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Regional differences in 5-HT receptors in cerebral and testicular arterioles of the rat as revealed by Ca^{2+} imaging of real-time confocal microscopy: variances by artery size and organ specificity*

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Summary. 5-hydroxytriptamine (5-HT) is an important transmitter for vessel constriction. The present study was performed to clarify the effect of 5-HT on smooth muscles in large- and small-sized cerebral and testicular arterioles by confocal microscopy, with special reference to intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) dynamics. In cerebral vessels, 5-HT induced a $[Ca^{2+}]_i$ increase and the contraction of smooth muscle cells in large- and mid-sized arterioles (external diameters >50 μm) but not in small-sized arterioles. Conspicuous $[Ca^{2+}]_i$ changes by 5-HT were especially observed in the portions close to the cerebral arterial circle, and the 5-HT-induced responses were caused by both Ca^{2+} influx and mobilization. Experiments using agonists and antagonists also revealed that cerebral arteriole smooth muscles possess 5-HT1a, 1b, 2 (G-protein-coupled type), and 3 (ion channel type) receptors; specifically, 5-HT2 plays a major role in these responses. On the other hand, in testicular vessels, there were few regional differences among responses to 5-HT, and both large- and small-sized arterioles responded to 5-HT. The responses were caused by only Ca^{2+} mobilization mediated 5-HT1a and 2. These results indicate that arterioles in different tissues may respond to 5-HT in different manners. Regional differences and the size-dependent manner of responses to 5-HT in cerebral blood vessels also indicate that the regulatory mechanism of blood circulation is highly differentiated in each region of the central nervous system.

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Introduction

Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) plays an essential role in stimulus-response coupling in various tissues as a wide variety of cell functions, including cell secretion, cell division, muscle contraction, neurotransmitter release, and gene expression are controlled by changes in $[Ca^{2+}]_i$ (Ozawa *et al*., 1967; Ebashi 1976; Bootman *et al*., 2001). The regulation of the calcium metabolism is also vital for vascular smooth cells and plays a role in maintaining the Ca^{2+} gradient between the extracellular space and

cytosolic free Ca^{2+}. Some stimuli promote the entry of Ca^{2+} from the extracellular medium, while others release Ca^{2+} from internal stores (Berridge *et al*., 2003).

Using digital imaging analysis of $[Ca^{2+}]_i$ dynamics in intact living tissues, we recently showed that adenosine triphosphate (ATP) participates in a variety of actions in arterioles of different tissues (Saino *et al*., 2002b). We also reported that, in small cerebral arterioles (< 50 μm in diameter), protease-activated receptor (PAR) 1-activating peptide (AP) induced an increase in $[Ca^{2+}]_i$ and PAR2-AP induced a decrease in $[Ca^{2+}]_i$ in smooth muscle cells (Misaki *et al*., 2006). In addition, testicular and cerebral arterioles reacted quite differently to some reagents. Consequently, we have been interested in $[Ca^{2+}]_i$ dynamics in individual cells in various intact arteriole specimens using real-time confocal microscopy in relation to the function of arteries and arterioles (Saino *et al*., 2002a, b; Saino and Satoh, 2004; Matsuura *et al*., 2004; Misaki *et al*., 2006).

The neurotransmitter 5-hydroxytriptamine (5-HT: serotonin), is also involved in a wide variety of physiological functions mainly in the central and peripheral nervous systems as well as in the gastrointestinal tract (Bradley *et al*., 1986 ; Racke *et al*., 1996; Baumgarten and Grozdanovic, 1997; Crowell *et al*., 2004). Because 5-HT also acts as a well-known potent vasoconstrictor agent in several arteries (Vanhoutte *et al*., 1984; Van Nueten, 1985: Van Nueten *et al*., 1985), a possible role for 5-HT in the control of brain perfusion has been widely discussed (Young *et al*., 1986, 1987; MacKenzie and Scatton, 1987). More specifically, 5-HT has been implicated in cerebrovascular dysfunctions such as vasospasm and migrane (Fozard, 1987, 1989). For example, the level of 5-HT in the cerebrospinal fluid (CSF) was elevated immediately after subarachnoid hemorrhage and involved in fatal vasospasmus (Saida *et al*., 1997). In addition, the accumulation of blood in the CSF alters the balance of the formation of vasoconstrictive and vasodilatory factors in cerebral circulation (Faraci and Heistad, 1998).

The role of 5-HT in cardiovascular regulation is usually explained on the basis of the knowledge of seven main types of 5-HT receptors (5-HT1 to 5-HT7) (Hoyer *et al*., 1994, 2002; Ramage and Villalón, 2008). With the exception of the 5-HT3 receptor, which is a ligand-gated ion channel, each of the identified 5-HT receptors is a seven-transmembrane domain, G-protein-coupled receptor (Hoyer *et al*., 1994; Hartig *et al*., 1996; Gerhardt and Heerikhuizen, 1997). The 5-HT1 and 5-HT4-7 receptor families are coupled to adenylyl cyclase. Binding of 5-HT to the Gq-coupled 5-HT2 receptor activates phospholipase C, resulting in the release of inositol triphosphate and an elevation of cytosolic calcium. The 5-HT2 family receptors are important for the mediation of a number of physiological functions, including vascular and nonvascular smooth muscle contraction (Roth *et al*., 1998).

However, there are only a few studies concerning $[Ca^{2+}]_i$ changes in intact arterioles in relation to the vasoactive properties of 5-HT. Thus, the aim of this study was to clarify the Ca^{2+} signalling mechanism of 5-HT in arteriole smooth muscle cells of cerebral and testicular arterioles. It reports on the investigation of $[Ca^{2+}]_i$ alteration of smooth muscle cells in arterioles with respect to 5-HT and selected modifying reagents, using real-time confocal laser scanning microscopy—which has excellent time and spatial resolution. We show that the reaction of smooth muscle cells to 5-HT differs between cerebral and testicular arterioles, a finding which emphasizes a different regulation of regional blood circulation.

Materials and Methods

Preparation of arterioles

Experiments were conducted according to the Guidelines of the Ethics Committee for Animal Treatment of Iwate

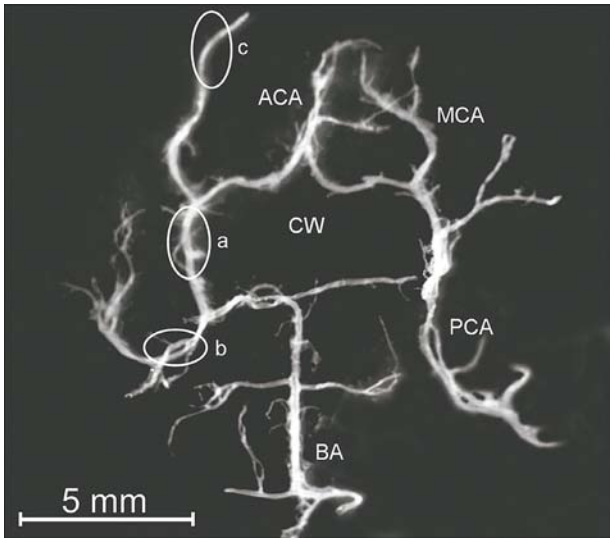

Fig. 1. Stereoscopic photographs of arteries in the rat cerebral arterial circle (the circle of Willis). CW: the circle of Willis, ACA: the anterior cerebral artery, MCA: the middle cerebral artery, PCA: the posterior cerebral artery, BA: the basilar artery. a, b and c are measured region in Figure 3. Bar=5 mm

Medical University. Adult male rats (Wistar, 8–12 weeks old, body weight 250–400 g) were killed by carbon dioxide gas. They were then perfused *via* the left cardiac ventricle with Ringer's solution (147 mM NaCl, 4 mM KCl and 2.25 mM $CaCl_2$) at 25℃ at a hydrostatic pressure of approximately 1 m of H_2O. After washing out blood cells from the vessels, the brain and testis were removed and placed in Hepes-buffered Ringer's solution (HR). The HR solution contained 118 mM NaCl, 4.7 mM KCl, 1.25 mM $CaCl_2$, 1.13 mM $MgCl_2$, 1 mM NaH_2PO_4, 5.5 mM D-glucose, MEM amino acids solution (Gibco, Grand Island, NY, USA), 0.2% bovine serum albumin (Sigma, St. Louis, MO, USA), and 10 mM Hepes; pH 7.4, adjusted with NaOH. Arterioles were isolated from the testis and brain and their connective tissues were then roughly removed by forceps. Arterioles were immersed in HR containing purified collagenase (100 U/m*l*; Elastin Products, Owensville, MO, USA) for 2 h at 37℃. After the digestion, connective tissues were then carefully removed by fine forceps. Ca^{2+}-deficient solutions were prepared by replacing $CaCl_2$ with EGTA (1.0 mM; Sigma).

Intracellular Ca^{2+} imaging

In order to measure $[Ca^{2+}]_i$, arterioles were placed on a glass cover slip, set in a perfusion chamber, and incubated with 10 μM Indo-1/AM (Dojindo, Kumamoto) in the HR solution for 12 h at 4℃. Indo-1 (excited by ultraviolet light) is a ratiometric dye that is used in the quantitative determination of $[Ca^{2+}]_i$. The emission maximum of Indo-1 shifts from 475 nm in a Ca^{2+}-free medium to 400 nm when the dye solution is saturated with Ca^{2+}. A ratio of emission intensity shorter than 440 nm to that longer than 440 nm can be used to estimate $[Ca^{2+}]_i$; a higher ratio indicates a higher $[Ca^{2+}]_i$. Artifacts, which include photobleaching and dye leakage, can be eliminated by this ratiometry.

A real-time confocal microscope (RCM/Ab; a modified version of a Nikon model RCM-8000, Tokyo) was used

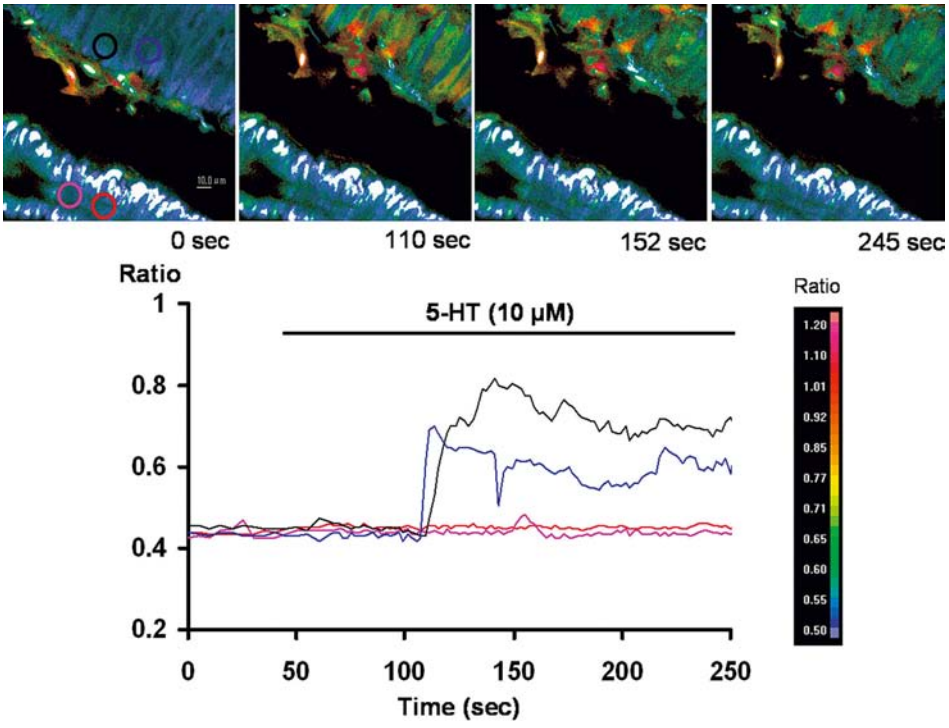

Fig. 2. Pseudocolor images and time course for $[Ca^{2+}]_i$ dynamics induced by 5-HT in cerebral large and small arteriole smooth muscle cells (**a**, **b;** blue and black lines and **c**, **d;** red and pink lines, respectively) in certain areas (about 3 μm^2). Arterioles were stimulated by 5-HT (10 μM) at room temperature. Color scale bar: fluorescence ratio represents $[Ca^{2+}]_i$.

to measure $[Ca^{2+}]_i$ changes. Cells loaded with Indo-1 were exposed to an ultraviolet-beam (351 nm). An argon-ion laser was equipped with an inverted microscope (TE300, Nikon), and the fluorescence emission was passed through a water-immersion objective lens (Nikon C Apo 40×, N.A. 1.15) to a pinhole diaphragm. To decrease electrical noise and to reduce photodamage, each image was integrated from four frames, and sequential images were intermittently acquired every 1 sec. Images were immediately stored on high-speed hard disks. The digital images in the laser scanning microscopic imaging were composed of 512 × 480 pixels with a density resolution of 8 bits/pixel. The fluorescent intensity was displayed in pseudocolor with 256 colors, with red representing high $[Ca^{2+}]_i$ and purple and blue representing low $[Ca^{2+}]_i$.

Stimulation by 5-HT and selected some reagents

The $[Ca^{2+}]_i$ dynamics of arterioles were examined in a perfusion chamber as quickly as possible after the dye-loading. After perfusion with the standard HR for a few minutes at room temperature, intact arterioles were selected and examined under the microscope. Specimens were continuously perfused with HR containing the following agonists and/or antagonists: 5-hydroxytryptamine (5-HT, Nacalai Tesque, 10 μM); $GdCl_3$ (a nonspecific cation channel blocker, Wako, 100 μM); diltiazem (a L-type Ca^{2+} channel blocker, Sigma, 40 μM); thapsigargin (a sarco/endoplasmic reticulum Ca^{2+}-ATPase inhibitor, Sigma, 5 μM); 8-hydroxy-DPAT (5-HT1a agonist, Sigma, 50 μM); CP93129 (5-HT1b agonist, TOCRIS, USA, 10 μM); α-methylserotonin maleate (5-HT2 agonist, Biomol International, USA, 100 μM); ketanserin (5-HT2 antagonist, Sigma, 1 μM); 2-methylserotonin maleate (5-HT3 agonist, Biomol, 100 μM); and H-89 (a protein kinase A (PKA) antagonist, Biomol, 20 μM).

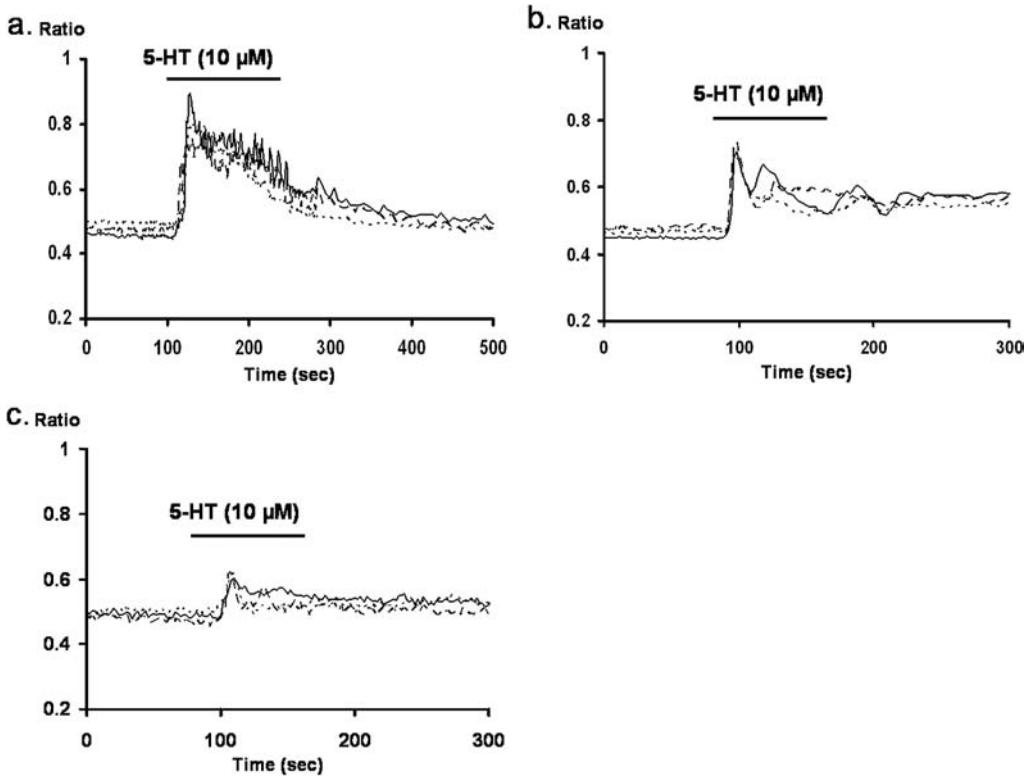

Fig. 3. Time courses of cerebral arteriole smooth muscle cells show $[Ca^{2+}]_i$ changes in Figure 1 (**a**–**c;** solid, dashed, and dotted lines). Cerebral arterioles were stimulated with 5-HT (10 μM) at room temperature. **a:** The circle of Willis. **b** and **c:** MCA and PCA periphery.

Results

The structure of cerebral arterioles

The structural integrity of arterioles was maintained in specimens prepared for the intracellular calcium imaging study. Arterioles branching out from the cerebral arterial circle (the circle of Willis)—especially the beginning portions of the anterior cerebral artery and middle cerebral artery—were used in this study (a, b, c in Fig. 1).

5-HT-induced $[Ca^{2+}]_i$ increase in cerebral arterioles

Arteriole specimens were perfused with normal Hepes-buffered Ringer's solution for 5 min before stimulation by the selected reagents. Under resting conditions, no spontaneous $[Ca^{2+}]_i$ changes were observed. Injured cells in the arterioles, which showed high $[Ca^{2+}]_i$ at resting conditions, were excluded from the subsequent analyses. No structural difference was detected between the controls and the specimens with respect to Indo-1 loading.

5-HT (10 μM) induced an increase in $[Ca^{2+}]_i$ in smooth muscle cells of cerebral large- and mid-sized arterioles (external diameters >50 μm) (Fig. 2; blue and black lines, and Fig. 4a), followed by contraction of the arterioles. The $[Ca^{2+}]_i$ increase was rapid in these vessels, and oscillatory fluctuation or persistent increases were sometimes observed. In addition, there were regional differences for the reaction to 5-HT in large- or mid-sized vessels. Conspicuous $[Ca^{2+}]_i$ changes and contractions

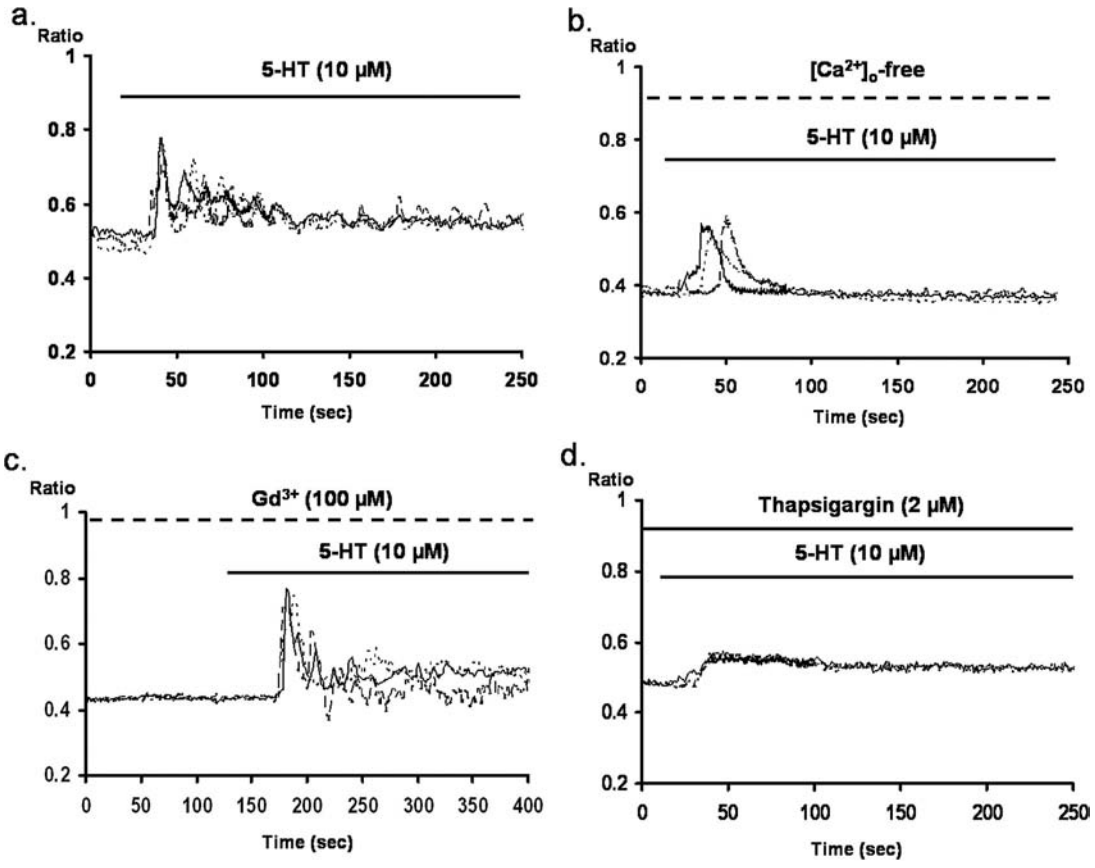

Fig. 4. Time courses for the 5-HT (10 μM)-induced $[Ca^{2+}]_i$ dynamics in cerebral large-sized arteriole (external diameters >50 μm) smooth muscle cells (**a**–**d**; solid, dashed and dotted lines) at certain areas (about 3 μm^2). **a:** 5-HT stimulation. **b:** Extracellular Ca^{2+}-free conditions ($[Ca^{2+}]_o$-free). **c:** Gd^{3+} (100 μM); a nonspecific cation channel blocker. **d:** Thapsigargin (2 μM); a sarco/endoplasmic reticulum Ca^{2+}-ATPase inhibitor resulting in the inhibition of Ca^{2+} release from the internal Ca^{2+} store.

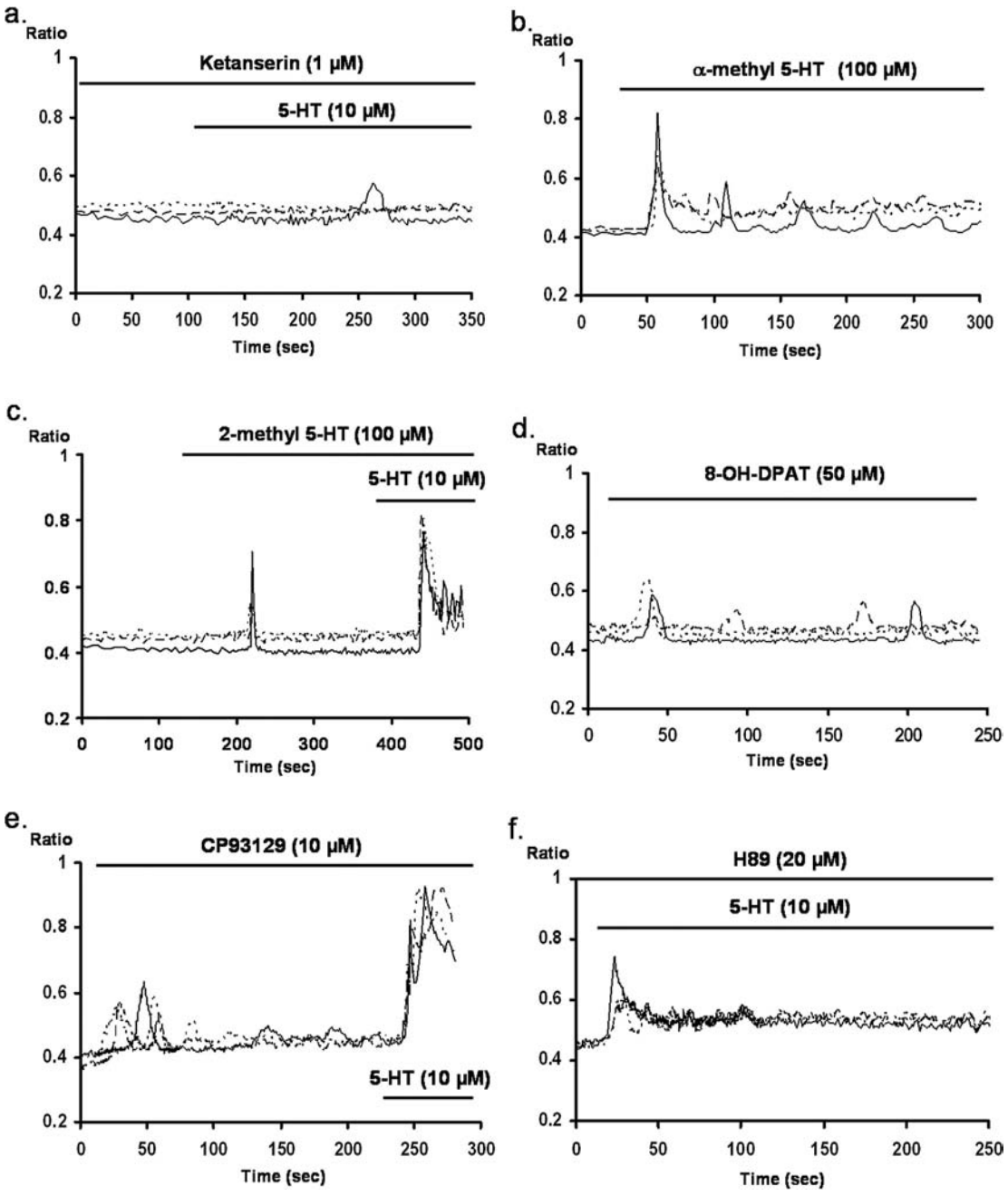

Fig. 5. Time courses for the 5-HT-induced $[Ca^{2+}]_i$ dynamics using selected modified drugs in cerebral large-sized arteriole (external diameters >50 μm) smooth muscle cells (**a**–**f;** solid, dashed, and dotted lines) in certain areas (about 3 μm^2). **a:** ketanserin (1 μM); a 5-HT2 antagonist. **b:** α-methyl 5-HT (100 μM); a 5-HT2 agonist. **c:** 2-methyl 5-HT (100 μM); a 5-HT3 agonist. **d:** 8-OH-DPAT (50 μM); a 5-HT1a agonist. **e:** CP93129 (10 μM); a-5-HT1b agonist. **f:** H89 (20 μM); a protein kinase A antagonist.

were observed in the portions close to the cerebral arterial circle. However, the farther the vessels were from the circle, the weaker were the reactions although the diameter of the reacted vessels was over 50 μm (n=22) (Fig. 3, compare a to b and c).

On the other hand, in the case of small-sized arterioles (a second or third branch of the cerebral arteries; external diameters < 50 μm), no significant 5-HT (10 μM)-induced $[Ca^{2+}]_i$ increases, or contractions, or dilatations of arteriole smooth muscle cells were detected (n=22) (Fig. 2; red and pink lines). Up to 1 mM 5-HT was used, but no $[Ca^{2+}]_i$ changes were observed (n=12)(data not shown).

In the absence of extracellular Ca^{2+}, the increase in $[Ca^{2+}]_i$ was not completely inhibited (n=16)(Fig. 4b). The effect of Gd^{3+} was the same as that observed in the absence of Ca^{2+} (n=10)(Fig. 4c). Diltiazem (40 μM) also inhibited 5-HT-induced $[Ca^{2+}]_i$ increases (n=10) (data not shown). However, 5-HT-induced $[Ca^{2+}]_i$ increases in the cells were not prevented by pretreatment with thapsigargin (n=14)(Fig. 4d). These results indicate that Ca^{2+} influx and Ca^{2+} release result in a 5HT-induced $[Ca^{2+}]_i$ increase in large and mid-sized arterioles; thus, putative receptors of smooth muscle cells are both ion-channel (5-HT3)and metabotropic (5-HT2: inositol 1, 4, 5-trisphosphate dependent, 5-HT1, 4-7: cAMP dependent) receptors.

Effect of 5-HT and its analogs on $[Ca^{2+}]_i$ dynamics

The effect of 5-HT and its analogs on cerebral arteriole smooth muscle cells was studied in order to observe whether they are mediated *via* any of the recognized subtypes of 5-HT receptors. In the presence of ketanserin, a 5-HT2 antagonist, 5-HT induced a slight increase in $[Ca^{2+}]_i$ in smooth muscle cells (n=10)(Fig. 5a). On the other hand, α-methyl serotonin, a 5-HT2 agonist, induced a strong increase in $[Ca^{2+}]_i$ in these cells (n=10) (Fig. 5b). However, 2-methyl serotonin, a 5-HT3 agonist, induced a little increase in $[Ca^{2+}]_i$ (n=8)(Fig. 5c).

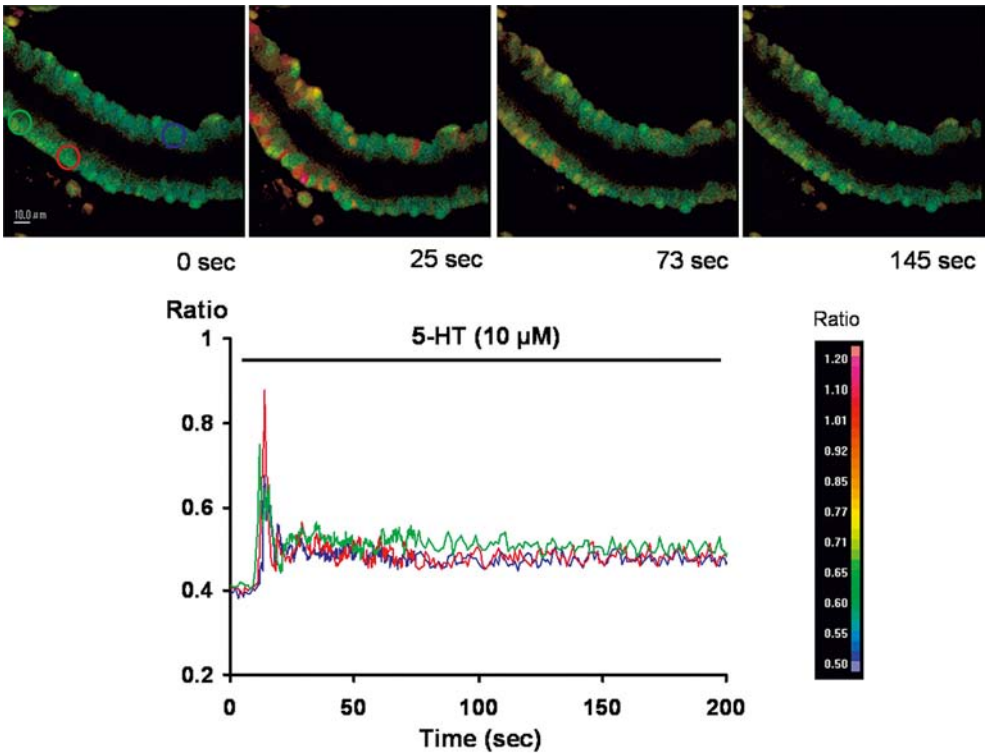

Fig. 6. Pseudocolor images and the time course of testicular arteriole smooth muscle cells show $[Ca^{2+}]_i$ changes, as measured by ratiometry of Indo-1, and time courses for the 5-HT (10 μM)-induced $[Ca^{2+}]_i$ dynamics in testicular arteriole smooth muscle cells (blue, red and green lines) in certain areas (about 3 μm^2). Color scale bar: fluorescence ratio represents $[Ca^{2+}]_i$.

8-hydroxy-DPAT, a 5-HT1a agonist (n=12)(Fig. 5d), and CP93129, a 5-HT1b agonist (n=8)(Fig. 5e), induced a weak $[Ca^{2+}]_i$ increase compared with 5-HT.

In contrast, H-89, a PKA antagonist, did not inhibit the 5-HT-induced $[Ca^{2+}]_i$ response (20 μM)(n=11)(Fig. 5f), suggesting that the cAMP dependent pathway (5-HT4-7) is insignificant in vasocontraction with respect to 5-HT. Therefore, it can be concluded that the response of cerebral arteriole smooth muscle cells is mediated by 5-HT1a, 1b, 2, and 3 receptors; specifically, 5-HT2 plays a major role in these responses.

5-HT-induced $[Ca^{2+}]_i$ increase in testicular arterioles

5-HT (10 μM)-induced an increase in $[Ca^{2+}]_i$ in smooth muscle cells of testicular arterioles (Fig. 6, 7a). Oscillatory fluctuations were often observed. In contrast to cerebral arterioles, neither any regional difference nor size-dependent reactivity was observed in testicular arterioles.

In the absence of extracellular Ca^{2+}, the increase in $[Ca^{2+}]_i$ was not inhibited (n=16) (Fig. 7b). The effect of Gd^{3+} was the same as that observed in the absence of Ca^{2+} (Fig. 7c). Diltiazem (40 μM) also inhibited 5-HT-induced $[Ca^{2+}]_i$ increases (n=8)(data not shown). However, 5-HT-induced $[Ca^{2+}]_i$ increases in the cells were almost prevented by pretreatment with thapsigargin (n=14)(Fig. 7d). These results indicate that increases in $[Ca^{2+}]_i$ caused by 5-HT result from releases from internal Ca^{2+} stores; only metabotropic receptors played a role in 5-HT induced $[Ca^{2+}]_i$ increase.

8-hydroxy-DPAT induced an increase in $[Ca^{2+}]_i$ in these cells (n=11)(Fig. 8a), and α-methyl serotonin induced a strong increase in $[Ca^{2+}]_i$ (n=12)(Fig. 8c). However, 2-methyl serotonin (n=12) and CP93129 (n=12) induced no increases in $[Ca^{2+}]_i$ (Fig. 8b, d). 5-HT-induced $[Ca^{2+}]_i$

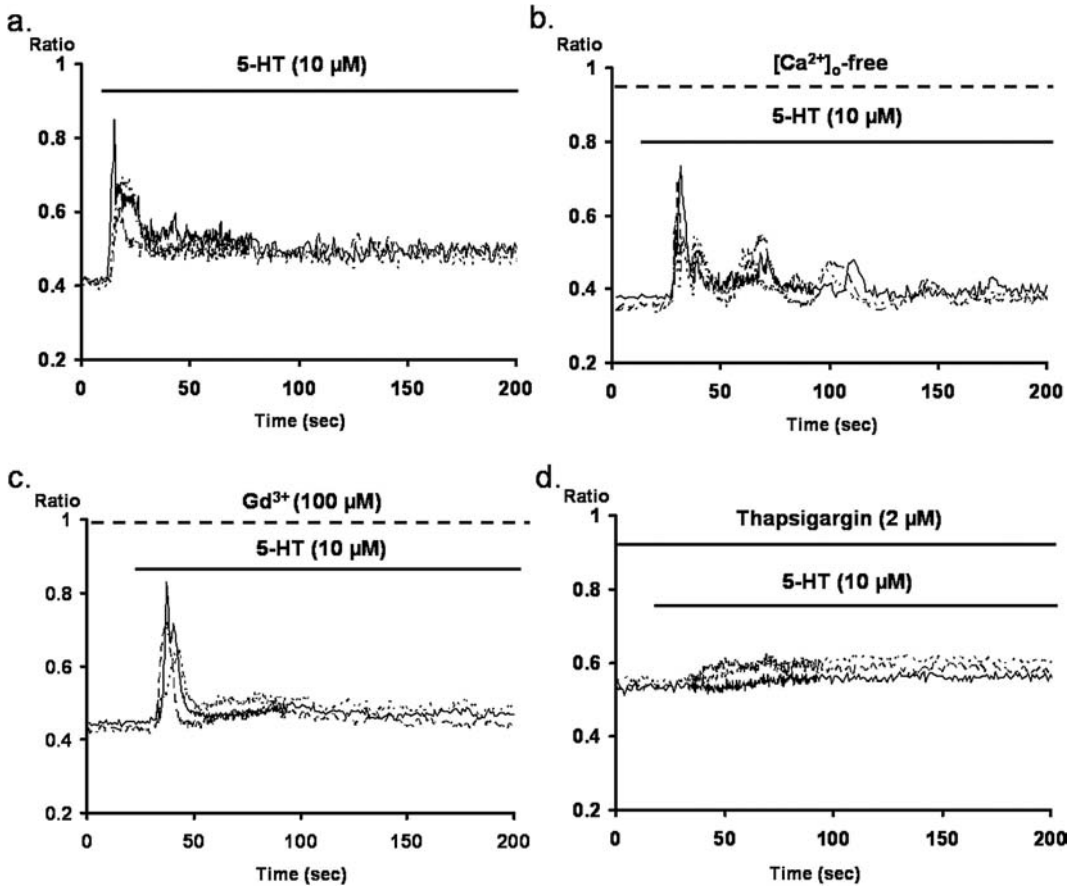

Fig. 7. Time courses for the 5-HT (10 μM)-induced $[Ca^{2+}]_i$ dynamics in testicular arteriole (external diameters >50 μm) smooth muscle cells (**a**–**d;** solid, dashed, and dotted lines) in certain areas (about 3 μm^2).

increases in the cells were not inhibited in the presence of H-89 (20 μM)(n=11)(Fig. 8e), suggesting that the 5-HT induced $[Ca^{2+}]_i$ mobilization of testicular arteriole can be mainly mediated by 5-HT2 and partly by 5-HT1a.

Discussion

The Ca^{2+} response of vascular smooth muscle cells has been examined by previous investigators using

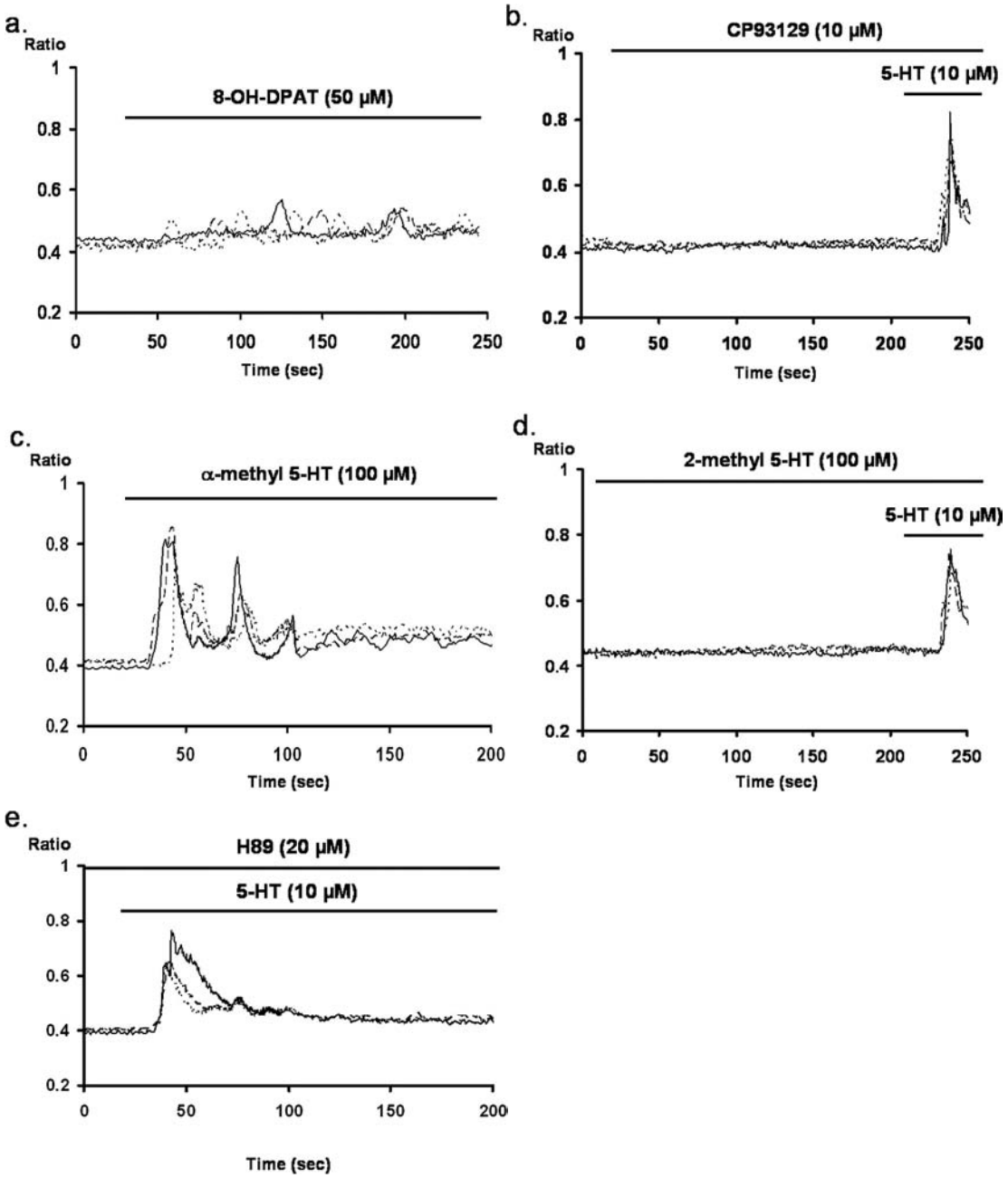

Fig. 8. Time courses for the 5-HT-induced $[Ca^{2+}]_i$ dynamics using selected modified drugs in testicular arteriole smooth muscle cells (**a–e;** solid, dashed, and dotted lines) in certain areas (about 3 μm^2).

various imaging techniques, but most of these studies used cultured or isolated smooth muscle cells from large vessels (Iino *et al*., 1994; Yip and Marsh, 1996; Li *et al*., 1997; Bolton and Gordienko, 1998; Jaggar *et al*., 1998). There are few data to analyze signaling systems in intact vessels, and it was hard to extrapolate the signaling mechanism of cultured cells to the cells *in vivo* (Ueki at al., 1987). Furthermore there are few pharmacophysiological studies on arterioles, even though these vessels play a pivotal role in tissue circulation. This is probably because of difficulties in analyzing the cell reactions in intact arterioles by conventional methods. Here we achieved a breakthrough with our living tissue imaging.

The present study revealed that the vasoactive properties of 5-HT in arteriole smooth muscles differ between cerebral and testicular arterioles; in cerebral arterioles they were induced by both the Ca^{2+} influx from extracellular spaces and the Ca^{2+} release from internal stores, whereas the $[Ca^{2+}]_i$ increase in testicular arterioles was dependent only on Ca^{2+} release from intracellular stores. In addition, the $[Ca^{2+}]_i$ increase in cerebral blood vessels was observed only in large- or mid-sized arterioles, especially in the portions close to the cerebral arterial circle and not in small-sized arterioles. On the other hand, the reaction observed in testicular arterioles was size- and region-independent.

Response to 5-HT in cerebral arterioles

Previous pharmacophysiological studies reported that 5-HT1 and 2 families most commonly mediated contractile responses to 5-HT in some vascular smooth muscles (Hamel *et al*., 1989; Hoyer *et al*., 1994, 2002; Miranda *et al*., 1995; Ullmer *et al*., 1995; Teng *et al*., 1998), but 5-HT induced $[Ca^{2+}]_i$ dynamics had not been studied. Under Ca^{2+} imaging, we demonstrated the details of 5-HT receptors in cerebral arterioles: there are 5-HT1a, 1b, 2, and 3 receptors, among which the 5-HT2 receptor plays a main role.

In the present study, we could not detect any changes in $[Ca^{2+}]_i$ dynamics of small arterioles during 5-HT stimulation, indicating that smooth muscle cells in peripheral cerebral blood vessels lack responses to 5-HT. In the hemorrhaged region, a considerable amount of 5-HT was released from platelets, and if small-sized arterioles of the brain have 5-HT receptors, the 5-HT released from brain hemorrhage may induce severe vasoconstriction in the whole cerebral vascular network, resulting in fatal brain damage. The lack of responses to 5-HT may be involved in reactive hyperemia, in which the cerebral blood flow increases transiently following occlusions of the large artery (Zimmer *et al*., 1971; Hossmann, 1997; Matsubara *et al*., 1998). In this context, previous physiological studies reported that 5-HT constricted large arteries and reduced the vascular resistance of small vessels (Rapport, 1949, Sjaastad, 1975, Cohen *et al*., 1983, Van Nueten 1985; Van Nueten *et al*., 1985; Faraci and Heistad, 1990). Another *in vivo* observation confirmed that 5-HT induced the contraction of the pial vessel (>200 μm in resting diameter), whereas it dilated small vessels (< 70 μm in diameter) (Harper and MacKenzie, 1977). Teng *et al*. (1998) reported that the ratio of 5-HT1 and 2 receptors decreases progressively to their smallest values in second branch middle cerebral arteries or smaller segments. It seems likely that the 5-HT induced dilatation of small vessels previously observed is one of the reactive hyperemia which is caused by various relaxant factors and not a relaxant effect of 5-HT directly on smooth muscle cells.

Response to 5-HT in testicular arterioles

There are no obvious differences in structure and function among arterioles in the testicular tissues. In the present study, no regional- or size-dependent differences were found in response to 5-HT. Thus, our results suggest that the receptor is mainly 5-HT2 and partly 5-HT1a, whereas the cerebral vasculature can possess 5-HT1a, 1b, 2, and 3 receptors. These findings also indicate that the relatively homogeneous tissue such as the testis does not require a delicate control mechanism for tissue circulation.

Prospect

It had been assumed that all smooth muscle cells react uniformly, but this does not appear to be the case. We observed arteriole responses to 5-HT under real-time confocal microscopy, and found that smooth muscle cells in the cerebral arterioles show characteristic regional differences in $[Ca^{2+}]_i$ dynamics. The regional differences for the functions of arterioles could not have been discovered using other methods. The central nervous system is a highly differentiated organ, and it is easily speculated that the blood circulation control mechanism is also specialized in each part, but to date few researchers have focused on this issue. Clarification of these specifics is essential for the development of therapies designed to improve blood circulation. To those ends, we are planning to develop a bioassay system using confocal microscopy.

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