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Role of nitric oxide in the contractile response to 5-hydroxytryptamine of the basilar artery from Wistar Kyoto and stroke-prone rats

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1 Isolated basilar arteries from spontaneously hypertensive stroke-prone rats (SHRSP) are more sensitive to the contractile effect of 5-hydroxytryptamine (5-HT) than those from normotensive Wistar Kyoto rats (WKY). This has been attributed to a different proportion of 5-HT receptor subtypes mediating these responses. In the present study we have examined if differences in nitric oxide release could also contribute to this difference in sensitivity to 5-HT.

2 At rest, the normalized internal diameter was significantly smaller in SHRSP (297.4 ± 3.5 μm, $n=88$) than in WKY (375.1 ± 4.0 μm, $n=62$, $P<0.01$) arteries. The contractile response to 100 mM KCl was higher in WKY (3.57 ± 0.15 mN mm^{-1}, $n=22$) than in SHRSP arteries (2.32 ± 0.20 mN mm^{-1}, $n=28$, $P<0.01$).

3 When added on the plateau of contraction to 5-HT (1 μM), acetylcholine (ACh, 3 μM) evoked significant relaxation in all preparations from WKY ($n=20$), but only in 15 out of 26 preparations from SHRSP. The mean relaxations were 55.4 ± 5.2% in WKY and 20.6 ± 4.6% in SHRSP (as % of the contractile tone evoked by 5-HT; $P<0.01$).

4 The NO synthase inhibitor N$^{\omega}$-nitro-L-arginine (L-NOARG, 0.1 mM) produced a similar increase in tone in both WKY and SHRSP. This tone was equal (in % of the contractile response to 100 mM KCl) to 70.8 ± 4.4% in WKY ($n=20$) and 67.6 ± 5.9% in SHRSP ($n=26$) and was reversed by L-arginine (1 mM) and by 1,4-dihydropyridine calcium channel blockers (10 nM nisoldipine, 10 nM lacidipine, 100 nM nifedipine). The L-NOARG-induced tone was absent when the arteries were bathed in phosphate-free Krebs (pH 7.4).

5 EC_{50} values of 5-HT were about four fold smaller in SHRSP than in WKY arteries ($P<0.01$). The maximal response to 5-HT (E_{max}) was higher than 100 mM KCl-contraction in SHRSP but not in WKY arteries. Removal of endothelium produced a shift to the left of the 5-HT curve in WKY, but not in SHRSP arteries.

6 When evoked in phosphate-free Krebs, the contractile responses to 5-HT showed tachyphylaxis, but the responses were reproducible by adding the agonist at 30 min intervals. In such conditions, EC_{50} values of 5-HT were about two fold smaller in SHRSP than in WKY arteries ($P<0.01$). In phosphate-free Krebs, the blockade of NO synthase did not change the contractile response to 100 mM KCl; it reduced EC_{50} and increased E_{max} of 5-HT in WKY, but not in SHRSP.

7 These results confirm that the sensitivity to 5-HT is higher in basilar artery isolated from SHRSP than in those from WKY. They show that endothelium-dependent vasorelaxation to ACh is impaired in SHRSP. The finding that removal of endothelium or blockade of NO synthase augmented the contractile response to 5-HT in WKY, but not in SHRSP basilar arteries indicates that the difference in responsiveness to 5-HT observed between WKY and SHRSP basilar arteries might be, at least in part, related to dissimilarities in NO release. Furthermore, the L-NOARG-induced contraction sensitive to calcium channel blockers indicates that, in basilar arteries, NO production might lower L-type calcium channel opening and thereby control the tone of the vessels.

Keywords: Basilar artery; 5-hydroxytryptamine; Wistar Kyoto rats (WKY); stroke-prone spontaneously hypertensive rats (SHRSP); endothelium; nitric oxide

Introduction

Stroke-prone spontaneously hypertensive rats (SHRSP) constitute an animal model of cerebrovascular disorders occurring in hypertension (Okamoto *et al*., 1974).

Nishimura (1996) has recently shown that basilar arteries isolated from SHRSP are more sensitive to the contractile effect of 5-hydroxytryptamine (5-HT) than those from normotensive Wistar Kyoto rats (WKY). He also found that, although the same mixed population of receptors (5-HT$_{1B}$ and 5-HT$_2$) seemed to mediate the response to 5-HT, the contraction mediated by the 5-HT$_{1B}$ receptor was greater in SHRSP than in WKY. It has been known for a long time that the contractile responses of isolated peripheral vessels to vasoconstrictor agonists is inhibited by endothelium-derived relaxing factor (Eglème *et al*., 1984; Godfraind *et al*., 1985; Martin *et al*., 1986) and that removal of endothelium increases contractile responses to 5-HT in rat basilar arteries from WKY (Yokota *et al*., 1994). The amount of nitric oxide basally released is different according to the vascular bed examined (Gao *et al*., 1995). Its role could be relatively important in rat cerebral arteries; for example, Kimura *et al*. (1994), when studying isolated preparations cannulated with glass pipettes, found that extraluminal application of NO synthase inhibitors evoked a large reduction of the internal diameter of cerebral penetrating arterioles of rat. It has been shown that the inhibitory activity of endothelium on arterial contraction is lower in hypertensive than in normotensive rats (Otsuka *et al*., 1988; Shirasaki *et al*., 1988) and, more recently, that a specific impairment of endo-

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thelium dependent vasorelaxation is associated and concomitant with stroke in SHRSP (Volpe *et al*., 1996). The purpose of the present experiments was to examine if the difference in responsiveness of basilar arteries from WKY and SHRSP to 5-HT could also be related to endothelial dysfunction. Therefore, we compared 5-HT concentration-contraction curves obtained in basilar arteries of WKY and SHRSP, either intact (unrubbed) or after removal (by rubbing) of endothelium. Furthermore, 5-HT curves were obtained with and without preincubation with the NO synthase inhibitor N^{ω}-nitro-L-arginine (L-NOARG).

The results show that removal of endothelium or blockade of NO synthase increased the contractile responses to 5-HT in WKY, but not in SHRSP, suggesting that the higher responsiveness to 5-HT of basilar artery isolated from SHRSP could partly be related to reduced endothelial release of NO.

Methods

Experimental animals

Wistar Kyoto rats (WKY) and spontaneously hypertensive stroke-prone rats (SHRSP) were purchased from Iffa Credo (L'arbresle, France). All rats were kept in the same environment and received water and food *ad libitum*. The systolic blood pressure was measured by the tail-cuff method in conscious animals prewarmed to 35°C in thermostatic cages (Physiograph Narco, Houston, TX, U.S.A.). Rats were killed at the age of 14 weeks. At this time systolic blood pressure was 136.4±4.4 mmHg in WKY ($n=19$) and 221.9±4.2 mmHg in SHRSP ($n=29$).

Measurement of contractile tension in isolated basilar artery

Rats were killed by decapitation, the brain was removed and immersed in physiological solution. The basilar artery was dissected under a binocular microscope (Leica Wild MZ8, Wetzlar, Germany). In some experiments, endothelium was removed by rubbing carefully the inner surface of basilar artery with a human hair. Arterial segments (1.5–2 mm long) were threaded onto 40 μm stainless steel wires and mounted in a dual isometric myograph (400A, J-P Trading, Denmark). After mounting, each preparation was equilibrated unstretched, for 20 min, in physiological solution, maintained at 37°C and aerated with a gas mixture of 95% O_2–5% CO_2. The normalized passive resting force and the corresponding diameter were determined for each preparation from its own length-pressure curve, according to Mulvany and Halpern (1977). In this way, the preparation was distended stepwise every minute while micrometre and force were recorded. The stepwise distension was stopped when the effective transmural pressure exceeded 100 mmHg (13.3 kPa). An exponential curve was then fitted to the internal circumference pressure data and the point of the curve corresponding to 100 mmHg was determined (IC_{100}). The preparation was then slightly relaxed to a circumference equal to 0.9 IC_{100} and held at this degree of stretch for the remainder of the experiment. Contractile responses were recorded with a computer, by using data acquisition hardware (MacLab) and data recording software (Chart v3.4.2, AD Instruments Pty Ltd., Castle Hill, Australia).

After normalization and 30 min equilibration in physiological solution, the preparations were stimulated with 100 mM KCl. After wash-out and 30 min recovery, they were exposed to 1 μM 5-HT; 3 μM acetylcholine was added on the plateau of contraction evoked by 1 μM 5-HT to assess endothelial function. After wash-out and 30 min recovery, 5-HT (1 nM–10 μM) was cumulatively added to the organ bath. Finally, after wash-out and 30 min recovery, L-NOARG (0.1 mM) was added to the organ bath. The first contractile response to 100 mM KCl was considered as an index of arterial contractility; the contractile responses to 5-HT and L-NOARG were expressed as % of the KCl-evoked contraction.

Solutions and drugs

Unless otherwise stated, experiments were performed in a Krebs solution (composition, mM: NaCl 122, KCl 5, $NaHCO_3$ 15, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 1.25 and glucose 11, pH 7.4 at 37°C). A phosphate-free Krebs modified solution was also used in some experiments (composition, mM: NaCl 122, KCl 5.9, $NaHCO_3$ 15, $MgCl_2$ 1.25, $CaCl_2$ 1.25 and glucose 11, pH 7.4 at 37°C). The 100 mM KCl-depolarizing solutions were obtained by isotonic replacement of NaCl with KCl (Godfraind & Kaba, 1969) either in the Krebs solution (composition, mM: NaCl 27, KCl 100, $NaHCO_3$ 15, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 1.25 and glucose 11, pH 7.4 at 37°C) or,

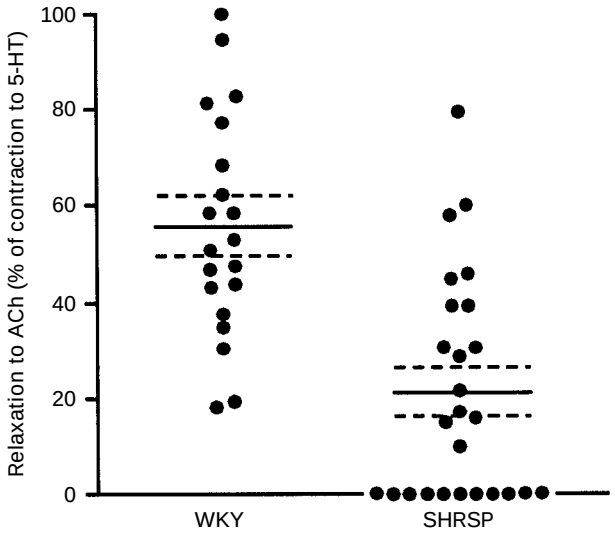


Figure 1 Dot diagram showing the distribution of relaxation to acetylcholine (ACh) 3 μM in basilar arteries from Wistar Kyoto (WKY) and stroke-prone (SHRSP) rats. Solid lines indicate the mean and dashed lines s.e.mean.

Table 1 Contractile responses to 100 mM KCl and to 5-HT, in basilar arteries from Wistar Kyoto rats (WKY) and spontaneously hypertensive stroke-prone rats (SHRSP), in Krebs physiological solution

	Endothelium intact		*Endothelium removed*	
	WKY ($n=22$)	*SHRSP* ($n=28$)	*WKY* ($n=9$)	*SHRSP* ($n=17$)
Contractile response to KCl (mN mm^{-1})	3.57±0.15	2.32±0.20**	2.76±0.32†	0.96±0.17**††
Contractile response to 5-HT				
EC_{50} (nM)	226.4±45.0	58.5±7.7**	49.9±5.9†	62.6±5.6
E_{max} (% of contraction to 100 mM KCl)	91.7±4.2	119.5±4.5**	102.9±4.1	118.4±8.6

EC_{50} is the concentration producing 50% of the maximum effect; E_{max} is the maximum effect. *$P<0.05$, **$P<0.01$ vs WKY; unpaired Student's t test. †$P<0.05$, ††$P<0.01$ vs endothelium unrubbed; unpaired Student's t test.

when stated, in the phosphate-free Krebs modified solution (composition, mM: NaCl 27, KCl 101, $NaHCO_3$ 15, $MgCl_2$ 1.25, $CaCl_2$ 1.25 and glucose 11, pH 7.4 at 37°C).

N^{ω}-nitro-L-arginine (L-NOARG), L-arginine and D-arginine were purchased from Sigma (St. Louis, MO, U.S.A.); 10 mM stock solutions were prepared in physiological solution, and further diluted as required. 5-Hydroxytryptamine creatinine sulphate was from E. Merck (Darmstadt, Germany), a 10 mM stock solution was prepared in water and further diluted as required. Nifedipine and nisoldipine were obtained from Bayer (Leverkusen, Germany), lacidipine was obtained from Glaxo (Verona, Italy); millimolar stock solutions were prepared in ethanol and further diluted in water as required. Experiments with 1,4-dihydropyridines were done under yellow light, in order to avoid photoinactivation.

Statistical analysis

Data are expressed as mean ± s.e.mean. EC_{50} is the drug concentration producing an effect of 50% of the maximum, it was estimated by linear regression from log concentration-effect curves. Tests of significance were made by unpaired Student's t test; P values less than 0.05 were considered statistically significant.

Results

Internal normalized diameter and contractile response to 100 mM KCl

Segments of basilar artery, mounted in wire myographs, were first submitted to the normalization protocol (see Methods)

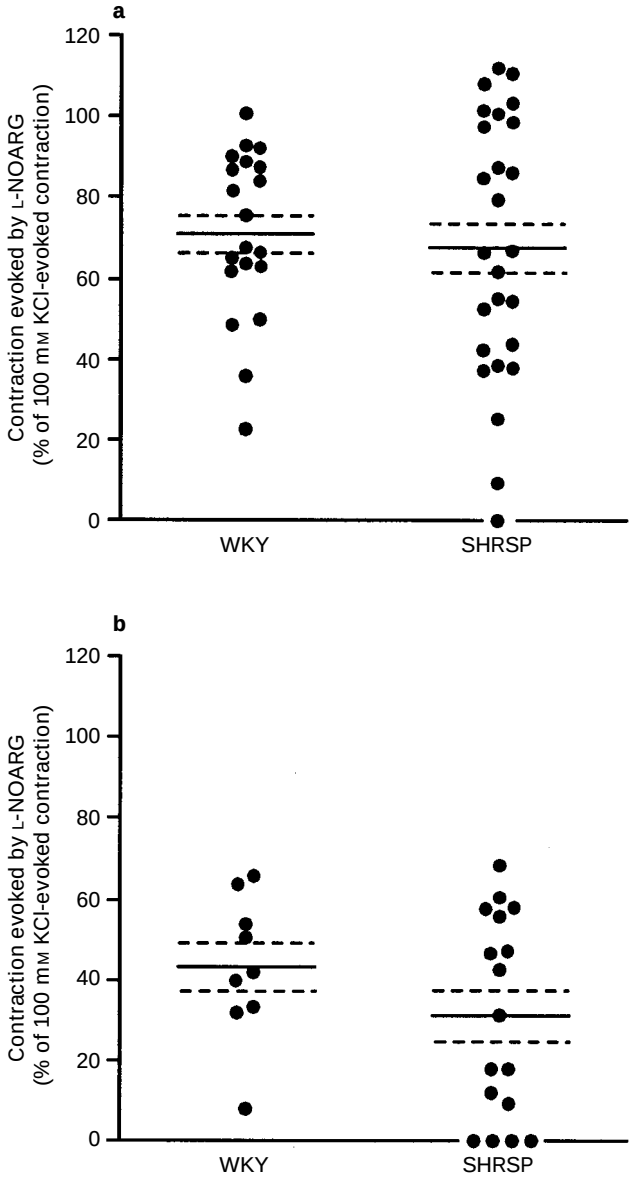


Figure 2 Dot diagrams showing the distribution of contraction to N^{ω}-nitro-L-arginine (L-NOARG, 0.1 mM), in endothelium-intact (unrubbed, a) and endothelium-removed (rubbed, b) basilar arteries from Wistar Kyoto (WKY) and stroke-prone (SHRSP) rats. Solid lines indicate the mean and dashed lines s.e.mean.

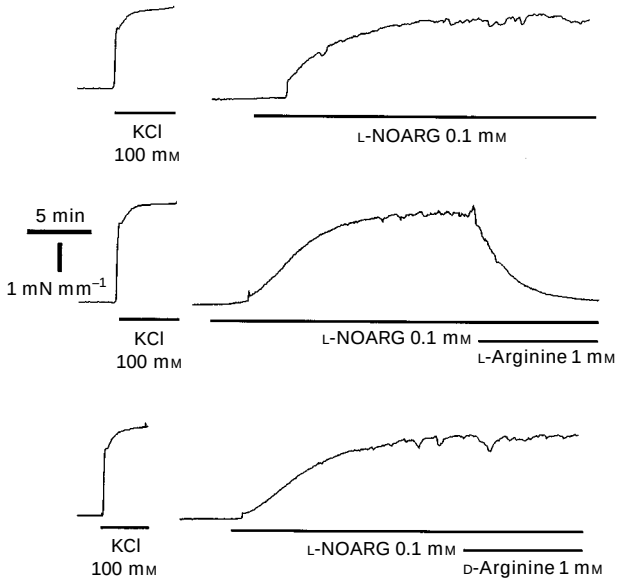


Figure 3 Effects of 1 mM L-arginine and 1 mM D-arginine on the contractile tone evoked by 0.1 mM N^{ω}-nitro-L-arginine (L-NOARG) in isolated basilar artery bathed in Krebs physiological solution. Upper trace: control; middle trace: L-arginine; lower trace: D-arginine. The contractile response of each preparation to 100 mM KCl is shown for comparison. This typical recording represents 1 of 3 experiments performed with basilar artery from Wistar Kyoto rats. The same results with L-arginine and D-arginine were obtained in 3 experiments with basilar artery from stroke-prone rats.

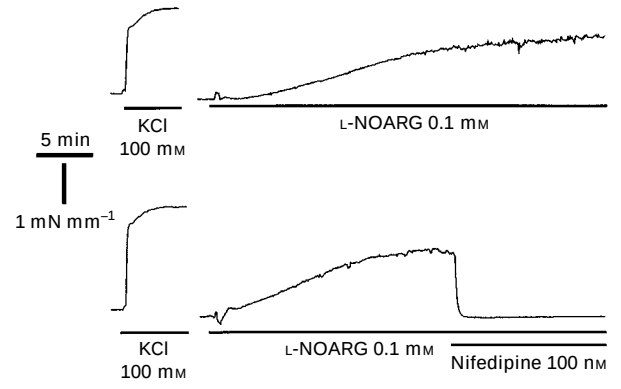
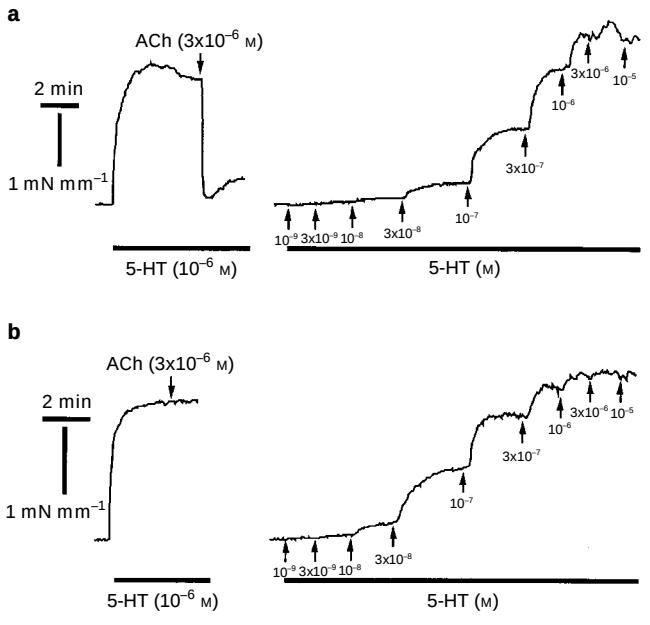


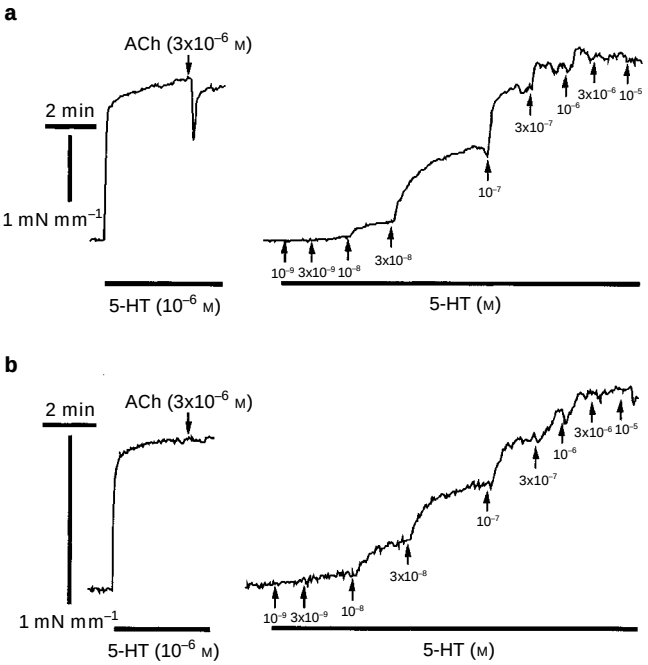
Figure 4 Effect of 100 nM nifedipine on the contractile tone evoked by 0.1 mM N^{ω}-nitro-L-arginine (L-NOARG), in isolated basilar artery bathed in Krebs physiological solution. Upper trace: control; lower trace: nifedipine. The contractile response of each preparation to 100 mM KCl is shown for comparison. This typical recording represents 1 of 3 experiments performed with nifedipine in basilar artery from Wistar Kyoto rat. The complete relaxation of L-NOARG-evoked tone was also observed with 10 nM nisoldipine and 10 nM lacidipine in basilar arteries from either Wistar Kyoto ($n=4$) or stroke-prone ($n=4$) rats.

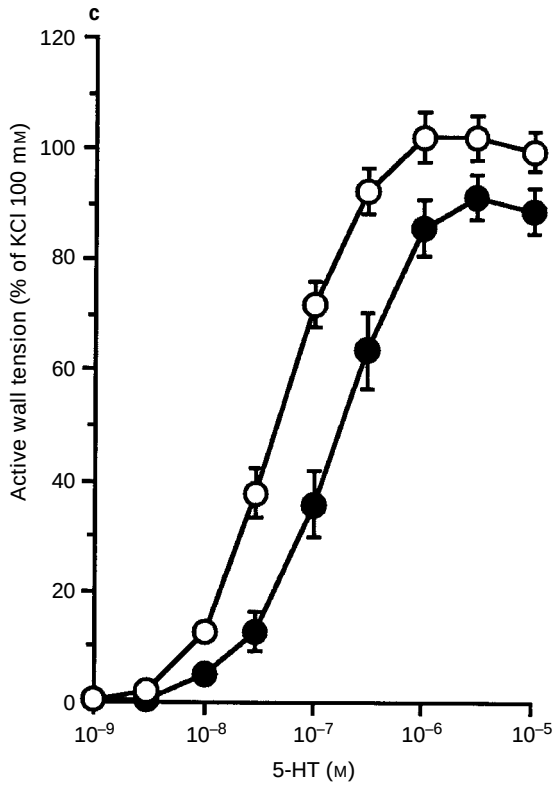
and the internal diameter was measured. At rest, the normalized internal diameter was significantly smaller in SHRSP (297.4 ± 3.5 μm, $n=88$) than in WKY (375.1 ± 4.0 μm, $n=62$, $P<0.01$). Table 1 shows that the contractile force in response to 100 mM KCl was higher in WKY than in SHRSP arteries ($P<0.01$).

Relaxation to acetylcholine of 5-HT evoked-contraction and effect of L-NOARG on basal tone

The presence of a functional endothelium was assessed by measuring the relaxing effect of 3 μM acetylcholine on the contraction evoked by 1 μM 5-HT. As shown in Figure 1,







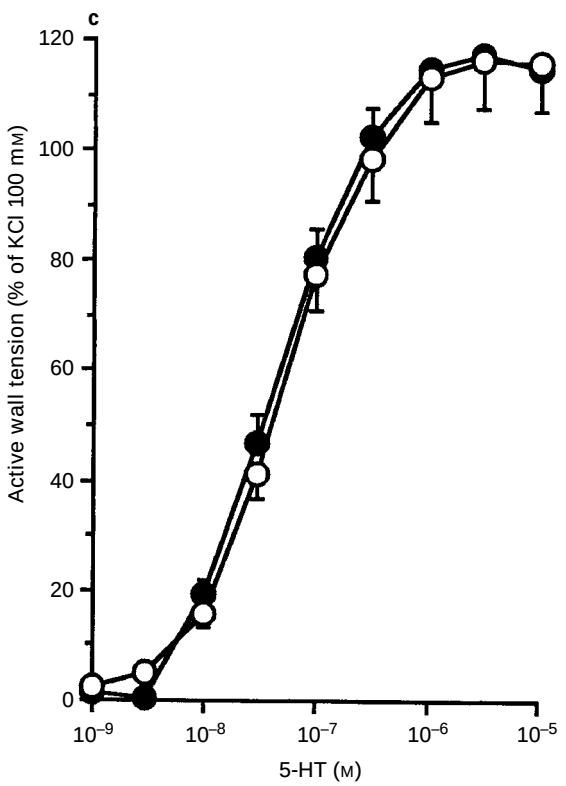


Figure 5 Relaxation to 3 μM acetylcholine (ACh) and concentration-contractile response curves to 5-HT, in basilar artery bathed in Krebs physiological solution, isolated from Wistar Kyoto rats. (a) Endothelium-intact (unrubbed) preparation, (b) endothelium-depleted (rubbed) preparation. (c) Concentration-effect curves to 5-HT from 22 endothelium-intact (●) and 9 endothelium-depleted (○) preparations. Data in (c) are expressed as mean, vertical lines show s.e.mean (when it exceeded the size of the symbol). Corresponding EC_{50} and E_{max} values are given in Table 1.

Figure 6 Relaxation to 3 μM acetylcholine (ACh) and concentration-contractile response curves to 5-HT, in basilar artery bathed in Krebs physiological solution, isolated from stroke-prone rats. (a) Endothelium-intact (unrubbed) preparation, (b) endothelium depleted (rubbed) preparation, (c) Concentration-effect curves to 5-HT from 28 endothelium-intact (●) and 17 endothelium-depleted (○) preparations. Data in (c) are expressed as mean, vertical lines show s.e. mean (when it exceeded the size of the symbol). Corresponding EC_{50} and E_{max} values are given in Table 1.

acetylcholine evoked significant relaxation in all the preparations from WKY ($n=20$), but only in 15 out of 26 preparations from SHRSP. The averaged relaxing effect of acetylcholine was $55.4\pm5.2\%$ in WKY and $20.6\pm4.6\%$ in SHRSP (as % of the contractile tone evoked by 5-HT; $P<0.01$).

When N^{ω}-nitro-L-arginine (L-NOARG, 0.1 mM), an inhibitor of NO synthase, was added to the Krebs physiological solution, basilar arteries developed a contractile tone which was not different between groups, when expressed as % of the 100 mM KCl-evoked contraction: $70.8\pm4.4\%$ in WKY ($n=20$) and $67.6\pm5.9\%$ in SHRSP ($n=26$, Figure 2a). This contractile tone evoked by L-NOARG was completely reversed by 1 mM L-arginine, but was unaffected by 1 mM D-arginine (Figure 3), indicating that it was due to the inhibition of NO synthase (Rees *et al*., 1990). Nevertheless, when 0.1 mM L-NOARG was added to the Krebs physiological solution bathing preparations in which endothelium had been removed by rubbing, they still developed a contractile tone (Figure 2b), although significantly less than in endothelium-intact preparations (as % of the 100 mM KCl-evoked contraction: $43.2\pm5.9\%$ in WKY and $30.9\pm6.0\%$ in SHRSP, $P<0.01$ vs endothelium intact preparations from both WKY and SHRSP). The addition of cumulative concentrations of L-NOARG (0.1 μM – 0.1 mM) allowed the estimate of its EC_{50} value, which was not affected by removal of endothelium (unrubbed 0.93 ± 0.29 μM, $n=9$; rubbed 0.89 ± 0.15 μM, $n=13$). The L-NOARG-induced tone was reversed by maximum active concentrations of 1,4-dihydropyridine calcium channel blockers (nifedipine 100 nM, nisoldipine 10 nM, lacidipine 10 nM), indicating that voltage-dependent L-type calcium channels were activated in the presence of L-NOARG (Figure 4).

The L-NOARG-induced tone was also reversed when the Krebs solution was changed to a phosphate-free Krebs solution; furthermore, L-NOARG did not produce any change of contractile tone when added to resting preparations bathed in phosphate-free Krebs solution.

Contractile responses to 5-HT

As shown in Figures 5 and 6, 5-HT evoked contractile responses in a concentration-dependent manner in both WKY and SHRSP. In endothelium-intact (unrubbed) preparations, the EC_{50} value was about four fold lower in SHRSP than in WKY ($P<0.01$). In WKY the amplitude of the maximum contraction in response to 5-HT was similar to that induced by 100 mM KCl, whereas in SHRSP the maximum response to 5-HT was 20% higher than the contraction induced by 100 mM KCl (Table 1).

In order to remove endothelium, some preparations were rubbed (see Methods); the successful removal of endothelium was confirmed by the lack of relaxation to acetylcholine (Figures 5b and 6b). Removal of endothelium produced a shift to the left of the 5-HT curve in WKY, but not in SHRSP basilar artery (Figures 5 and 6, Table 1).

Effect of L-NOARG on 5-HT-evoked contraction in phosphate-free Krebs solution

The contractile tone evoked by L-NOARG in Krebs solution did not allow quantification of the responses to 5-HT, therefore the study of the effect of L-NOARG on the contractile response to 5-HT was done in phosphate-free Krebs solution, in which L-NOARG did not change resting tone. The absence of phosphate did not reduce the contractile force evoked by 100 mM KCl solution (Table 2). However, the responses to 5-HT were tachyphylactic, in such a way that 5-HT could not be added cumulatively to the organ chamber. Reproducible contractile responses were observed when successive additions of 5-HT were spaced by prolonged drug-free intervals. A 30 min rest between successive additions of 5-HT was maintained through all the experimental protocol in phosphate-free Krebs solution. Under such a condition, reproducible concentration-effect curves to 5-HT were obtained. As shown in Table 2 and Figure 7, the maximal responses to 5-HT (E_{max}) relative to the 100 mM KCl-evoked contraction were lower in phosphate-free Krebs than in normal Krebs solution, while EC_{50} values were larger in phosphate-free Krebs than in normal Krebs solution. Nevertheless, the difference in EC_{50} values between WKY and SHRSP was still marked ($P<0.01$). In the presence of L-NOARG, EC_{50} values were significantly reduced in WKY arteries; they were not significantly changed in SHRSP, in such a way that EC_{50} values of 5-HT were not significantly different between WKY and SHRSP after blockade of NO synthase activity. Also, E_{max} of 5-HT was increased in the presence of L-NOARG in WKY but not in SHRSP arteries, whereas the contractions developed by KCl-depolarized arteries were similar in the absence and presence of L-NOARG.

Discussion

The purpose of this study was to examine the role of NO release in the responsiveness to 5-HT of basilar arteries from WKY and SHRSP. The results show that the relaxing effect of acetylcholine was reduced or absent in SHRSP arteries and that removal of endothelium or inhibition of NO synthase activity enhanced the responsiveness to 5-HT in WKY but not in SHRSP arteries, suggesting that NO release could, at least in part, account for the difference in responsiveness to 5-HT between WKY and SHRSP.

Differences in morphology of basilar artery between WKY and SHRSP lie in a geometric rearrangement of smooth muscle cells in the media (Arribas *et al*., 1996). This rearrangement correlates with the inherent contractility of isolated basilar artery which is lower in SHRSP than in WKY. Therefore, responses to 5-HT of vessels from WKY and SHRSP were normalized in relation to the contractile response to 100 mM KCl.

We found that endothelium-dependent relaxation evoked by acetylcholine was absent or significantly impaired in arteries from SHRSP when compared to arteries from WKY. Endo-

Table 2 Effect of N^{ω}-nitro-L-arginine (L-NOARG, 0.1 mM) on contractile responses to 100 mM KCl and to 5-HT, in basilar arteries from Wistar Kyoto rats (WKY) and spontaneously hypertensive stroke-prone rats (SHRSP), in phosphate-free Krebs solution.

	Without L-NOARG		*With L-NOARG*	
	WKY ($n=14$)	*SHRSP* ($n=27$)	*WKY* ($n=16$)	*SHRSP* ($n=16$)
Contractile response to KCl (mN mm^{-1})	4.25 ± 0.13	2.76 ± 0.21**	4.01 ± 0.12	2.19 ± 0.29**
Contractile response to 5-HT				
EC_{50} (nM)	628.4 ± 84.3	385.9 ± 43.9**	282.4 ± 36.5††	336.1 ± 62.7
E_{max} (% of contraction to 100 mM KCl)	49.5 ± 3.4	79.0 ± 3.3**	69.9 ± 1.9††	81.7 ± 3.9*

EC_{50} is the concentration producing 50% of the maximum effect; E_{max} is the maximum effect. *$P<0.05$, **$P<0.01$ vs WKY; unpaired Student's t test. ††$P<0.01$ vs without L-NOARG; unpaired Student's t test.

thelial dysfunction could participate in the development of cerebrovascular lesions in SHRSP, as suggested by a recent study showing that impairment of endothelium-dependent vasorelaxation is associated and concomitant with stroke, in SHRSP (Volpe *et al*., 1996).

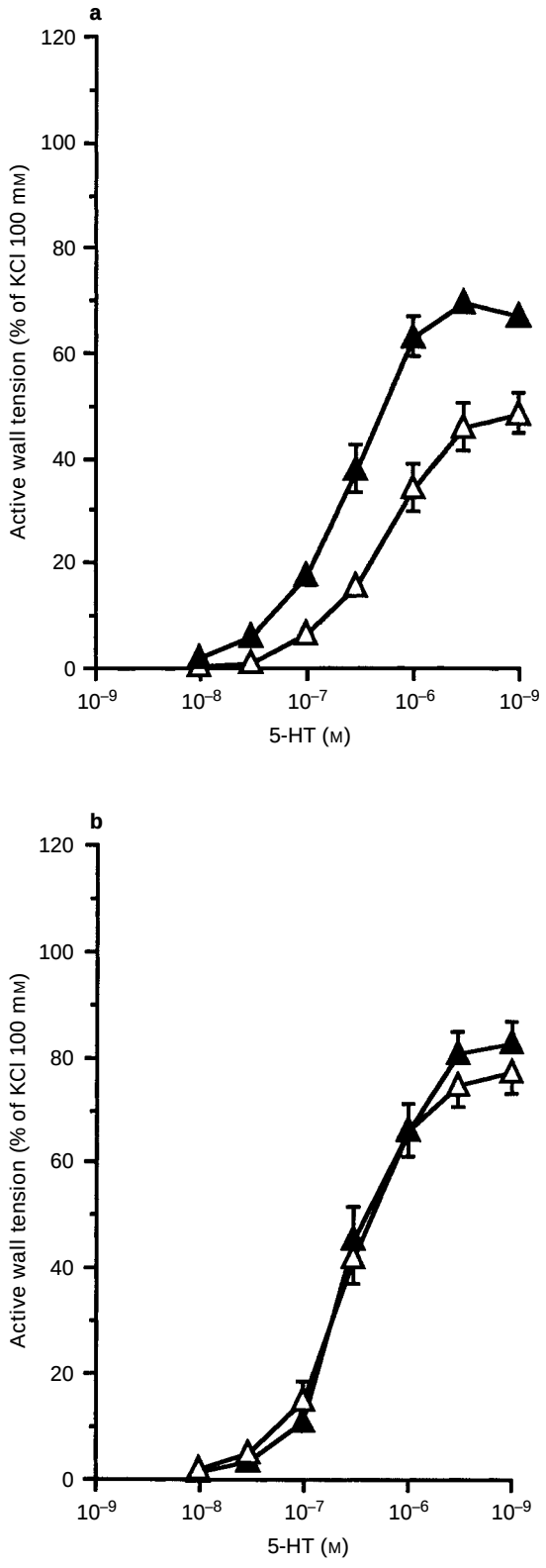


Figure 7 Effect of 0.1 mM N^{ω}-nitro-L-arginine (L-NOARG) on concentration-contractile response curves to 5-HT, in basilar arteries bathed in phosphate-free Krebs solution. (a) Basilar artery from Wistar Kyoto rats in the absence ($n=14$, △) and presence ($n=16$, ▲) of L-NOARG; (b) basilar artery from stroke-prone rats in the absence ($n=27$, △) and presence ($n=16$, ▲) of LNOARG. Data are expressed as mean, vertical lines show s.e.mean (when it exceeded the size of the symbol). Corresponding EC_{50} and E_{max} values are given in Table 2.

It has been shown that acetylcholine-induced dilatation of rat basilar artery mostly depends on endothelial release of nitric oxide (Faraci, 1991). We found that the NO synthase inhibitor L-NOARG evoked a similar increase in tone in resting basilar arteries from WKY and SHRSP. This effect was reversed by L-arginine and not by D-arginine, indicating that it was due to NO synthase inhibition. The increase in tone induced by L-NOARG was reduced, but not abolished, after removal of endothelium, suggesting that this NO synthase activity was only in part accessible to mechanical rubbing. Katusic (1991) demonstrated an increase in contractile tone and a decrease of guanosine 3′:5′-cyclic monophosphate (cyclic GMP) content in the presence of NO synthase inhibitors, in endothelium-denuded canine basilar arteries. Our finding together with this result suggest that, in isolated basilar arteries, NO synthase could also be located in cellular types different from endothelial cells. L-NOARG in the millimolar range is known to be active on all NO synthase isoforms, whereas, in the micromolar range, its inhibitor effect is limited to the endothelial (eNOS or NOS III) and the neuronal (nNOS or NOS I) isoforms, the inducible isoform (iNOS or NOS II) being about two orders of magnitude less sensitive (Southan *et al*., 1996). In the present study L-NOARG evoked a contraction with an EC_{50} in the submicromolar range, its contractile effect could therefore be related to inhibition of eNOS and/or nNOS. The existence of NO synthase-immunoreactive nerve fibres has been demonstrated in the adventitia of different vessels, where they are considered to be an important source of NO for modulation of vascular tone (Toda & Okamura, 1993). The L-NOARG-induced tone observed in rubbed preparations could be related to this neuronal NOS, since these perivascular fibres have been shown to be particularly abundant in cerebral arteries (Nozaki *et al*., 1993) and they are responsible for the constriction of basilar artery observed in anaesthetized dogs after intracisternal application of L-NOARG (Toda *et al*., 1993).

It is noteworthy that the L-NOARG-evoked contractile tone in basilar arteries was completely reversed by 1,4-dihydropyridine calcium channel blockers; this indicates that this tone resulted from calcium entry through voltage-dependent L-type calcium channels. Basal NO production in basilar arteries could tonically lower L-type calcium channel opening by controlling resting membrane potential of smooth muscle cells. This hypothesis is supported by the observation that NO has a hyperpolarizing effect in other vascular preparations (Tare *et al*., 1990; Krippeit-Drews *et al*., 1992; Rand & Garland, 1992) and has been ascribed to activation of K^+ channels in vascular smooth muscle cells (Murphy & Brayden, 1995). Consistent with this view, a recent study has shown that, *in vivo*, inhibitors of Ca^{2+}-activated K^+ channels decrease the dilatation of rat basilar artery induced by acetylcholine or sodium nitroprusside (Kitazono *et al*., 1997).

In an initial series of experiments, in normal Krebs solution, we found that 5-HT was about four fold more potent in arteries from SHRSP than in those from WKY, confirming the observation by Nishimura (1996). Mechanical removal of endothelium produced a significant drop in the active wall tension of isolated basilar arteries, as attested by the contractile responses to 100 mM KCl presented in Table 1, probably because damage of some smooth muscle cells occurred during the rubbing procedure. Nevertheless, as indicated by the EC_{50} values, the responses to 5-HT were increased by removal of endothelium in rubbed arteries from WKY and not significantly modified in rubbed arteries from SHRSP. This indicates that endothelial release of vasorelaxant factors inhibited the responsiveness to 5-HT of WKY arteries. It is known that NO reduces the contractile response of vessels to various agonists (Alosachie & Godfraind, 1986). Therefore, in order to determine if NO was the main relaxant factor, we designed an experimental protocol in which the concentration-contractile response curve to 5-HT was carried out in the presence of the

NO synthase inhibitor L-NOARG. In this protocol, we used a phosphate-free Krebs solution, in which L-NOARG did not evoke any increase of contractile tone in resting preparations. In this solution, the contractile response to 5-HT was tachyphylactic. This modification of the contractile effect of 5-HT cannot be related to the pH of the solution, since pH values measured in phosphate-containing and in phosphate-free solutions were identical (pH = 7.4 at 37°C, in the presence of 5% CO_2, in both solutions). It could be due to a decrease of the cytosolic free calcium concentration, as this has been found in other cellular types bathed in phosphate-free solution (Korc & Schöni, 1987). Consistently, tachyphylaxis to 5-HT has been shown to occur in Ca^{2+}-depleted solutions (Fasciolo, 1984).

In phosphate-free Krebs the responsiveness of WKY basilar artery to 5-HT was increased by L-NOARG. This shows that NO release inhibited the contractile response to 5-HT. Such an effect was not observed in SHRSP arteries, indicating that NO release in these arteries was much lower than in WKY arteries, a view consistent with the difference in the vasodilator response to acetylcholine. Indeed, the possibility that the effect of NO, instead of its release, could be impaired in SHRSP is unlikely, since it has been shown that, by contrast with acetylcholine, relaxations to sodium nitroprusside are preserved in SHRSP (Volpe *et al*., 1996). Whether the responsiveness to 5-HT in WKY arteries is inhibited by basal NO release or by NO release evoked by 5-HT itself remains to be determined. Indeed, the possibility that in WKY arteries, additional NO release resulted from the action of 5-HT on endothelial 5-HT receptors cannot be ruled out. 5-HT_{1D} (Schoeffter & Hoyer, 1990) and, more recently, 5-HT_{2B} receptors (Ullmer *et al*., 1996; Schmuck *et al*., 1996) have been found in endothelial cells where they stimulate NO release. In isolated arteries with functional endothelium, such as WKY basilar artery, the activation of these 5-HT endothelial receptors could therefore partially mask the contraction evoked by the action of 5-HT on vascular smooth muscle cells. Further studies are obviously required in order to verify this hypothesis.

In conclusion, the findings that the vasorelaxant effect of acetylcholine was reduced or absent in SHRSP and that removal of endothelium or blockade of NO synthase augmented the contractile response to 5-HT in WKY, but not in SHRSP basilar arteries, suggest that the difference in responsiveness to 5-HT observed between WKY and SHRSP basilar arteries might be, at least in part, related to dissimilarities in NO release. They also indicate that in basilar arteries, NO production might lower L-type calcium channel opening and thereby control the tone of the vessels.

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References

ALOSACHIE, I. & GODFRAIND, T. (1986). Role of cyclic GMP in the modulation by endothelium of the adrenolytic action of prazosin in rat isolated aorta. *Br. J. Pharmacol*., **89,** 525 – 532.

ARRIBAS, S.M., GORDON, J.F., DALY, C.J., DOMINICZAK, A.F. & MCGRATH, J.C. (1996). Confocal microscopic characterization of a lesion in a cerebral vessel of the stroke-prone spontaneously hypertensive rat. *Stroke*, **27,** 1118 – 1123.

EGLÈME, C., GODFRAIND, T. & MILLER, R.C. (1984). Enhanced responsiveness of rat isolated aorta to clonidine after removal of the endothelial cells. *Br. J. Pharmacol*., **81,** 16 – 18.

FARACI, J.C. (1991). Role of endothelium-derived relaxing factor in cerebral circulation: large arteries vs microcirculation. *Am. J. Physiol*., **261,**H1038 – H1042.

FASCIOLO, J.C. (1984). Calcium pools in the contraction of arterial smooth muscle induced by several agonists. *J. Hypertens*., **2,** 515 – 521.

GAO, Y., ZHOU, H. & RAJ, J.U. (1995). Heterogeneity in role of endothelium-derived NO in pulmonary arteries and veins of full-term fetal lambs. *Am. J. Physiol*., **268,** H1586 – H1592.

GODFRAIND, T., EGLÈME, C. & ALOSACHIE, I. (1985). Role of endothelium in the contractile response of rat aorta to α-adrenoceptor agonists. *Clin. Sci*., **68,** 65s – 71s.

GODFRAIND, T. & KABA, A. (1969). Blockade or reversal of contraction induced by calcium and adrenaline in depolarized arterial smooth muscle. *Br. J. Pharmacol*., **36,** 459 – 460.

KATUSIC, Z.S. (1991). Endothelium-independent contractions to N^G-monomethyl-L-arginine in canine basilar artery. *Stroke*, **22,** 1399 – 1404.

KIMURA, M., DIETRICH, H.H. & DACEY, R.G.JR. (1994). Nitric oxide regulates cerebral arteriolar tone in rats. *Stroke*, **25,** 2227 – 2234.

KITAZONO, T., IBAYASHI, S., NAGAO, T., FUJII, K. & FUJISHIMA, M. (1997). Role of Ca^{2+}-activated K^+ channels in acetylcholine-induced dilatation of the basilar artery *in vivo*. *Br. J. Pharmacol*., **120,** 102 – 106.

KORC, M. & SCHÖNI, M.H. (1987). Modulation of cytosolic free calcium levels by extracellular phosphate and lanthanum. *Proc. Natl. Acad. Sci. U.S.A*., **84,** 1282 – 1285.

KRIPPEIT-DREWS, P., MOREL, N. & GODFRAIND, T. (1992). Effect of nitric oxide on membrane potential and contraction of rat aorta. *J. Cardiovasc. Pharmacol*., **20**(supp.12), S72 – S75.

MARTIN, W., FURCHGOTT, R.F., VILLANI, G.M. & JOTHIANANDAN, D. (1986). Phosphodiesterase inhibitors induce endothelium-dependent relaxation of rat and rabbit aorta by potentiating the effects of spontaneously released endothelium-derived relaxing factor. *J. Pharmacol. Exp. Ther*., **237,** 539 – 547.

MULVANY, M.J. & HALPERN, W. (1977). Contractile properties of small arterial resistance vessel in spontaneously hypertensive and normotensive rats. *Circ. Res*., **41,** 19 – 26.

MURPHY, M.E. & BRAYDEN, J.E. (1995). Nitric oxide hyperpolarizes rabbit mesenteric arteries via ATP-sensitive potassium channels. *J. Physiol*., **486,** 47 – 58.

NISHIMURA, Y. (1996). Characterization of 5-hydroxytryptamine receptors mediating contractions in basilar arteries from stroke-prone spontaneously hypertensive rats. *Br. J. Pharmacol*., **117,** 1325 – 1333.

NOZAKI, K., MOSKOWITZ, M.A., MAYNARD, K.I., KOKETSU, N., DAWSON, T.M., BREDT, D.S. & SNYDER, S.H. (1993). Possible origins and distribution of immunoreactive nitric oxide synthase-containing nerve fibers in cerebral arteries. *J. Cereb. Blood Flow Metab*., **13,** 70 – 79.

OKAMOTO, K., YAMORI, Y. & NAGAOKA, A. (1974). Establishment of the stroke-prone spontaneously hypertensive rat (SHR). *Circ. Res*., **34 & 35**(suppl.1), 143 – 153.

OTSUKA, Y., DIPIERO, A., HIRT, E., BRENNEMAN, B. & LOCKETTE, W. (1988). Vascular relaxation and cGMP in hypertension. *Am. J. Physiol*., **254,** H163 – H169.

RAND, V.E. & GARLAND, C.J. (1992). Endothelium-dependent relaxation to acetylcholine in the rabbit basilar artery: importance of membrane hyperpolarization. *Br. J. Pharmacol*., **106,** 143 – 150.

REES, D.D., CELLEK, S., PALMER, R.M.J. & MONCADA, S. (1990). Dexamethasone prevents the induction by endotoxin of a nitric oxide synthase and the associated effects on vascular tone: an insight into endotoxin shock. *Biochem. Biophys. Res. Commun*., **173,** 541 – 547.

SCHMUCK, K., ULLMER, C., KALKMAN, H.O., PROBST, A. & LÜBBERT, H. (1996). Activation of 5-HT2B receptors: an early step in the generation of migraine headache. *Eur. J. Neurosci*., **8,** 1996, 959 – 967.

SCHOEFFTER, P. & HOYER, D. (1990). 5-Hydroxytryptamine (5-HT)-induced endothelium-dependent relaxation of pig coronary arteries is mediated by 5-HT receptors similar to the 5-HT_{1D} receptor subtype. *J. Pharmacol. Exp. Ther.*, **252,** 387–395.

SHIRASAKI, Y., KOLM, P., NICKOLS, G.A. & LEE, T.J.F. (1988). Endothelial regulation of cyclic GMP and vascular responses in hypertension. *J. Pharmacol. Exp. Ther.*, **245,** 53–58.

SOUTHAN, G.J., ZINGARELLI, B., O'CONNOR, M., SALZMAN, A.L. & SZABO, C. (1996). Spontaneous rearrangement of aminoalkylisothioureas into mercaptoalkylguanidines, a novel class of nitric oxide synthase inhibitors with selectivity towards the inducible isoform. *Br. J. Pharmacol.*, **117,** 619–632.

TARE, M., PARKINGTON, H.C., COLEMAN, H.A., NEILD, T.O. & DUSTING, G.J. (1990). Hyperpolarization and relaxation of arterial smooth muscle caused by nitric oxide derived from endothelium. *Nature*, **346,** 69–71.

TODA, N., AYAJIKI, K. & OKAMURA, T. (1993). Neural mechanism underlying basilar arterial constriction by intracisternal 1-NNA in anesthetized dogs, *Am. J. Physiol.*, **265,** H103–H107.

TODA, N. & OKAMURA, T. (1993). Response to perivascular nerve stimulation of distal temporal arteries from dogs and monkeys. *J. Cardiovasc. Pharmacol.*, **22,** 744–749.

ULLMER, C., BODDEKE, H.G.W.M., SCHMUCK, K. & LÜBBERT, H. (1996). 5-HT_{2B} receptor-mediated calcium release from ryanodine-sensitive intracellular stores in human pulmonary artery endothelial cells. *Br. J. Pharmacol.*, **117,** 1081–1088.

VOLPE, M., IACCARINO, G., VECCHIONE, C., RIZZONI, D., RUSSO, R., CONDORELLI, G., GANTEN, U., GANTEN, D., TRIMARCO, B. & LINDPAINTNER, K. (1996). Association and cosegregation of stroke with endothelium-dependent vasorelaxation in stroke-prone, spontaneously hypertensive rats. *J. Clin. Invest.*, **98,** 256–261.

YOKOTA, Y., IMAIZUMI, Y., ASANO, M., MATSUDA, T. & WATANABE, M. (1994). Endothelium-derived relaxing factor released by 5-HT: distinct from nitric oxide in basilar arteries of normotensive and hypertensive rats. *Br. J. Pharmacol.*, **113,** 324–330.

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