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Moderate Elevation of Extracellular K^+ Concentration Induces Vasorelaxation in Isolated Rat, Rabbit and Human Cerebral Arteries: Role of Na Pump and Ba-Sensitive Process

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Cerebral blood vessels relax when extracellular K^+ concentrations ($[K^+]_e$ are elevated moderately (2∼15 mM, K^+-induced vasorelaxation). We have therefore studied the underlying mechanism for this K^+-induced vasorelaxation in the isolated middle cerebral arteries (MCAs). The effects of ouabain and Ba^{2+} on K^+-induced vasorelaxation were examined to determine the role of sodium pump and/or Ba-sensitive process (possibly, inward rectifier K current) in the mechanism. Mulvany myograph was used to study 24 rats, 18 rabbits, and 10 humans MCAs (216±3 μm, 347±7 μm, and 597±39 μm in diameter when stretched to a tension equivalent to 55 mmHg). High K^+ (125 mM) and $PGF_{2\alpha}$ (1∼10 μM) induced concentration-dependent contractions in all 3 species, while histamine (10∼50 μM) evoked contraction only in the rabbits and induced relaxation in the rats and humans. Addition of K^+ (2∼10 mM) to the control solution induced vasorelaxations. These effects were inhibited by the pretreatment with both ouabain (10 μM) and Ba^{2+} (0.1∼0.3 mM) in the rat, but only with ouabain (10 μM) in the rabbit and human. These results suggest that K^+-induced vasorelaxation occurs via the stimulation of electrogenic Na pump in the rabbit and human MCAs, while in the rat MCAs via the activation of both Na pump and Ba-sensitive process.

Key Words: Cerebral artery, Vasorelaxation, Myograph, Potassium, Ouabain, Barium

INTRODUCTION

Previous investigations on the contractile properties of the vascular smooth muscle have included the studies on complete vascular beds and isolated vessels having lumen diameters greater than 500 μm. Thanks to the progress in biological technology, myograph, which was developed by Mulvany and Halpern in 1976, made possible the experiments on small resistance artery (about 100∼400 μm diameter) in vitro (Mulvany & Halpern, 1976).

Small vessels could be mounted as ring preparations without damages on a myograph, which measures highly isometric responses. Contractions should be determined under "isometric" condition. Main reasons for that are: (a) The force generation is dependent on the extent of stretch, according to the well-known active tension-length relation of all muscles; (b) The sensitivity of vessels to different agonists is also strongly dependent on the extent of stretch (Mulvany, 1983).

This myograph technique is suitable for the vessels with internal diameters between 100∼400 μm. It appears that in some vascular beds, at least 50% or more of the precapillary pressure drop occurs in vessels with internal diameters greater than 100 μm. Thus the vessels with internal diameters in the range of 100∼400 μm must contribute substantially to the peripheral resistance (Mulvany & Aalkjaer, 1990).

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Considering the size of resistant vessels, this myograph technique is useful in studying the physiological role of vascular smooth muscle in hypertension and blood flow.

Cerebral blood flow is influenced by brain metabolism, and it is also reported that changes in extracellular potassium ion (K^+) concentration are important in linking brain metabolic activity with blood supply (Sykova, 1983). Moreover, it was reported that increase in extracellular K^+ concentration (1~15 mM) induced hyperpolarization and relaxation in cerebrovascular smooth muscles (Cameron & Caronna, 1976; Hermsmeyer & Harder, 1986; Edwards et al, 1988).

Lots of experimental data on K^+-induced vasodilatation and hyperpolarization from a variety of tissue types have shown consistently the involvement of the sodium pump as an underlying mechanism (Chen et al, 1972; Hendrickx & Casteels, 1974; Webb & Bohr, 1978). In other experiments on potassium-induced dilation, two components have been observed: One of them was, as has been expected, the activated sodium pump; and the other was barium sensitive process which might be inward rectifier potassium current. To determine the role of two components in the response of K^+-induced vasodilation, ouabain (sodium pump inhibitor) and Ba^{2+} (inward rectifier K^+ channel blocker), were used (McCarron & Halpern, 1990a; 1990b).

Up to now, K^+-induced vasorelaxation and its underlying mechanisms in human cerebral artery have never been investigated *in vitro*. This study presents firstly the compared responses of K^+-induced vasorelaxations in rat, rabbit and human middle cerebral arteries using Mulvany myograph.

METHODS

Rabbits of either sex (New Zealand white rabbit, 4~5 weeks old, 0.7~1.5 kg) were anesthetized with sodium pentobarbital (50 mg/kg, i.v.) and exsanguinated. Rats of either sex (Sprague-Dawley rat, 8~16 weeks old, 0.3~0.5 kg) were anesthetized with 20% urethane (5 ml/kg, i.p.) and exsanguinated. Their brains were then rapidly removed and placed in 95% O_2~5% CO_2 saturated, bicarbonate-buffered physiological salt solution (PSS; NaCl 119, KCl 4.7, $CaCl_2 \cdot 2\ H_2O$ 2.5, $MgSO_4 \cdot 7\ H_2O$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, EDTA 0.027, Glucose 5.5 mM). Then, the segment of cerebral artery was gently dissected from the surface of the brain and placed in bicarbonate-buffered PSS. Tunica adventitia, surrounding connective tissue, and side branches were removed gently under a stereomicroscope. Then, the segment was divided into 1~2 mm wide vascular rings, and they were mounted on myograph (Model 410A, J-P Trading, Denmark) as ring preparations to measure isometric tension (Mulvany & Halpern 1976).

Human arteries were dissected from the temporal pial surface of brain biopsy specimens removed during neurosurgical dissection, as distantly as possible from the diseased area and placed in a dissecting dish containing cold pregassed PSS. After that, arterial segments were cleaned off surrounding connective tissue and set up in a myograph as previously described. All patients (aged 27 to 55 years; mean, 37.2± 5.1 years) were normotensive and had no indications of other cardiovascular diseases.

The arteries were put in the PSS of pH 7.4 at 37°C and bubbled with 95% O_2 containing 5% CO_2. The vessels were equilibrated in PSS for 1 hour, and then stretched to determine the passive and active tension-internal circumference characteristics in some experiments. From the tension-internal circumference curve, appropriate transmural pressure and equivalent internal circumference were determined (the maximum active tension for the minimum resting tension developed approximately at this circumference). Then, vessels were stretched to a predetermined internal circumference through "normalization" process, which is part of myograph software (Myosight, J-P trading, Denmark).

Arteries were equilibrated at this internal circumference for 1~1.5 hours and kept at this level of internal circumference throughout the experiment. To estimate generalized contraction response, 125 mM potassium-PSS (high K^+-PSS) was used. Arteries were maximally contracted by high K^+-PSS. Experiments were aborted if the effective active pressure was less than 100 mmHg. The mechanical responses of the arteries were expressed as active wall tension. To evaluate the degree and mechanism of K^+-induced vasorelaxation, K^+ (1~15 mM) was added to the normal PSS, which already contained 5.9 mM K^+. All data were expressed as mean with standard errors. Drugs were administered to the bath in a single dose to reach the final required concentrations, and concentration-response curves were made for all the agents used. The viability of the vessels was verified

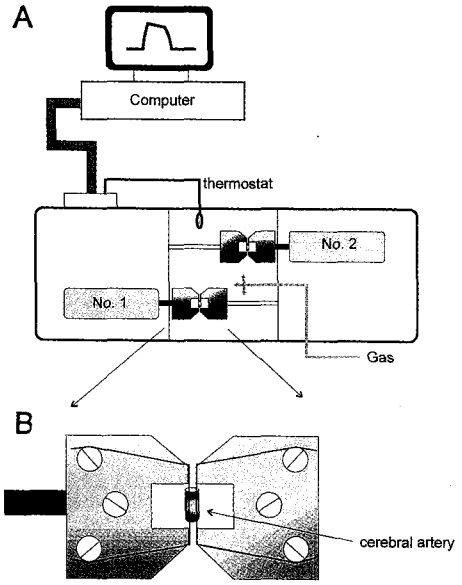

Fig. 1. A. Overview of dual myograph model. Dissected vessel rings were mounted in the middle chamber (10 ml size) which was bubbled by mixed gas (95% O_2 and 5% CO_2) and heated by a thermostat. No. 1 and No. 2 indicate force transducers, respectively. **B.** One end of myograph and mounted vessel.

by comparing the response of high K^+ or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) at the end of each experiment with that at the start. The endothelium was not removed in this experiment.

For the Myograph method of mounting vessels, refer to the reports by the following authors: Mulvany and Halpern, 1977; Mulvany and Warshaw, 1981; Mulvany et al 1982; Aalkjaer & Mulvany, 1985; Angus et al 1988; Nielsen et al 1989; Aalkjaer & Cragoe, 1989; Videbaek et al 1990; and Jensen et al 1992. Fig. 1 shows the diagram of myograph apparatus.

The drugs used in this study are: histamine, vasopressin, $PGF_{2\alpha}$, $BaCl_2$, and ouabain (all purchased from Sigma).

RESULTS

Twenty four branches of the middle cerebral artery obtained from rats, eighteen from rabbits and ten from human were used in this study. The responses to agonists were reproducible for 10~12 hours from the beginning.

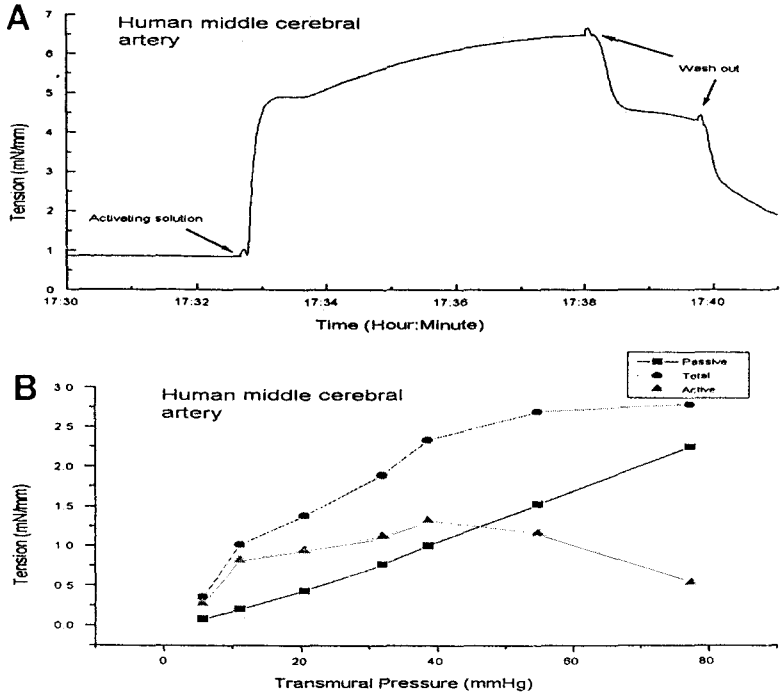

Fig. 2. A. Response of human middle cerebral artery to activating solution (5 mM Ca^{2+} in 125 mM K^+-PSS). B. Resting, total and active wall tension- transmural pressure relationship of human middle cerebral arteries when activating solution (5 mM Ca^{2+} in 125 mM K^+-PSS) was introduced.

Tension-internal circumference characteristics

The wall tension-internal circumference curves of rats, rabbits and human were obtained. Maximum active tension for the minimum resting tension was developed between 40 and 60 mmHg transmural pressure and equivalent internal circumference was obtained from the tension-internal circumference curve of each vessel. Fig. 2 demonstrates the wall tension-internal circumference relationship of human middle cerebral artery (MCA). In continual experiments, vessels were stretched to transmural pressure of 55 mmHg, which is within physiological range. Normalized intraluminal diameters were 216±3 μm in MCAs of rats, 347±7 μm in those of rabbits, and 597±39 μm in human MCAs, respectively.

Resting tone and contractile responses

Main experimental procedure started 1.5~2 hours after vessels were mounted on myograph. MCAs of rats showed spontaneous phasic activity, which were characteristic of repetitive spontaneous contraction-relaxation phenomena, even in the resting state with normal PSS. This phenomenon, which was not observed in MCA of rabbit or human, was accentuated by adding K^+ (4~10 mM) to the bath (Fig. 3). MCAs of rats were characterized by maintaining considerable tension even in the resting state without stimulus. The resting tone in MCAs of rats was 1.6± 0.13 mN/mm and was much higher than that in rabbits (0.3±0.03 mN/mm). In contrast, rabbit MCAs showed lower resting tone (0.3±0.03 mN/mm) without phasic activity under control condition.

Among the ten vessels of human, two showed high resting tone without phasic activity (4.55, 4.89 mN/mm), four showed moderate tone (1.74, 1.92, 1.49, 1.88 mN/mm); and the rest four showed low tone (0.67, 0.69, 0.69, 0.72 mN/mm). Four out of the 10 specimens were excluded due to their irregular high tone state and irregular response to several drugs, but all tested vessels showed contractile response to 125 mM K^+. Six vessels showed somewhat relaxed or a little contractile response, which were similar to

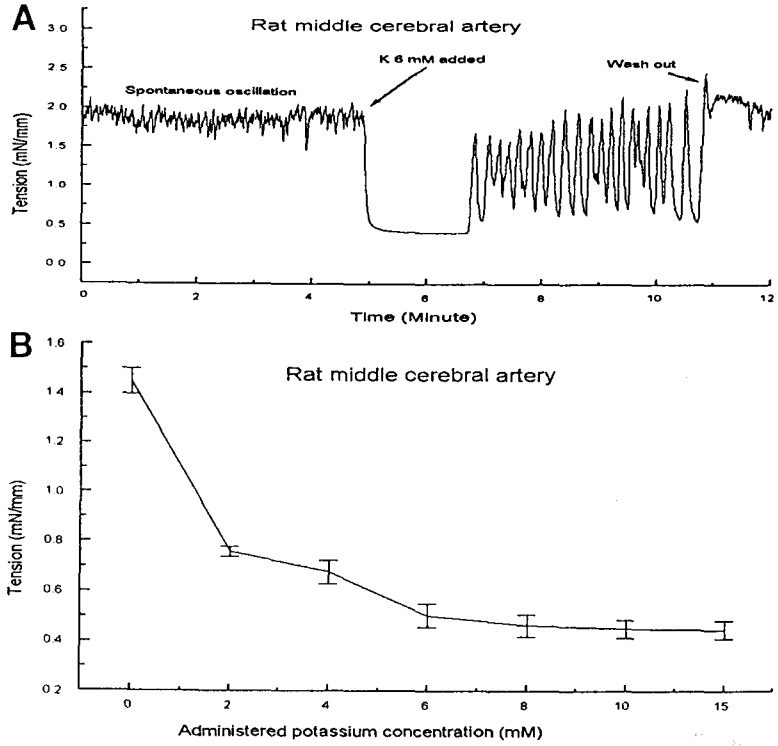

Fig. 3. A. Spontaneous oscillation and K^+-induced vasorelaxation of rat middle cerebral artery. The oscillations were exaggerated after K^+ was introduced. B. Dose-response curve of K^+-induced vasorelaxation in rat middle cerebral arteries.

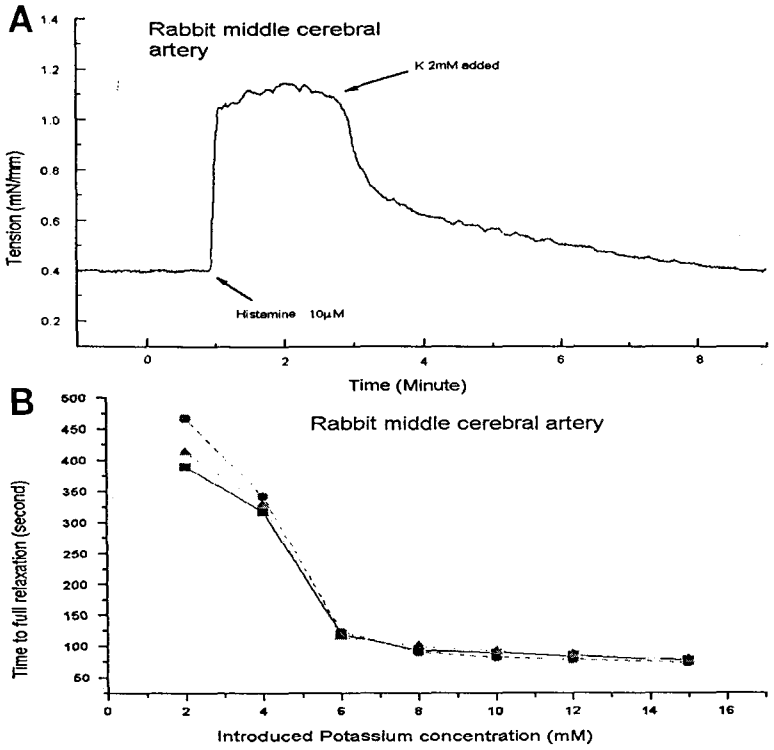

Fig. 4. A. K^+ 2 mM-induced vasorelaxation in rabbit middle cerebral artery. B. Dose-Time to full relaxation curve of K^+-induced vasorelaxation in rabbit middle cerebral arteries.

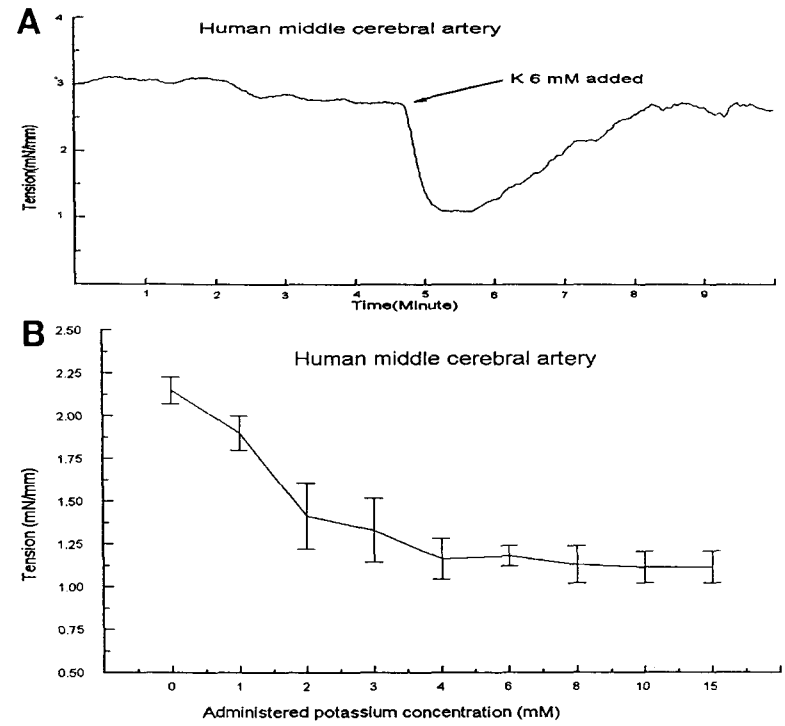

Fig. 5. A. K^+ 6 mM-induced vasorelaxation in human middle cerebral artery. B. Dose-response curve of K^+-induced vasorelaxation in human middle cerebral arteries.

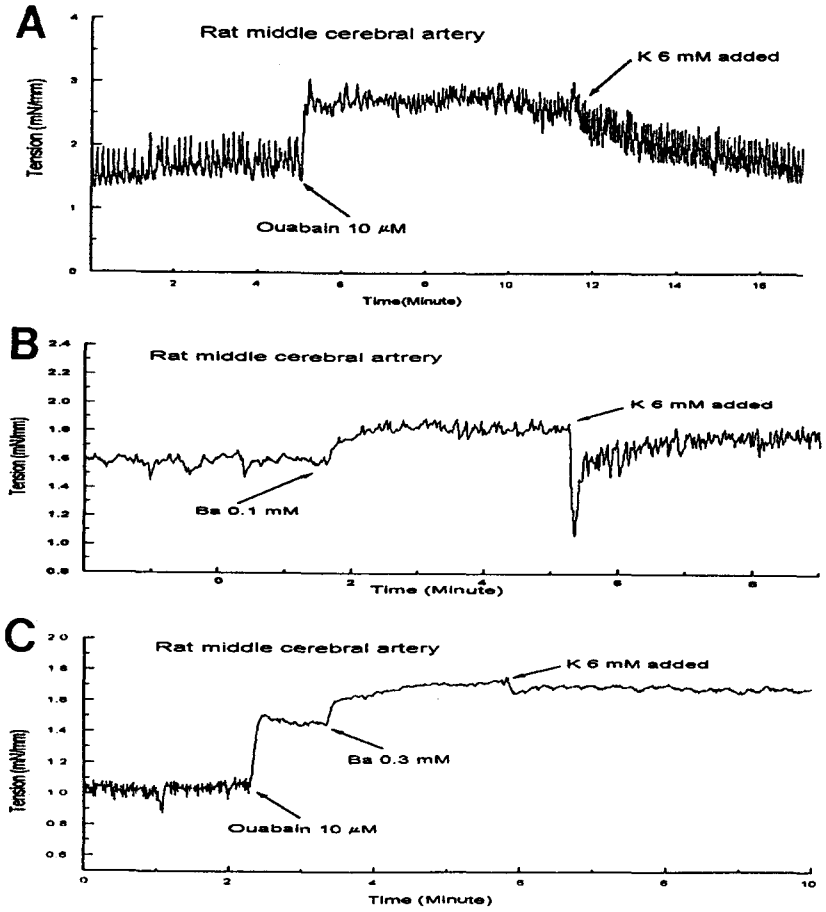

Fig. 6. A. Partial blockade of K^+-induced vasorelaxation in rat middle cerebral artery with ouabain. B. Partial blockade of K^+-induced vasorelaxation in rat middle cerebral artery with $BaCl_2$. C. Blockade of K^+-induced vasorelaxation in rat middle cerebral artery with ouabain and $BaCl_2$.

resting state of rabbit MCAs.

All vessels used for this study revealed reversible contractions when depolarization was evoked by adding high concentration of K^+-PSS (K^+ 125 mM). Course of contractile response consisted of abrupt increase of tension and subsequent gradual increase or maintenance of tone (see Fig. 2A). Mean values of maximal response to 125 mM K^+-solution were 1.7 ± 0.1 mN/mm in rats, 1.8 ± 0.2 mN/mm in rabbits, and 4.6 ± 0.5 mN/mm in humans.

In rabbit MCAs, sustained or slowly decaying contraction was induced by histamine (Fig. 4 & Fig. 7). $PGF_{2\alpha}$ or vasopressin was applied to induce control contraction for K^+-induced vasorelaxation in human or rat MCAs. PGF2α (1~10 μM, Fig. 8) or vasopressin induced stable and reproducible contractions of MCAs of all the species tested.

K^+-induced vasorelaxation

In vessels with low resting tone in the rabbit and human MCA, K^+-induced relaxation was attempted after inducing the contraction with appropriate agonist as a pretreatment. K^+-induced relaxation of cerebral artery was evaluated after the contraction was induced with histamine (5~10 μM) in rabbits, and with $PGF_{2\alpha}$ or vasopressin in humans as an agonist. In vessels maintaining high resting tone as in the rat MCAs and in some human MCAs, K^+-induced relaxation was observed by adding small amount of K^+ to the extracellular fluid without agonist pretreatment (Fig. 3 & Fig. 5). Dose-response curves related to K^+-induced relaxation of each vessel are shown together in Figs. 3, 4, and 5.

In rat MCAs, vasorelaxation reached maximal level rapidly with administration of K^+ (2~15 mM), and

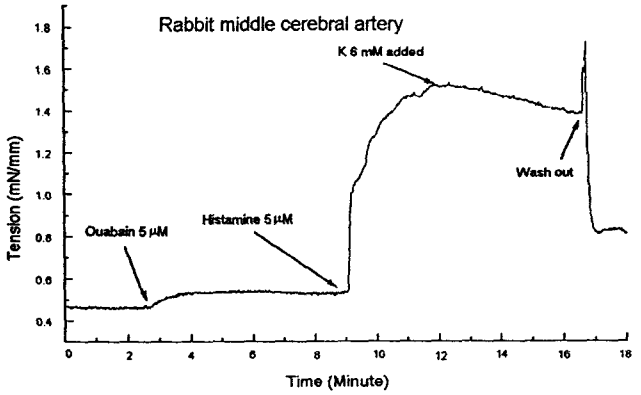

Fig. 7. Blockade of K^+-induced vasorelaxation in rabbit middle cerebral artery with ouabain.

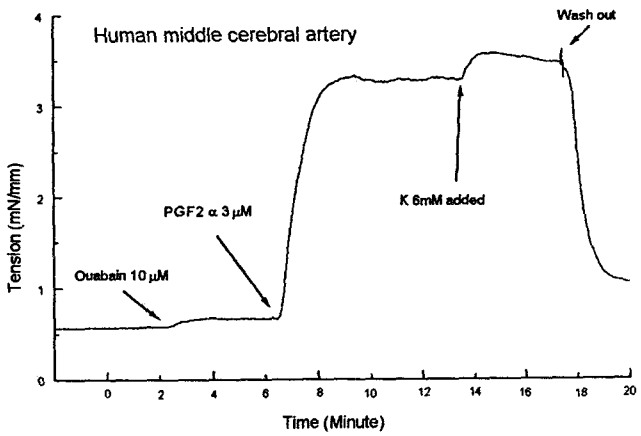

Fig. 8. Blockade of K^+-induced vasorelaxation in human middle cerebral artery with ouabain.

then tension returned gradually to original level. Spontaneous phasic contractions developed subsequently after vasorelaxation until K^+ was washed out (Fig. 3A). Attempts were made to evaluate dose-response relationship between extracellular concentration of K^+ and relaxation, but a variety of spontaneous recovery pattern after maximal relaxation thwarted the attempts. As a results, maximums of relaxation were compared instead (Fig. 3B). Concentration-response relationship was obtained at each concentration of K^+ by observing the K^+ response at new concentration after washout of previous excessive K^+. The relaxation reached maximum when 6 mM K^+ was added (n=3), and time to reach maximum relaxation was not different between each concentration.

In rabbit MCAs, K^+-induced relaxation was observed in histamine-induced contraction. Relaxation was maintained continuously without recovery to original contracted state. Although the time to reach maximal relaxation was dependent on the concentration of K^+, the extent of maximal relaxation was not different in the range of 2~15 mM of K^+ (Fig. 4A). Time to reach maximal relaxation according to concentration was not different when concentration of K^+ was higher than 6 mM of K^+ (n=3) (Fig. 4B).

In human MCAs, vessels were abruptly relaxed within tens of seconds when treated with K^+, and then recovered gradually to original level within several minutes. Maximum relaxation was compared because contraction-recovery time and pattern after maximal relaxation was various (Fig. 5). Relaxation was maximum at 4 mM of K^+ (n=4), and time for maximal relaxation was not different between each concentration of K^+.

Although all vessels showed relaxation when 2~15 mM of K^+ was added, relatively constant relaxation close to maximal relaxation was induced when 4~8 mM of K^+ was added. Therefore, relaxation response was observed by using these concentrations, and its mechanism was studied.

Mechanisms for K^+-induced vasorelaxation

The mechanism was investigated, although indirectly, using known inhibitors of Na^+-K^+ ATPase (ouabain) and inwardly rectifying K^+ channel (Ba^{2+}). In most of the vessels investigated, direct effect of $BaCl_2$ (0.1~0.3 mM) or ouabain (5~10 μM) on resting tone was relatively negligible; the cotractile tone increased gradually and revealed its peak within 1 to 2 minutes (Fig. 7).

Effects of Na^+-K^+ ATPase inhibitor: As shown in Fig. 6A, in rat MCAs after pretreatment with ouabain 5~10 μM, rate of rapid relaxation response to K^+ was slower, and vessels relaxed more gradually than without ouabain (n=20). In human MCAs, relaxation by potassium was completely blocked, and vessels contracted slightly after pretreatment with ouabain 5~10 μM (n=6, Fig. 8). Also in rabbit MCAs, relaxation response to K^+ was completely blocked by pretreatment with ouabain 5~10 μM (n=15, Fig. 7).

Effects of inwardly rectifying K^+ channel blocker: In rat MCAs, gradually increasing contractile response was induced by pretreatment with 0.1~0.3 mM of Ba^{2+}. When 4~8 mM of K^+ was added

subsequently, rapid relaxation period was similar to that with a single treatment of potassium only, but subsequent recovery phase to contracted state was much faster than that with K^+ single treatment (Fig. 6B). K^+-induced relaxation was blocked when both barium 0.1~0.3 mM and ouabain 5~10 μM were added as pretreatments (n=20, Fig. 6C).

Human vessels showed gradually increasing contractile response to the pretreatment with barium 0.1~0.3 mM like murine vessels. However, when 4~8 mM of K^+ was added to barium-induced contracted state, relaxation response was the same as when vessels were not treated with barium (data not shown).

When pretreatment was performed with 0.1~0.3 mM Ba^{2+} in rabbit, relaxation response to K^+ was similar to the response to K^+ only without Ba^{2+} pretreatment (data not shown).

DISCUSSION

The cerebral vascular autoregulation and the pathophysiology underlying its disturbances are areas of intense research activity. The *in vitro* technique makes possible the investigation for the action of selective drugs on small cerebral arteries. Rat and rabbit middle cerebral arteries are similar to pial arteries on the human cortical surface in size and structure.

It has been well known that cerebral blood vessels respond to increases in potassium concentration (1~15 mM) with smooth muscle hyperpolarization and relaxation (Hermsmeyer & Harder, 1986; McCarron & Halpern, 1990a; McCarron & Halpern, 1990b). In particular. Edwards et al (1988) showed that 10 mM of K^+ hyperpolarizes small cerebral arteries and arterioles of rats, compared with large cerebral arteries showing depolarization to 10 mM of K^+. In their study, they examined this K^+-induced hyperpolarization using the voltage-clamp technique, and it was noted that the increased current carried when the membrane was hyperpolarized was sensitive to external K^+ concentration and antagonized by low concentrations of barium. Since these are the characteristics of inward rectifier K^+ channels, the authors suggested that inward rectifier K^+ current was responsible for the hyperpolarization they observed rather than sodium pump. However, it should be mentioned here that they examined only membrane potential, and others examined contractile responses in K^+-induced vasorelaxations. Thus, comparisons of their results are indirect. This study directly compared and analyzed the machanical responses of K^+-induced vasorelaxation in rat, rabbit and human MCAs.

Tension-internal circumference characteristics and resting tone

Tension-response relationships showed that the basal tension corresponding to 40~60 mmHg in pressure was optimal to perform experiments in rat, rabbit, and human MCAs. This condition agreed well with the previous report that blood pressure of cerebral artery *in vivo* was about 1/2 of systolic blood pressure (McCarron & Halpern, 1990). Therefore, all procedures were performed under this condition, where vessels were stretched to receive tension corresponding to 55 mmHg in order to compare each vessel. In most of other experiments using myograph, internal circumference was fixed at 0.8 or 0.9 times of internal circumference corresponding to 100 mmHg (Mulvany & Halpern, 1977; Woolfson et al, 1990; Laing et al, 1995).

This study compared normalized intraluminal diameters of MCA. Vessels of rat and rabbit were separated from the second order branch under microscopic examination. Relatively large arteries located at the surface of cerebral cortex were isolated from the human samples. Therefore, there were significant differences in the diameters of vessels. When considered the fact that caliber and pressure of vessels are closely related to the contractile response (Harder, 1988), this study might be incomplete in that it did not compare the vessels of the same caliber. However, comparison of the response in the same region and under constant and physiological pressure seemed more useful to the purpose of this study.

Vessels of rats under resting condition showed spontaneous high tone and oscillation, which previous investigators have observed (McCarron & Halpern, 1990; 1990b). Whereas, resting tone of vessel in rabbit maintained relaxed state, and measurement of tension was much easier than in rats because oscillation did not develop even in contractile response to agonist. Human vessels were obtained from the brains removed at neurosurgical process. Because cortical surface of brain was exposed to dry external environment and electrocautery was performed on adjacent vessels before harvesting sample during operation, viability could not be preserved constantly. In

fact, in some human vessels, resting tone and response to agonist was so irregular that it was difficult to interprete the results.

Mechanisms for K^+-induced relaxation

As K^+-induced relaxation was blocked when both 0.1~0.3 mM Ba^{2+} and ouabain 5~10 μM were added as pretreatment in rats, both sodium pump and barium sensitive inward rectifier potassium channels may contribute to K^+-induced relaxation. It had been known that rats are less sensitive to ouabain than other animals (Aalkjaer & Mulvany, 1984). In rats, contraction of MCA was induced by relatively low concentration (5~10 μM) of ouabain. Form the point on, it started to relax gradually and then completely relaxed in 20 to 30 minutes. K^+-induced relaxation was not blocked even at higher concentration of ouabain. In human and rabbit MCAs, Ba^{2+} did not significantly inhibit K^+-induced relaxation though it elevated the resting tone slightly. However, the sodium pump was considered as having a major role because K^+-induced relaxation was completely blocked by ouabain 5~10 μM unlike in rats.

McCarron & Halpern (1990b) proposed mechanisms of K^+-induced relaxation, which were divided into sodium pump at 0 to 5 mM of K^+ and ouabain-, barium-, and cesium-sensitive process at a higher concentration than 7 mM. In this study, responses to 0-5 mM K^+ solution were not observed because K^+ was simply added to normal PSS unlike them who added K^+ cumulatively to K^+-free solution. It was because cumulative administration of drug showed quite varied responses, depending on the time of addition. This has made the comparison irrelevant.

The mean of maximal relaxation response in human was larger than the ones in rats and rabbits. It seemed to be related to the caliber of vessel and amount of vascular smooth muscle, but thickness and volume of vascular media were not determined histologically in this study.

In addition to the differences in underlying mechanisms for K^+-induced vasorelaxation, the contractile responses to histamine were different; according to species, in rats and humans, relaxation was concentration-dependent while in rabbits, contraction was concentration-dependent. In contrast, $PGF_{2\alpha}$ or vasopressin induced tonic contractions irrespective of animal species tested. Such differences were not further investigated here.

Although the number of samples was insufficient and viability was uncertain in human MCA, it was meaningful to observe the contraction-relaxation responses and to compare those in other animals. For example, responses of human vessels were similar to those of rabbits in that resting tone was not high under pressure given in test. In addition, K^+-induced relaxation was blocked by ouabain, but those were similar to those of rat in the fact that K^+-induced relaxation did not last long. Also in the response of histamine, human MCAs showed similar response to that of rat MCAs. Since agonists stimulate endothelial cell to release various vasoactive materials in addition to acting on vascular smooth muscle, it is uncertain whether differences in vascular response between each species were due to the differences in vascular smooth muscle.

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