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The Vasocontractive Action of Norepinephrine and Serotonin in Deep Arterioles of Rat Cerebral Gray Matter

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TAKAHASHI, R., SAKAI, T., FURUYAMA, Y., KONDO, Y., INOUE, C.N., ONUMA, S. and IINUMA, K. *The Vasocontractive Action of Norepinephrine and Serotonin in Deep Arterioles of Rat Cerebral Gray Matter.* Tohoku J. Exp. Med., 2000, **190** (2), 129-142 ——— To examine the direct effects of norepinephrine (NE) and serotonin (5-HT) on the contractility of arterioles in the gray matter of the rat cerebrum, we micro-perfused arterioles in vitro and observed the changes in luminal diameter under the stop-flow condition with constant intraluminal pressure. While the average diameter of the lumen of arterioles was $39.9 \pm 9.7\ \mu m$ ($n=7$) in Hepes-buffered saline, the average in 10^{-7} M NE in the extraluminal solution changed into smaller in saline by $21.1 \pm 5.4\%$ ($n=7$). The contractile effect of NE shows a dose-dependent curve between the 10^{-7} and 10^{-5} M. The contractile response to 10^{-6} M NE was significantly reduced by yohinbin, an α_2 blocker. 10^{-6} M NE applied to the lumen also caused contraction of arterioles by $12.4 \pm 5.3\%$ in diameter ($n=5$). 5-HT at 10^{-7} M in the extraluminal solution caused contraction of arterioles by $10.9 \pm 4.4\%$ in diameter ($n=7$). 5-HT in the extraluminal solution caused contraction of arterioles in a dose dependent manner between 10^{-10} and 10^{-6} M. The contractile effect of 5-HT at 10^{-6} M was strongly reduced by 10^{-6} M ketanserin, a 5-HT_2 receptor antagonist. 5-HT applied to the lumen had no effect at all ($n=6$), however NE applied to the lumen caused contraction. These results strongly suggest that 5-HT plays a significant role in arteriolar contractility only from the cerebrospinal fluid (CSF) side, while NE is an important regulator of arteriolar contractility from both the CSF and blood circulation sides. ——— norepinephrine; serotonin; cerebral blood flow; in vitro microperfusion; vasocontraction

Among various types of vasoactive substances, norepinephrine (NE) and serotonin (5-hydroxytryptamine, 5-HT) are the most well-known and well-investigated compounds. NE has thus far been reported to cause contraction of arteries and arterioles in various tissues.

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Cerebral arteries are richly innervated by sympathetic nerves, however, they are relatively unresponsive to norepinephrine, compared with the response of vessels in other organs (Bevan et al. 1987). Bohr et al. (1961) first described the vasoactive effect of NE on the cerebrovascular system in vitro. They reported that there are marked differences in the sensitivity of isolated dog cerebral and peripheral arteries to vasoactive substances including NE. After this report, there were many experiments on the precise location and mechanism of NE's cerebrovascular reactivity.

It then became obvious that NE dose not always contract vessels strongly concerning cerebral vessels. While contractile responses to NE via α receptors were observed in isolated cerebral arteries of guinea pig (Chang et al. 1988), monkey (Chang et al. 1987; Toda 1991), and beagle (Toda et al. 1986), relaxant responses were also observed in isolated bovine caudal arteries (Ayajiki and Toda 1992), and porcine cerebral arteries (Winquist et al. 1982). Furthermore, Hempelmann and Ziegler (1993) reported a relaxant response to NE of isolated rat cerebral arteries, which depended upon the presence of an intact endothelium. Kitazono et al. (1993) showed that the topical application of NE increased the diameter of the basilar artery in a cranial window preparation. They suggested that the dilatation was due to activation of the β_1 receptors and that it was not mediated by endothelium-derived relaxing factor (EDRF). On the other hand, Chang et al. (1988) reported that NE had no effect on isolated rat basilar arteries from rats. These controversial results regarding the sensitivity of the cerebrovascular system to NE lead us to the idea that cerebrovascular system is heterogeneous in its response to NE.

Another well-known classical vasoactive substance is 5-HT. Its physiological role remains unclear. Toda and Fujita (1973) found in isolated spiral strips of cerebral arteries from the dog and man, that 5-HT caused markedly contraction of arteries, the magnitude of which was greater than in the case of NE. Similar results for isolated artery preparations were reported in the rat (Forster and Whalley 1982; Chang et al. 1988), cat (Hardebo et al. 1978), guinea pig (Chang et al. 1988), monkey (Chang et al. 1987; Toda 1991), and man (Hardebo et al. 1978). Therefore, the effects of 5-HT on isolated cerebral arteries thus far reported are in good agreement with each other in that 5-HT is a potent contractile agent in different species and brain regions.

Grome and Harper (1983), however, showed that the intracarotid infusion of 5-HT into rats did not change the cerebral blood flow except for that in the caudal nucleus. Although there are several hypotheses, the most possible explanation for the discrepancy between in vivo and in vitro studies is that 5-HT exerts its vasoconstrictive effect only from the extraluminal side. This idea is supported by some studies demonstrating that intravenously administered 5-HT receptor agonists exhibit poor ability to penetrate the cerebrovascular intimal layer (Connor et al. 1992; Ferrari 1993). If this is the case, the classical vascular

theory of migraine (Silberstein 1992), which suggests that an increase in plasma 5-HT induced by some catalyst plays a major role in the constriction of cerebral vessels and that the vasodilatation which subsequently occurs may be a cause of head pain, must be reconsidered.

Recently, the efficacy of sumatriptan, a 5-HT_1 receptor agonist, in the treatment of migraine has revived interest in the vascular theory. However, it is debatable whether the mechanism of action of sumatriptan is due to a direct vascular effect or to the inhibition of trigeminal nerve terminals (Lance 1993). This debate has continued because of a lack of studies that have directly examined the constrictive effects of 5-HT on intracerebral vessels.

It is therefore important to examine the vasoreactivity of the cerebrovascular system to NE and 5-HT with an in vitro preparation which allows the detection of the intraluminal and extraluminal effects of these substances separately. Considering the lack of a report demonstrating directly the laterality of the vasoreactivity of NE and 5HT in cerebral arterioles, we conducted a series of experiments on the direct effects of intraluminal and extraluminal NE and 5-HT on an in vitro microcannulated preparation. Our results strongly suggested that NE is a potent vasoconstrictive substance for cerebral arterioles from both the systemic blood flow and cerebrospinal fluid (CSF) sides, whereas 5-HT exerts its vasoconstrictive effect on cerebral arterioles only from the CSF side.

Materials and Methods

The techniques used for the dissection and cannulation of intracerebral arterioles were those of Dacey and Duling (1982) with the modification of in vitro renal tubule microperfusion technique (Kondo and Frömter 1987).

Isolation of arterioles

Male Wister rats weighing 300–400 g were anesthetized with pentobarbital sodium (Nembutal®, Abbott Lab., North Chicago, IL, USA; 50 mg/kg i.p.) and then decapitated with a small-animal guillotine. The top of the calvarium was removed with a drill, and then the temporal and parietal bones were fractured laterally with ophthalmologic surgical scissors. The dura was removed and the brain was rapidly immersed in a dissection chamber at 4°C containing Hepes-buffered solution, the composition of which was as follows, in mM; 135 NaCl, 3 KCl, 2 KH_2PO_4, 1.5 $CaCl_2$, 1.0 $MgCl_2$, 10 Hepes, 5.5 glucose, 5 l-alanine titrated to pH 7.4 with NaOH. Under a stereomicroscope (SZ40R, Olympus, Tokyo), a piece of cerebral cortex approximately 2 mm thick was removed from the lateral cerebral hemispheric surface. The pia mater and the attached penetrating arterioles were separated from the parenchyma with fine forceps. An unbranched segment of intracerebral arterioles of approximately 0.7 to 1.0 mm in length and 20 to 40 μm in diameter was cut with a 29 G needle.

The arterioles were transferred to a temperature-controlled chamber, contain-

ing the above Hepes-buffered solution. The chamber was mounted on the stage of an inverted microscope (IMT-2, Olympus). The temperature of this chamber was maintained at 37°C.

In vitro cannulation of arterioles

The isolated arterioles were cannulated using a system of concentric glass pipettes mounted on micromanipulators (in vitro microperfusion manipulators, Narishige, Tokyo) attached to the microscope stage. By applying gentle suction, one end of an arteriole was drawn into a holding pipette through the constriction. A perfusion pipette was then inserted into the lumen of the arteriole. The inner diameters of the holding and perfusion pipettes were 30 to 36 and 3 to 5 μm, respectively. To stop the luminal flow completely, the other end was wedged with another holding pipette, the inner diameter of which was 30–40 μm. After cannulation, the intraluminal pressure was constantly maintained at 30 mmHg by means of hydrostatic pressure. Hepes-buffered solution described above was used to perfuse both the luminal and extraluminal sides of arterioles.

To ensure the stop-flow of the lumen, arterioles with holes or branches were discarded. After an equilibration period of approximately 30 minutes, spontaneous tone developed. Images of the arterioles were monitored at the magnification of 200 with a video camera (C-2400-08, Hamamatsu Photonics, Hamamatsu). The luminal diameters of three random points were measured with an image-analysis system (ARGUS-10, Hamamatsu Photonics).

The viability and biological responsiveness of the arterioles were then assessed by applying a high K^+ solution instead of 124 mM NaCl with the same molar concentration of KCl to the extraluminal side. To test the viability of the endothelial cells in preparation, lissamine green was applied to the lumen of the arterioles in another series of arterioles preparation. No leakage or staing of cells with lissamine green was observed, indicating that the viability of both endothelial and smooth muscle cells were kept intact in our preparation.

Chemicals

Serotonin (5-HT), L-norepinephrine hydrochloride (NE), prazosin hydrochloride and yohinbin hydrochloride were purchased from Sigma Chemical Company (St. Louis, MO, USA). Ketanserin tirtrate and methiothepin mesylate were from RBI (Boston, MA, USA). All other chemicals were of reagent grade and were purchased from Wako Pure Chemical (Osaka).

Statistics

All values were expressed as means$\pm$S.D. All statistical analyses were performed using percent changes from control values. Paired t-test and Student's t-test were used to compare mean values between two groups. Values of $p<0.05$ in a two-tailed test was considered significant.

Results

Basal contractile response assessed as the high K^+-induced contractility of arterioles

After a 30 minute preequilibration period, the average luminal diameter of the arterioles was 39.9 ± 9.7 μm ($n=7$).

It is well known that the depolarization of arteriolar smooth muscle cells by increasing extracellular K^+ induces an increase in intracellular Ca^{2+} followed by their contraction. To confirm the viability and biological contractility of the arterioles, the extraluminal K^+ concentration was increased to 124 mM by substituting the same molar concentration of Na^+, and then the percent contractility of the arteriolar luminal diameter in 5 minutes was determined. The change in the luminal diameter in response to the extraluminal high K^+ solution is depicted in Fig. 1. The contractility response to high K^+ proves that cells retain their physiological intracellular environment. Therefore, the group of arterioles exhibiting low contractility was regarded as an inappropriate preparation and thus discarded. To eliminate this group used in the present study, all arterioles exhibiting less than 20% contractility were discarded.

Contractile response to extraluminal NE

The maximal steady-state contraction of the vessel diameter was observed at

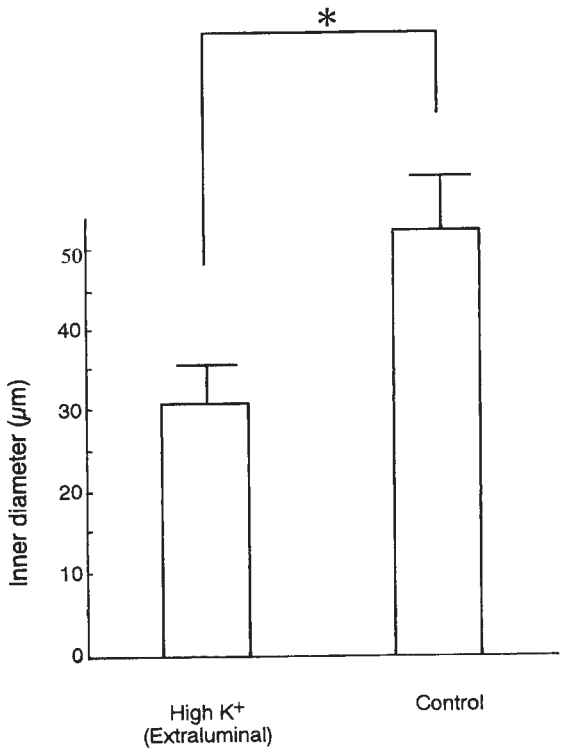

Fig. 1. Contractile response of intracortical arterioles to an extracellular high K^+ solution. In controls, the inner diameter of arterioles was 52.0 ± 5.0 μm. After 5 minutes exposure of the arterioles to the high K^+ solution containing 124 mM K^+, the inner diameter was reduced to 31.2 ± 4.6 μm ($n=5$, $p<0.05$), corresponding to 42.4 ± 8.2% contraction.

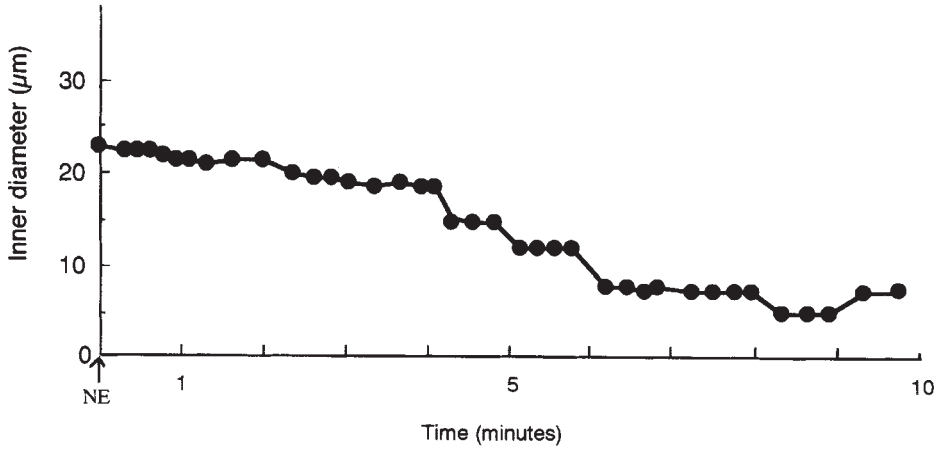

Fig. 2. Representative time course of contraction induced by extraluminal NE. Contractile responses, as inner diameters of the arterioles, were plotted against time in minutes for two arterioles. As depicted, the maximal contractile responses were observed at approximately 5 to 10 minutes after the administration of 10^{-6} M NE.

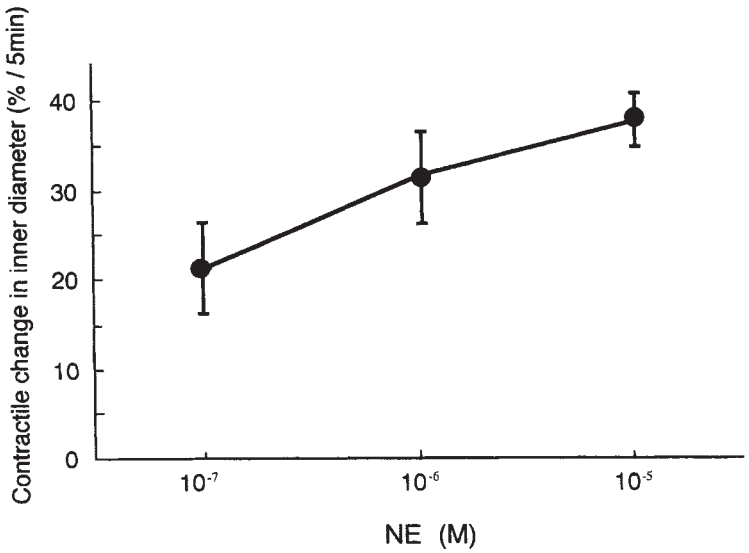

Fig. 3. Dose-dependent contractility of arterioles in response to extraluminal NE. NE, from 10^{-7} to 10^{-5} M, was applied to extraluminal side, and then the contractile responses, as percent changes of the inner diameter, were observed. NE at 10^{-7}, 10^{-6}, and 10^{-5} M caused contraction of the arterioles in 5 minutes by 21.1 ± 5.4, 31.6 ± 5.0, and $37.6 \pm 3.0\%$ ($n = 7$), respectively.

5 minutes after the administration of NE.

A representative time course of the contractile response of arterioles to 10^{-6} M NE is shown in Fig. 2. As shown in Fig. 3, 10^{-7}, 10^{-6}, 10^{-5} M NE decreased the arteriolar diameter by 21.1 ± 5.4, 31.6 ± 5.0, and $37.6 \pm 3.0\%$ (each $n = 7$), respectively.

To determine whether or not α_1 and α_2 adrenergic receptors were involved in the contractile responses to NE, arterioles were first incubated with 10^{-8} M prazosin, a selective α_1 antagonist, or 10^{-6} M yohinbin, an α_2 antagonist, for 5

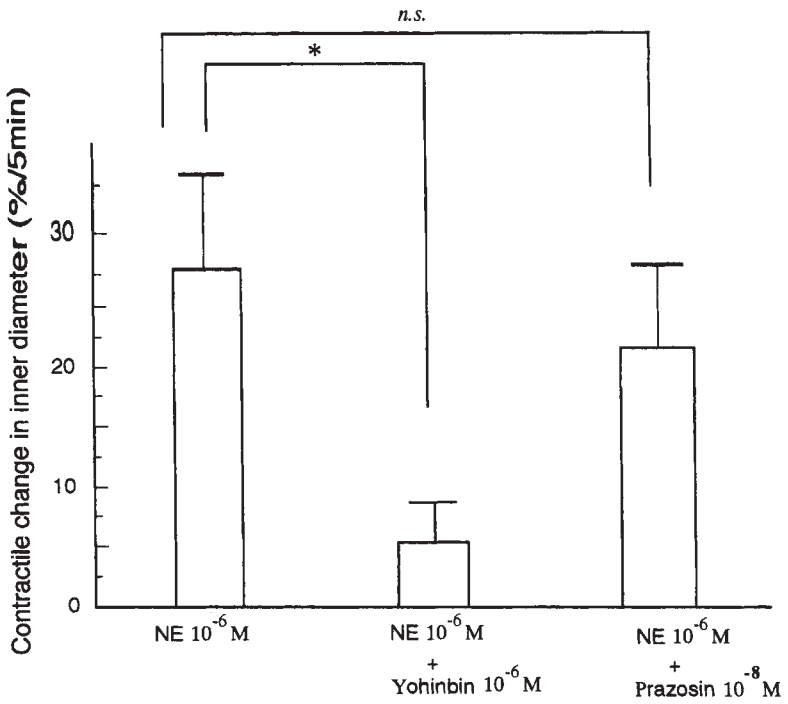

Fig. 4. Effects of prazosin and yohinbin on NE-induced contraction of arterioles. In controls, 10^{-6} M NE caused contraction of the arterioles by $28.3\pm6.1\%$ ($n=5$). Preincubation of the arterioles with 10^{-6} M yohinbin reduced the contractility to $4.9\pm3.9\%$ in 5 minutes ($n=5$, $p<0.05$ vs. control), whereas NE, with preincubation of 10^{-8} M prazosin for 5 minutes, still caused contraction of the arterioles by $21.1\pm8.7\%$ ($n=5$, n.s. vs. control).

minutes in the extraluminal solution. Then the contractile responses to 10^{-6} M NE were observed in the presence of these antagonists.

Neither prazosin nor yohinbin itself affected the luminal diameter of the arterioles. In the presence of prazosin, 10^{-6} M NE caused contraction of the arterioles by $21.1\pm8.7\%$, which was not different from the response in the absence of prazosin ($28.3\pm6.1\%$, $n=5$) (Fig. 4). In the presence of yohinbin in the extraluminal solution, the contractile response significantly decreased to $4.9\pm3.9\%$ ($n=5$, $p<0.05$) (Fig. 4).

These results clearly show that NE caused contraction of the arterioles via α_2 adrenergic receptors, which may be located on either the plasma membrane of the smooth muscle cells or the extraluminal surface of the endothelium.

Contractile response to luminal NE

The intraluminal application of 10^{-6} M NE also decreased the arteriolar diameter by $12.4\pm5.3\%$ ($n=5$), this magnitude being less than that of the extraluminal response ($p<0.05$, Fig. 5).

Contractile response to extraluminal 5-HT

The maximal steady-state contraction of the vessel diameter was observed at 5 minutes after the administration of 5-HT.

A representative time course of the responses of arterioles to 5-HT is shown

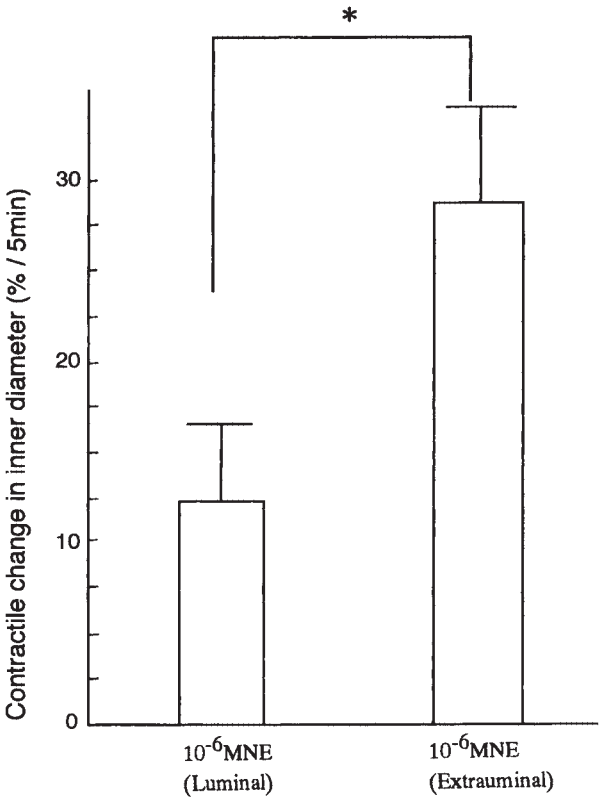

Fig. 5. Contractile effect of NE applied from the lumen. To determine whether or not NE was capable of causing contraction of arterioles from the intraluminal side, 10^{-6} M NE was applied to the lumen by simple diffusion from the tip of a perfusion pipette without perfusing the lumen. To accomplish the diffusion, only the inside of the perfusion pipette was exchanged to the solution containing to 10^{-6} M NE, without any change in the hydrostatic pressure, carefully. As is shown in the figure, NE applied to the intraluminal side caused contraction of the arterioles by $12.4 \pm 5.3\%$ ($n=5$) in 5 minutes.

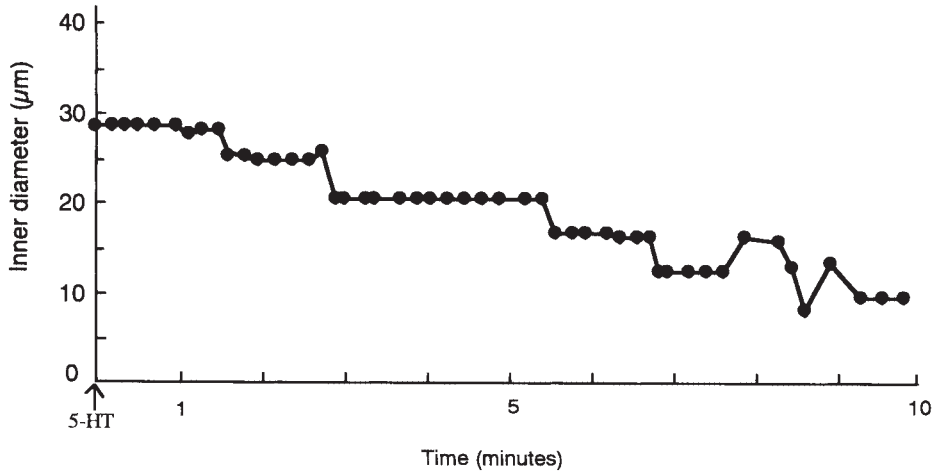

Fig. 6. Representative time course of contraction induced by extraluminal 5-HT. Contractile responses, as inner diameters of the arterioles, were plotted against time in minutes for two arterioles. As depicted, the maximal contractile responses were observed at approximately 5 to 10 minutes after the administration of 10^{-6} M 5-HT.

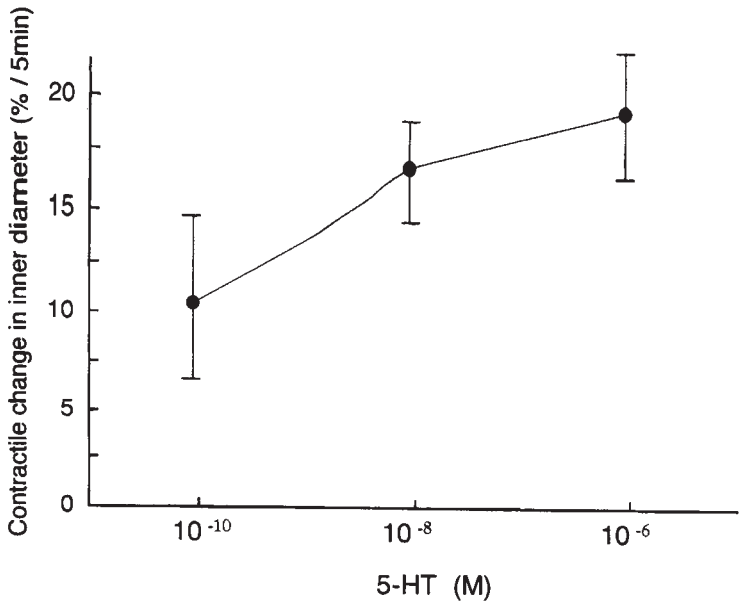

Fig. 7. Dose-dependent contractility of arterioles in response to extraluminal 5-HT. 5-HT, from 10^{-10} to 10^{-6} M, was applied to the extraluminal side, and then the contractile responses ,as percent changes of the inner diameter, were observed. 5-HT at 10^{-10}, 10^{-8}, 10^{-6} M caused contraction of the arterioles in 5 minutes by 10.9 ± 4.4, 16.2 ± 2.4, and $18.5\pm2.8\%$ ($n=7$), respectively.

in Fig. 6. 10^{-10}, 10^{-8}, and 10^{-6} M 5-HT decreased the arteriolar diameter by 10.9 ± 4.4, 16.2 ± 2.4, and $18.5\pm2.8\%$ ($n=7$), respectively (Fig. 7).

To determine whether or not 5-HT_1 and 5-HT_2 receptors are involved in the contractile response to 5-HT, the arterioles were first incubated with either 10^{-6} M methiothepin, a 5-HT_1 antagonist, or 10^{-6} M ketanserin, a 5-HT_2 antagonist, for 5 minutes in the extraluminal solution, and then the contractile response to 5-HT were observed in the presence of these antagonists.

Neither methiothepin nor ketanserin itself affected the luminal diameters of the arterioles. In the presence of methiothepin, 10^{-6} M 5-HT caused contraction of the arterioles by $9.7\pm5.6\%$, which was not different from the response in the absence of methiothepin ($13.9\pm3.2\%$, $n=5$), as shown in Fig. 8. In the presence of ketanserin in the extraluminal solution, the contractile response decreased to $2.9\pm2.0\%$ ($n=5$), as shown in Fig. 8.

These results clearly show that 5-HT caused contraction of the arterioles via 5-HT_2 receptors, which may be located on either the plasma membrane of the smooth muscle cells or the extraluminal surface of the endothelium.

Contractile response to luminal 5-HT

The intraluminal application of 10^{-6} M 5-HT decreased the luminal diameter of arteriole by $0.1\pm0.1\%$ ($n=6$) in 5 minutes, which was not a statistically significant change (Fig. 9).

It is clear from these results that 5-HT has no receptor on the luminal membrane of the arteriolar endothelium, and thus it modulates contractility only

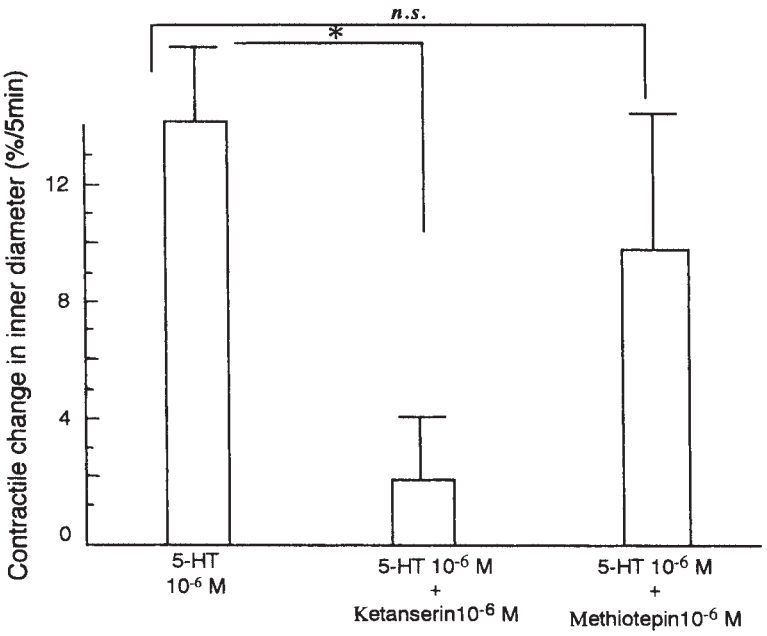

Fig. 8. Effects of methiothepin and ketanserin on 5-HT induced contraction of arterioles. In controls, 10^{-6} M 5-HT caused contraction of the arterioles by $13.9 \pm 3.2\%$ ($n=5$). Preincubation of the arterioles with 10^{-6} M ketanserin reduced the contractility to $2.0 \pm 2.0\%$ in 5 minutes ($n=5$, $p<0.05$ vs. control), whereas 5-HT, with preincubation of 10^{-6} M methiothepin for 5 minutes, still caused contraction of the arterioles by $9.7 \pm 5.6\%$ ($n=5$, n.s. vs. control).

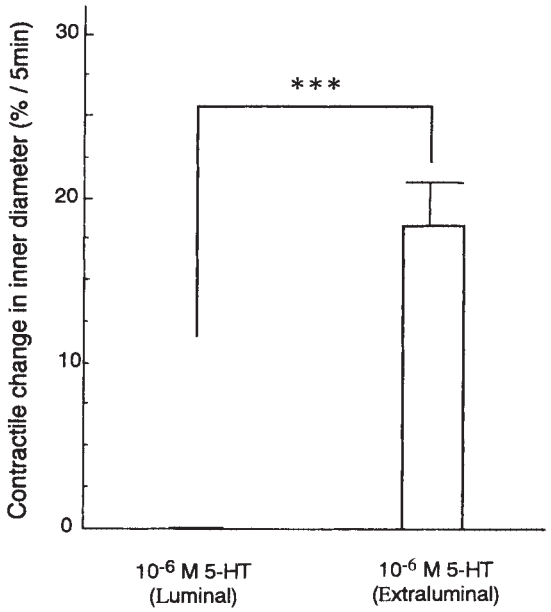

Fig. 9. Contractile effect of 5-HT applied from the lumen. To determine whether or not 5-HT was also capable of causing contraction of arterioles from the intraluminal side, 10^{-6} M 5-HT was applied to the lumen by simple diffusion from the tip of a perfusion pipette without perfusing the lumen. As shown in the figure, 5-HT applied to the intraluminal side caused contraction of the arterioles by only $0.1 \pm 0.1\%$ ($n=6$, n.s. vs. 0) in 5 minutes.

from the cerebral blood circulation side.

Discussion

In the present study, we demonstrated the precise laterality of the effects of classical vasoactive substances, NE and 5-HT. Our data indicate differences in the physiological roles of these two substances in light of the locations of their receptors.

As for the effect of NE on the cerebrovascular system, in vivo experiments in the man (Greenfield and Tindal 1968) and dog (Ekstrom-Jordal and Haggendal 1974) demonstrated that the intravenous infusion of NE induced a reduction in cerebral blood flow (CBF) via α adrenergic receptors. On the other hand, the same experimental protocol in the rat showed that NE increased CBF via β_2 adrenergic receptors. This type of experiment dose not always allow a clear conclusion as to the contractile response of cerebral arteries to NE, because CBF is determined not only by vascular resistance but also by regional blood pressure. Although direct analysis of the effect of NE was performed on in vitro isolated vessels, including basilar, pial and carotid arteries, it must be noted that all in vitro helical strips and ring preparations examined exhibited the mixed-up phenomena caused by the stimulation of both intraluminal and extraluminal adrenergic receptors.

It is also important that the majority of studies have been on the arterial contractility induced by NE in relatively large arteries, including basilar, pial, and carotid arteries. As for intracerebral arterioles, Dacey and Duling (1982, 1984), and their groups (Duling et al. 1981) performed the first experiment using the in vitro microperfusion technique. They reported that a physiological dose of NE caused contraction of the arterioles from the extraluminal side via α adrenergic receptors (Dacey and Duling 1984). Their data are in good accordance with ours in that cerebrovascular NE caused contraction of the arterioles at a physiological concentration. Our data further demonstrated that the contractile effect of NE is mediated by α_2 adrenergic receptors.

Thus far, there has been no study on the effect of intraluminal NE on the vascular contractility of intracerebral arterioles as to NE. Our data demonstrate clearly that NE in the cerebrovascular system is also a potent vasoconstrictive factor for the intracerebral arterioles and regulates the cerebrovascular circulation. At present, no information is available on how intraluminal NE causes contraction of smooth muscle cells, which have no direct contact with an intraluminal solution. Another series of experiments on the mechanism of signal transduction of the NE effect is required.

In the present study, we did not examine whether or not the β adrenergic receptors were also involved in the vasocontractile response of intracerebral arterioles, as was reported in previous studies (Berntman et al. 1978; Kitazono et al. 1993). In the case of our data, inhibition of α_2 adrenergic receptors by

yohinbin did not change the effect of NE on arterioles from vasoconstriction to dilatation. Although this result implies the absence of β adrenergic receptors in intracerebral arterioles, more direct examination of the effects of NE are required to elucidate whether or not functional β receptors are present in intracerebral arterioles.

5-HT is another classical vasoconstrictive substance, the role of which in regulation of the cerebrovascular system remains unclear. In migraine, antagonists to 5-HT receptors are often applied for therapeutic use, because one of the causes of migraine is thought to be a disturbance of a 5-HT related vasoconstrictive system in the brain (Ferarri 1993). In anesthetized baboons and rats, direct intravenous infusion of 5-HT in vivo had no effect on cerebrovascular blood flow except for that in the caudal nucleus (Grome and Harper 1983). Our data that 5-HT exerts no vasoconstrictive effect on the cerebral circulation from the luminal side are in good agreement with those of the previous in vivo studies demonstrating the absence of an intraluminal receptor for 5-HT in whole brain vessels. It is well established that the brain-blood-barrier is impermeable to 5-HT. Several in vitro studies demonstrated the occurrence of a vasoconstrictive effect of 5-HT in arteries and arterioles, including basilar, carotid arteries and intracerebral arterioles (Chang et al. 1988; Saxena and Villalon 1990). The preparations used were in vitro helical strips or simply isolated vessels. Therefore, these data do not necessarily indicate the presence of intraluminal vasoconstrictive receptors for 5-HT. Rather, it seems more reasonable that all the observed vasoconstrictive effects of 5-HT are exerted from the extraluminal side. There has only been one preliminary report demonstrating the contractile effect of 5-HT on in vitro microperfused intracerebral arterioles from the lumen (Ogura et al. 1991). Considering the discrepancy of our results, their results might be due to the direct effects on the smooth muscle cells of luminal 5-HT leaked out of the lumen. Further studies will be necessary to elucidate the issue.

While the present results clearly refute the possibility that systemic 5-HT plays an important role in the cerebral arterial vasoconstrictive phenomena observed in migraine, our technique implies that in vitro microcannulation of intracerebral vessels is highly useful in elucidating the function of intracerebral vessels. We believe that our technique will be important in clarifying the basic role of intracerebral vessels in the cranial circulation.

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