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Decreased Voltage Dependent K^+ Currents in Cerebral Arterial Smooth Muscle Cells of One-Kidney, One-Clip Goldblatt Hypertensive Rat

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The Kv channel activity in vascular smooth muscle cell plays an important role in the regulation of membrane potential and blood vessel tone. It was postulated that increased blood vessel tone in hypertension was associated with alteration of Kv channel and membrane potential. Therefore, using whole cell mode of patch-clamp technique, the membrane potential and the 4-AP-sensitive Kv current in cerebral arterial smooth muscle cells were compared between normotensive rat and one-kidney, one-clip Goldblatt hypertensive rat (1K,1C-GBH rat). Cell capacitance of hypertensive rat was similar to that of normotensive rat. Cell capacitance of normotensive rat and 1K,1C-GBH rat were 20.8 ± 2.3 and 19.5 ± 1.4 pF, respectively. The resting membrane potentials measured in current clamp mode from normotensive rat and 1K,1C-GBH rat were -45.9 ± 1.7 and -38.5 ± 1.6 mV, respectively. 4-AP (5 mM) caused the resting membrane potential hypopolarize but charybdotoxin (0.1 μM) did not cause any change of membrane potential. Component of 4-AP-sensitive Kv current was smaller in 1K,1C-GBH rat than in normotensive rat. The voltage dependence of steady-state activation and inactivation of Kv channel determined by using double-pulse protocol showed no significant difference. These results suggest that 4-AP-sensitive Kv channels play a major role in the regulation of membrane potential in cerebral arterial smooth muscle cells and alterations of 4-AP-sensitive K_V channels would contribute to hypopolarization of membrane potential in 1K,1C-GBH rat.

Key Words: Goldblatt hypertensive rat, Cerebral arterial smooth muscle, K^+-channel

INTRODUCTION

Hypertension is one of main causes of cerebrovascular disease. Chronic exposure of the vessel to high pressure exhibits anatomical, biochemical and biophysical changes in hypertensive animal. Anatomically, blood vessel hypertrophy, arteriosclerosis and abnormal endothelial cell function are exhibited in hypertension. These anatomical changes make the animal more hypertensive and more susceptable to cerebrovascular disease. Biochemical and biophysical alterations in Na^+-K^+ ATPase, Ca^{2+}-ATPase, Na^+-Ca^{2+} exchange activities and ion channel function have been reported in blood vessels and isolated cell preparations of various hypertensive models.

The development of systemic hypertension is associated with gradual and sustained increase in peripheral resistance (Dobrin, 1983; Bohr & Webb, 1988). Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is a major determinant of blood vessel tone and peripheral resistance. The primary source of Ca^{2+} for contraction is the influx via voltage-gated Ca^{2+} channel. Therefore voltage-gated Ca^{2+} channel activity and membrane potential of vascular smooth muscle play a key role in the regulation of blood vessel tone. From the electrophysiological studies in various hypertensive models, it was demonstrated that ion channels, especially Ca^{2+} channels or K^+ channels, were altered and mem-

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brane potential was hypopolarized (Van de Voorde et al, 1992; Rusch & Runnells 1994), even though other laboratories reported that no change in membrane potential existed in hypertensive model (Lamb & Webb, 1989; Rush et al, 1992). It has become apparent that K^+ channels of vascular smooth muscle are important determinants of membrane potential (Nelson & Quayle, 1995; Faraci & Sobey, 1996; Kitazono et al, 1995). At least, four types of K^+ channels have been identified in arterial smooth muscle. These include the ATP-sensitive K^+ channel (K_{ATP}), Ca^{2+}-activated K^+ channel (K_{Ca}), voltage-dependent or delayed rectifier K^+ channel (K_V), and the inward rectifier K^+ channel (K_{ir}). Among these Kv channel is thought to be a primary determinant of resting membrane potential of vascular smooth muscle cell (Robertson & Nelson, 1994; Knot & Nelson, 1995).

However, no study to date has shown alterations in Kv current in renovascular hypertensive model to the best of my knowledge. In renovascular hypertension, it is also well known that increased peripheral resistance is one of the causes of hypertension. Thus alteration of Kv channel activity may be involved as a cause of increased blood vessel tone. The primary goal of this study is to examine the alteration of membrane potential and Kv channel activity in cerebral arterial smooth muscle cells (CASMC) of the renovasular hypertensive model, 1K,1C-GBH rat.

METHODS

Preparation of 1K,1C-GBH rats

4~5 week-old Sprague-Dawley (SD) male rats were used for the preparation of 1K,1C-GBH rat. Rats were anesthetized with ketamine hydrochloride (100 mg/kg) given peritoneally. 1K,1C-GBH rats were made by partial ligation of left renal artery combined with contralateral nephrectomy. Renal arteries were exposed through laparotomy and dissected from renal vein. Right kidney was removed. A sterile stainless steel needle of 0.25 mm in diameter was placed alongside the left renal artery. The left renal artery was tied together with the needle using 4-0 silk. Drawing the needle out from the tie leaves the renal artery stenotic with the inner diameter of about 0.25 mm. 1K,1C-GBH rats at the age of 12~16 weeks whose systolic blood pressure raised about 200 mmHg were used in the patch clamp experiment.

Measurement of blood pressure

The systolic blood pressure was measured weekly 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 electro-spygmomanometer (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

The neck of the rat was dislocated and exsanguinated. The cerebral artery (<200 μm in diameter) was isolated under stereomicroscope in normal physiological salt solution (PSS) and then was recovered in nominally Ca^{2+}-free PSS at 4°C for 20 minutes. Artery was digested in Ca^{2+}-free PSS containing papain (0.2 mg/ml), collagenase (1 mg/ml), bovine serum albumin (1 mg/ml), and dithiothreitol (1 mg/ml) at 35°C for 20 minutes. The digested arteries were washed twice with Ca^{2+}-free PSS without enzyme. Single cells are dispersed by agitation with a wide-bore Pasteur pipette in Kraft-Bruehe (KB) solution at room temperature.

Electrophysiological recordings

Single cells were voltage-clamped, and whole-cell membrane currents were measured using the conventional whole-cell patch-clamp technique at room temperature. Cell membrane was ruptured at the tip of the pipette by additional negative pressure after giga-seal formation. Whole-cell membrane currents were recorded using patch-clamp amplifier (Axopatch-1D, Axon instruments), monitored on an oscilloscope (MD5441, Tektronix). Data were digitalized on-line (0.5~2 KHz) using data acquisition system (Digitata 1200, Axon instruments), and stored in the computer. Cell membrane potentials were measured in current clamp configuration (the 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 resistances formed

Table 1. General characteristics of 12~16 week-old normotensive rat (NTR) and one-kidney, one-clip Goldblatt hypertensive (1K,1C-GBH) rat

Group	n	Body weight (g)	Heart (g/kg of BW)	Kidney (g/kg of BW)	SBP (mmHg)
NTR	12	370±5.2	3.15±0.02	3.40±0.02	121.0±4.50
1K,1C-GBH	20	390±6.5	4.25±0.04	5.48±0.10	202.5±10.90

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

gigaseal (above 10 GΩ). Membrane area was estimated by integrating capacitive currents generated by 5 mV of hyperpolarizing pulses for 5 millisecond after electronic cancellation of the patch pipette capacitance. Whole-cell membrane current amplitudes were individually calculated in pA/pF to normalize for differences in cell size. All data analyses were performed with pClamp 6.0.3 software (Axon instruments) and Sigmaplot.

Solutions

Normal 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 potassium current, bath solution was nominally Ca^{2+}-free PSS of the following composition (mM) : NaCl 134, KCl 6, $MgCl_2$ 1, $CaCl_2$ 0, HEPES 10, Glucose 5, pH 7.4. The internal solution for patch electrode contained the following composition (mM) : KCl 107, KOH 33, $MgCl_2$ 0.5, EGTA 10, MgATP 5, HEPES 10, pH 7.2. KB solution for storage of single cells consisted of the following composition (mM) : L-glutamic acid 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, HEPES 10, EGTA 0.5, pH 7.35.

4-aminopyridine (4-AP), tetraethylammonium (TEA), and charybdotoxin were purchased from Sigma chemicals. 4-AP was dissolved in 0.1N HCl and pH was adjusted to 7.4. All drugs were diluted in bath solution before use.

RESULTS

Blood pressure and general characteristics of 1K,1C-GBH rats

The systolic blood pressure of 1K,1C-GBH rat after operation was increased markedly. The systolic blood pressure of 1K,1C-GBH rat was 202.5±10.9 mmHg and that of normotensive rat was 121.0±4.5 mmHg. The body weights of 1K,1C-GBH rats were not different from those of normotensive rats. However, wet weights of heart and kidney of 1K,1C-GBH rats were greater than those of normotensive rats. The ratio of wet weights of heart and kidney in 1K,1C-GBH rats to those in normotensive rat were 1.24 and 1.61, respectively (Table 1).

Cell capacitance and resting membrane potential

Cell capacitances were measured by 5 ms-voltage ramp from −70 to −75 mV at a holding potential of −70 mV. Cell capacitances of 1K,1C-GBH rats and normotensive rats were 20.8±2.3 and 19.5±1.4 pF, respectively. Cells of both groups had similar membrane capacitances.

The resting membrane potentials of the CASMC from 1K,1C-GBH rats and normotensive rats were measured using the whole-cell current clamp configuration (I=0). Seal resistance for recording membrane potentials was above 10 GΩ. Resting membrane potentials (−38.5±1.6 mV) of 1K,1C-GBH rats were significantly less than those (45.9±1.7 mV) of normotensive rats (Fig. 1A). To examine what kind of K^+ channel is an important regulator of resting membrane potential, the change of resting membrane potential by the treatment of K^+ channel blocker, 4-AP or charybdotoxin was measured. The resting membrane potential was significantly decreased by 4-AP (5 mM) but not by charybdotoxin (0.1 μM). The membrane potential of normotensive rat was hypopolarized to −28.7±3.2 from −45.9±1.7 by 4-AP. And the membrane potential of 1K,1C-GBH rat was hypopolarized to −26.0±5.8 from −38.5±1.6 by 4-AP (Fig. 1B).

Identification of K^+ current

In order to identify the major ion current by an activation of K^+-selective channel, the reversal potential (E_{rev}) was measured at various extracellular potassium concentrations (6, 60, 140 mM) in CASMC of normotensive rat. Deactivating tail currents were recorded at various repolarizing potentials in a 10 mV step increment after a 100 ms prepulse voltage of +50 mV (Fig. 2A). Measured E_{rev} at 6, 60, and 140 mM, was -69.0 ± 3.7, -16.2 ± 4.0 and 5.1 ± 2.0 mV, respectively (Fig. 2B). E_{rev} did not completely correspond to the theoretical potassium equilibrium potential (E_k) calculated by the Nernst equation, -80.3, -21.6 and 0.0 mV for each extracellular potassium concentrations, respectively. Although E_{rev} did not coincide with E_k completely, it was confirmed that outward current recorded was mainly carried by an activation of K^+-selective channel.

Effects of 4-AP and charybdotoxin on voltage dependent K^+ (Kv) current

To identify voltage dependent K^+ currents, cells

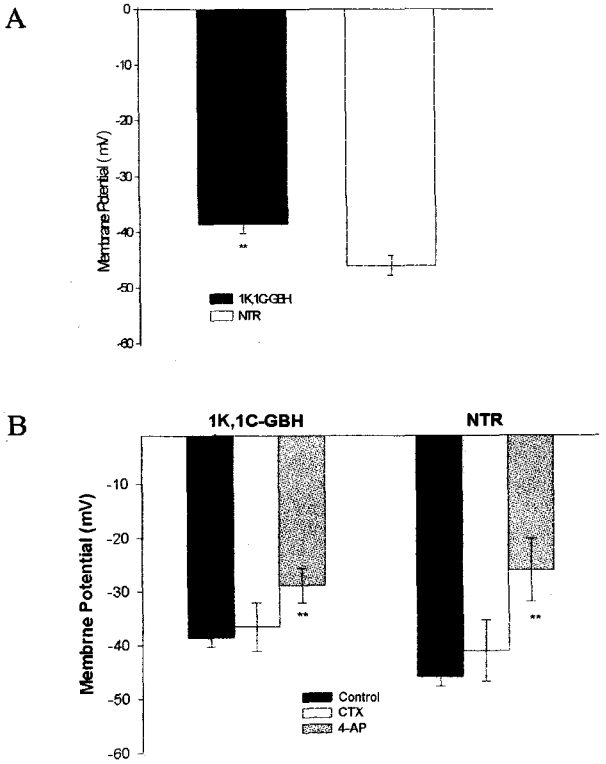

Fig. 1. Resting membrane potential of cerebral arterial smooth muscle cell from normotensive rats (NTR, n=20) and one-kidney, one-clip Goldblatt hypertensive (1K,1C-GBH, n=17) rats (A). Membrane potential was measured in current clamp configuration (I=0). Effect of 4-aminopyridine (4-AP 5 mM) or charybdotoxin (CTX 0.1 μM) on the resting membrane potential in NTR and 1K,1C-GBH rat (B). *value significantly different from corresponding value in NTR ($P<0.05$). **value significantly different from corresponding value in resting membrane potential ($P<0.001$).

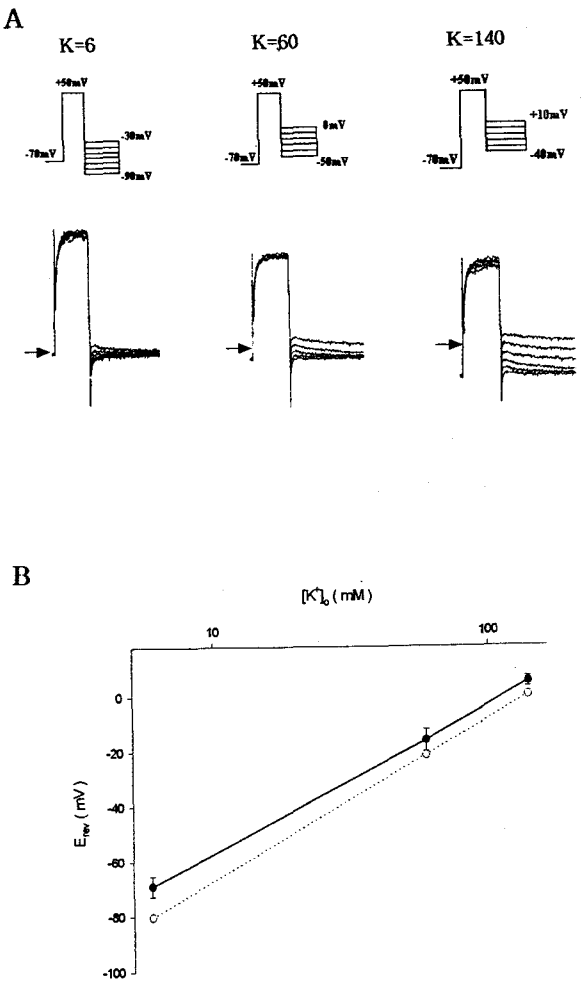

Fig. 2. The reversal potentials (E_{rev}) were measured at various extracellular potassium concentrations ($[K^+]_o$) (6, 60, 140 mM) in cerebral arterial smooth muscle cells from normotensive rat. Deactivating tail currents were recorded at various repolarizing potentials in 10 mV step increment after a 100 ms prepulse voltage of +50 mV. → represent zero current (A). Obtained E_{rev} (●) was plotted against $[K^+]_o$, the lower line (○) represented the theoretical E_k calculated from Nernst equation (B). Symbols represents mean values and vertical bars are ± S.E.M. (n=6).

were dialyzed with pipet solution buffered with 10 mM EGTA and with 5 mM ATP to minimize the K_{Ca} channel activity and K_{ATP} channel activity. Bath solution was nominally Ca^{2+}-free PSS. K^+ currents were elicited by the 2 s voltage step depolarization to various potentials (from -140 to $+60$ mV) from a holding potential of -70 mV (Fig. 3A). Small amount of inward K^+ currents were recorded at negative potential and outward K^+ current was activated at about -50 mV. Inward K^+ currents were not inhibited by charybdotoxin (0.1 μM), K_{Ca} channel blocker, or 4-AP (5 mM), K_V channel blocker. Outward K^+ currents were slightly inhibited by charybdotoxin from 862.5 ± 64.42 pA to 701.0 ± 55.48 pA at 60 mV. 4-AP suppressed dominantly outward K^+ currents from 701.0 ± 55.48 pA to 283.7 ± 28.64 pA at 60 mV (Fig. 3B).

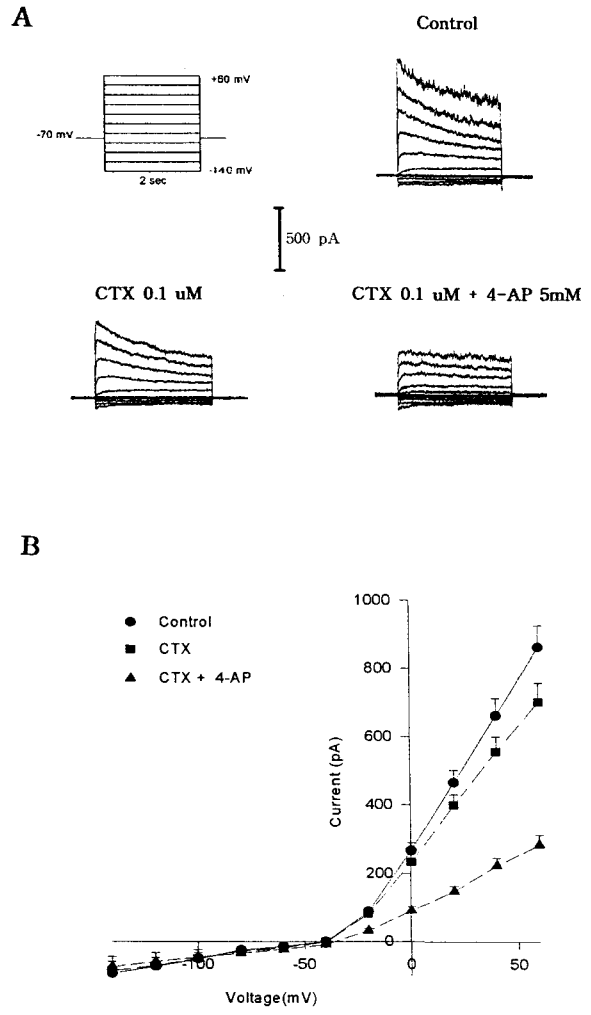

Fig. 3. Effects of 4-aminopyridine (4-AP, 5 mM) or charybdotoxin (CTX, 0.1 μM) on voltage dependent K^+ current in cerebral arterial smooth muscle cells of normotensive rat. Traces show voltage dependent K^+ current elicited by 2 s voltage step from -140 to $+60$ mV in a 10 mV increment at a holding potential of -70 mV (A). Mean current-voltage relationships of voltage dependent K^+ current were recorded from 10 different cells (B). Symbols represents mean values and vertical bars are $\pm$S.E.M..

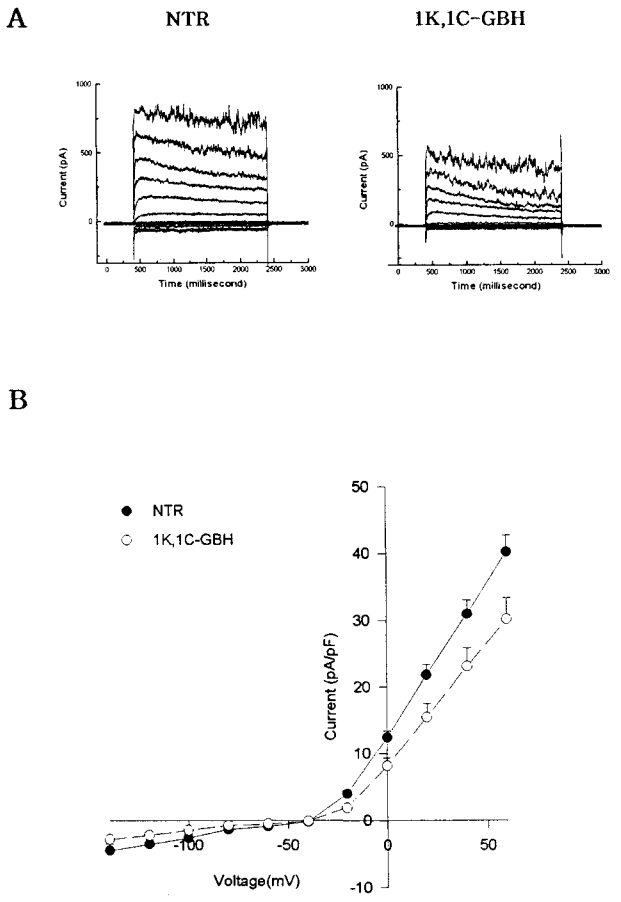

Fig. 4. Voltage dependent K^+ current in cerebral arterial smooth muscle cells from normotensive rats (NTR, ●) and One-kidney, One-clip Goldblatt hypertensive (1K,1C-GBH, ○) rats. Traces show voltage dependent K^+ currents recorded from cells of NTR (20.1 pF) and 1K,1C-BGH (21.2 pF) rat in strongly Ca^{2+}-buffered (10 mM EGTA) internal solution (A). Current-voltage relationship of K^+ current elicited by step depolarization from -140 to $+60$ mV in a 20 mV increment at a holding potential of -70 mV. Peak currents were determined at each test voltage, divided by cell capacitance, and than averaged (B). Symbols represents mean values and vertical bars are $\pm$S.E.M. (n=10).

4-AP-sensitive, voltage dependent K^+ (Kv) currents in normotensive rats and 1K,1C-GBH rats

Outward K^+ currents of CASMC were recorded and the amplitude of those currents of 1K,1C-GBH rats was compared to those of normotensive rats. K^+ currents were elicited by applying 2 seconds-step depolarization from −140 to +60 mV in a 20 mV increment from a holding potential of −70 mV (Fig. 4A). Peak currents elicited by step depolarization were determined at each test voltage, and divided by cell capacitance. Fig. 4B shows mean current-voltage relationship obtained from outward K^+ current during voltage-step depolarization. The amplitudes of outward K^+ currents in 1K,1C-GBH rats were significantly reduced as compared with those in normotensive rats.

To isolate the Kv currents from K_{Ca} currents in the present experiment, charybdotoxin, specific K_{Ca} channel blocker, was treated and the current-voltage relationship was analyzed in the same manner of Fig. 4. Though the components of charybdotoxin-sensitive K^+ currents recorded in both groups were very small, involvement of charybdotoxin-sensitive K^+ current could be confirmed (Fig. 5). Therefore, the 4-AP-sensitive Kv currents of 1K,1C-GBH rats were compared to those of normotensive rats after pretreatment of charybdotoxin (0.1 μM). As shown in Fig. 5, 4-AP-sensitive Kv currents of 1K,1C-GBH rats were significantly smaller than those of normotensive rats. 4-AP-sensitive Kv currents of 1K,1C-GBH rats were 16.7 pA/pF and those of normotensive rats were 27.5 pA/pF.

Voltage dependence of 4-AP-sensitive, voltage dependent K^+ (Kv) channel in normotensive rats and 1K, 1C-GBH rats

In order to analyze the voltage-dependence of 4-AP-sensitive Kv channel, the activation curve and inactivation curve were drawn according to Boltzmann equation. The voltage dependence of steady-state activation of 4-AP-sensitive Kv channel was determined by using double-pulse protocol. Currents were elicited by 100 ms depolarization to various test potentials 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 400 ms (Fig. 6A). The instantaneous current gives a measure of the

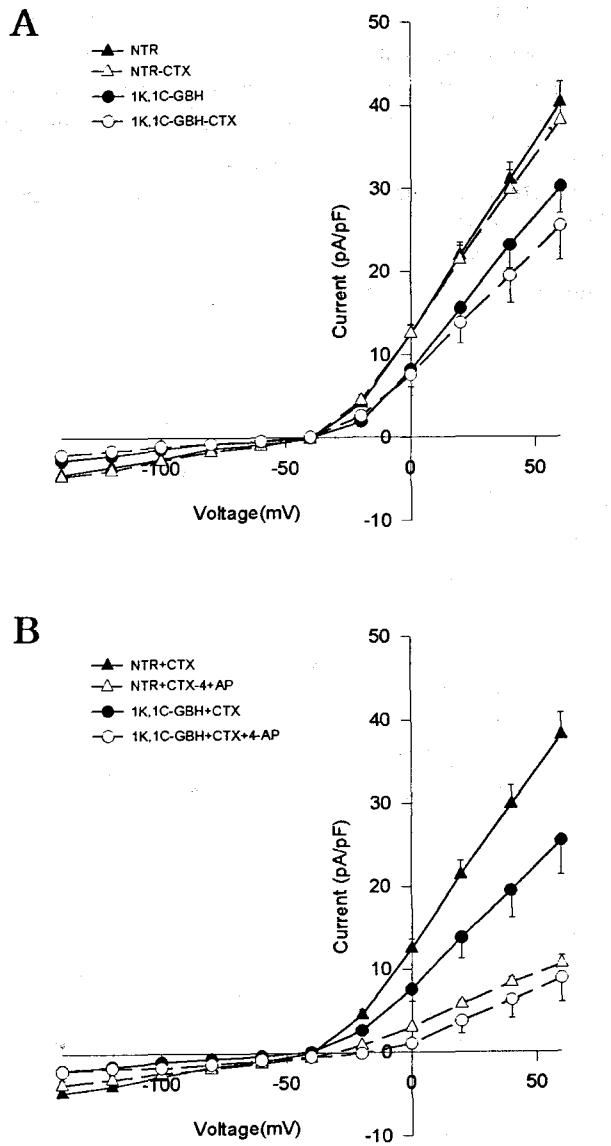

Fig. 5. Effects of Charybdotoxin (CTX, 0.1 μM) (A) and 4-aminopyridine (4-AP, 5 mM) (B) on voltage dependent K^+ current in cerebral arterial smooth muscle cells of normotensive rat (NTR) and one-kidney one-clip Goldblatt hypertensive (1K,1C-GBH) rat during step depolarizations. Current-voltage relationship of K^+ current elicited by step depolarization from −140 to +60 mV in a 20 mV increment from a holding potential of −70 mV. Peak currents determined at each test voltage were, divided by cell capacitance, and than averaged. Symbols represents mean values and vertical bars are ±S.E.M. (n=10).

instantaneous conductance of $I_{K(V)}$ activated at test potential. The amplitudes of instantaneous tail currents were normalized to the maximal tail current and plotted (Fig. 6B). The plotted data from two groups

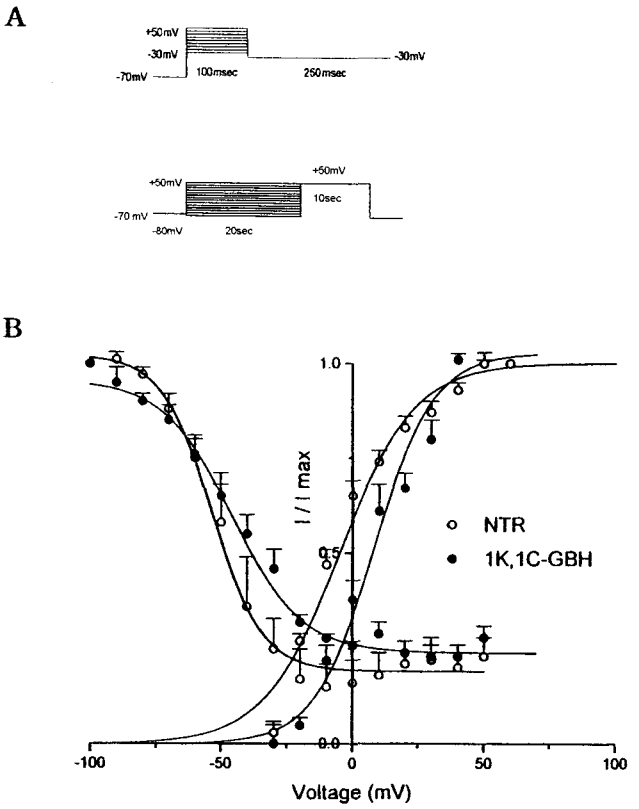

Fig. 6. Voltage dependence of steady-state activation (NTR, ○; 1K,1C-GBH, ●) and steady-state inactivation (NTR, △; 1K,1C-GBH, ▲) of voltage dependent K^+ current in normotensive rat (NTR) and one-kidney one-clip Goldblatt hypertensive (1K,1C-GBH) rat. The voltage dependence of steady-state activation was determined by using a double-pulse protocol (A). Tail current values (I) were determined at each voltage, normalized by being divided by the maximum value (I_{max}) for the cell, and then averaged at each voltage. Symbols represent mean values and vertical bars are ±S.E.M. n=10). The smooth line through these data points is the best fit to the Boltzmann function.

were fitted by following Boltzmann equation $I / I_{max} = 1/(1+\exp((V_t - V_{1/2})/k_l)$, where $V_{1/2}$ is half activation potential and k_l is slope factor. The activation curves of both groups were saturated above +30 mV. There was no significant difference in values of half activation voltage ($V_{1/2}$) between normotensive rats and 1K,1C-GBH rats, which were $+4.9\pm1.0$ and $+6.6\pm1.4$ mV respectively. And the values of slope factor (k_l) in normotensive rats and 1K,1C-GBH rats, which were -14.9 ± 0.9 and -13.2 ± 1.0 mV, were not significantly different.

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 of −70 mV for 20~40 s and then measured the amplitude of the peak current at a constant test potential of +50 mV for 10 seconds (Fig. 6A). Amplitudes of each tail currents were normalized to the maximal tail current and plotted (Fig. 6B). The current was not completely inactivated to zero current level even at high membrane potentials for 20~40 s prepulse. The plotted data from two groups were fit with Boltzmann equation. There was no significant difference in values of half inactivation voltage ($V_{1/2}$) between normotensive rats and 1K,1C-GBH rats, which were -53.9 ± 2.5 and -45.8 ± 2.8 mV respectively. The values of slope factor (k_l) between normotensive rats and 1K,1C-GBH rats, which were $+9.1\pm0.9$ and $+13.2\pm2.1$ mV, showed no significant difference. The voltage dependences of K_V channel were virtually similar in CASMC of both groups.

DISCUSSION

Alterations of activity of ion channels have been associated with an increased vascular reactivity in hypertension. Increase of Ca^{2+} current (Ohya et al, 1993; Wilde DW et al, 1994) has been demonstrated in spontaneously hypertensive rats. Increased $[Ca^{2+}]i$ causes vascular smooth muscle to contract, thereby increasing peripheral resistance. Alteration of K^+ current has been reported in various forms of hypertension. Some investigators (Rush et al, 1992; England et al, 1993) reported that K^+ current was enhanced in various forms of hypertension. They suggested that this enhanced K^+ permeability might provide a negative-feedback mechanism by which an increased arterial contractility may be limited in hypertension. On the contrary, other investigators reported that K^+ current was impaired or decreased in hypertension (Kitazono et al, 1993; Martens & Gelband, 1996) and this decreased K^+ permeability caused the membrane to hypopolarize. Alterations of activity of ion channels in blood vessel, especially in small resistance vessel (200< μm in diameter, renal, mesenteric, cerebral artery) are very important in regulation of peripheral resistance and blood pressure. Therefore, present study describes the electrophysiological properties of physiologically relevant vascular beds, resistance-sized cerebral artery.

Membrane potential is a major determinant of blood vessel tone. Membrane hypopolarization opens

the voltage-gated Ca^{2+} channel of vascular smooth muscle. Opening of voltage-gated Ca^{2+} channel results in contraction of the vascular smooth muscle due to an increase in $[Ca^{2+}]_i$, thus increasing vessel tone (Trapani et al, 1981; Haeusler, 1983). Normotensive vascular smooth muscle cells, including those obtained from coronary artery, portal vein and cerebral artery, have resting membrane potential between −30 and −55 mV (Fujiwara et al, 1982; Hume & Leblanc, 1989; Matsuda et al, 1990; Kang et al, 1997; Kim et al, 1997). These variations in membrane potential value were shown according to vascular bed and species. In this study, membrane potential was 45.9± 1.7 mV in cerebral arterial smooth muscle cells (CASMC) from normotensive rats. Resting membrane potential of CASMC from 1K,1C-GBH rat was less negative than that of normortensive rat. Using the conventional microelectrodes as well as patch-clamp method, many investigators reported that membrane potentials were 10 to 20 mV more hypopolarized in genetic (Hermsmeyer, 1976) and nongenetic hypertensive rats (Martens & Gelband, 1996) than in normortensive rats. These results suggest that membrane hypopolarization may contribute to the increase of peripheral resistance in hypertension.

It is postulated that the resting membrane potential of vascular smooth muscle is determined by the membrane permeability of ions, such as K^+, Ca^+, Na^+, Cl^- (Nelson et al, 1990; Daut et al, 1994; Kuriyama et al, 1995). Among these ion channels, K^+ channels play the most important role in the regulation of membrane potential. Thus, the decrease of K^+ conductance lead to membrane hypopolarization and contraction of arterial smooth muscle, thereby increasing blood pressure. K^+ channels identified in most vascular smooth muscle cells are K_{Ca} channel, Kv channel, K_{ATP} channel and Kir channel. All of these K^+ channels have their own function. Among theses K^+ channels, Kv channel is thought to contribute to the regulation of the steady state resting membrane potential. It is well postulated that Kv channel influences the myogenic response of resistance vessels through its contribution to membrane potential and Kv channels blocker, 4-AP caused hypopolarization and vasoconstriction in rabbit cerebral artery (Knot & Nelson, 1995). Therefore, K^+ currents through Kv channel in CASMC of 1K,1C-GBH rats were recorded and compared to those of control rats. In this study, 4-AP (5 mM) caused 10 to 20 mV depolarization in normortensive rat and 1K,1C-GBH rat but K_{Ca} blocker, charybdotoxin did not cause any membrane potential to change. 4-AP-sesitive Kv currents were significantly decreased in 1K,1C-GBH rat when compared to normotensive rat. However, amplitude of charybdotoxin-sensitive K_{Ca} current did not show any difference between two groups. These results suggest that 4-AP-sensitive K^+ currents are involved in a set of resting membrane potential in CASMC. Membrane hypopolarization of CASMC from 1K,1C-GBH may be due to alteration in resting conductance through 4-AP-sensitive K^+ channels.

The voltage dependent gating and kinetics of K_V channels in 1K,1C-GBH rats were similar to those of normotensive rats. These results suggest that decreased components of 4-AP-sensitive Kv currents in 1K,1C-GBH rats may not be due to changes of electrophysiological properties of 4-AP-sensitive K^+ channels. Decreased activity of 4-AP-sensitive K^+ channels in 1K,1C-GBH rats may be caused by decrease in a density of channels or their single channel conductance. Therefore, further study is required for more information about undelying mechanism of decreased 4-AP-sensitive K^+ channel activity in hypertension.

Renal blood flow is reduced in 1K,1C GBH rat, thereby increasing plasma volume. Increased plasma volume cause the animal to be hypertensive and local blood flow is increased. In order to return the plasma volume and local blood flow to normal state, peripheral resistance must be elevated. In the resistance artery where systemic blood pressure and organ blood flow are regulated, transmural pressure is elevated. Elevated intravascular pressure may affect Kv channel activity and causes the membrane potential hypopolarization which result in contraction of arteries (Knot & Nelson, 1995) and increase in peripheral resistance. In summary, it is suggested that the increase of intravascular pressure causes the decrease of the 4-AP-sensitive Kv channel activity in 1K,1C-GBH rat and this would contribute to the hypopolarization of membrane potential, thereby developing vascular tone in 1K,1C-GBH rat.

REFERENCES

Bohr DH, Webb RC. Vascular smooth muscle membrane in hypertension. *Annu Rev Pharmacol Toxicol* 28: 389 −409, 1988

Daut JN, Standen NB, Nelson MT. The role of the mem-

brane potential of endothelial and smooth muscle cells in the regulation of coronary blood flow. *J Cardiovas Electrophysiol* 5: 154−181, 1994

Dobrin BP. Vascular mechanisms. In: Shepherd JT, Abboud FM (Eds.) *Handbook of Physiology, Section 2, The Cardiovascular System, Vol. III, Part I.* p 65−102 Bethesda, MD: American Physiological Society

England SK, Wooldridge TA, Stekeil WJ, Rush NJ. Enhanced single-channel K-current in arterial membranes from genetically hypertensive rats. *Am J Physiol* 264: H1337−H1345, 1993

Faraci FM, Sobey CG. Potassium channels and cerebral circulation. *Clin Exp Pharmacol Physiol* 23: 1091−1095, 1996

Fujiwara S, Itoh T, Suzuki H. Membrane properties and excitatory neuromuscular transmission in the smooth muscle of dog cerebral arteries. *Br J Pharmacol* 77: 197−208, 1982

Haeusler G. Contraction, membrane potential and calcium fluxes in rabbit pulmonary arterial muscle. *Fed Proc* 42: 263−268, 1983

Hermsmeyer K. Electrogenesis of increased norepinephrine sensitivity of arterial vascular muscle in hypertensive. *Circ Res* 38: 362−367, 1976

Hume JR, Leblanc N. Macroscopic K^+ currents in single smooth muscle cells of the rabbit portal vein. *J Physiol* 413: 49−73, 1989

Kang TM, So I, Uhm DY, Kim KW. Two types of voltage-dependent outward currents in smooth muscle cells of rabbit basilar artery. *Korean J Physiol Pharmacol* 1: 169−183, 1997

Kim SH, Choi KM, Kim HS, Jeon BH, Chang SJ. Mechanism of membrane hyperpolarization by extracellular K^+ in resistance-sized cerebral arterial muscle cell of rabbit. *Korean J Physiol Pharmacol* 3: 1−10, 1999

Kitazono T, Faraci FM, Taguchi H, Heistad DD. Role of potassium channels in cerebral blood vessels. *Stroke* 26: 1713−1723, 1995

Kitazono T, Heistad DD, Faraci FM. ATP-sensitive potassium channels in the basilar artery during chronic hypertension. *Hypertension* 22: 677−678, 1993

Knot HJ, Nelson MT. Regulation of membrane potential and diameter by voltage-dependent K^+ channels in rabbit myogenic cerebral arteries. *Am J Physiol* 269: H348−H355, 1995

Kuriyama H, Kitamura K, Nabata H. Pharmacological and physiological significance of ion channels and factors that modulate them in vascular tissues. *Pharmacol Rev* 47: 387−573, 1995

Lamb FS, Webb RC. Regenerative electrical activity and arterial contraction in hypertensive rats. *Hypertension* 13: 70−76, 1989

Matsuda JJ, Volk KA, Shibata EF. Calcium current in isolated rabbit coronary arterial smooth muscle myocytes. *J Physiol* 427: 657−680, 1990

Martens JR, Gelband CH. Alterations in rat interlobar artery membrane potential and K^+ channels in genetic and nongenetic hypertension. *Circ Res* 79: 295−301, 1996

Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol* 268: C799−C822, 1995

Nelson MT, Patlak JB, Worley JF, Standen NB. Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am J Physiol* 259: C3−C18, 1990

Ohya Y, Abe I, Fujii K, Takata Y, Fujishima M. Voltage-dependent Ca^{2+}-channels in resistance arterises from spontaneously hypertensive rats. *Circ Res* 73: 1090−1099, 1993

Robertson BE, Nelson MT. Aminopyridine inhibition and voltage dependence of K^+ currents in smooth muscle cells from cerebral arteries. *Am J Physiol* 267: C1589−C1597, 1994

Rusch NJ, Runnells AM. Remission of high blood pressure reverses arterial potassium channel alterations. *Hypertension* 23: 941−945, 1994

Rush NJ, De Lucena RG, Wooldridge TA, England SK, Cowley AW Jr. A Ca^{2+}-dependent K^+ current is enhanced in arterrial membranes of hypertensive rats. *Hypertension* 19: 301−307, 1992

Trapani A, Matsuki N, Abel PW, Hermsmeyer K. Norepinephrine produces tension through electromechanical coupling in rabbit ear artery. *Eur J Pharmacol* 72: 87−91, 1981

Van de Voorde J, Vanheel B, Leusen I. Edothelium-dependent relaxation and hyperpolarization in aorta from control and renal hypertensive rats. *J Pharmacol Exp Ther* 257: 555−561, 1992

Wilde DW, Furspan PB, Szocik JF. Cacium current in smooth muscle cells from normotensive and genetically hypertensive rats. *Hypertension* 24: 739−746, 1994