Korean J Physiol Pharmacol
Vol 3: 1−10, February, 1999

Mechanism of Membrane Hyperpolarization by Extracellular K^+ in Resistance-sized Cerebral Arterial Muscle Cell of Rabbit

Se Hoon Kim[1], Kun Moo Choi, Hoe Suk Kim, Byeong Hwa Jeon, and Seok Jong Chang

[1]Department of Physiology, Konyang University Medical College, Chungnam 320−711, Korea; Department of Physiology, Chungnam National University Medical College, Chungnam 301−131, Korea

We sought to find out the mechanism of vascular relaxation by extracellular K^+ concentration ($[K^+]_o$) in the cerebral resistant arteriole from rabbit. Single cells were isolated from the cerebral resistant arteriole, and using voltage-clamp technique barium-sensitive K^+ currents were recorded, and their characteristics were observed. Afterwards, the changes in membrane potential and currents through the membrane caused by the change in $[K^+]_o$ was observed. In the smooth muscle cells of cerebral resistant arteriole, ion currents that are blocked by barium, 4-aminopyridine (4-AP), and tetraethylammonium (TEA) exist. Currents that were blocked by barium showed inward rectification. When the $[K^+]_o$ were 6, 20, 60, and 140 mM, the reversal potentials were -82.7 ± 1.0, -49.5 ± 1.86, -26 ± 1.14, -5.18 ± 1.17 mV, respectively, and these values were almost identical to the calculated K^+ equilibrium potential. The inhibition of barium-sensitive inward currents by barium depended on the membrane potential. At the membrane potentials of -140, -100, and -60 mV, K_d values were 0.44, 1.19, and 4.82 μM, respectively. When $[K^+]_o$ was elevated from 6 mM to 15 mM, membrane potential hyperpolarized to -50 mV from -40 mV. Hyperpolarization by K^+ was inhibited by barium but not by ouabain. When the membrane potential was held at resting membrane potential and the $[K^+]_o$ was elevated from 6 mM to 15 mM, outward currents increased; when elevated to 25 mM, inward currents increased. Fixing the membrane potential at resting membrane potential and comparing the barium-sensitive outward currents at $[K^+]_o$ of 6 and 15 mM showed that the barium-sensitive outward current increased at 15 mM K^+. From the above results the following were concluded. Barium-sensitive K^+ channel activity increased when $[K^+]_o$ is elevated and this leads to an increase in K^+-outward current. Consequently, the membrane potential hyperpolarizes, leading to the relaxation of resistant arteries, and this is thought to contribute to an increase in the local blood flow of brain.

Key Words: Membrane hyperpolarization, Barium-sensitive K^+-channel, Cerebral artery

INTRODUCTION

Cerebral blood flow is controlled by the autonomic nervous system and metabolites, but it is known (Hill et al, 1986) that there are few sympathetic nerves distributed in cerebral arterioles. It is believed that metabolic regulation rather than nervous regulation plays more important role in the regulation of local blood flow in the brain. If cerebral activity such as motor activity, sensory stimulation, or seizure increases, metabolism around the area, as well as blood flow, increases (Plume et al, 1968; Invar, 1975; Fox and Raichle, 1984). This is because when metabolism in a locality in the brain increases, many metabolites diffuse out into the extracellular fluid, and it is thought that these metabolites dilate the arterioles in the surrounding area.

Metabolites that affect blood flow rate are reported to be H^+, metabolic products of purine, and K^+ (Kuschinsky et al, 1972; Whal and Kuschinsky, 1976), and among these K^+ is thought to play a major role in metabolic autoregulation of blood flow (Cameron & Cameron, 1976; Somjen, 1979; Sieber et al, 1993). When cerebral metabolism increases K^+ fluxes out of astrocytes (Newman, 1986; Paulson & Newman, 1987), and this elevates K^+ concentration of the extracellular fluid of vascular smooth muscle cells and leads to relaxation of arterioles. Most of the vascular smooth muscle hypopolarize and contract when $[K^+]_o$ is elevated (Casteels et al, 1979), but

Corresponding to: Se Hoon Kim, Department of Physiology, Konyang University Medical College, 30 Nae-Dong, Nonsan, Chungnam 320-711, Korea. (Tel) 0461-730-5344

smooth muscle in cerebral and cardiac arterioles, on the other hand, relaxes unlike the others (Kuschinsky et al, 1972; McCarron & Halpen, 1990). This suggests that there may be different types of ion channel or transmembrane protein in the membranes of cerebral and coronary artery smooth muscle cells that react with K^+ in a different manner from other smooth muscle cells, and many studies are currently in progress on them.

Hyperpolarization of smooth muscle cell due to an elevation in the $[K^+]_o$ is believed to be caused when inward rectifier K^+ channel (K_{ir} channel) is activated and K^+ permeability is increased, or when Na^+-K^+ pump is activated. Presence of K_{ir} channel and Na^+-K^+ pump in the smooth muscle of cerebral arterioles is well known, and close relationship between relaxation/hyperpolarization and K_{ir} channel/Na^+-K^+ pump has been demonstrated by the recording of mechanical tension or the conventional recording of membrane potential (Edwards et al, 1988; McCarron & Halpen, 1990; Knot et al, 1996).

This study, therefore, by making recordings of K_{ir} channel in single cell, strived to find out whether K_{ir} channel plays a role in the regulation of membrane potential in smooth muscle cells from resistance-sized cerebral artery, and whether hyperpolarization after the elevation of $[K^+]_o$ plays a role in the increase of K^+ permeability of the membrane through the increase in the K_{ir} channel activity.

METHODS

Isolation of single smooth muscle cell

For this study New-Zealand white rabbits weighing 2.5~3 Kg were used regardless of sex. Sodium pentobarbital (20 mg/kg) was injected into the ear vein, then common carotid artery was cut and exsanguinated. The brain was removed, then the basilar artery and its branches were carefully separated while supplying 100% O_2 in the preparation chamber containing physiological salt solution (PSS). Using a stereomicroscope (Olympus) blood within the vessels as well as the connective tissue surrounding the vessels were removed. Arteries with diameters smaller than 150 μm from the branches of middle cerebral artery and posterior cerebral artery were selected and cut out. These were placed in 4°C Ca^{2+}-free PSS for 20 minutes. Next, they were incubated in Ca^{2+}-free PSS (35°C) containing papain (200 μg/ml), collagenase (1 mg/ml), dithioerythritol (1 mg/ml), and bovine serum albumin (1 mg/ml) for 20 minutes. After this they were placed in modified-KB solution and agitated with a blunt glass pipette to obtain single cells. Separated cells were stored at 4°C and used for study within 10 hours.

Voltage-clamping of single cell

Using the method of Hamill et al (1981) single smooth muscle cells were whole-cell voltage clamped, and at room temperature (15~20°C) ion current and membrane potential were recorded. Pipettes were prepared by using microelectrode puller (PP-83, Narishige Scientific Instrument), and after being filled with pipette solution the pipettes with resistance of 2~3 MΩ were chosen. Resistance between the pipette and cell were made to be 10 Ω and over. Cell membrane was ruptured by applying negative pressure, then whole-cell currents were recorded. Using an amplifier (Axopatch 200 A, Axon Instrument, USA) ionic current was amplified and observed on an oscilloscope and recorded on a physiograph. A personal computer installed with pClamp software (v. 6.0.2) was used along with an analogue-digital converter (Digidata 1200, Axon Instrument, USA) to hold the membrane potential and apply the stimulating pulse. The amplified membrane currents were stored and analyzed. The data obtained from voltage-clamp technique were analyzed and processed with pClamp and Origin softwares.

Solutions

The composition of each solution used were as follows (all units in mM). Physiological salt solution (PSS) contained 134 NaCl, 6 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 5 glucose, and pH was made to 7.4 by titrating with Tris. PSS with no Ca^{2+} added were used as Ca^{2+}-free PSS. For high K^+ concentration solution an equal amount of Na^+ was replaced by K^+, thus keeping the osmolarity unchanged. Pipette solution contained 107 KCl, 1 $MgCl_2$, 10 HEPES, 0.1 EGTA, 3 Na_2ATP, 0.1 NaADP, and pH was made to 7.2 by titrating with KOH; K^+ concentration due to KCl and KOH was made to 140. The composition of modified KB (Kraftbruhe) solution was 50 L-glutamate, 40 KCl, 20 KH_2PO_4, 20 taurine, 3 $MgCl_2$, 10 HEPES, 0.5 EGTA, 10 glucose. pH was 7.3 by titration with KOH.

Drugs

The drugs used in this study were 4-aminopyridine (sigma), glibenclamide (sigma), tetraethylammonium

chloride (sigma), collagenase (Wako), papain (sigma), bovine serum albumin (sigma), and dithioerythritol (sigma). Glibenclamide was dissolved in dimethylsulfoxide (DMSO) and achieved a concentration of 10 mM. All the other drugs were dissolved in distilled water. When using 4-aminopyridine, pH of the solution was modified by titrating with HCl.

RESULTS

Various types of K^+ channel exist in smooth muscle cells of arteries, and depending on the vessel diameter and the organ or tissue where the arteries are found the distribution of K^+ channels varies. Accordingly, to find out which types of K^+ channel exist in smooth muscle cells of cerebral artery, ramp pulses from -140 mV to $+50$ mV for 3.5 seconds were applied on whole cells. Membrane currents were observed, and the resulting currents after applying several different K^+ channel blockers were compared with that of the control. Under the condition of K^+ concentration of 140 mM inside and 6 mM outside the cell, reversal potential was found to be -37.8 ± 1.12 mV (n=6), which was much lower than the K^+ equilibrium potential (-80 mV). At below -80 mV

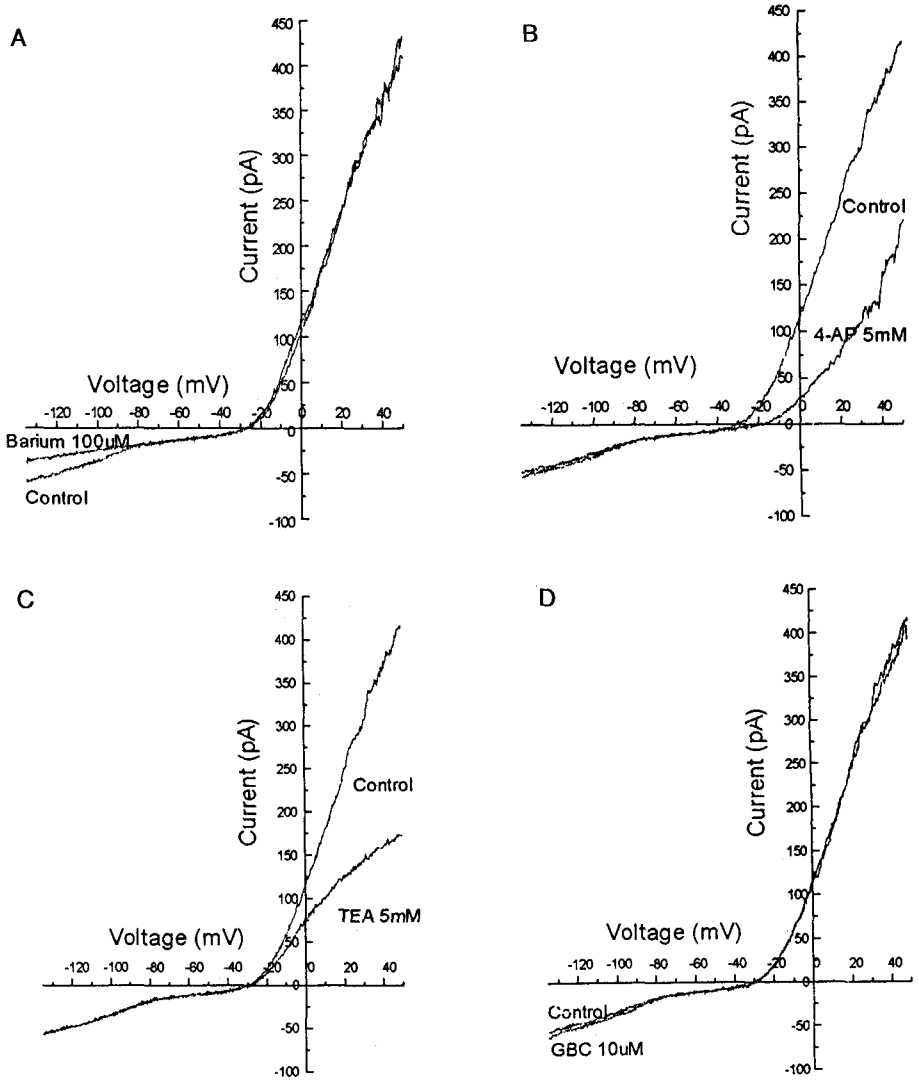

Fig. 1. Effects of K^+ channel blockers on K^+ currents. Barium (A), glibenclamide (GBC, B), tetraethylammonium chloride (TEA, C), and 4-aminopyridine (4-AP, D) were applied and membrane currents were recorded in response to voltage ramps of 3.5 second duration from -140 to $+50$ mV. Two currents were superimposed in each case, one in control solution and the other in solution containing K channel blockers.

inward currents increased, but at above −20 mV noise-like outward currents were observed (Fig. 1). Above procedures were repeated by separately adding K^+ channel blockers barium, 4-aminopyridine (4-AP), tetraethylammonium (TEA), glibenclamide (GBC) to the test solution. Barium (100 μM) showed no change in reversal potential nor outward current, but inward current that appeared below -80 mV was suppressed (Fig. 1A). 4-AP (5 mM) had no effect on inward currents but decreased outward currents, and also decreased the reversal potential from −37.8± 1.12 mV to −27.4±1.95 (n=6) (Fig. 1B). TEA (5 mM), however, did not affect reversal potential and inward currents; it did selectively suppress noise-like outward current (Fig. 1C). GBC (10 μM) had no effect at all on ionic currents or reversal potential (Fig. 1D).

To find out the characteristics of barium-blocked inward currents, the cells were stimulated and observed under the different K^+ concentrations (6 mM, 20 mM, 60 mM, 140 mM) with or without 100 μM barium (Fig. 2). Inward currents increased due to the elevation of $[K^+]_o$, but outward currents showed no big change (Fig. 2A). Barium selectively inhibited inward currents at each $[K^+]_o$ (Fig. 2B). All currents that were inhibited by barium at each $[K^+]_o$ showed inward rectification; outward currents inhibited by barium were extremely small, thus they practically were not observed on the voltage-current curve (Fig. 2C). Reversal potential for the barium-sensitive

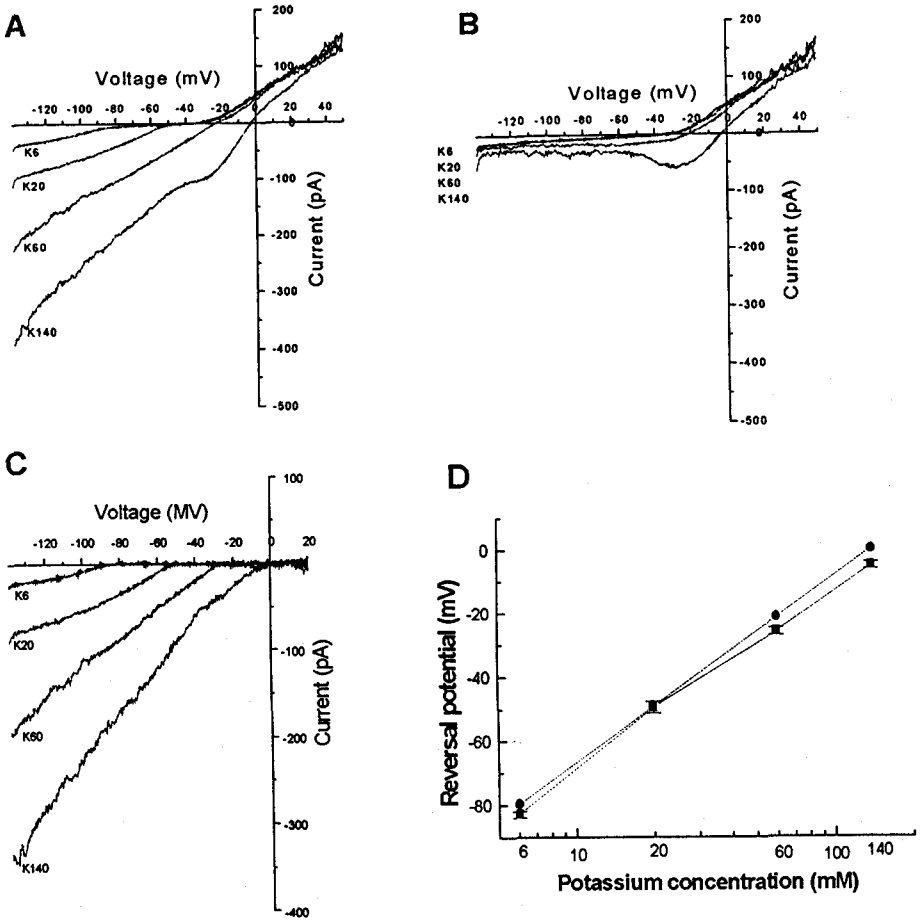

Fig. 2. Current-voltage relationships of K^+-currents in different extracellular K^+ concentration ($[K^+]_o$). $[K^+]_i$ was 140 mM and $[K^+]_o$ were 6, 20, 60 and 140 mM, respectively. A and B: membrane currents recorded from a cell in response to voltage ramps of 3.5 second duration from −140 to +50 mV in control solution(A), and in solution cotaining 100 μM barium(B). C: 100 μM barium-sensitive currents obtained from data of A and B. Four current traces are superimposed in each case. D: reversal potentials of barium-sensitive currents (■). Data points represent mean±SE (n=5). Theoretical equilibrium potential for K^+ obtained from the Nernst equation (●).

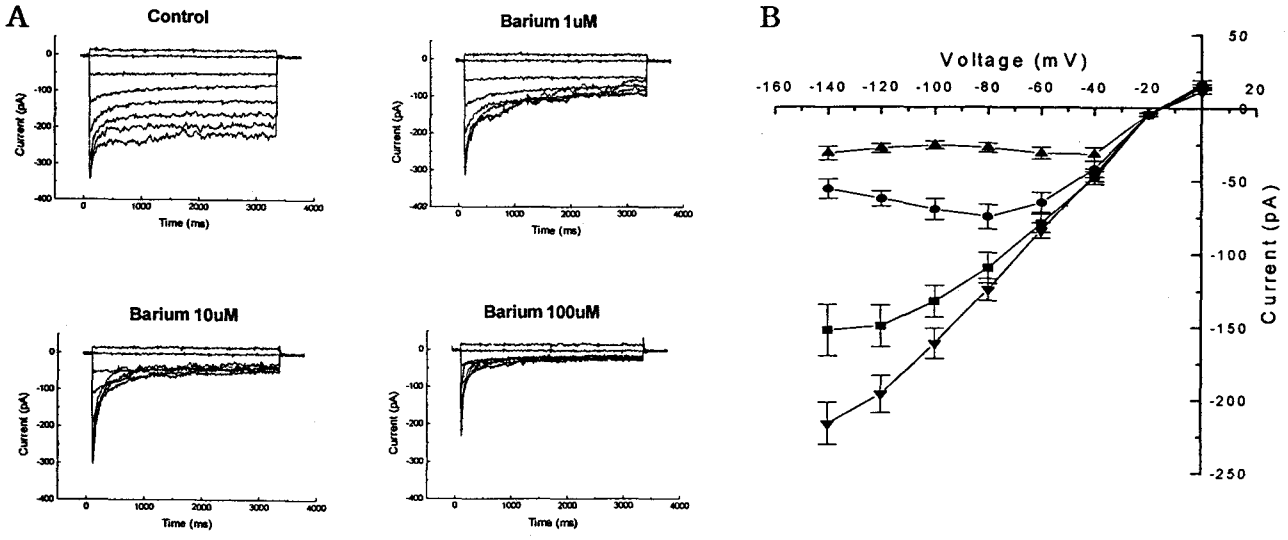

Fig. 3. Barium inhibition of inward currents. Intracellular K^+ concentration ($[K^+]_i$) and ($[K^+]_o$) were 140 and 60 mM, respectively. A: Membrane currents recorded from a cell in response to voltage steps of 3.5 second duration from holding potential of −20 mV to test potentials between −140 and 0mV in 20 mV increments. B: mean current-voltage relationships from 5 cells in control solution (▼) and solution containing 0.3 (■), 3 (●), 30 uM (▲) barium. Currents measured at the end of 3.5-s pulse.

currents were -82.7 ± 1.0 (n=5, $E_k=-79.6$), -49.5 ± 1.86 (n=5, $E_k=-49.2$), -26 ± 1.14 mV (n=5, $E_k=-21.4$), -5.18 ± 1.17 mV (n=5, $E_k=0$) at 6, 20, 60, and 140 mM K^+, respectively, and these correspond well to the calculated values of K^+ equilibrium potential. Inward conductance increased, too, as $[K^+]_o$ was elevated.

Barium is known to inhibit K_{ir} channels that are found in all tissues, including smooth muscle cells, at μM level. Therefore, it is used as a useful channel blocker in studies that seek to find out physiological role of Ki_{ir} channel in various tissues. At $[K^+]_o$ of 60 mM membrane potential was held at −20 mV, a value close to the K^+ equilibrium potential ($[K^+]i$: 140 mM; $[K^+]_o$: 60 mM; $E_k=-21.4$ mV), and step-pulse was given for 3.5 seconds at potentials from -140 mV to 0 mV at increments of 20 mV. Under the same conditions but varying barium concentrations from 0.3 μM to 100 μM the same stimuli were given, and the membrane currents were compared with those of the control (Fig. 3). The control showed initial inactivation after the step-pulse, but steady-state currents were recorded after 1 second. When barium was present currents decreased time-dependently (Fig. 3A). Because the decrease in inward currents caused by barium was dependent on time, steady state currents were recorded 3.5 seconds after stimulation at each fixed potential and barium concentration, and degree of inhibition on the inward current due to barium was compared with the control. The extent of inward current inhibition by barium was dependent on membrane potential. Fitting the results in fig. 4A with the equation $I_{Ba}/I_{Con}=1(1+[Ba^{2+}]/Kd)$, dissociation constants (K_d) were found to be 0.44, 0.67, 1.19. 2.57, and 4.82 μM at potentials of −140, −120, −100, −80, and −60 mV, respectively. As membrane potential increased inward currents were inhibited at lower barium concentration. The relationship between K_d and the membrane potential was graphed and fitted with the equation $K_d(V)=K_d(0)$ exp [zF μ V/RT] (Fig. 4B), where $K_d(0)$ is the dissociation constant at 0mV, R is the gas constant, T is the absolute temperature, F is Faraday's constant, z is the valence of the ion, and μ is the sensitivity of K_d to the membrane potential. At 0 mV K_d was 28.8 μM, and as the membrane potential increased by 33 mV K_d decreased e-fold.

To find out what effects the various K^+ channel blockers had on the membrane potential of single smooth muscle cell, current was held at 0 with the use of an amplifier, and 5 mM TEA, 100 μM barium, and 5 mM 4-AP were separately introduced. The resting membrane potential of single cell (with $[K^+]_o$ of 6 mM) was -35.3 ± 1.49 mV (n=9), which was much reduced from K^+ equilibrium potential (−80 mV), but when the $[K^+]_o$ was elevated to 60 mM membrane potential changed to -17.6 ± 1.1 mV (n=9), a value similar to K^+ equilibrium potential (−21.4 mV). Membrane potential did not seem to be affected by TEA but decreased to -32.5 ± 1.26 mV

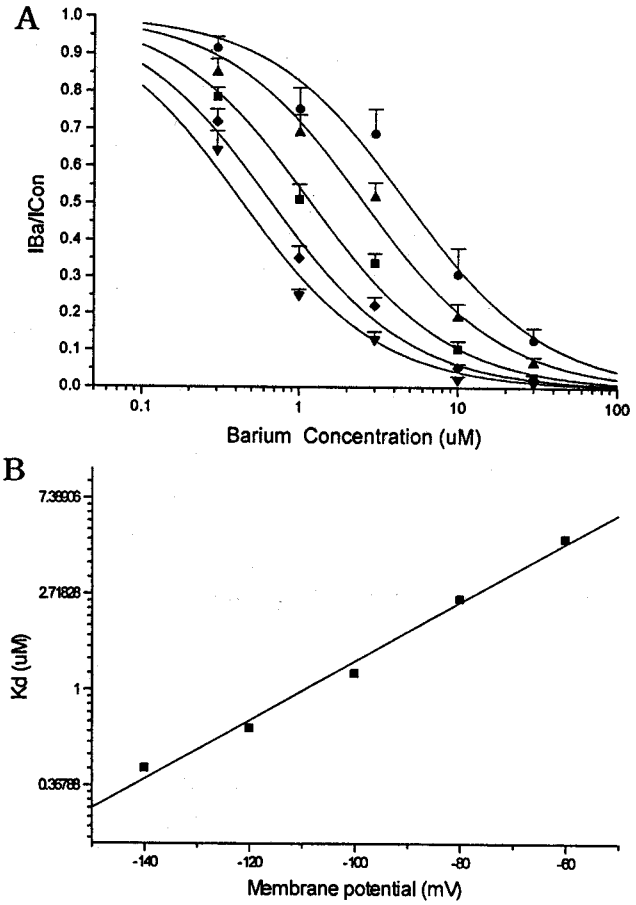

Fig. 4. Voltage dependence of barium block of inward K^+-currents. A: dose-response curves for fractional inhibition of current by barium at different membrane potentials −60 mV (●), −80 mV (▲), −100 mV (■), −120 mV (◆), and −140 mV (▼). Data points represent mean±SE(n=5). B: voltage dependence of K_d from Fig. 2A.

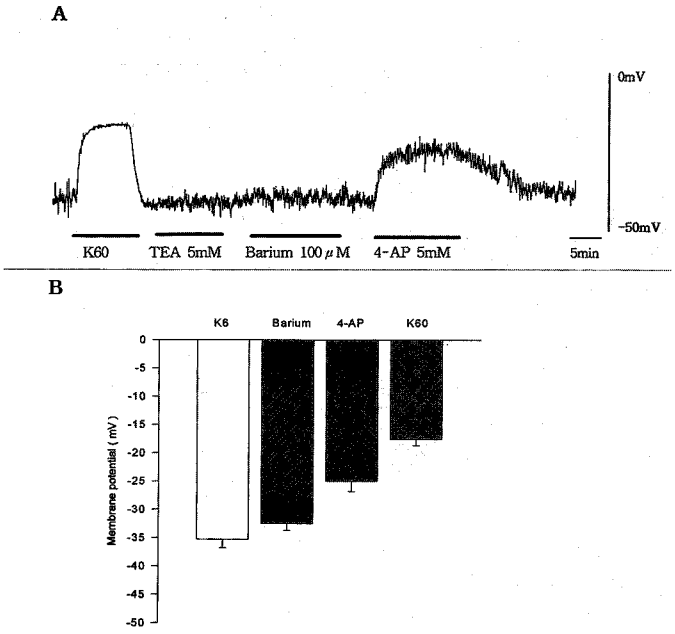

Fig. 5. Effects of K^+ channel blockers on the resting membrane potential. A: the membrane potential recorded from a cell in physiological salt solution. Addition of barium or 4-AP (4-aminopyridine) depolarized the membrane potential but TEA (tetraethylammonium) did not affect on membrane potential. B: mean (±SE) membrane potential from 7 cells.

(n=9) by barium, and to −25±1.83 mV by 4-AP (Fig. 5).

The mechanism involved for the change in membrane potential after an elevation in the $[K^+]_o$ was sought. When the $[K^+]_o$ is elevated from 6 mM to 15 mM with the membrane current held at 0, membrane potential increased from −40 mV to −50 mV, but relative to the K^+ equilibrium potential (when $[K^+]_o$ is 15 mM, K^+ equilibrium potential is −56.4 mV) membrane potential was yet lower (Fig. 7A). After increasing the membrane potential (by 15 mM K^+), administration of 100 μM barium lowered the membrane potential to −25 mV, but 100 μM ouabain did not. The increase in membrane potential when $[K^+]_o$ was elevated implies that outward currents increased by K^+. Accordingly, to confirm this idea, the currents were recorded with the membrane potential at resting membrane potential, and also after elevating the $[K^+]_o$ from 6 mM to 15 mM and to 25 mM. When the $[K^+]_o$ was 15 mM membrane current changed from 0 pA (at 6 mM K^+) to 1.5 pA, an increase in the outward current, but at 25 mM K^+ the current changed to −5.0 pA, an increase in the inward current. To see if the outward current that increases as $[K^+]_o$ is elevated has any relationship to the barium-sensitive ion channel, 100 μM barium was given at 6 mM and 15 mM K^+ while fixing the membrane potential at the resting membrane potential. At 15 mM K^+, despite the fact that the K^+ concentration gradient was decreased compared to at 6 mM, the outward currents that are inhibited by barium increased instead (Fig. 6C).

DISCUSSION

A variety of K^+ channels exist in smooth muscle cells of arteries, including Ca^{2+}-activated K^+ channel (K_{Ca} channel, Benham et al, 1986; Brayden and Nelson, 1992), voltage-dependent K^+ channel (K_v channel, Beech & Bolton, 1989; Bonnet et al, 1991), ATP-sensitive K^+ channel (K_{ATP} channel, Standen et al, 1989; Nagano et al, 1996), and inward rectifier K^+ channel (K_{ir} channel, Edwards and Hirst, 1988;

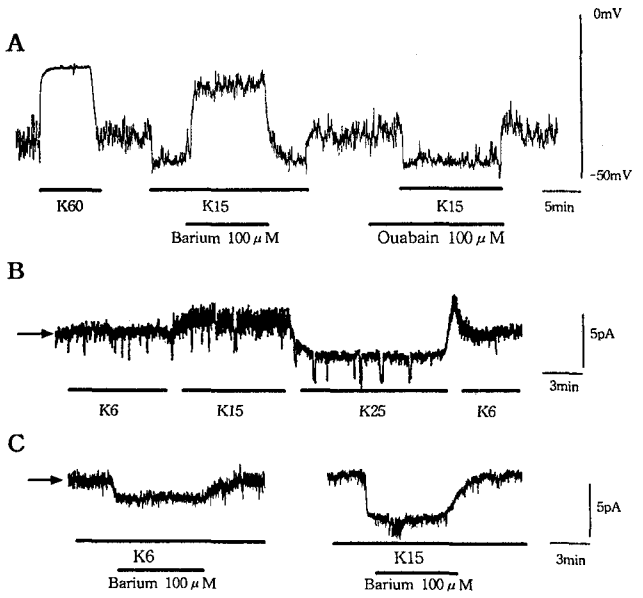

Fig. 6. Effect of changing extracellular K^+ concentration ($[K^+]_o$) on the membrane potential and current of single cell. A: the membrane potential recorded in trace was obtained from single cell. Increase of $[K^+]_o$ hyperpolarized the membrane potential. Hyperpolarization of membrane potential was blocked by barium but not ouabain. B and C: membrane current recorded in trace was obtained from single cell at holding potential (−51 mV). Arrow, 0 pA. B: when $[K^+]_o$ was raised from 6 mM to 15 mM, outward current was enhanced. However, inward current was enhanced by raising $[K^+]_o$ from 6 to 25 mM(B). C: barium (100 uM)-sensitive curents increased by raising $[K^+]_o$ from 6 to 15 mM.

Quayle et al, 1993; Robertson et al, 1996). Their distribution varies according to the size of the vessel and the organ in which the vessels are located. In cerebral arteries, especially in the small resistant arterioles, K_{Ca}, K_v, and K_{ir} channels have been found (Quayle et al, 1993; Robertson and Nelson, 1994; Gokina et al, 1996), and in large cerebral arteries like basilar artery, K_{ATP} channels in addition to the above three types have been identified (Nagano et al, 1996). In this study resistance-sized cerebral arteries with diameters smaller than 150 μm were used. The experiment was also carried out with the ATP concentration of the solution within the pipette at 3 mM. Under these conditions K^+ current that is inhibited by the selective K_{ATP} channel blocker glibenclamide was not recorded, and there was no increase in the inward or outward currents by K_{ATP} channel openers cromakalim and pinacidil. McCarron et al (1991) noted that cerebral arterioles were not relaxed by K_{ATP} channel activator cromakalim and suggested that, unlike other types of arterial smooth muscle cell, cerebral arterioles do not possess K_{ATP} channels.

K^+ currents that are selectively inhibited by TEA, 4-AP, and barium were observed. Consequently, it is thought that there are at least three types of K^+ channels in smooth muscle cells of resistance-sized cerebral artery. K_{Ca} channel activity is regulated by intracellualr Ca^{2+} concentration and has noise-like K^+ current (Langton et al, 1991). Also, it is known to be very effectively blocked at lower concentration of TEA compared to other types of K^+ channels. In the current study TEA-inhibited current showed noise-like characteristics, and was activated near the membrane potential of −20 mV. This is thought to be since intracellular Ca^{2+} concentration was lower due to 0.1 mM EGTA in the pipette solution. Thus, it is believed that TEA-sensitive K_{Ca} channel will not have any significant effect on producing resting membrane potential under the conditions of this study. The ionic current that is blocked by 4-AP, however, is dependent on membrane potential, and seeing that it has outward-rectified characteristics, it is probably due to currents through K_v channels. This K^+ channel starts to activate around −40 mV which is almost identical to the resting membrane potential of single cell. Furthermore, membrane potential was hypopolarized, and the reversal potential moves toward 0 mV by 4-AP. All these suggest that under the conditions of this study the membrane potential of cerebral artery is controlled by K_v channel. Robertson & Nelson (1994) reported that K_v channel in rabbit cerebral arteriole is inhibited by 4-AP and is activated around the resting membrane potential of the cerebral arteriole. Ion currents that are blocked by barium showed inward rectification and dependent on membrane potential and $[K^+]_o$. In addition, not only are the currents blocked at very low concentration of barium, but as membrane potential increased K_d of barium inhibition decreased. These characteristics are similar to those of the K_{ir} channel found in starfish egg (Hagiwara et al, 1978), skeletal muscle (Standen & Starfield, 1978; Leech & Stanfield, 1981), and other types of smooth muscle (Quayle et al, 1993; Quayle et al, 1996). At resting state the membrane potential of single cell is decreased only slightly by barium, and this suggests barium-sensitive K^+ channel contributes little to the resting membrane potential. Generally, the resting membrane potential of arteriolar smooth muscle is −50 ~ −60 mV, which is much lower than the K^+ equilibrium potential. This phenomenon arises because cell membrane of smooth muscle cell at rest has a permeability to K^+ that is much lower than nerve, skeletal muscle, or cardiac

muscle. The reason for this seems to be that K_{ir} channel that is activated when hyperpolarized and contributes to formation of resting membrane potential is less developed compared to other tissues, or has different characteristics. In resistance-sized cerebral artery at $[K^+]_o$ of 6 mM, inward current through barium-sensitive K^+ channel was large but outward current was small. This signifies that barium-sensitive K^+ channel has low conductance at resting potential. Therefore, it is presumed that barium-sensitive K^+ channel in resistance-sized cerebral artery is less developed relative to other tissues, and because the outward current is extremely low it contributes little to forming resting potential. It has been reported that in cerebral arteries types of K^+ channels are different depending on the location, and the K^+ channels that form resting membrane potential are different since each type of K^+ channel has different conductance. According to Edwards et al (1988), cerebral arteries proximal to the basilar artery in the brain has resting membrane potential near -70 mV, and in these arteries the resting potential is controlled mainly by K_{ir} channel; in distal cerebral arteries the resting potential is around -40 mV, mainly by K_v channel. On the other hand, according to McPherson & Keily (1995), the membrane potential of cerebral arteriole changes according to the magnitude of passive tension applied on the arterial wall, because the types of K^+ channel that is activated by a change in passive tension changes.

Cerebral and coronary blood flow depends on nervous and metabolic controls, and metabolic control plays more important role than nervous control. Metabolic control of blood flow relies on the metabolites K^+, H^+, adenosine, and others (Kuschinsky et al, 1972; Whal & Kuschinsky, 1976). Especially, autoregulation of blood flow by an elevation in $[K^+]_o$ plays an important role (Kuschinsky et al, 1972; Cameron & Carrona, 1976; Somjen, 1979; Edwards et al, 1988; Sieber et al, 1993). The fact that distal cerebral artery has a membrane potential that is more hypopolarized than that of proximal cerebral artery carries great significance in the control of cerebral blood flow. To restate, since the membrane potential of proximal cerebral artery is controlled by K_{ir} channel, it is regulated to be near the K^+ equilibrium potential at normal $[K^+]_o$. When $[K^+]_o$ is elevated the membrane potential is hypopolarized and smooth muscle contracts. On the other hand the membrane potential of distal cerebral artery is controlled by K_v channel at rest and is maintained around -40 mV at normal $[K^+]_o$. This is much lower than the K^+ equilibrium potential. Therefore, if by some factor K_{ir} channel is activated, there is a possibility that the membrane potential will be hyperpolarized. Known as an important regulating factor of blood flow among metabolites, K^+ activates K_{ir} channel in various types of cells. Consequently, when metabolism is increased in the brain or in the heart, the elevated $[K^+]_o$ enhances K_{ir} channel activity, leading to the relaxation of the distal cerebral arteries and so playing a major role in increasing the blood flow. In proximal cerebral arteries and the arteries that are involved in systemic circulation, an elevation of $[K^+]_o$ has no effect on K^+ conductance, but rather decreases the K^+ concentration gradient within the cell and causing hypopolarization. In the distal arteries, however, K_{ir} channel is activated, leading to the increase in K^+ conductance. The ensuing hyperpolarization due to K^+ conductance increase overcomes the effect of hypopolarization by the decrease in K^+ concentration gradient, and hyperpolarization occurs overall. In this study, not only is the membrane potential hyperpolarized at $[K^+]_o$ of 15 mM compared to at $[K^+]_o$ of 6 mM, but also outward currents increased when the potential was fixed at resting potential and the $[K^+]_o$ was elevated from 6 mM to 15 mM. The increase in outward currents by elevating $[K^+]_o$ has strong ties to barium-sensitive K^+ current. In other words, at resting potential there is greater barium-sensitive outward current at $[K^+]_o$ of 15 mM K^+ than at $[K^+]_o$ of 6 mM K^+. This signifies that the elevation of $[K^+]_o$ results in hyperpolarization through an increase in K^+ conductance more than in hypopolarization through a decrease in the K^+ concentration gradient.

In resistance-sized cerebral artery the membrane potential at resting state is regulated mainly by K_v channel, and the membrane potential is below the K^+ equilibrium potential. When metabolism in the brain increases, however, K^+ diffuses into the artery from the surrounding nerve cells, and the $[K^+]_o$ is elevated in the periphery of the arterial cells. The elevated $[K^+]_o$ then activates barium-sensitive K^+ channels of arterial smooth muscle cells. An increase in K^+ conductance follows, which then causes hyperpolarization. Next, the voltage-dependent Ca^{2+} channels are inhibited and the influx of Ca^{2+} into the smooth muscle cells is decreased. It is thought that the decrease in Ca^{2+} influx into the arterial cells relaxes the smooth muscle and thus increases the local cerebral blood flow.

REFERENCES

Beech DJ, Bolton TB. Two components of potassium

current activated by depolarization of single smooth muscle cells from the rabbit portal vein. *J Physiol* 418: 293–309, 1989

Bonnet P, Rusch NJ, Harder DR. Characterization of an outward K^+ current in freshly dispersed cerebral arterial muscle cells. *Pfluegers Arch* 418: 292–296, 1991

Brayden JE, Nelson MT. Regulation of arterial tone by activation of calcium-dependent K^+ channels. *Science* 256: 532–535, 1992

Benham CD, Bolton TB, Lang RJ, Takewaki T. Calcium activated potassium channels in single smooth muscle cells of rabbit jejunum and guinea pig mesenteric artery. *J Physiol* 371: 45–67, 1986

Cameron IR, Caronna J. The effects of local changes in potassium and bicarbonate concentrations on hypothalamic blood flow in the rabbit. *J Physiol* 262: 445–430, 1976

Casteels R, Kitamura K, Kuriyama H, Suzuki H. Excitation-contraction coupling in the smooth muscle cells of the rabbit main pulmonary artery. *J Physiol* 271: 62–79, 1979

Edwards FR, Hirst GDS. Inward rectification of submucosal arterioles of guinea-pig ileum. *J Physiol* 404: 437–454, 1988

Edwards FR, Hirst GDS, Silverberg GD. Inward rectification in rat cerebral arterioles: involvement of potassium ions in autoregulation. *J Physiol* 404: 455–466, 1988

Fox PT, Raichle ME. Stimulus rate dependence of regional cerebral blood flow in human striate cortex, demonstration by positron emission tomography. *J Neurophysiol* 51: 1109–1120, 1984

Gokina NI, Wellman TD, Bevan RD, Walters CL, Penar PL, Bevan JA. Role of Ca^{2+}-activated K^+ channels in the regulation of membrane potential and tone of smooth muscle in human pial arteries. *Circ Res* 79: 881–886, 1996

Hagiwara S, Myazaki S, Moody W, Patlak J. Blocking effects of barium and hydrogen ions on the potassium current during anomalnous rectification in the starfish egg. *J Physiol* 279: 167–185, 1978

Hamill OP, Marty A, Neher E, Sakman B, Sigworth FJ. Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patchs. *Pfluegers Arch* 391: 85–100, 1981

Hill CE, Hirst GDS, Silverberg GD, van Halden GF. Sympathetic innervation and excitability of arterioles originating from the rat middle cerebral artery. *J Physiol* 371: 305–316, 1986

Invar DH. Correlation between the cerebral blood flow and cerebral function in man. In: *Cerebral Circulation and Metabolism*, edited by G Tramer. Milan, Italy: Farmitalia, P1–15, 1975

Knot HJ, Nelson MT. Regulation of membrane potential and diameter by voltage dependent potassium channels in rabbit myogenic cerebral arteries. *Am J Physiol* 269: H348–355, 1995

Kuschinsky W, Wahl M, Bosse O, Thurau K. Perivascular potassium and pH as determinants of local pial arterial diameter in cats. *Circ Res* 31: 240–247, 1972

Langton PD, Nelson MT, Huang Y, Standen NB. Block of calcium-activated potassium channels in mammalian arterial myocytes by tetraethylammonium ions. *Am J Physiol* 260: H927–H934, 1991

Leech CA, Stanfield PR. Inward rectification in frog skeletal muscle fibers and its dependence on membrane potential and external potassium. *J Physiol* 319: 295–309, 1981

McCarron JG, Halpern W. Potassium dilates rat cerebral arteries by two independent mechanisms. *Am J Physiol* 259: H902–908, 1990

McCarron JG, Quayle JM, Halpern W, Nelson MT. Cromakalim and pinacidil dilate small mesenteric arteries but not small cerebral arteries. *Am J Physiol* 261: H287–H291, 1991

McPherson GA, Keily SG. Electrophysiological properties of the rat middle cerebral artery at different levels of passive wall tension. *Clin Exp Pharmacol Physiol* 22: 724–731, 1995

Nagao T, Ibayashi S, Sadoshima S, Fujii K, Fujii K, Ohya Y, Fugishima M. Distribution and physiological roles of ATP-sensitive K^+ channels in the vertebrobasilar system of the rabbit. *Circ Res* 78: 238–243, 1996

Newman EA. High potassium conductance in astrocyte endfeet. *Science* 233: 453–454, 1986

Paulson OB, Newman EA. Does the release of potassium from astrocyte endfeet regulate cerebral blood flow? *Science* 237: 896–899, 1987

Plum F, Posner JF, Troy B. Cerebral metabolic and circulatory response to induced convulsion in man. *Arch Neurol* 18: 1–13, 1968

Quayle JM, Dart C, Standen NB. The properties and distribution of inward rectifier potassium currents in pig coronary arterial smooth muscle. *J Physiol* 494: 715–726, 1996

Quayle JM, McCarron JG, Brayden JE, Nelson MT. Inward rectifier K^+ currents in smooth muscle cells from rat resistance-sized cerebral arteries. *Am J Physiol* 265: C1363–C1370, 1993

Robertson BE, Bonev AD, Nelson MT. Inward rectifier K^+ currents in single smooth muscle cells from rat coronary arteries: block by Mg^{2+}, Ca^{2+}, and Ba^{2+}. *Am J Physiol* 271: H696–H705, 1996

Robertson BE, Nelson MT. Aminopyridine inhibition and voltage dependnece of K+ currents in smooth muscle cells from cerebral arteries. *Am J Physiol* 267: C1589–1597, 1994

Sieber FE, Wilson DA, Hanley DE, Traystman RJ. Extracellular potassium activity and cerebral blood flow during moderate hypoglycemia in anesthetized dogs. *Am J Physiol* 264: H1774–1780, 1993

Somjen GG. Extracellular potassium in the mammalian central nervous system. *Annu Rev Physiol* 41: 159–

177, 1979

Standen NB, Quayle JM, Davies NM, Brayden JE, Huang Y, Nelson MT. Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science* 245: 177−180, 1989

Standen NB, Stanfield PR. A potential- and time-dependent blockade of inward rectification in frog skeletal muscle fibers by barium and strontium ions. *J Physiol* 280: 169−191, 1978

Wahl M, Kuschinsky W. The dilatory action of adenosine on pial vessels and its inhibition by theophylline. *Pfluegers Arch* 362: 55−59, 1976