Ca\textsuperscript{2+}–Sensitivity of Portal Vein Circular Muscle from Normotensive and Hypertensive Rats

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Abstract

Contractile sensitivity to Ca\textsuperscript{2+} was studied in rat portal vein circular muscle isolated from normotensive Wistar Kyoto rats (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP). There was no difference in resting membrane potential between WKY and SHRSP. Elevation of extracellular K\textsuperscript{+} concentration induced similar increase in tension in preparations from WKY and SHRSP. High K\textsuperscript{+}-depolarized preparation developed tension increase when extracellular Ca\textsuperscript{2+} concentration was increased. The sensitivity to extracellular Ca\textsuperscript{2+} was similar in preparations from WKY and SHRSP. Permeabilized preparation contracted when free Ca\textsuperscript{2+} concentration was increased. Sensitivity of permeabilized preparation to Ca\textsuperscript{2+} was similar in preparations from WKY and SHRSP. These results indicate that regulation of contraction by Ca\textsuperscript{2+} was similar between WKY and SHRSP. Since there was no change in high K-induced contraction in intact preparations or Ca-induced contraction in permeabilized preparations, there seemed to be no difference in Ca\textsuperscript{2+} influx via voltage-dependent channels in portal vein circular muscle from WKY and SHRSP.

Key words: portal vein, circular muscle, SHRSP, skinned fiber, Ca-sensitivity

Introduction

Clinical and animal studies on genetic hypertension have indicated importance of Ca\textsuperscript{2+} in pathological cardiovascular functions (Lau and Eby, 1985). Although cardiac mechanisms may be involved in development of arterial hypertension (Tarazi et al., 1983), vascular system is responsible in maintenance of hypertension by mechanisms such as increase of total peripheral resistance (Folkow 1982).

Because Ca\textsuperscript{2+} plays a key role in smooth muscle contraction (Van Breemen, 1989; Horowitz et al., 1996; Karaki et al., 1997), changes in Ca\textsuperscript{2+} sensitivity of vascular smooth
muscle is one of fundamental items to understand pathophysiological mechanism of hypertension (Daniel and Kwan, 1981; Bohr and Webb, 1988).

In arterial smooth muscles, contractile response to extracellular Ca\(^{2+}\) in depolarized mesenteric artery was not different between Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) (Mulvany and Halpern, 1976; Aoki and Asano 1986). In permeabilized preparations from hypertensive rats, response to intracellular Ca\(^{2+}\) has been shown to be not different from WKY in tail artery (Mrwa et al., 1986; Nghiem and Rapp, 1983) and increased in aorta (Soloviev and Bershtein, 1992).

Although increase in venous tone may contribute to the blood pressure regulation by increasing cardiac output and thus increasing arterial pressure, less information is available on venous smooth muscle from hypertensive rats. Most studied on venous smooth muscle was performed in portal vein. Depolarized preparation of longitudinal muscle in rat portal vein was sensitive to extracellular Ca\(^{2+}\) (Biamino and Johansson, 1970). In longitudinal preparation of portal vein from SHR, sensitivity to extracellular Ca\(^{2+}\) was reported to be increased (Harris et al., 1984) or same (Greenberg and Bohr, 1975) when compared with that of WKY. We have reported that the Ca\(^{2+}\)-induced contraction in K\(^{+}\)-depolarized preparation was greater in the preparation from stroke-prone SHR (SHRSP; Okamoto et al., 1974) compared with that of preparation from WKY, without change of sensitivity to extracellular Ca\(^{2+}\) (Shimamura et al., 1989). In permeabilized preparations of longitudinal smooth muscle of portal vein from hypertensive rats, response to intracellular Ca\(^{2+}\) has been shown to be increased (Soloviev and Bershtein, 1992).

In circular muscle of portal vein from hypertensive rats, no difference was observed in response to KCl (Greenberg and Wilborn, 1982) and no information is available on Ca\(^{2+}\) sensitivity. In the present study we studied on the sensitivity to Ca\(^{2+}\) in circular muscle of portal vein from normotensive and hypertensive rats. Part of this study was reported in a meeting abstract (Shimamura et al., 1997).

Materials and methods

Portal vein was dissected under ether anesthesia from 16-week-old male normotensive WKY and SHRSP. Ring preparation of 1 mm width was made from the portal vein by fine scissors under a binocular microscope. Usually two ring preparations were made from a portal vein. Endothelium was removed by gentle rubbing of luminal surface.

In the experiment of intact preparation, each ring preparation was suspended horizontally with a pair of tungsten wire (50 μm diameter) in a 5 ml organ bath filled with a modified Tyrode’s solution. Isometric contraction was measured by a force-displacement transducer (Minebea UL–10GR, Karuizawa, Japan) and a carrier amplifier (NEC–San-ei, Transducer Ampl 1829, Tokyo, Japan) and recorded on a thermal pen recorder (NEC–San-ei, RECTI-HORIZ-8K). Composition of the modified Tyrode’s solution was (in mM): NaCl 137, KCl 5.4, NaHCO\(_3\) 11.9, NaH\(_2\)PO\(_4\) 0.4, CaCl\(_2\) 2.0, MgCl\(_2\) 1.0, glucose 5.6, aerated with 95% O\(_2\) and 5% CO\(_2\) pH7.3 at 37°C. High K\(^{+}\) Tyrode solution was made by equimolar replacement of NaCl with KCl. Preparation was set in a nominally Ca\(^{2+}\)-free Tyrode solution (made by omitting CaCl\(_2\)
under a resting tension of 4 mN which stretched preparation to around 150% of resting length. Experiment was started after equilibration for 1 hour in the normal Tyrode solution and exposure to 80 mM K⁺ Tyrode solution for 10 min twice. Experiment in the presence of high K⁺ Tyrode solution was performed in the presence of 1 µM of atropine and 10 µM of guanethidine to exclude contribution of cholinergic and adrenergic nerves.

In experiment with permeabilized preparation, both ends of a circular muscle thin strip (50 µm thick, 100 µm width, 2 mm long) were tied to tungsten wire rings with silk thread. The preparation was set horizontally in a chamber (1 ml) at 25°C under a binocular microscope. One of the wire ring was connected to a micro-manipulator (Narishige, MM-31, Tokyo, Japan) and another was connected to an isometric force-displacement transducer (Minebea, UL-2GR). After equilibration in a physiological salt solution for 1 hr, the preparation was contracted by an 80 mM KCl solution and then permeabilized according to a previous method (Gonzalez et al., 1995). Briefly, the strip was incubated in a relaxing solution containing 10 µM β-escin with pCa 6.0 for 30 min. The treatment was considered successful when the amplitude of contraction exceeded the 80 mM KCl solution-induced contraction (Jensen 1994). After removal of β-escin, response to intracellular free Ca²⁺ was examined using Ca²⁺-buffered solutions according to a previous report (Kerrick and Hoar 1994). Calculation of free Ca²⁺ concentration based on association constants was made in accordance with the temperature and pH of the solution (Fabiato and Fabiato, 1979). Composition of the physiological salt solution used in this experiment was (in mM); NaCl 130, KCl 5.6, glucose 11, Tris 20, MgCl₂ 1, CaCl₂ 2.5. The 80 mM KCl solution was made by equimolar replacement of NaCl with KCl. Composition of the relaxing solution was (in mM); K propionate 130, Tris-malate 20, EGTA 10, MgCl₂ 1, ATPMg 3, Creatine phosphate Na₂ 10 and Creatine phosphokinase 10 unit/ml pH 6.8 which was essentially the same as a previous report (Saida et al., 1991).

Membrane potential was recorded in isolated circular muscle layer with the endothelial side uppermost by a conventional microelectrode technique in a chamber (2 ml volume) which was perfused by the Tyrode's solution (37°C) at a rate of 4 ml/min. A borosilicate glass microelectrode (WPI, 1B120F-3, Sarasota, USA) was made by a puller (Narishige, PA-91) and had a resistance of 40–80 MΩ. Potential difference between the electrode and a reference electrode was measured through a mico-electrode amplifier (Nihon Kohden MEZ 7101, Japan) and displayed on a digital oscilloscope (Kikusui, COR 5521, Japan). Data were stored on a videotape recorder after pulse code modulation (SONY, PCM 501ES, Japan) and recorded on an ink–pen oscillograph (Nihon-Kohden, RJG 4022, Japan).

Drugs used were; β-escin (Sigma, USA), ATPMg (Sigma), EGTA (Dojin, Japan), Creatine phosphate Na₂ (Wako, Japan), maleic acid (Wako), Tris (Wako), K propionate (Nacalai tesque, Japan), Creatine phosphokinase (EC 2.7.3.2, Oriental Yeast, Japan)

Data are presented as mean±SEM with number of preparations in parenthesis. Statistical evaluation was performed using Student t-test. P value smaller than 0.05 was considered significant. Data in graphs were fitted to dose-response curves using a computer program (Prism; GraphPad Software Inc., San Diego, USA).
Results

The circular preparation of rat portal vein did not show spontaneous mechanical activity in the modified Tyrode's solution. Increase in extracellular K\(^+\) concentration higher than 20 mM induced sustained contraction rather than tension oscillation (Fig. 1). High K\(^+\) solution sometimes induced blunted phasic contraction which formed the initial maximum tension, although the phasic contraction was usually unclear. Dominance of tonic contraction in circular muscle preparation was different from the phasic contraction-dominated high K\(^+\) contraction in longitudinal muscle preparation. In 80 mM K\(^+\)-induced contraction, amplitude of the phasic contraction measured at the initial peak was 3.8±0.2 mN (n=20) and 3.9±0.2 mN (n=20) in WKY and SHRSP, respectively. Amplitude of the tonic contraction measured at sustained plateau was 2.9±0.2 mN (n=20) and 3.3±0.2 mN (n=20) in WKY and SHRSP, respectively. There was no difference in the phasic or tonic contraction amplitude of 80 mM K\(^+\)-induced contraction between WKY and SHRSP.

When amplitude of the tonic tension of each contraction was measured and normalized by the maximum contraction height, it increased concentration-dependently as K\(^+\) concentration of the solution was increased up to 60 mM in the preparations both from WKY and SHRSP. Further increase in K\(^+\)-concentration, however, caused decrease of the amplitude of the phasic contraction. ED\(_{50}\) of K\(^+\) concentration calculated between 5.4 to 60 mM K\(^+\) for the initiation of the phasic contraction was 26.6±0.6 mM (n=12) and 27.3±0.4 mM (n=12) in preparations from WKY and SHRSP, respectively. There was no difference in the relation between K\(^+\) concentration and induced contraction in WKY and SHRSP (Fig. 2).

Replacement of the modified Tyrode's solution with the nominally Ca\(^{2+}\)-free solution did not induce change in basal tension. It was also demonstrated that no change in tension was induced when the nominally Ca\(^{2+}\)-free solution was changed to the nominally Ca\(^{2+}\)-free 80 mM K\(^+\) solution. High K\(^+\)-depolarized preparations contracted concentration-dependently when Ca\(^{2+}\) concentration of the solution was increased stepwise from nominally Ca\(^{2+}\)-free to 5 mM Ca\(^{2+}\) solution.

![Fig. 1](image)

Fig. 1. Typical traces of high K\(^+\)-induced contraction in intact circular preparations from WKY (upper trace) and SHRSP (bottom trace). K\(^+\) concentration of the perfusate was changed from 5.4 mM during the period indicated by horizontal bar. Concentration of K\(^+\) is presented by number under each bar.
Ca-sensitivity of SHRSP portal vein

Fig. 2. Summarized graph of high K⁺-depolarization-induced contraction of intact circular preparations from WKY (open circle) and SHRSP (filled circle). Amplitude of contraction was expressed as percent of the maximal tension which was attained at 60 mM K⁺. Each circle with vertical bar represents mean±S.E.M. Number of each data is 12 in the preparations both of WKY and SHRSP.

Fig. 3. Typical traces of CaCl₂-induced contraction in depolarized circular preparations from WKY (upper trace) and SHRSP (bottom trace). The preparation was depolarized in nominally Ca²⁺-free K⁺ 80 mM Tyrode solution (Ca⁻). CaCl₂ was applied cumulatively at arrows with number indicating concentration of CaCl₂.

(Fig. 3). Both in the preparations from WKY and SHRSP, the threshold concentration to induce contraction was around at 5 μM. The contraction did not reach the maximum in the range of Ca²⁺ concentration employed in the present study, although the response almost saturated at 5 mM. When the response was expressed relative to that at 2 mM Ca²⁺, there was no remarkable difference in the contractile response to extracellular Ca³⁺ between preparations from WKY and SHRSP (Fig. 4). The pD₂ (-logED₅₀) of Ca²⁺ to induce the response in preparations of WKY and SHRSP was 3.32±0.11 (n=8) and 3.52±0.08 (n=8) respectively, being no difference between them.

Response of contractile proteins to Ca²⁺ ion was examined in the β-escin permeabilized preparation. The preparation contracted concentration-dependently when free Ca²⁺ concentration of the buffering solution was elevated from 0.1 to 100 μM (Fig. 5). There was no remarkable difference between preparations from WKY and SHRSP in the Ca²⁺ sensitivity of
Fig. 4. Summarized graph for CaCl$_2$-induced contraction in 80 mM K$^+$-depolarized circular preparations from WKY (open circle) and SHRSP (filled circle). Amplitude of contraction was expressed as percent of the tension which was attained at 2 mM CaCl$_2$. Each circle with vertical bar represents mean±S.E.M. Number of each data is 8 in both of WKY and SHRSP.

Fig. 5. A typical trace of a circular muscle preparation from WKY portal vein before and after β-escin-treatment. (upper trace). Bath solution was changed from physiological salt solution to the relaxing solution at the arrow. The preparation was incubated with β-escin at pCa 5.5 in the relaxing solution until developed tension grew comparable to the contraction induced by 80 mM KCl before treatment (indicated by a horizontal bar). Increase of free Ca$^{2+}$ concentration (indicated with number at each arrow) induced tonic contraction in the β-escin-skinned preparation (bottom trace).

The resting membrane potential of smooth muscle cells in circular muscle layer was $-47.4±1.6$ mV (n=63) and $-48.9±1.0$ mV (n=30) in preparations from WKY and SHRSP, respectively (Fig. 6). The resting membrane potential was

the fiber. Fifty percent of the maximum contraction was observed at pCa of $6.27±0.04$ (n=6) and $6.28±0.03$ (n=7) in preparations from WKY and SHRSP, respectively (Fig. 6).
Ca-sensitivity of SHRSP portal vein

Fig. 6. Dose-response curve for Ca\textsuperscript{2+}-induced contraction of \( \beta \)-escin skinned fiber made from circular muscle of portal vein of WKY (open circle) and SHRSP (filled circle). Contraction amplitude is expressed in percent of the maximum contraction of each preparation. Each circle with vertical bar represents mean±S.E.M. Number of each data is 6 and 7 in WKY and SHRSP, respectively.

![Dose-response curve](image)

Fig. 7. Membrane potential of circular layer smooth muscle cells from WKY and SHRSP at various K\textsuperscript{+} concentration. Each circle with vertical bar represents mean±S.E.M. Number of each data is from 3 to 64.

![Membrane potential](image)

interrupted with sporadic irregular spikes. However, when the longitudinal muscle layer was not isolated from the preparation, rhythmic spontaneous spike burst which was commonly recorded from longitudinal layer cells was observed. Membrane potential was recorded in preparations which did not show rhythmic spontaneous spike burst. In the solution with increased K\textsuperscript{+} concentration, membrane was depolarized in the preparations both from WKY and SHRSP. Sporadic spikes appeared more frequently in the presence of increased K\textsuperscript{+} concentration and, when depolarization was large, spike amplitude was decreased. There was no difference in the depolarized membrane potential between preparations from WKY and SHRSP at each concentration of K\textsuperscript{+} (Fig. 7).
Discussion

The muscle wall of rat portal vein is composed of longitudinal outer layer and circular inner layer (Ts’ao et al., 1970). The circular preparation of rat portal vein did not show spontaneous mechanical activity, as reported previously (Cohen and Weily, 1977; Mathison, 1983; Bratveit et al., 1987). Responses to agonists has also been reported to be different between longitudinal and circular muscle of rat portal vein (Mathison, 1983; Bratveit et al., 1987; Rydningen et al., 1987; see Sutter, 1990).

Most mechanical or morphological studies on portal vein of arterial hypertensive rats have been conducted in the longitudinal muscle (Greenberg and Bohr, 1975; Sutter and Ljung, 1977; Mulvany et al., 1980; Pegram and Ljung, 1981; Westfall et al., 1984; Reilly et al., 1989; Sutter, 1990). Although ionic transport has been shown to be abnormal in a number of plasma membranes of primary hypertension (Postnov and Orlov, 1985), no abnormality in ion transport was reported in portal vein from hypertensive rats (Jones, 1974).

In longitudinal muscle of portal vein, phasic mechanical activity has been shown to be associated with spike burst activity (Funaki and Bohr, 1964). We have previously reported increase in duration and spike number of spontaneous action potential, while no difference in the resting membrane potential was observed (Shimamura et al., 1989). No difference in the membrane potential has also been reported in SHR (Kuriyama and Suzuki, 1978). In the present study on circular muscle, no spontaneous spike burst was observed and there was no difference in resting membrane potential of circular smooth muscle cells between preparations from WKY and SHRSP.

In the present experiment, it was shown that membrane potential at various extracellular K+ concentration was identical in the preparation from WKY and SHRSP as reported in WKY and SHR (Kuriyama and Suzuki, 1978). Theses results indicate that potassium equilibrium potential at all extracellular K+ concentration would be the same in both preparations. Although the concentration of extracellular Ca2+ was changed in the subsequent experiment, the resting membrane potential may not be influenced by this change, since it was reported that membrane potential of smooth muscle from WKY and SHRSP was not modified differently by extracellular Ca2+ (Chai and Webb, 1992). High K+-induced contraction of circular muscle of portal vein demonstrated in the present experiment would be initiated by this membrane depolarization. The result that the dependency on K+ concentration of the contraction was not different between the preparations from WKY and SHRSP, was in agreement with a previous report in SHR portal vein (Greenberg and Wilborn, 1982).

Small phasic phase or predominant tonic phase in high K+-induced contraction in portal circular muscle both in preparations from WKY and SHRSP was a remarkable difference from that we have previously observed in those of longitudinal muscle (Shimamura et al., 1989). It is possible that Ca2+ influx or extrusion mechanisms is different between circular and longitudinal muscle tonic contraction. However, further detailed study is necessary to clarify the mechanism of difference in tonic contraction of circular and longitudinal muscle.

The Ca2+-sensitivity in the preparation from longitudinal muscle of K+-depolarized portal vein has been reported to be higher in SHR than that from WKY (Harris et al., 1984) or same
Ca-sensitivity of SHRSP portal vein

Our previous report on K⁺-depolarized longitudinal muscle from SHRSP did not show any increase in sensitivity to Ca²⁺ (Shimamura et al., 1989). In the present study with circular muscle, we also could not observe difference in sensitivity to Ca²⁺ in K⁺-depolarized preparation.

In smooth muscle cells of mesenteric artery (Ohya et al., 1993; Cox and Lozinskaya 1995; Kubo et al., 1998), basilar artery and cerebral arteries (Wilde et al., 1994) from SHR, amplitude of voltage-dependent Ca²⁺ current has been reported to be augmented. In azygos venous smooth muscle cells from SHR, total Ca²⁺ current was not different but proportion of L and T current was different (Rusch and Hermsmeyer, 1988). Voltage-dependent Ca²⁺ channels of venous smooth muscle cells of SHR may be modulated differently from arterial smooth muscle cells. There seemed to be no difference in Ca²⁺ influx via voltage-dependent channels in WKY and SHRSP portal vein circular muscle, because there was no change in high K⁺-induced contraction in intact preparations or Ca²⁺-induced contraction in permeabilized preparations.

In arterial preparation permeabilized by Triton X, it has been reported that Ca²⁺ sensitivity of tail artery from SHR (Nghiem and Rapp, 1983) or SHRSP (Mrwa et al., 1986) was not different from that from WKY. However, in saponin-skinned preparation of aorta, Soloviev and Bershtein (1992) have reported that preparation from SHR showed higher sensitivity to Ca²⁺ than that from WKY. In the report, they also observed that the Ca²⁺ sensitivity of the smooth muscle from portal vein was also higher in the preparation from SHR when compared with that from WKY (Soloviev and Bershtein, 1992). Permeabilized condition may differ among detergents used (Kerrick and Hoar, 1994). β-escin-treated muscle was reported to lose larger intracellular molecules when higher concentration of β-escin was used (Konishi and Watanabe, 1995). The present study showed that permeabilized circular muscle preparation contracted when free Ca²⁺ concentration was increased and that there was no difference in response to Ca²⁺ between preparation from WKY and SHRSP. The response of permeabilized preparation in the present study was very similar to that reported in Triton X-permeabilized longitudinal muscle of rat portal vein in the presence of calmodulin 1 μM (Arner, 1983) and to that reported in saponin-skinned, longitudinal muscle under inhibition of protein kinase C (Soloviev and Bershtein, 1992). Our result does not itself guarantee that any important intracellular molecules which may regulate contraction are not lost. However, simultaneous recording studies of intracellular Ca²⁺ concentration and force in mesenteric artery and aorta from SHR also showed that Ca²⁺-sensitivity of myofilament is not different from that of WKY (Sada et al., 1990; Bian and Bukoski, 1995).

As there was no difference in the dose-response curves to K⁺ and Ca²⁺ in the contractions of preparation from WKY and SHRSP, it may be reasonable to conclude that Ca²⁺ sensitivity of portal vein circular muscle from SHRSP is normal. However, enhanced response to agonists in circular muscle (Greenberg and Wilborn, 1982) as well as longitudinal muscle layer (Greenberg and Bohr, 1975; Sutter and Ljung 1977; Kuriyama and Suzuki, 1978; Westfall et al., 1984; Reilly et al., 1989; Kamata et al., 1990) of portal vein may contribute to the development or maintenance of hypertension.
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