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Alteration of 4-Aminopyridine-Sensitive, Voltage-Dependent K^+-Channel in Arterial Smooth Muscle Cells of One-Kidney, One-Clip Goldblatt Hypertensive Rats

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Using the patch-clamp technique, we investigated the alteration of 4-aminopyridine(4-AP)-sensitive, voltage-dependent K^+ channel (Kv) in the mesenteric arterial smooth muscle cell (MASMC) of renovascular hypertensive model, one-kidney one-clip Goldblatt hypertensive rat (GBH). To isolate K_V current, internal pipette solution contained 5 mM ATP and 10 mM EGTA. Under these condition, MASMC was depolarized by 4-AP, but charybdotoxin did not affect membrane potential. Membrane potential of hypertensive cell (-40.3 ± 3.2 mV) was reduced when compared to that of normotensive cell (-59.5 ± 2.8 mV). Outward K^+ current of hypertensive cell was significantly reduced when compared to normotensive cell. At 60 mV, the outward currents were 19.10 ± 1.91 and 14.06 ± 1.05 pA/pF in normotensive cell and hypertensive cell respectively. 4-AP-sensitive K^+ current was also smaller in hypertensive cell (4.28 ± 0.38 pA/pF) than in normotensive cell (7.65 ± 0.52 pA/pF). The values of half activation voltage ($V_{1/2}$) and slope factor (*kl*) as well as the values of half inactivation voltage ($V_{1/2}$) and slope factor (*kl*) were virtually similar between GBH and NTR. These results suggest that the decrease of 4-AP-sensitive K^+ current contributes to a depolarization of membrane potential, which leads to development of vascular tone in GBH.

Key Words: Mesenteric artery, Vascular smooth muscle, 4-aminopyridine, K^+ channel, One-kidney, One-clip Goldblatt hypertensive rat

INTRODUCTION

Peripheral resistance is increased in hypertension and increased vascular tone is associated with intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Sugiyama et al, 1986; Zidek et al, 1987). A primary source of Ca^{2+} for contraction in vascular smooth muscle cell is influx via voltage-gated Ca^{2+} channel. Therefore membrane potential may play a key role in regulation of contraction of vascular smooth muscle cell. In recent years, it has become apparent that K^+ channels located on vascular smooth muscle are important determinants of membrane potential and vascular tone. The decrease in the conductance of K^+ depolarizes the cell membrane potential, which leads to the opening of the voltage-gated Ca^{2+} channel. Opening of voltage-gated Ca^{2+} channel results in vasoconstriction due to an increase in $[Ca^{2+}]_i$, thereby altering blood pressure.

The major types of K^+ channels in VSMC are ATP-sensitive K^+ channel (K_{ATP} channel), Ca^{2+}-activated K^+ channel (K_{Ca} channel), voltage-dependent or delayed rectifier K^+ channel (K_V channel), and inward rectifier K^+ channel (K_{ir} channel) (Nelson et al, 1990). Among these K^+ channels, K_V channel plays an important role in the regulation of resting membrane potential and vascular tone in many different vessels, i.e. coronary (Volk & Shibata, 1993), cerebral (Bonnet et al, 1991), renal (Gelband &

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Hume, 1992), mesenteric (Smironov & Aaronson, 1992) and pulmonary artery (Okabe et al, 1987). It has been reported that alteration of K_V channel is associated with hypertension such as in spontaneously hypertensive rat (SHR) (Cox, 1996) and deoxycorticosterone (DOCA) hypertensive rat (Martens & Gelband, 1996), and with pulmonary hypertension (Yuan et al, 1998). In renovascular hypertension, it is also well known that increased peripheral resistance is one of the cause of hypertension. However, the alteration of Kv in renovascular hypertension is not investigated yet. Therefore, we demonstrated that the membrane potential and 4-aminopyridine(4-AP)-sensitive K_V channel was changed in vascular smooth muscle cell from the renovascular hypertensive model, GBH.

METHODS

Surgical procedures

4 to 5 week-old male Sprague-Dawley rats were used for preparation of GBH. Rats were anesthetized with ketamine hydrochloride (100 mg/Kg) peritoneally. GBH was made by partial ligation of left renal artery combined with contralateral nephrectomy. GBH at the age of 12 to 16 weeks, of which a systolic blood pressure was raised about 180 mmHg, were used. Sham operated rat, of which systolic blood pressure was not changed, were used as NTR.

Measurement of blood pressure

The systolic blood pressure was measured in conscious restrained rats by the tail-cuff method. The rats were pre-warmed for 10 minutes in rat holder by placing them on a hot plate with its surface temperature of 35°C. They became sedated within 10 minutes of being restrained in the rat holder. The cuff of 15 mm in width was placed at the base of the rat tail for blood pressure measurement. Systolic blood pressure was monitored using the electrosphygmomanometer (PE-300, Narco-Biosystens, Huston, Texas). For each rat, three consecutive recordings were taken and averaged to obtain the individual blood pressure.

Single cell isolation and electrophysiological recordings

Neck of the rats were dislocated and exsanguinated. Mesenteric artery was enzymatically dissociated using collagenase and papain and we obtained single smooth muscle cell. Single cells were stored at 4°C and were used in the experiment within 10 h. Single cells were voltage-clamped, and whole-cell membrane currents were measured using the conventional whole-cell configuration of patch-clamp technique at room temperature. Cell membrane was ruptured at the tip of the pipette with additional negative pressure after the gigaseal formation. Whole-cell membrane currents were recorded using patch-clamp amplifier (Axopatch-1D, Axon instruments), and monitored on an oscilloscope (MD5441, Tektronix). Data were digitized on-line (0.5~2 KHz) using data acquisition system (Digitata 1200, Axon instruments), and stored on a computer. Cell membrane potentials were measured in current clamp configuration (I=0 position of the Axopatch-1D). The liquid junction potentials were corrected with an offset circuit before each experiment. The resistance of patch pipette filled with internal solution was 3~5 MΩ and seal resistance formed gigaseal (above 10 GΩ). Membrane area was estimated by integrating capacitive currents generated by 5 mV hyperpolarizing pulses for 5 ms after electronic cancellation of the patch pipette capacitance. Whole-cell membrane currents were individually calculated in pA/pF to normalize for difference in cell size. All data acquisition and analysis were performed using pClamp 6.0.3 software. Data were expressed as mean standard error with n representing the number. Student's t test was used and $p < 0.05$ was considered to be statistically significant for the test.

Solutions

Normal physiological salt solution (PSS) consisted of the following composition (mM): NaCl 134, KCl 6, $MgCl_2$ 1, $CaCl_2$ 1, HEPES 10, Glucose 5, pH 7.4. For the recording K^+ current, nominal Ca^{2+}-free PSS (normal PSS without Ca^{2+}) was used as bath solution. The internal solution for patch pipette contained the following composition (mM): KCl 107, KOH 33, $MgCl_2$ 0.5, EGTA 10, MgATP 5, HEPES 10, pH 7.2. Solution for storage of single cells consisted of the following composition (mM): L-glutamic acid (free acid) 50, KCl 40, taurine 20, KH_2PO_2 20, $MgCl_2$ 3, glucose 10, HEPES 10, EGTA 0.5, pH 7.35. 4-AP, and charybdotoxin (CTX) purchased from Sigma chemicals. 4-AP was dissolved in 0.1 N HCl and pH was adjusted to 7.4. All drugs were diluted in bath

solution before use.

RESULTS

The systolic blood pressure of GBH was increased markedly and was 195.5±15.50 mmHg at 8 weeks after operation. The body weights were not different between two groups but the heart and kidney wet weights of GBH were increased significantly compared to NTR (Table 1). Cell capacitances were similar between two groups. Capacitances of mesenteric arterial smooth muscle cell (MASMC) were 14.3 ±0.8 pF in NTR and 14.5±1.5 pF in GBH.

5 mM ATP was present in the recording pipette to inhibit K_{ATP} channel. To decrease contribution to the outward currents by K^+ efflux through K_{Ca} channels, cells were bathed in Ca^{2+}-free PSS and pipette solution contained 10 mM EGTA. Under these conditions, 4-AP caused the membrane potential to depolarize in MASMC of NTR, but CTX did not affect the membrane potential. The membrane potential depolarized from −59.5±2.8 mV to −28.6±6.3 mV by 4-AP (Fig. 1). Significant difference of

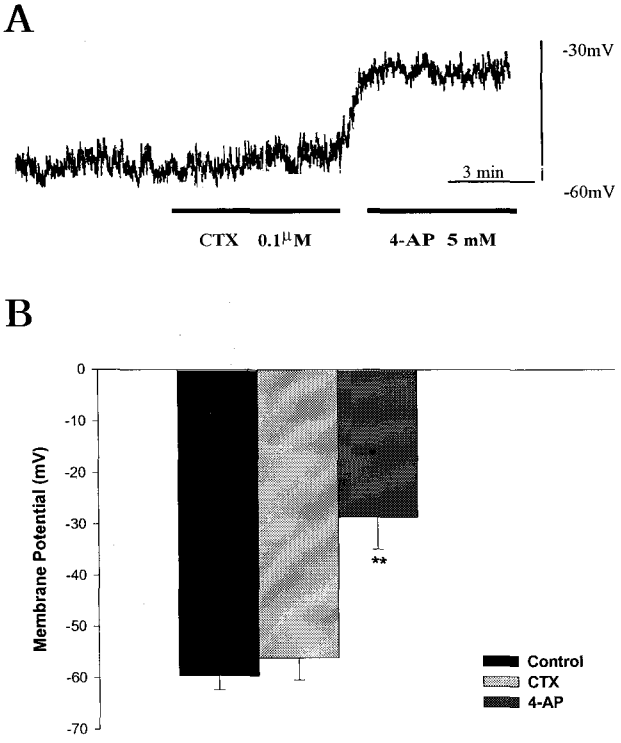

Fig. 1. Effect of K^+ channel blockers on the membrane potential. Panel A: Tracing of membrane potential change was shown in mesenteric arterial smooth muscle cells of normotensive rat. Membrane potential was measured in current clamp configuration. Panel B: Effect of 4-aminopyridine (4-AP, 5 mM), or charybdotoxin (CTX, 0.1 μM) on membrane potential in mesenteric arterial smooth muscle cells from normotensive rat. Values are means±SEM (n=15). **represents $p<0.01$

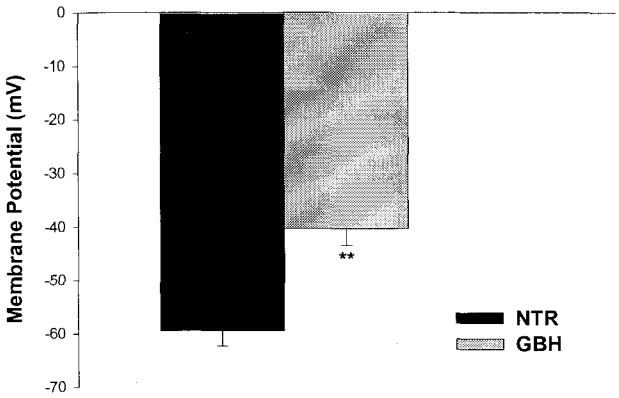

Fig. 2. Membrane potentials of mesenteric arterial smooth muscle cell from normotensive rat (NTR) and one-kidney, one-clip Goldblatt hypertensive rat (GBH). Membrane potentials were measured in current clamp configuration. Values are means±SEM (n=15). **represents $p<0.01$.

Table 1. General characteristics of 12~16 week-old normotensive rat and one-kidney, one-clip Goldblatt hypertensive rat

Group	N	Body weight (g)	Heart (g/Kg of BW)	Kidney (g/Kg of BW)	SBP (mmHg)
NTR	15	360±6.2	3.10±0.03	3.30±0.03	119.0±5.50
GBH	20	370±6.0	4.23±0.05	5.68±0.10	195.5±15.50

Values are mean±S.E.M.. n indicates number of individuals.
BW: body weight, SBP: systolic blood pressure

membrane potential was observed between hypertensive cells and normotensive cells (Fig. 2). Hypertensive mesenteric artery was more depolarized than normotensive artery. Membrane potential of MASMC was -59.5 ± 2.8 mV in NTR and -40.3 ± 3.2 mV in GBH. Fig. 3 shows difference of outward K^+ current between GBH and NTR. K^+ current was elicited by 2s-step depolarization of MASMC of both groups. Outward K^+ current of hypertensive cell was significantly reduced when compared to that of normotensive cell. At 0 mV, the outward currents were 5.02 ± 0.78 (NTR) and 2.86 ± 0.28 pA/pF. At 60 mV, the currents were 19.10 ± 1.91 (NTR) and 14.06 ± 1.05 pA/pF (GBH). Reduced K^+ conductance of GBH may be related to the membrane depolarization of hypertensive artery.

4-AP-sensitive, voltage dependent K^+ current was compared between NTR and GBH. K^+ current was elicited by 2s-step depolarization from -80 to $+60$ mV in a 20 mV increment at a holding potential of -70 mV in the presence of CTX (0.1 μM) or CTX+4-AP (5 mM). 4-AP-sensitive K^+ current was obtained by extracting CTX-sensitive current from 4-AP- and CTX-sensitive current. Each current determined at each test voltage was, divided by cell capacitance, and than averaged. Fig. 4 illustrates mean current-voltage relationships for 4-AP-sensitive K^+ current during step-depolarization. In ten cells tested, 4-AP-sensitive K^+ current at each test potential was

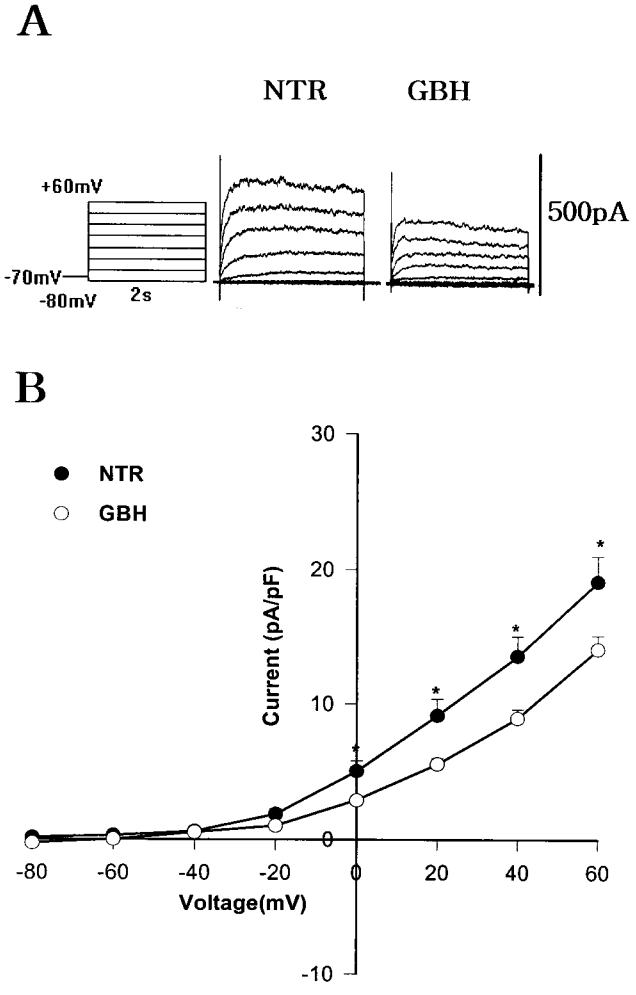

Fig. 3. Comparison of outward K^+ currents between normotensive rat (NTR) and one-kidney one-clip Goldblatt hypertensive rat (GBH). K^+ current was recorded in strongly Ca^{2+}-buffered (10 mM EGTA) cells. Panel A: K^+ currents elicited by 2s-steps depolarization from -80 to $+60$ mV at a holding potential of -70 mV in NTR (12.7 pF) and GBH (12.2 pF). Panel B: Current-voltage relationship of K^+ current in mesenteric arterial smooth muscle cells of NTR and GBH during step depolarizations. Data were obtained from panel A. Peak currents determined at each test voltage were, divided by cell capacitance, and than averaged. Each point represents the mean $\pm$ SEM (n=10). *represents $p<0.05$.

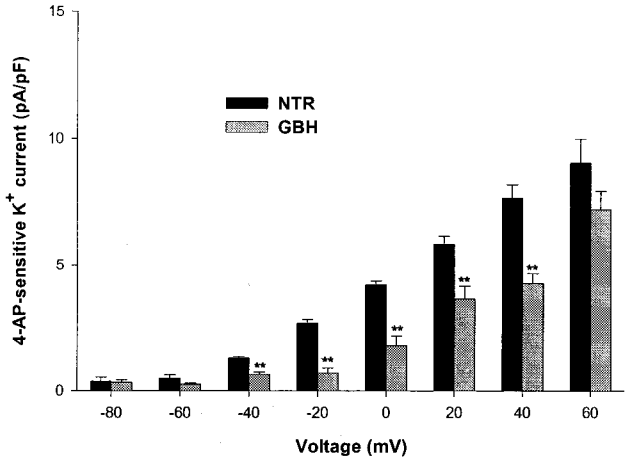

Fig. 4. Comparison of 4-aminopyridine(4-AP)-sensitive K^+ current between normotensive rat (NTR) and one-kidney one-clip Goldblatt hypertensive rat (GBH). K^+ current was elicited by 2s-step depolarization from -80 to $+60$ mV at a holding potential of -70 mV in the presence of charybdotoxin (CTX, 0.1 μM) or CTX+4-AP (5 mM). 4-AP-sensitive K^+ current was obtained by extracting CTX-sensitive current from 4-AP- and CTX-sensitive current. Peak currents determined at each test voltage were, divided by cell capacitance, and than averaged. Values are means $\pm$ SEM (n=10). **represents $p<0.01$.

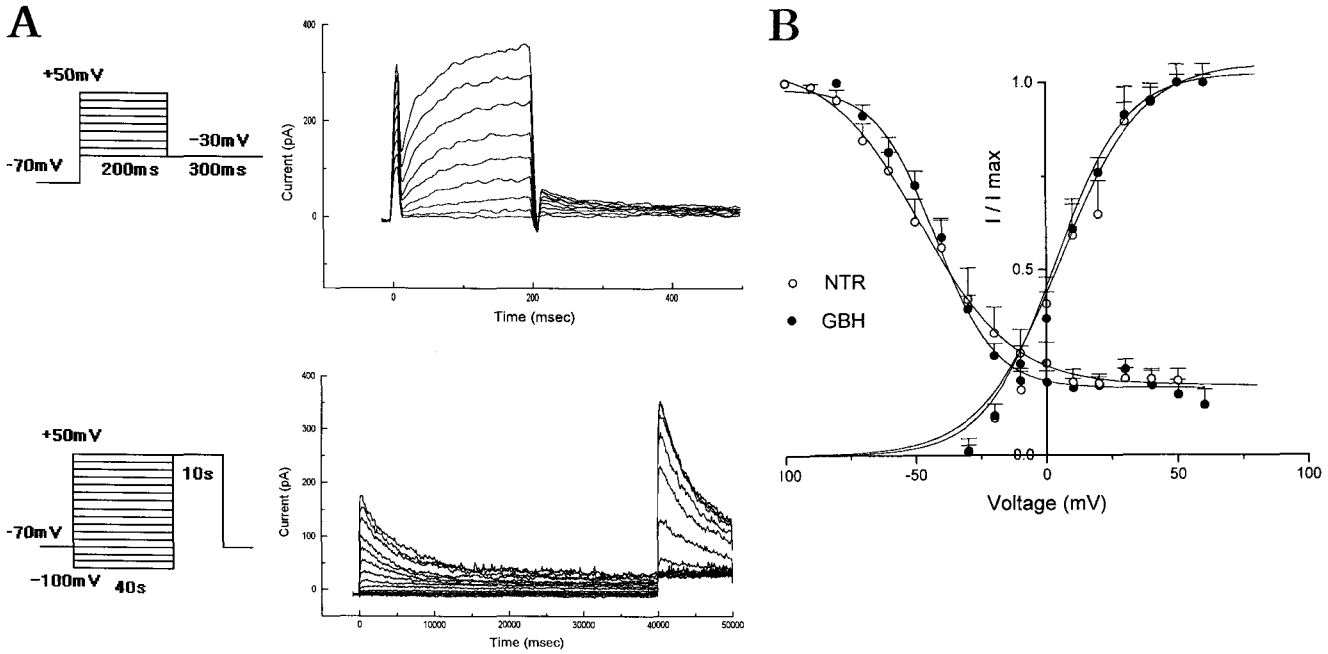

Fig. 5. Voltage dependence of steady-state activation and steady-state inactivation of voltage dependent K^+ current in normotensive rat (NTR, ○) and one-kidney one-clip Goldblatt hypertensive rat (GBH, ●). Panel A represent pulse proctocol for activation and inactivation of K^+ channel. Panel B: Tail current values (I) were determined at each voltage, normalized by dividing by the maximum value (Imax) for the cell, and then averaged at each voltage. Each point represents the mean ± SEM (n=10). The smooth line through these data points is the best fit to the Boltzmann function.

smaller in hypertensive cell when compared to normotensive cell. At 0 mV, 4-AP-sensitive K^+ current were 4.21±0.16 (NTR) and 1.8±0.38 pA/pF (GBH). At 40 mV, those were 7.65±0.52 (NTR) and 4.28± 0.38 pA/pF (GBH).

Voltage dependence of 4-AP-sensitive, voltage dependent K^+ channel in NTR and GBH was investigated. The voltage dependence of steady-state activation and inactivation in 4-AP-sensitive K^+-channel was determined by using double-pulse protocol. Currents were elicited by 200 ms-depolarization to a various test potential from −30 to +50 mV in a 10 mV increment at a holding potential of −70 mV, and then deactivating tail currents were elicited at a constant repolarizing potential of −30 mV for 300 ms (Fig. 5A). Tail currents were fitted by single exponential function, and the amplitudes of instantaneous current of each tails were calculated. Amplitude of instantaneous current gives a measure of the instantaneous conductance of 4-AP-sensitive K^+-channel activated at test potential. The amplitudes of instantaneous tail currents were normalized to the largest tail current and plotted (Fig. 5B). The plotted data from the two groups were fitted into the following Boltzmann equation I/Imax=1 (1+exp ((Vt−V1/2)/*k1*. In MASMC, the values of half activation voltage (V1/2) (GBH=+4.4±3.4 mV and NTR = +3.4±1.1 mV) and slope factor (*k1*) (GBH=−14.9 ±3.4 and NTR=−13.6±1.1 mV) were virtually similar between the two groups. Steady-state inactivation curve was obtained by varying the potential of the conditioning pre-pulse from −100 to +50 mV in a 10 mV increment from a holding potential of −70 mV for 40 s and then measured the amplitude of the peak current at a constant test potential of +50 mV for 10 s (Fig. 5A). Amplitudes of tail currents fitted by single exponential function were normalized to the largest tail current and plotted (Fig. 5B). The current was not completely inactivated to zero current level even at high membrane potentials for 40 s pre-pulse. The plotted data from the two groups were fitted into the following Boltzmann equation I/Imax= 1(1+exp((Vt−V1/2)/*k1*. In MASMC, the values of half inactivation voltage (V1/2) (−49.4±6.2 mV in GBH and −42.0±2.3 mV in NTR) and slope factor (*k1*) (+17.4±4.5 mV in GBH and +11.2±1.7 mV in NTR), were virtually similar in two groups.

DISCUSSION

Renal blood flow is known to influence plasma volume regulation and arterial resistance, which may be involved in the etiology or maintenance of hypertension. In GBH, renal blood flow is reduced and plasma volume is enhanced. As a result, arterial blood pressure is increased. In order to return the plasma volume to original state, peripheral resistance must be increased. From the electrophysiological studies in various hypertensive models, it is demonstrated that depolarization in resting membrane potential and alteration in ion channel, especially Ca^{2+} channels and K^+ channels, were associated with a high vascular tone. We previously demonstrated that vascular tone was increased in GBH (Jeon et al, 1996). In this study, our results are the first to show that alterations of 4-AP-sensitive K-channel contribute to the depolarization of membrane potential and may contribute to the increase in the vascular tone in GBH.

The membrane potential is regulated by a dynamic balance between steady-state inward current and steady-state outward current through Ca^{2+} channel, non-selective cation channels, Cl^- channel and K^+ channel (Daut et al, 1994; Kuriyama et al, 1995; Nelson & Quayle, 1995). The range of resting membrane potentials was from -50 to -75 mV in VSMC of normotensive animals (Fleischmann et al, 1993). In this study, it was shown that the membrane potential of MASMC was about -40 to -60 mV. One reason for the difference in membrane potential may be the different composition of internal solution and bathing solution. In the present study, internal solution contained the 5 mM ATP and 10 mM EGTA. This condition inhibits the K_{ATP} and K_{Ca}, thereby decrease the K^+ conductance. Decreased K^+ conductance depolarizes the membrane potential. It was previously demonstrated that membrane potential of VSMC was more depolarized in genetic and nongenetic hypertnesion (Hermsmeyer, 1976; Martens & Gelband, 1996). Membrane potentials of cells in GBH were significantly depolarized when compared with those of NTR. These suggest that the depolarization of membrane potential may contribute to the regulation of peripheral resistance in hypertension.

It is well known that K^+ channels play a significant role in setting the level of resting membrane potential of VSMC. The participation of K_v, K_{Ca}, K_{ATP} and K_{ir} in resting membrane conductance appears to vary with tissue source and physiological condition (Asano et al, 1993; O'Rourke, 1996). The Kv are thought to be the primary determinant of resting membrane potential (Boyle et al, 1992; Yuan, 1995). The contribution of Kv to membrane potential in VSMC is suggested by the ability of 4-AP, a blocker of this channel. 4-AP causes depolarization and vasoconstriction in many vessels (Hara et al, 1980; Knot & Nelson, 1995; Cole et al, 1996). In this study, 4-AP caused significant depolarization of membrane potential of MASMC, but K_{Ca} blocker, CTX, did not significantly. These results would suggest that 4-AP-sensitive Kv is a major determinant of resting membrane potential in both types of arterial smooth muscle cells.

It was previously demonstrated that K_{Ca} currents were increased (Rusch & Runnells, 1994; Martens & Gelband, 1996; Rusch et al, 1997) and Kv currents were decreased (Cox, 1996; Martens & Gelband, 1996; Martens & Gelband, 1998) in several hypertensive models. However, there is no report that 4-AP-sensitive K^+-channel was altered in the renovascular hypertensive models, GBH. For the first time, our results show that the intrinsic properties of 4-AP-sensitive K^+-channel in MASMC from GBH were altered. The components of 4-AP-sensitive K^+ current at each test potential were decreased significantly in GBH compared to NTR. However the voltage dependent gating and kinetics of 4-AP-sensitive K^+ channel were similar between two groups. We suggest that the decrease of 4-AP-sensitive K^+ current in GBH may be due to the result of a decrease in the number of K^+-channel or their single channel conductance. In order to identify a cause of decrease in 4-AP-sensitive K^+ current, single channel studies for determining single channel conductance and molecular work for determining difference of expression level in the 4-AP-sensitive K^+-channel gene would be required.

In summary, it is suggested that 4-AP-sensitive K^+-channel play a major role in the regulation of membrane potential in MASMC. The decrease of 4-AP-sensitive K^+ current contributes to a depolarization of membrane potential, which leads to an increase in Ca^{2+} influx through Ca^{2+} channel, thereby developing vascular tone in GBH.

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