or mesenteric artery of spontaneously hypertensive rats (Cox & Lozinskaya, 1995).

The augmented calcium current may also be accounted for by a bigger size of the cell. Single relaxed cell with 70 to 100 μm in length and 10 μm in width was used in both hypertensive and control groups. This size of VSM cells in hypertensive rats is similar to that in normotensive rats (Archer et al, 1996) and spontaneously hypertensive rats (Bolzen & Cheung, 1989). Cell surface area may be directly esteemed by a normalization of the current with its capacitance. When the magnitude of the calcium

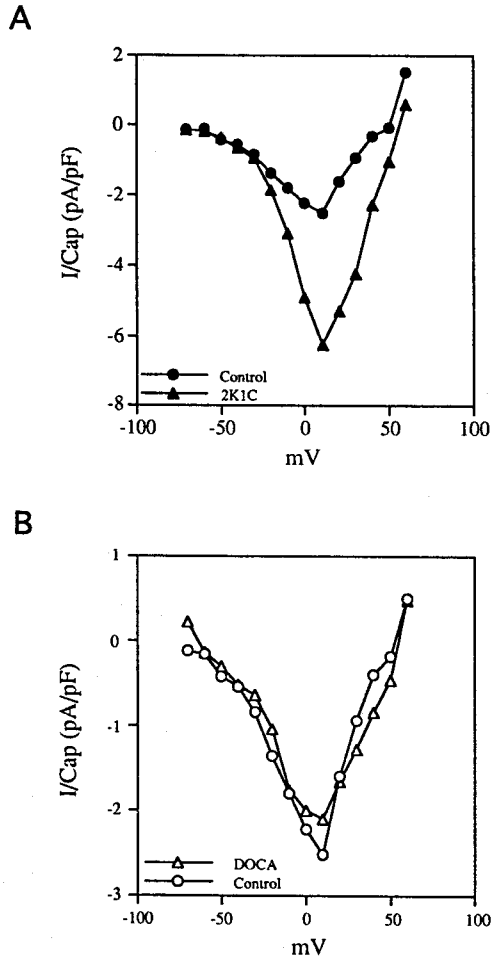

Fig. 5. I-V relationships of the normalized high-threshold calcium current. The amplitude of the steady-state calcium current was normalized with cell capacitance and plotted against membrane potentials in the control, 2K1C (A) and DOCA-salt (B) hypertension. No significant difference in voltage dependence was noted between before and after normalization.

current was normalized with the cell capacitance in 2K1C hypertension, it was still larger than that in the control, indicating that the cell size is not responsible for the larger calcium current.

By comparison, DOCA-salt hypertension showed no changes in the magnitude of calcium currents. Furthermore, there were no significant differences in properties of calcium currents after normalization with cell capacitance. Our results in DOCA-salt hypertension may provide an important insight to understand its hypertensive mechanism. The mechanisms recruiting intracellular calcium ions may differ between 2K1C and DOCA-salt hypertension. The speculation is supported by the different modes of vascular calcium uptake after functional depletion of the cellular calcium store between them (Kim et al, 1997). Alterations of Na^+-K^+ pump activity in vascular smooth muscle cells have also been observed in DOCA-salt hypertension (Soltis & Field, 1986; Hamlyn et al, 1988; Soszynski et al, 1997). Taken together, the calcium ions may increase in the cell by way of inactivation of plasamalemmal Na^+-K^+ pump or by release from the intracellular calcium stores rather than through the voltage-dependent calcium channels in DOCA-salt hypertension.

In summary, an augmented high-threshold calcium current in VSM was observed in 2K1C hypertension, while electrophysiological properties of the high-threshold calcium current were not altered in DOCA-salt hypertension. These results suggest that the calcium influx in VSM occurs in different manners between 2K1C and DOCA-salt hypertension. Further studies will be needed to understand the precise role of the altered calcium current.

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