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Sensitivity of Rabbit Cerebral Artery to Serotonin is Increased with the Moderate Increase of Extracellular K^+

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$[K^+]_o$ can be increased under a variety of conditions including subarachnoid hemorrhage. The increase of $[K^+]_o$ in the range of 5~15 mM may affect tensions of blood vessels and can change their sensitivity to various vasoactive substances. Therefore, it was examined in the present study whether the sensitivity of cerebral arteries to vasoactive substances can be changed with the moderate increase of $[K^+]_o$, using Mulvany-type myograph and $[Ca^{2+}]_c$ measurement. The contractions of basilar artery and branch of middle cerebral artery induced by histamine were not increased with the elevation of $[K^+]_o$ from 6 mM to 9 mM or 12 mM. On the contrary, the contractions induced by serotonin were significantly increased with the elevation of $[K^+]_o$. The contractions were also significantly increased by the treatment with nitro-L-arginine (10^{-4} M for 20 minutes). In the nitro-L-arginine treated arteries, the contractions induced by serotonin were significantly increased with the elevation of $[K^+]_o$ from 6 mM to 12 mM. K^+-induced relaxation was evoked with the stepwise increment of extracellular K^+ from 0 or 2 mM to 12 mM by 2 mM in basilar arterial rings, which were contracted by histamine. But $[K^+]_o$ elevation from 4 or 6 mM to 12 mM by the stepwise increment evoked no significant relaxation. Basal tension of basilar artery was increased with $[K^+]_o$ elevation from 6 mM to 12 mM by 2 mM steps or by the treatment with ouabain and the increase of basal tension was blocked by verapamil. The cytosolic free Ca^{2+} level was not increased by the single treatment with serotonin or with the elevation of $[K^+]_o$ from 4 mM to 8 or 12 mM. In contrast to the single treatment, the Ca^{2+} level was increased by the combined treatment with serotonin and the elevation of $[K^+]_o$. The increase of free Ca^{2+} concentration was blocked by the treatment with verapamil. These data suggest that the sensitivity of cerebral artery to serotonin is increased with the moderate increase of $[K^+]_o$ and the increased sensitivity to serotonin is due to the increased $[Ca^{2+}]_i$ induced by extracellular Ca^{2+} influx.

Key Words: Rabbit cerebral artery, Arterial sensitivity to serotonin, Moderate elevation of $[K^+]_o$

INTRODUCTION

It is supposed that the cerebral subarachnoid hemorrhage (SAH) induces various alterations of extracellular environment. Among the alternations, the increase of $[K^+]_o$ and blood cell-derived vasoactive substances (e.g. serotonin) are highly plausible. The increase of $[K^+]_o$ may affect tensions of blood vessels and can change their sensitivity to various vasoactive substances. Wilkins & Levitt (1971) suggested that the intracranial cerebral arterial spasm after SAH might be due to the localized elevation of K^+ originating from the hemolysis of the periarterial clot. Other investigators have demonstrated that vessels isolated from hemorrhaged cerebral tissue have an increased sensitivity to a number of vasoactive agents, including K^+ (Wilkins & Levitt, 1971; Hossman et al, 1977), norepinephrine and serotonin (Hossman et al, 1977; Svendgaard et al, 1977),

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prostaglandins (Kontos et al, 1980; Sasaki et al, 1981; Schumacher & Alksne, 1981), and superoxide free radicals (Asano et al, 1980). On the contrary to these reports, Toda et al (1977) and Simeone et al (1979) found that the sensitivity of cerebral artery to serotonin did not change after subarachnoid hemorrhage or, indeed, was reduced.

Most arteries constrict in response to the severe increase of extracellular K^+, such as more than 30 mM. Small cerebral arteries, however, are hyperpolarized and dilated if extracellular K^+ is increased moderately, such as between 6 and 15 mM range (Kuschinsky et al, 1972; McCarron & Halpern, 1990). It is well- known that the external K^+-induced relaxation is caused by the stimulation of electrogenic Na^+-K^+ pump and by the activation of inward rectifier K^+ current (McCarron & Halpern, 1990; Quayle et al, 1993). From above reports, it is likely that the sensitivity of vascular smooth muscle to vasoactive agents might decrease with the increase of extracellular K^+ in the moderate range.

In the present study, we investigated whether the moderate increase of $[K^+]o$ could change the contractions induced by vasoactive substances in rabbit cerebral arteries. The changes in cytosolic Ca^{2+} concentration was measured in isolated cerebral arterial myocytes under the same condition as mechanical experiment to exclude the indirect effects from endothelium and/or perivascular nerves.

METHODS

Animal preparation

Rabbits of either sex, weighing about 2.5 kg, were killed by exsanguination from the femoral artery under sodium pentobarbital (40 mg/kg) anesthesia. The basilar artery, middle cerebral artery, and superior cerebellar artery were excised and immersed in the Krebs Ringer bicarbonate solution at room temperature and cleaned by removing connective tissues surrounding the vessels.

Isometric contraction recording

Mechanical responses were recorded from the ring segments (3.0~4.0 mm) using home-made myograph, which was made similarly to the myograph made by Mulvany. Each ring was threaded onto two pieces of 40 μm stainless wire. One wire was anchored in organ chamber (1 ml) and the other connected to a mechano-transducer (Grass, FT-03), which was connected to a three dimensional manipulator. The rings were mounted under optimal resting tensions (0.6~1 g) and the muscle chamber was perfused with modified Krebs Ringer bicarbonate solution at 36.5°C, at a constant flow rate of 2.5 ml/min using peristaltic pump. The optimal resting tensions were determined by comparing the tension developed by 30 mM K^+ solution under different resting tension. The tissues were equilibrated for 60 min at the optimal resting tension for maximal tension development in response to high-K^+ solution.

Single myocyte isolation

Both right and left middle cerebral arteries were dissected in a Ca^{2+}-free physiological salt solution (PSS). Isolated vessels were transferred to Ca^{2+}-free PSS containing collagenase (1.5 mg/ml, Wako), bovine serum albumin (2 mg/ml; essentially fatty acid free, Sigma) and dithiothreitol (DTT, 1 mg/ml) and incubated at 35°C for 20 min. After collagenase treatment, segments were transferred to fresh Ca^{2+}-free PSS and single myocytes were dispersed by gentle agitation with a fire-polished wide-bored glass pipette.

Isolated single cells were loaded with acetoxymethyl ester form of fura 2 (2 μM diluted from 1 mM stock in dimethyl sulfoxide) in Ca^{2+}-free PSS for 15 minutes at room temperature. After then, the cell suspension was briefly centrifuged (800 r.p.m., 2 min) and washed with Ca^{2+}-free PSS twice. Fura-2 loaded cells were stored at 4°C until use. Experiments were done within 8 hours after isolation of cells.

$[Ca^{2+}]_c$ measurement

The recording of single cell $[Ca^{2+}]_c$ was performed with a microfluorometric system consisting of an inverted fluorescence microscope (Diaphot 300, Nikon, Japan) with a dry-type fluorescence objective lens (x40, NA 0.85), a photomultiplier tube (type R 1527, Hamamatsu, Japan) and PTI deltascan illuminator (Photon Technology International Inc, USA). One drop of cell suspension was put on a superfusion chamber (100 μl). Cells were allowed to settle down and thereafter superfused at a constant flow of 2 ml/min. Light was provided by a 75 W xenon lamp (Ushino, Japan) and to control excitation frequency,

a chopper wheel alternated the light path to monochromators (340 and 380 nm) with a frequency of 5 or 10 Hz. A short-pass dichroic mirror passed emission light of < 570 nm onto the photomultiplier tube, and intensity at 510 nm was measured. A mechanical image mask was placed in the emission path, thus limiting measurement to a single cell. Both data acquisition and control of light application were done by using a computer software (Felix v. 1.1, PTI).

Because of uncertainties in calibrating the fura 2 signals in intact cells, no attempt was made to calibrate $[Ca^{2+}]_c$, and all results were instead reported as changes in the 340 nm/380 nm signal ratio ($R_{340/380}$).

Solutions and drugs

The ionic composition of the Krebs Ringer bicarbonate solution was as follows (in mM): NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.22, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, CaEDTA 0.016, and glucose 11.1. The solution was aerated with 95% O_2-5% CO_2 (pH 7.3~7.4). High-K^+ solution (30 mM KCl) was prepared by replacing NaCl with KCl.

PSS contained (in mM) NaCl 140, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, 4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid (HEPES) 10, glucose 10 (pH was adjusted to 7.4 with NaOH). A small chamber (100 μl) was continously perfused (2 ml/min) by PSS and all experiments were performed at 30°C.

The fluorescence indicator fura 2-AM was purchased from Molecular Probes, Inc (USA) and all other chemicals and drugs used in this study were purchased from Sigma Chemical Co. (USA).

Statistics

Experimental values were expressed as means± SEM for n separate experiments. Statistical significances were determined using paired Student's t-test, and probabilities of less than 5% ($p<0.05$) were considered significant.

RESULTS

The effect of the elevation of $[K^+]_O$ on contractile response

Basilar artery and carotid artery were contracted by applying serotonin or histamine and the magnitudes

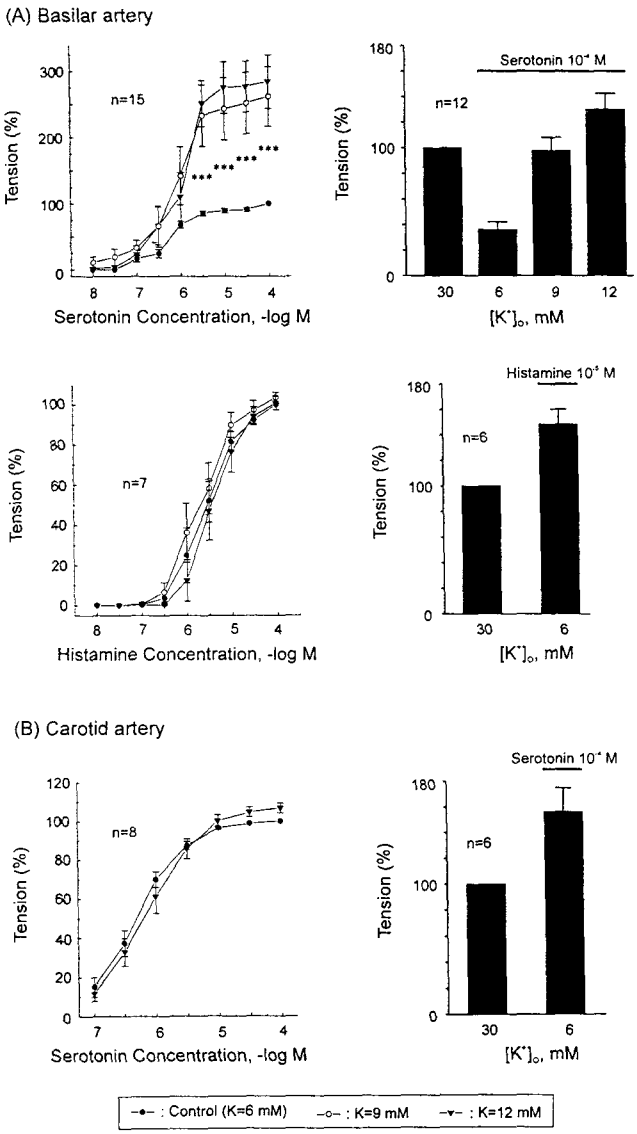

Fig. 1. Effect of the elevation of extracellular K^+ on the contraction of basilar or of carotid artery by serotonin or histamine. A: With the elevation of extracellular K^+, serotonin-induced contraction of basilar artery was markedly increased, whereas histamine-induced contraction was not increased. B: In carotid artery, there was no significant increase of the contraction by serotonin with the elevation of extracellular K^+. The data were expressed as percentages of the contraction by 10^{-4} M serotonin at 6 mM $[K^+]_O$ (left panels) or of the contraction by 30 mM K^+ (right panels). Each point represents the mean value and the vertical bar the SE. ***P <0.01.

of the contractions in 9 mM or 12 mM $[K^+]_O$ were compared to those of the contractions in 6 mM $[K^+]_O$ (Fig. 1. left panels). When extracellular K^+ was

increased from 6 mM to 9 mM and 12 mM, the contraction of basilar artery by serotonin was significantly increased in a dose-dependent manner. Whereas, the contraction induced by histamine was not increased with the elevation of extracellular K^+ (Fig. 1A). On the contrary to basilar artery, the contraction of carotid artery by serotonin was not augmented with the elevation of extracellular K^+ from 6 mM to 12 mM (Fig. 1B). In the arteries, the magnitudes of the contractions by serotonin or histamine were compared with those of the contraction by 30 mM K^+ (Fig. 1, right panels). In basilar artery, the magnitude of the contraction by serotonin in 6 mM K^+ was significantly lower than that of the contraction by 30 mM K^+. Whereas, the magnitude of the contraction by histamine in 6 mM K^+ was greater than that of the contraction by 30 mM K^+. With the elevation of extracellular K^+ from 6 mM to 9 mM and 12 mM, the contraction by serotonin was gradually increased to a greater magnitude than that of the contraction by 30 mM K^+. On the contrary to basilar artery, serotonin evoked a greater contraction of carotid artery than 30 mM K^+ did.

As serotonin is known to release NO from endothelial cells (Ohnuki & Ogawa, 1997), there was a possibility that the sensitivity was modified by the effect of serotonin on endothelial cells. Therefore nitro-L-arginine, a competitive inhibitor of NO synthesis, was used to inhibit NO synthesis from endothelial cells. The contractile response of basilar artery to serotonin was significantly augmented by the treatment with nitro-L-arginine (10^{-4} M for 20 min) (Fig. 2A). This result shows that serotonin stimulates NO release from endothelial cells. Under the condition in which basilar artery was treated with nitro-L-arginine, the magnitude of the contraction by serotonin was also significantly increased with the elevation of $[K^+]_o$ from 6 mM to 12 mM (Fig. 2B). Middle cerebral artery showed diverse responses to the elevation of extracellular K^+. In many cases (4 out of 7 cases), there was no elevation of basal tension with the elevation, but in some cases, the basal tension was increased (1 out of 7 cases) or decreased (2 out of 7 cases). The magnitude of the contraction by serotonin was slightly increased with the elevation of $[K^+]_o$ from 6 mM to 12 mM (Fig.

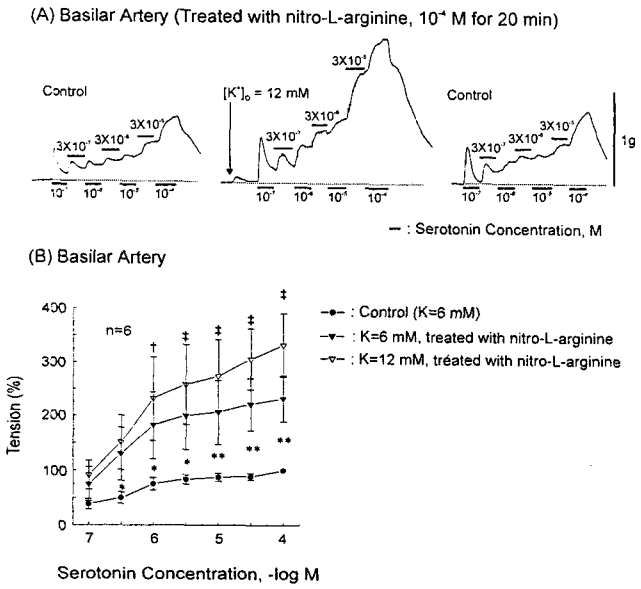

Fig. 2. Effect of the elevation of extracellular K^+ on the sensitivity of nitro-L-arginine-treated basilar artery to serotonin. A: Serotonin-induced contraction of basilar artery treated with nitro-L-arginine was markedly increased with the elevation of extracellular K^+. B: The data were expressed as percentages of the contraction by 10^{-4} M serotonin at 6 mM $[K^+]_o$. Each point represents the mean value and the vertical bar the SE. *$p<0.05$, **$p<0.025$ between control and arterial rings treated with nitro-L-arginine at 6 mM $[K^+]_o$. † $p<0.05$, ‡ $p<0.025$ between arterial rings treated with nitro-L-arginine at 6 mM $[K^+]_o$ and those at 12 mM $[K^+]_o$.

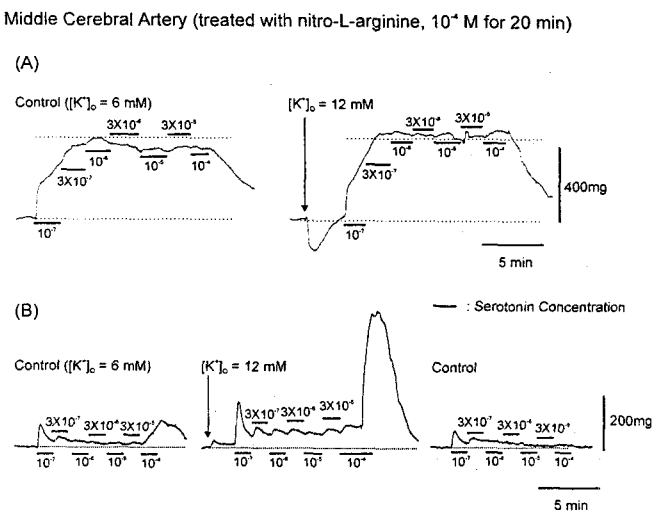

Fig. 3. Representative recordings showing the effect of the elevation of extracellular K^+ on the sensitivity of nitro-L-arginine-treated middle cerebral artery to serotonin. Serotonin-induced contraction of middle cerebral artery treated with nitro-L-arginine was slightly increased with the elevation of extracellular K^+ (A, B).

3A & B). Therefore, it could be suggested that extracellular K^+ increases the sensitivity of smooth muscle of cerebral artery to serotonin.

K^+-induced relaxation of cerebral arteries

It is well-known that the increase of extracellular K^+ in a moderate range can relax arterial smooth muscles. It suggests that the sensitivities of vascular smooth muscle to various vasoactive agents may be decreased by the increase of extracellular K^+. On the contrary to the suggestion, our results demonstrated that the sensitivity to serotonin was increased by the increase of extracellular K^+. This discrepancy suggested that extracellular K^+-induced relaxation was not occurred in cerebral arteries.

K^+-induced relaxation was evoked by the increase of extracellular K^+ in basilar artery which was contracted by application of histamine. The increase of extracellular K^+ evoked relaxation of the contracted basilar arterial ring in various magnitudes from complete relaxation to no relaxation (Fig. 4A).

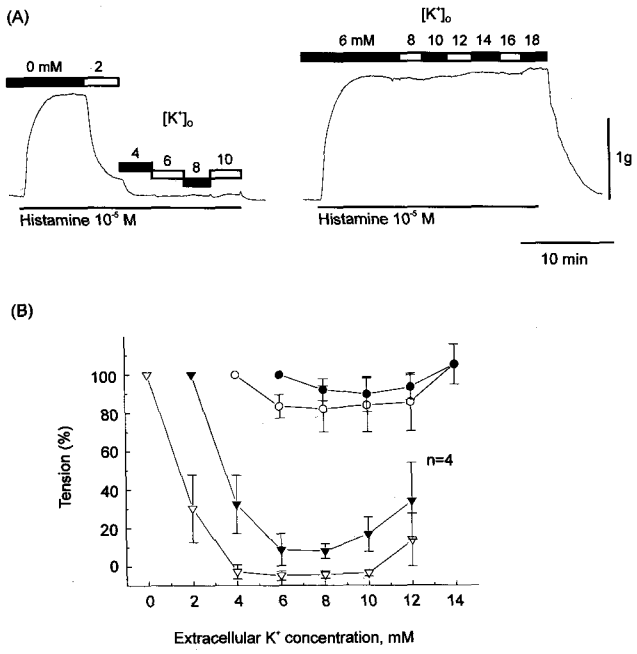

Fig. 4. Effect of the elevation of extracellular K^+ on basilar arterial rings contracted by histamine. When the initial $[K^+]_O$ was 0 or 2 mM, basilar arterial ring relaxed markedly with the elevation of extracellular K^+ by 2 mM steps. Whereas, when the initial $[K^+]_O$ was 4 or 6 mM, basilar arterial ring did not relaxed with the elevation of extracellular K^+ (A, B).

The magnitude of the relaxation was dependent on initial $[K^+]_O$. When initial $[K^+]_O$ was 0 mM or 2 mM, the tension was markedly decreased by the increment of extracellular K^+ by 2 mM steps. The arterial rings contracted by histamine were relaxed almost to basal level with the elevation of $[K^+]_O$ to 12 mM. On the contrary to these results, when initial $[K^+]_O$ was 4 mM or 6 mM, there was no significant relaxation with the gradual increase of extracellular K^+ (Fig. 4B). In addition to basilar artery, middle cerebral artery and superior cerebellar artery showed similar responses to the increase of extracellular K^+.

The effect of the increase of extracellular K^+ on basal tension of cerebral arteries

K^+-induced relaxation is well-known to be due to hyperpolarization of membrane potential by activation of Na^+-K^+ pump or inward rectifier K^+ channels. As K^+-induced relaxation was not occurred by the

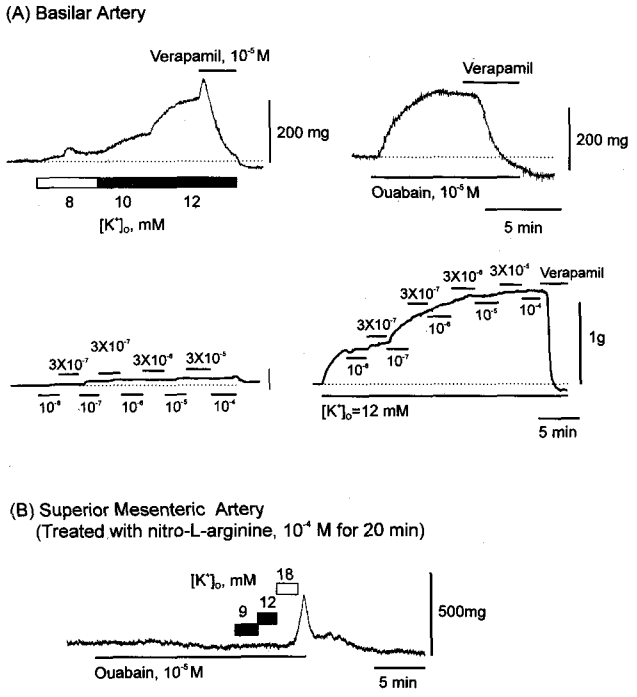

Fig. 5. Effect of the elevation of extracellular K^+ or ouabain on basal tension. A: Basal tension in basilar artery was increased by the stepwise increment and verapamil blocked completely the enhanced tension below initial basal level. B: In superior mesenteric artery, however, the effect of elevated extracellular K^+ on basal tension was much less significant compared to that in basilar artery.

increase of extracellular K^+ from 6 mM, it could be suggested that the increase of extracellular K^+ did not cause hyperpolarization of the membrane potential. Therefore, it was investigated the effect of the agents on basal tensions of cerebral arteries, which could depolarize the membrane potential, such as ouabain and the increase of extracellular K^+.

The basal tension of basilar artery was affected by the treatment with the agents (Fig. 5A). When extracellular K^+ was gradually increased from 6 mM to 12 mM by 2 mM steps, the basal tension was also gradually increased according to the elevation of $[K^+]_o$. The increased basal tension was blocked by verapamil below the initial basal tension. The contraction by serotonin was augmented with the elevation of extracellular K^+ and the elevated contraction was completely blocked by the treatment with verapamil below the initial tension. Ouabain (10^{-5} M) was applied to inhibit Na^+-K^+ pump of these arteries. When basilar artery was treated with ouabain, basal tension was increased immediately after the treatment. The magnitude of the increase by ouabain was similar to that of the increase by the increase of extracellular K^+ to 12 mM. The increased basal tension was blocked by the treatment with verapamil below the initial tension. The effect of ouabain or that of the increase of extracellular K^+ was not blocked by the treatment with nitro-L-arginine. On the contrary to basilar artery, the basal tensions of superior mesenteric artery and carotid artery were not immediately increased by the

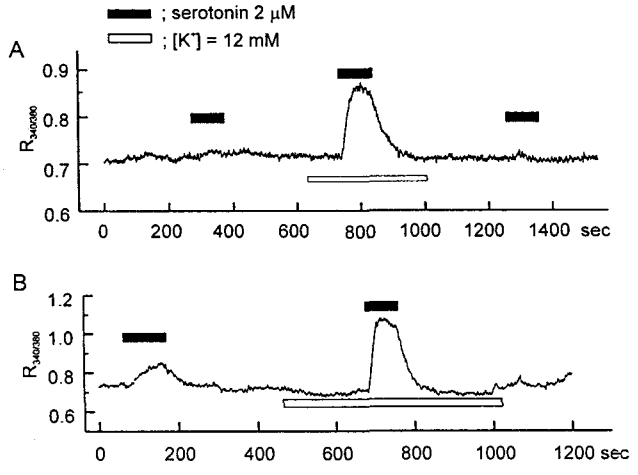

Fig. 6. Effects of combined application of serotonin and 12 mM KCl on fluorescence ratio of intracellular fura-2. A: Serotonin (5 uM) alone could not increase the fluorescence ratio ($R_{340/380}$). Under the perfusion of 12 mM KCl solution, however, the addition of serotonin induced prominent increase of $R_{340/380}$. B: In this myocyte, serotonin alone could induce weak increase of $R_{340/380}$. The increase was markedly enhanced by the underlying perfusion of 12 mM KCl.

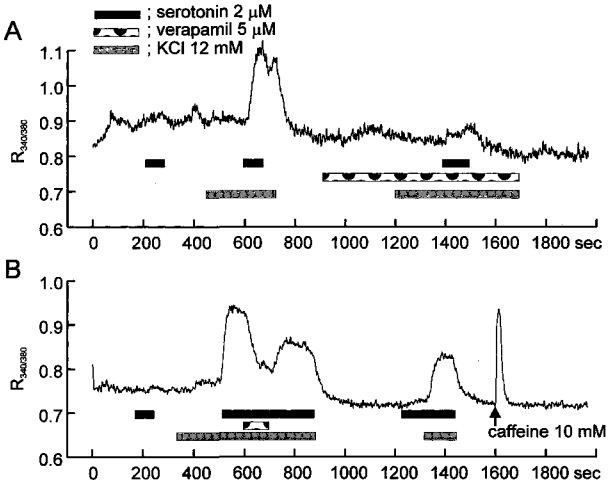

Fig. 7. Blocking effect of verapamil on the increase of R340/380 induced by combined application of serotonin and 12 mM KCl. Verapamil (5 uM), a Ca channel blocker, was applied before (A) and during (B) the application of serotonin and 12 mM KCl. The increase of R340/380 was reversibly suppressed by verapamil.

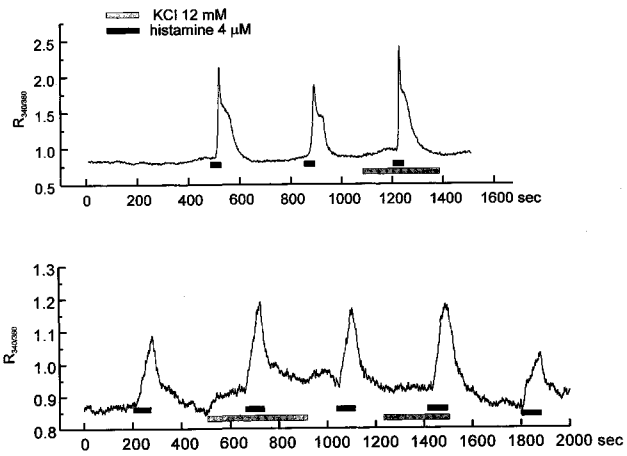

Fig. 8. Effect of 12 mM KCl on histamine-induced increase of fluorescence ratio. Responses of two different cells were shown. Repetitive application of histamine could induce prominent increase of $R_{340/380}$. The moderate increase of $[K^+]o$ (12 mM) could not significantly enhance the histamine-induced increase of $R_{340/380}$.

treatment with ouabain and the increase of extracellular K^+.

The effect of the moderate increase of $[K^+]_O$ and serotonin on $[Ca^{2+}]_C$

$[Ca^{2+}]_C$ of isolated single arterial myocyte was measured by using fura-2 fluorimetry as before (Kim et al, 1998). In isolated myocytes, bath application of serotonin rarely induced the increase of $[Ca^{2+}]_C$ (2 out of 15 cells tested, see Fig. 6B). The moderate increase of $[K^+]_O$ (from 5 mM to 12 mM) itself did not change (10 out of 20 cells, see Fig. 6A and Fig. 7) or slightly increased (3 out of 20 cells, see Fig. 8) or decreased (4 out of 20 cells, see Fig. 6B).

When the moderate increase of $[K^+]_O$ and serotonin was combined, however, prominent increase of $[Ca^{2+}]_C$ was observed. Two representative results were shown in Fig. 6. Such increase of $[Ca^{2+}]_C$ was not affected by the order of application; moderate increase of $[K^+]_O$ after the application of serotonin could induce similar increase of $[Ca^{2+}]_C$ (Fig. 7B).

As the effect of combined application on mechanical contraction was blocked by Ca^{2+} channel blocker, we also tested whether verapamil could block the increase of $[Ca^{2+}]_C$. Both pretreatment and aftertreatment of verapamil (5 uM) could suppress the effect of combined application (Fig. 7A & B). This data indicate that the increase of $[Ca^{2+}]_i$ was due to extracellular Ca^{2+} influx through voltage-operated Ca^{2+} channel.

We also tested whether the moderate increase of $[K^+]_O$ could increase the effect of histamine on $[Ca^{2+}]_C$. In cerebral arterial myocyte, the single application of histamine could induce prominent increase of $[Ca^{2+}]_C$ (Fig. 8) as already reported (Kim et al, 1998). Moderate increase of $[K^+]_O$, however, could not significantly affect the histamine-induced increase of $[Ca^{2+}]_C$ (Fig. 8).

DISCUSSION

This study shows that the sensitivity of cerebral arteries to serotonin is increased with the moderate increase of extracellular K^+. As $[Ca^{2+}]_i$ is prominently increased by the combined treatment with serotonin and the increase of extracellular K^+ and the increased $[Ca^{2+}]_i$ is blocked by verapamil, it could be suggested that the increased sensitivity is due to increased Ca^{2+} influx through voltage-operated Ca^{2+} channel by the moderate elevation of extracellular K^+.

In cerebral arteries, serotonin did not increase $[Ca^{2+}]_i$ at normal $[K^+]_O$ and the magnitude of the contraction by serotonin at normal $[K^+]_O$ was significantly smaller than that of the contraction by 30 mM K^+. On the contrary to serotonin, $[Ca^{2+}]_i$ was prominently increased by histamine and the contraction by histamine was significantly greater than 30 mM K^+-induced contraction. When extracellular K^+ was moderately increased to 12 mM, $[Ca^{2+}]_i$ was prominently increased by serotonin and serotonin evoked greater contraction than 30 mM K^+ did. From these data, it could be suggested that the responsiveness to serotonin was suppressed at normal $[K^+]_O$ and enhanced with the increase of extracellular K^+.

This suggestion is supported by the findings shown in Fig. 4 and Fig. 5. The elevation of extracellular K^+ has diverse effects on blood vessels. The activations of Na^+-K^+ pump or inward rectifier K^+ channels by the elevation hyperpolarize the membrane potential of vascular smooth muscle to be relaxed. As K^+-induced relaxation of basilar artery was not evoked by the increase of extracellular K^+ from 6 mM, the increase of extracellular K^+ seemed not to hyperpolarize the membrane potential. Another effect of the elevation is depolarization of membrane potential by Nernst equation. Na^+-K^+ pump blockade by ouabain also has diverse effects on blood vessels. The immediate effect of Na^+-K^+ pump blockade is depolarization of membrane potential, because Na^+-K^+ pump is electrogenic. The late effect of Na^+-K^+ pump blockade results from the increase of intracellular Na^+ concentration by Na^+-K^+ pump blockade. Na^+-K^+ pump blockade causes to accumulate Na^+ in intracellular space and then to increase $[Na^+]_i$. The increased $[Na^+]_i$ activates Na^+-Ca^{2+} exchanger, which increases $[Ca^{2+}]_i$. The increased $[Ca^{2+}]_i$ can evoke contraction of smooth muscle. This is the well-known mechanism of the contraction occurring after Na^+-K^+ pump blockade. When smooth muscles are contracted in this mechanism, it takes time because $[Na^+]_i$ and $[Ca^{2+}]_i$ is not immediately increased after Na^+-K^+ pump blockade. Most arterial smooth muscles start to be contracted several minutes or more after Na^+-K^+ pump blockade. On the contrary, basilar artery was immediately contracted after Na^+-K^+ pump blockade. Therefore, it could be suggested that the mechanism of the immediate contraction is different from that of

the late contraction by Na^+-Ca^{2+} exchange. As membrane potential can be immediately depolarized after electrogenic Na^+-K^+ pump blockade, the immediate contraction may be evoked by the depolarization. Therefore, it is suggested that the basal tension of basilar artery could be increased by the depolarization of membrane potential. The contraction induced by the increase of extracellular K^+ or by ouabain was blocked by verapamil and verapamil also blocked the increased contraction by serotonin with the elevation of extracellular K^+. In addition, it was reported that serotonin increased voltage-operated Ca^{2+} current in parapodial swim muscle from Aplysia brasiliana (Laurienti & Blankenship, 1997). These data suggest that the depolarized membrane potential may cause to increase Ca^{2+} influx through voltage-operated Ca^{2+} channel and that the Ca^{2+} influx can be further facilitated by the treatment with serotonin. This suggestion can be supported by the finding that $[Ca^{2+}]_C$ was prominently increased not by the single treatment with the moderate increase of $[K^+]_O$ or serotonin but by the combined treatment, and the increase of $[Ca^{2+}]_C$ was blocked by verapamil.

As shown in Fig. 2, serotonin stimulates NO release from endothelial cells. The production and release of NO is initiated by the increase of $[Ca^{2+}]_i$. In endothelial cells, functional voltage-operated Ca^{2+} channels are lack (Whorton et al, 1984; Hallam & Pearson, 1986; Whitmer et al, 1988) and membrane depolarization induced by raising $[K^+]_O$ leads to a decrease in ^{45}Ca uptake (Johns et al, 1987). Therefore, it could be suggested that NO production by serotonin is decreased with the elevation of extracellular K^+ and the increased contraction by serotonin with the elevation was due to the decreased NO production. On the contrary to this suggestion, there is no significant change of endothelium-dependent relaxation by acetylcholine in basilar artery with the elevation of extracellular K^+ (data was not shown in this paper).

What makes the difference of the effects of extracellular K^+ and of ouabain between cerebral arteries and other arteries? As shown in Fig. 5, verapamil inhibited basilar arterial contraction under various conditions even below basal tension, which suggests that there is Ca^{2+} influx on the resting state of basilar artery and voltage-operated Ca^{2+} channels can be activated by the resting membrane potential. Therefore, voltage-operated Ca^{2+} channel can be further activated by the depolarization induced by the moderate increase of extracellular K^+ or by ouabain. On the contrary to cerebral arteries, superior mesenteric artery and carotid artery were not immediately contracted by the increase of extracellular K^+ or ouabain.

The extracellular K^+ concentration in the brain is 3 to 5 mM under normal conditions (Wilkins & Levitt, 1971). The K^+ concentration has been noted to increase in various pathologic conditions, such as ischemic injury or hemorrhage. During conditions of hemorrhage, K^+ and serotonin may be discharged from the lysed cellular components present in the clot adjacent to the site of bleeding. After subarachnoid hemorrhage, major branches of the circle of Willis are often encased in clotted blood (Arutiunov et al, 1974). Regional K^+ concentration adjacent to these vessels may be much higher than the normal potassium concentrations of the cerebrospinal fluid pooled at sites remote from the hemorrhaged area. Our data suggest that the sensitivity of cerebral vessels to serotonin is increased after subarachnoid hemorrhage. The increased sensitivity may contribute hemorrhage-induced cerebral vasospasm.

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