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Characterization of 5-hydroxytryptamine receptors mediating contractions in basilar arteries from stroke-prone spontaneously hypertensive rats

Yoshitaka Nishimura

Department of Pharmacology, Kinki University School of Medicine, 377-2, Ohno-Higashi, Osaka-Sayama, Osaka 589, Japan.

1 The 5-hydroxytryptamine (5-HT) induced-contraction in ring preparations of basilar arteries from Wistar-Kyoto rats (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP) was pharmacologically characterized *in vitro*.

2 Contractile responses to 5-HT (1 nM - 100 nM) and their pD_2 values in arteries from SHRSP at 6 months of age were significantly greater than those in age-matched WKY, although the maximum response did not differ between the two groups.

3 There were no significant differences in contractile responses to U-44619, endothelin-1, neuropeptide Y, and angiotensin II between WKY and SHRSP arteries.

4 Spiperone (1 nM - 1 μM, a 5-HT_2 receptor antagonist), produced biphasic displacement of the 5-HT curves in WKY and SHRSP arteries. The response to high concentrations of 5-HT was concentration-dependently antagonized by spiperone, while the response to low concentrations of 5-HT was resistant to blockade by spiperone, and the spiperone-resistant contractile responses induced by 5-HT were greater in SHRSP than in WKY. Ketanserin (1 - 100 nM, 5-HT_2) also produced a biphasic shift of the 5-HT curves for both arteries.

5 Methiothepin (10 and 100 nM, 5-HT_1 and 5-HT_2) potently inhibited 5-HT-induced contractions in both groups. In addition, methiothepin (100 nM) produced a parallel shift to the right of the component of 5-HT-induced contractile responses that was resistant to blockade by spiperone in both groups.

6 The contractile effects of 5-HT in WKY and SHRSP arteries were not affected by MDL 72222 (1 μM, 5-HT_3) and SDZ 205-557 (1 μM, 5-HT_4). In addition, cocaine (10 μM), pargyline (50 μM), prazosin (10 μM), indomethacin (3 μM) and SQ 29,548 (1 μM) did not affect the contractile effects of 5-HT in either artery.

7 Contractile responses to 5-carboxamidotryptamine, CGS 12066B, pindolol and propranolol were greater in SHRSP arteries than in WKY arteries, whereas contractions in response to 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), α-methyl-5-HT and 2-methyl-5-HT did not differ between the two groups. Cisapride failed to contract basilar arteries in both groups. Furthermore, a correlation analysis showed a highly significant correlation between the pD_2 values of 5-HT agonists in WKY and SHRSP arteries and their published binding affinities at the 5-HT_{1B} subtype.

8 These findings suggest that 5-HT elicits vasoconstriction in rat basilar arteries by stimulation of a mixed receptor population of 5-HT_2 and 5-HT_1-like receptors (similar to the 5-HT_{1B} receptor subtype), and that the contraction mediated by 5-HT_1-like receptors is enhanced in the basilar artery from SHRSP.

Keywords: Basilar artery; 5-hydroxytryptamine (5-HT); 5-HT_1-like receptors; 5-HT_{1B} receptors; 5-HT_2 receptors; stroke-prone spontaneously hypertensive rat (SHRSP)

Introduction

5-Hydroxytryptamine (5-HT), a potent vasoconstrictor in the large cerebral arteries, is considered to be involved in the regulation of the cerebral circulation, and to be implicated in the aetiology of cerebrovascular disorders such as migraine, vasospasm following acute subarachnoid haemorrhage, and ischaemic brain disease (Van Zwieten, 1987; Bonvento *et al.*, 1991).

Hypertension, a risk factor for cerebrovascular diseases, produces functional and morphological changes of the cerebral vascular bed (Heistad & Baumbach, 1992). From functional studies using hypertensive animal models, some researchers have demonstrated increased reactivity to 5-HT in isolated basilar arteries from spontaneously hypertensive rats (SHR) (Winquist & Bohr, 1983; Yokota *et al.*, 1994) and deoxycorticosterone acetate (DOCA)-salt hypertensive rats (Soltis & Bohr, 1987). However, the detailed pharmacological characterization of 5-HT-induced contraction in the cerebral arteries from these hypertensive animal models remains unknown.

Recently, from the findings obtained in functional, radioligand, second messenger and molecular biological studies, 5-HT receptors have been classified into several subtypes (reviewed in Hoyer *et al.*, 1994). Thus, to characterize pharmacologically the 5-HT receptor mediating contraction in the basilar arteries from hypertensive animal model, I examined the contractile effects of various substances, including 5-HT receptor agonists, and the effects of various blocking agents, including 5-HT-receptor antagonists, on 5-HT-induced contractions in isolated basilar arteries from Wistar-Kyoto rats (WKY) and stroke-prone SHR (SHRSP).

Methods

Animals

Male WKY and SHRSP at 3 and 6 months of age, bred and maintained in the Department of Pharmacology, Kinki University School of Medicine, were used in this study. They were housed in stainless steel cages in a room illuminated from 07 h

00 min to 19 h 00 min and maintained at 23±1°C. All animals were provided with food and water *ad libitum*.

Experimental procedure

The rats were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.), and then exsanguinated from the abdominal aorta. The brains were quickly removed and immersed in physiological salt solution (PSS) with the following composition in mM: NaCl 120, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5 and glucose 10 (pH 7.4). Under a dissecting microscope, the basilar artery was carefully dissected from the brain, and then cut into rings, 1 mm in length. The ring preparations were mounted horizontally on L-shaped tungsten wires (50 μm in diameter) in an organ chamber filled with 2 ml PSS, maintained at 37°C, and bubbled with a mixture of 95% O_2 and 5% CO_2 (Nishimura *et al.*, 1992). Changes in isometric tension were measured by a force displacement transducer (UL-2GR, Minebea Co., Ltd., Nagano, Japan) and recorded on a polygraph (366, NEC San-ei Instruments, Ltd., Tokyo, Japan). The ring preparations were maintained at an initial resting tension of 150 mg, and were allowed to equilibrate for at least 90 min during which the PSS was changed at 15 min intervals and the tension was repeatedly readjusted to 150 mg.

5-HT and other agonists were added cumulatively to the organ chamber. To study the effects of blocking agents on 5-HT-induced contractions, two reproducible concentration-response curves to 5-HT were constructed in each preparation, and then the concentration-response curve to 5-HT was obtained in the presence of the blocking agent. The blocking agent was added to the organ chamber 30 min prior to the application of 5-HT. In studies with the blocking agents, the concentration-response curves for 5-HT in the presence of blocking agents were expressed as a percentage of the maximum contraction induced by 5-HT in the control curve.

Analysis of data

All results are expressed as means±s.e.mean. The pD_2 values (negative logarithm of EC_{50}; EC_{50}=concentration producing half the maximal response) of each agonist were calculated on individual curves. Where appropriate, pK_B values were calculated according to the formula: $pK_B = \log(DR-1) - \log[B]$, where DR is the ratio of equiactive concentrations of agonist in the presence and absence of antagonist and [B] the concentration of antagonist (Kenakin, 1993), and pA_2 values were determined from a Schild plot analysis (Arunlakshana & Schild, 1959). Significant differences were assessed by Student's paired or unpaired *t* test between two groups, and analysis of variance followed by the Newman-Keuls' multiple range test for comparison of more than two groups. In each case, *P* values less than 0.05 were considered significant.

Drugs

5-HT creatinine sulphate, (−)-propranolol hydrochloride, spiperone, pargyline hydrochloride, prazosin hydrochloride and indomethacin were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.; 5-carboxamidotryptamine maleate (5-CT), α-methyl-5-HT maleate, 2-methyl-5-HT maleate, 8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT), 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo[1,2-a]quinoxaline maleate (CGS 12066B), (−)-pindolol, ketanserin tartrate, methiothepin mesylate, [1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate (MDL 72222), [1S-[1α,2α(Z), 3α,4α]]-7-[3-[[2-[(phenylamino)carbonyl]-hydrazino]-methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptanoic acid (SQ 29,548) and 9,11,dideoxy - 11α,9α - epoxy - methano - prostaglandin $F_{2\alpha}$ (U-46619) from Research Biochemicals Inc., Natic, MA, U.S.A.; cocaine hydrochloride from Takeda Chemical Industries, Osaka, Japan; neuropeptide Y, endothelin-1 and angiotensin II from Peptide Institute Inc., Osaka, Japan; 2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino) ethyl ester (SDZ 205-557) was kindly provided by Sandoz Pharma Ltd., Basel, Switzerland; cisapride by Yoshitomi Pharmaceutical Industries, Osaka, Japan.

Results

Body weight and systolic blood pressure in WKY and SHRSP

The body weight of the animals at 3 and 6 months of age was 226±5 g (n=6) and 353±5 g (n=46) for WKY, and 224±5 g (n=6) and 322±3 g (n=45) for SHRSP, respectively. Systolic blood pressure was measured in the conscious state by the tail cuff method; at 3 and 6 months of age it was 137±3 mmHg (n=6) and 146±1 (n=46) mmHg for the WKY, and 218±4 mmHg (n=6) and 262±4 mmHg (n=45) for the SHRSP, respectively. Body weight was significantly lower in SHRSP at 6 months of age than in age-matched WKY ($P<0.01$), and systolic blood pressure was significantly higher in SHRSP at 3 and 6 months of age than in respective age-matched WKY ($P<0.01$).

Contractile effects of 5-HT on basilar arteries from WKY and SHRSP

Figure 1 shows the contractile effects of 5-HT in basilar arteries from WKY and SHRSP at 3 and 6 months of age and Table 1 summarizes the pD_2 values and the maximum contractions (E_{max}). 5-HT produced concentration-dependent contractions with a similar E_{max} in basilar arteries from WKY and SHRSP at both ages studied. The 5-HT-induced contractile responses were not different between WKY and SHRSP at 3 months of age, whereas the contractions induced by 5-HT at lower concentrations and pD_2 values in SHRSP aged 6 months were significantly greater than those in age-matched WKY and 3-month-old SHRSP. The contractile response to 5-HT in WKY basilar arteries did not differ between 3 and 6 months of age.

Contractile effects of other vasoconstrictor substances on basilar arteries from WKY and SHRSP

To determine whether the enhanced response of basilar arteries to 5-HT in SHRSP was related to nonspecific increases in vascular reactivity, the contractile effects of a thromboxane A_2 receptor agonist U-46619 (0.1 nM−3 μM), neuropeptide Y (0.1 nM−300 nM), endothelin-1 (0.1 nM−300 nM), and angiotensin II (0.1 nM−1 μM) were examined in basilar arteries obtained from WKY and SHRSP at 6 months of age. However, there was no significant difference in contractile response to vasoconstrictor substances tested in the present study between WKY and SHRSP (Table 2).

Effects of several 5-HT receptor antagonists and other drugs on 5-HT-induced contraction in basilar arteries from WKY and SHRSP

The effects of several 5-HT receptor antagonists and other drugs were examined against contractions produced by 5-HT in basilar arteries from WKY and SHRSP at 6 months of age.

Spiperone (1 nM−1 μM), a 5-HT_2 receptor antagonist, produced a biphasic shift of the concentration-response curves for 5-HT in basilar arteries from WKY and SHRSP (Figure 2). The concentration-response curves at high concentrations of 5-HT were concentration-dependently antagonized by spiperone, resulting in parallel shifts of the curves to the right, while the responses to low concentrations of 5-HT were resistant to blockade by spiperone. Both components of the 5-HT curves were evidently distinguishable when spiperone was used at a concentration of 1 μM. The pD_2 and E_{max} values determined from the first component of 5-HT-induced contractions in the

presence of 1 μM spiperone were 7.42±0.07 and 73±19 mg (24±5% of maximal 5-HT effects in the absence of spiperone) in WKY arteries, and 7.81±0.05 and 187±15 mg (58±4%) in SHRSP arteries, respectively. The pD_2 and E_{max} values in SHRSP arteries were significantly greater than those in WKY arteries ($P<0.01$). Ketanserin (1–100 nM) also produced a biphasic shift of the concentration-response curves for 5-HT in WKY and SHRSP basilar arteries (Figure 3). From 5-HT_2 receptor antagonist-sensitive components of 5-HT-induced contractions in WKY basilar arteries, calculated pA_2 values for spiperone and ketanserin were 9.78±0.16 (slope, 0.94±0.06) and 9.29±0.08 (slope, 0.99±0.05), respectively, assuming that the antagonism was competitive. For SHRSP arteries, the pA_2 values were determined at the 80% level of maximum 5-HT effects; they were 9.78±0.15 (slope, 1.00±0.06) for spiperone and 9.36±0.17 (slope, 1.01±0.11) for ketanserin. There were no significant differences in pA_2 values of ketanserin and spiperone between WKY and SHRSP arteries.

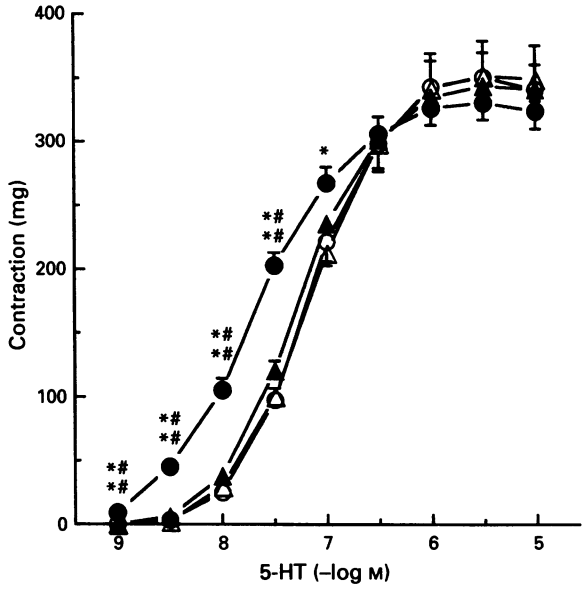

Figure 1 Contractile effects of 5-HT in basilar arteries from WKY (open symbols) and SHRSP (solid symbols) at 3 (△,▲) and 6 months of age (○,●). Each point is the mean with s.e.mean, $n=6$ (3 months of age) or 10 (6 months of age). $^*P<0.05$ and $^{**}P<0.01$, compared with respective age-matched WKY, and $^{\#\#}P<0.01$, compared with SHRSP at 3 months of age.

Furthermore, to determine whether 5-HT_1 receptors are involved in 5-HT-induced contractile responses of WKY and SHRSP arteries, the effects of methiothepin (an antagonist with affinity for 5-HT_1 and 5-HT_2 receptors) on 5-HT-induced contractions were examined in the absence or presence of 1 μM spiperone (Figure 4). Methiothepin (10 and 100 nM) produced both a rightward shift of the concentration-response curves and a decrease in the maximal contractile effect of 5-HT in WKY and SHRSP arteries (Figure 4a). The degree of inhibition of the maximum contractions produced by methiothepin was larger in WKY arteries than in SHRSP arteries. In the presence of spiperone, 5-HT produced monophasic contractions in WKY and SHRSP arteries at concentrations up to 30 μM, and methiothepin (100 nM) shifted the 5-HT concentration-response curves to the right in both groups (Figure 4b). Calculated pK_B values for methiothepin in WKY and SHRSP arteries were 8.08±0.12 and 8.11±0.07, respectively. No significant difference was found in the pK_B values between WKY and SHRSP. On the other hand, neither the 5-HT_3 receptor antagonist, MDL 72222 (1 μM) nor the 5-HT_4 receptor antagonist, SDZ 205-557 (1 μM) modified the concentration-effect curves for 5-HT in basilar arteries from WKY and SHRSP (Figure 5).

Reliable receptor analysis necessitates that agonist uptake and degradative processes are either negligible or inhibited. Therefore, the effects of cocaine (a monoamine uptake inhibitor) and pargyline (a monoamine oxidase inhibitor) on 5-HT-induced contractions were examined in WKY and SHRSP basilar arteries. Cocaine (10 μM) and pargyline (50 μM) did not affect the contractions of SHRSP and WKY arteries to 5-HT (each group, $n=5$). In addition, neither an α_1-adrenoceptor antagonist, prazosin (10 μM), a cyclo-oxygenase inhibitor, indomethacin (3 μM) nor a thromboxane A_2 receptor antagonist, SQ 29,548 (1 μM) altered the concentration-effect curves for 5-HT in either WKY or SHRSP basilar arteries (each group, $n=5$).

Contractile effects of various 5-HT receptor agonists in basilar arteries from WKY and SHRSP

Various 5-HT receptor agonists were examined for contractile activity in basilar arteries from WKY and SHRSP at 6 months of age.

The 5-HT_1 receptor agonist, 5-CT, produced biphasic con-

Table 1 The pD_2 values and maximal responses (E_{max}) for the concentration-response curves with 5-HT in basilar arteries from WKY and SHRSP at 3 and 6 months of age

	pD_2		E_{max} (mg)	
	WKY	*SHRSP*	*WKY*	*SHRSP*
3-months-old	7.19±0.04	7.30±0.04	352±27	344±11
6-months-old	7.20±0.04	7.73±0.04$^{**\#\#}$	351±19	331±13

The values are means±s.e.mean, $n=6$ (3-months-old) or 10 (6-months-old). $^{**}P<0.01$, compared with the respective age-matched WKY, and $^{\#\#}P<0.01$, compared with the 3-months-old SHRSP.

Table 2 The pD_2 values and maximal responses (E_{max}) for the concentration-response curves with U-46619, endothelin-1, neuropeptide Y and angiotensin II in basilar arteries from WKY and SHRSP at 6 months of age

Substances	pD_2		E_{max} (mg)	
	WKY	*SHRSP*	*WKY*	*SHRSP*
U-46619	7.76±0.07	7.70±0.05	259±16	274±20
Endothelin-1	8.14±0.06	8.12±0.06	395±22	341±21
Neuropeptide Y	8.02±0.03	8.00±0.08	93±12	105±20
Angiotensin II	8.65±0.06	8.69±0.04	98±7	73±14

The values are means±s.e.mean, $n=6$–8.

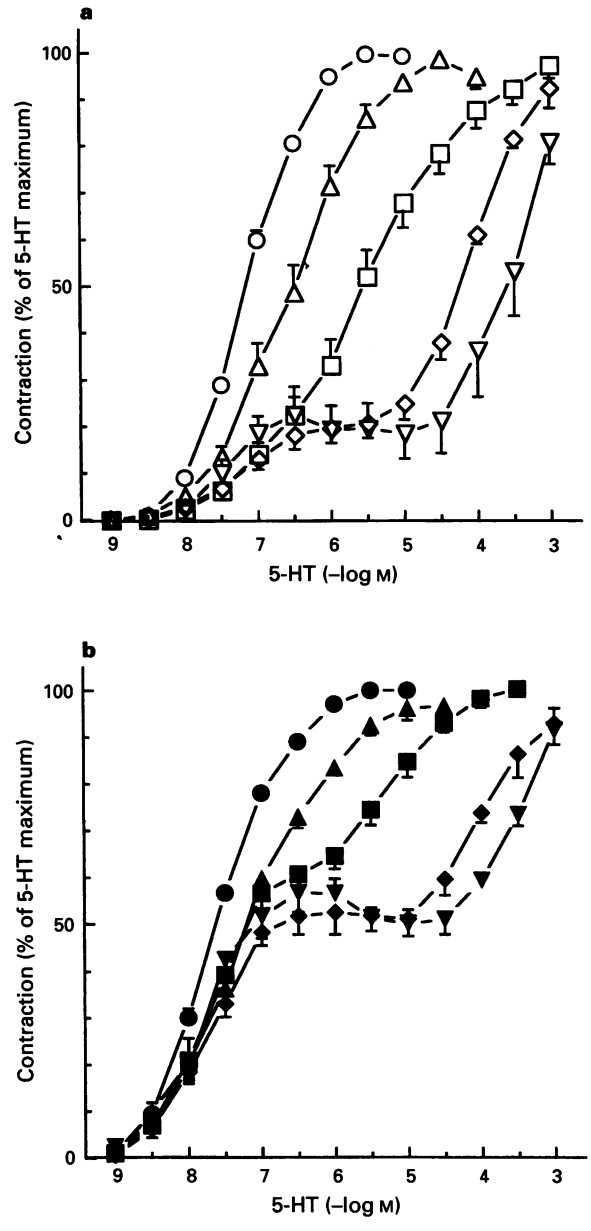

Figure 2 Effect of spiperone on the contractile responses to 5-HT in basilar arteries from WKY (open symbols) (a) and SHRSP (solid symbols) (b) at 6 months of age. The concentrations of spiperone used are represented by control (○,●; each group, $n=20$), 1 nM (△,▲; $n=5$), 10 nM (□,■; $n=5$), 100 nM (◇,◆; $n=5$), and 1 μM (▽,▼; $n=5$). The maximum contraction induced by 5-HT before treatment with spiperone was taken as 100%. Each value is the mean with the s.e.mean.

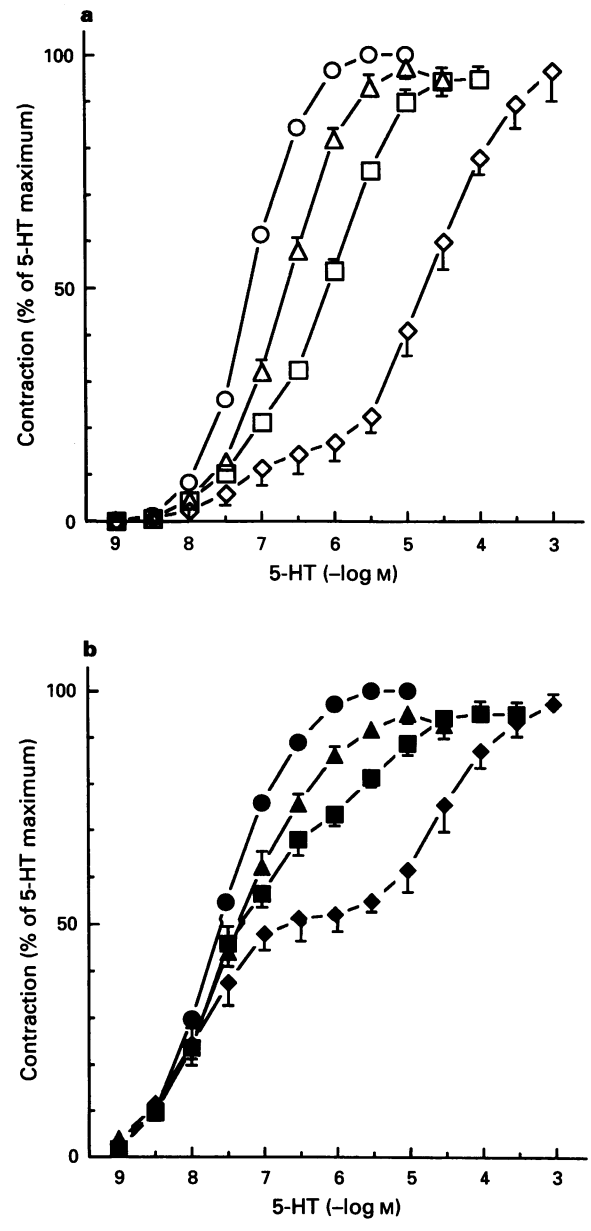

Figure 3 Effect of ketanserin on the contractile responses to 5-HT in basilar arteries from WKY (a) and SHRSP (b) at 6 months of age. The concentrations of ketanserin used are represented by control (○,●; each group, $n=15$), 1 nM (△,▲; $n=5$), 10 nM (□,■; $n=5$), and 100 nM (◇,◆; $n=5$). The maximum contraction induced by 5-HT before treatment with ketanserin was taken as 100%. Each value is the mean with the s.e.mean.

centration-response curves in basilar arteries from WKY and SHRSP (Figure 6a). The first phase of contraction was significantly greater in SHRSP arteries than in WKY arteries. Pretreatment with ketanserin (100 nM) for 30 min inhibited the second phase of contraction, but without modifying the first phase of contraction in both WKY and SHRSP arteries (Figure 6b), indicating that the second phase of 5-CT-induced contraction resulted from the stimulation of 5-HT_2 receptors. During blockade of 5-HT_2 receptors, pD_2 values of 5-CT were greater than those of 5-HT in WKY and SHRSP arteries, although the contractions were less than those seen with 5-HT in both groups (Table 3). In addition, contractile responses and pD_2 values of 5-CT were significantly larger in SHRSP arteries than in WKY arteries (Figure 6b and Table 3).

CGS 12066B, a 5-HT_1 receptor agonist, induced a smaller vasoconstrictor response than 5-HT, and contractile responses and pD_2 values of CGS 12066B were significantly increased in SHRSP when compared to WKY (Figure 7a and Table 3). In addition, propranolol and pindolol, which possess affinity for 5-HT_{1A} and 5-HT_{1B} receptors, caused concentration-dependent contraction in basilar arteries from WKY and SHRSP, but the contractions induced by these two agents were smaller than those by 5-HT. Contractile responses and pD_2 values of pindolol and propranolol were significantly greater in SHRSP than in WKY (Figure 7b and c, and Table 3).

8-OH-DPAT, a 5-HT_{1A} receptor agonist, and 2-methyl-5-HT, a 5-HT_3 receptor agonist, elicited contraction in basilar arteries from WKY and SHRSP, but relatively high concentrations of 8-OH-DPAT and 2-methyl-5-HT were required to elicit these effects. The contractile responses to these agonists were less than those to 5-HT in both groups (Figure 8a and c, and Table 3). On the other hand, α-methyl-5-HT, a 5-

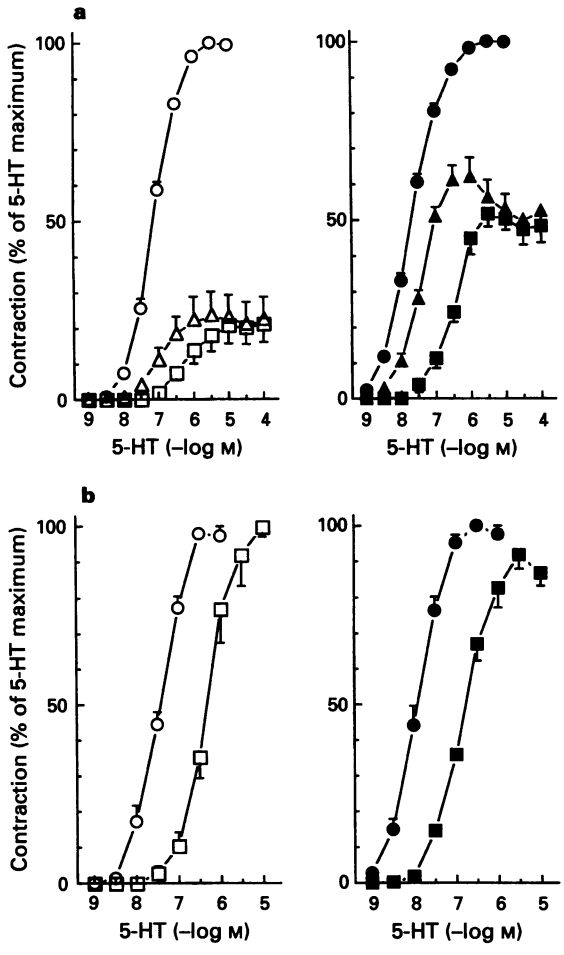

Figure 4 (a) Effects of methiothepin on the contractile responses to 5-HT in basilar arteries from WKY (left) and SHRSP (right) at 6 months of age. The concentrations of methiothepin used are represented by control (○, $n=11$; ●, $n=10$), 10 nM (△, $n=5$; ▲, $n=5$), and 100 nM (□, $n=6$; ■, $n=5$). The maximum contraction induced by 5-HT before treatment with methiothepin was taken as 100%. Each value is the mean with the s.e.mean. (b) Effects of methiothepin on spiperone-resistant component of contractile responses to 5-HT in basilar arteries from WKY (left) and SHRSP (right) at 6 months of age. (○) and (●) Control; (□) and (■) 100 nM methiothepin. Experiments were performed in the constant presence of spiperone (1 μM). The maximum contraction in spiperone-resistant component of contractions induced by 5-HT before treatment with methiothepin was taken as 100%. Each value is the mean with the s.e.mean; in each group, $n=5$.

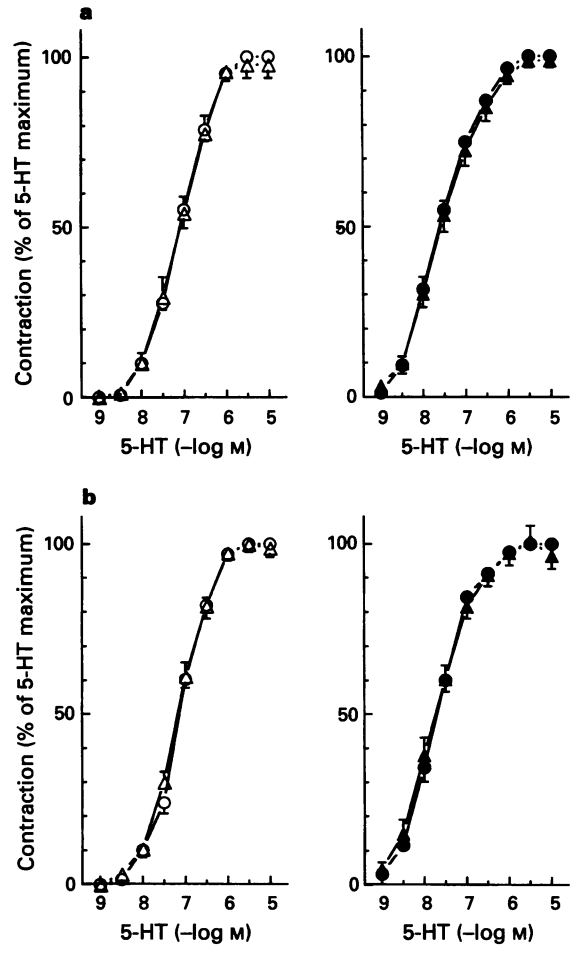

Figure 5 Effects of MDL 72222 (a) and SDZ 205-557 (b) on the contractile responses to 5-HT in basilar arteries from WKY (left) and SHRSP (right) at 6 months of age. The concentrations of the antagonists used are represented by control (○, ●; in each group, $n=5$) and 1 μM (△, ▲; $n=5$). The maximum contraction induced by 5-HT before treatment with the antagonist was taken as 100%. Each value is the mean with the s.e.mean.

HT_2 receptor agonist, produced potent contractions in WKY and SHRSP basilar arteries, but the pD_2 values of α-methyl-5-HT were less than those of 5-HT in both groups (Figure 8b and Table 3). Furthermore, no significant differences were found in the contractile response to 8-OH-DPAT, α-methyl-5-HT and 2-methyl-5-HT between WKY and SHRSP arteries (Figure 8 and Table 3). Cisapride, a $5\text{-}HT_4$ receptor agonist, in concentrations ranging from 10 nM – 10 μM, caused no significant contraction in WKY and SHRSP arteries (each group, $n=4$).

As described above, during blockade of $5\text{-}HT_2$ receptors, 5-HT at low concentrations still produced contractions in WKY and SHRSP arteries, and spiperone (1 μM) evidently distinguished the two components of contractile responses induced by 5-HT. Table 3 summarizes the first component of contractile responses produced by 5-HT in the presence of spiperone (1 μM) in WKY and SHRSP arteries (Figure 2 and 4b). Their pD_2 values and E_{max} values were similar to those of 5-CT.

Furthermore, attempts were made to correlate the pD_2 values of 5-HT receptor agonists which possess the high affinities at $5\text{-}HT_1$ binding sites taken from Table 3 (in the case of 5-HT, the pD_2 values of the first component of contractile response to 5-HT in the presence of 1 μM spiperone were used) in basilar arteries from WKY and SHRSP with the published affinities (pK_D values) of $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$ and $5\text{-}HT_{1D}$ binding sites (Schoeffter & Hoyer, 1989a; Hoyer & Schoeffter, 1991). A highly significant correlation was found between the pD_2 values of agonists in WKY and SHRSP and their binding affinities at the $5\text{-}HT_{1B}$ subtype (WKY, correlation coefficients (r) = 0.93, $P<0.01$; SHRSP, $r=0.96$, $P<0.01$), but not at $5\text{-}HT_{1A}$ (WKY, $r=0.19$; SHRSP, $r=0.08$) and $5\text{-}HT_{1D}$ (WKY, $r=0.42$; SHRSP, $r=0.36$) subtypes (Figure 9).

Discussion

The present findings demonstrate that the basilar arteries from SHRSP exhibit enhanced reactivities to 5-HT, when compared to WKY. This altered vascular response in SHRSP arteries to 5-HT is not related to nonspecific increases in vascular reactivity, since the basilar artery from SHRSP exhibited no alteration in the contractile response to other vasocontractile substances tested in this study. In addition, the enhanced vascular response to 5-HT observed in SHRSP basilar arteries may be a secondary change due to chronic hypertension, since the alteration in response to 5-HT in SHRSP arteries was found at 6 months of age, but not at 3 months when hy-

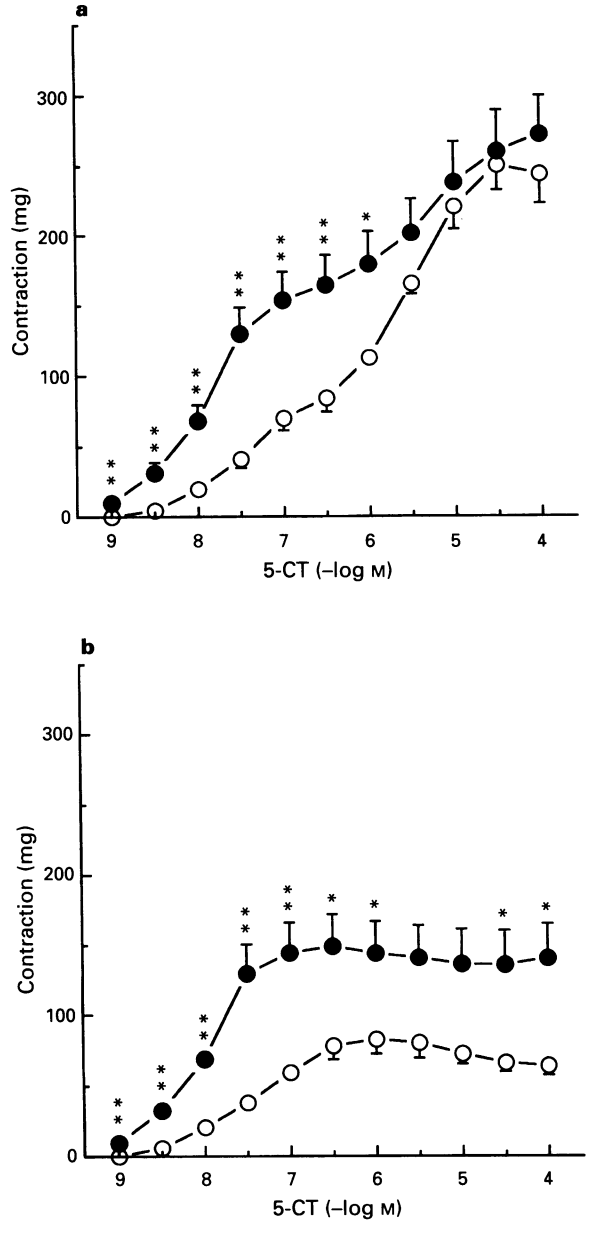

Figure 6 Contractile effects of 5-CT in the absence (a) or presence (b) of ketanserin in basilar arteries from WKY (○) and SHRSP (●) at 6 months of age. Ketanserin (100 nM) was added 30 min prior to the application of 5-CT. Each point is the mean with s.e.mean, $n=5$ or 6. $^{*}P<0.05$ and $^{**}P<0.01$, compared with WKY.

pertension is first established. Increased vascular reactivity to 5-HT has also been demonstrated in basilar arteries from DOCA-salt hypertensive rats which are experimental hypertensive animal models (Soltis & Bohr, 1987).

5-HT has been known to cause vasoconstriction through mainly activation of 5-HT_2 receptors in peripheral vascular beds (Saxena, 1989). However, in cerebral arteries or arterioles from humans (Parsons *et al.*, 1989; Hamel & Bouchard, 1991; Hamel *et al.*, 1993a; Jansen *et al.*, 1993), monkeys (Connor *et al.*, 1989), cows (Hamel *et al.*, 1993a, b), pigs (Miyamoto *et al.*, 1994), sheep (Gaw *et al.*, 1990), dogs (Peroutka *et al.*, 1986; Connor *et al.*, 1989; Frenken, 1989), cats (Hamel *et al.*, 1989), rabbits (Parsons & Whalley, 1989; Deckert *et al.*, 1994) and guinea-pigs (Chang *et al.*, 1988), stimulation of 5-HT_1 receptors either contributes to, or is primarily responsible for, the vasocontractile response to 5-HT. On the other hand, in rat basilar arteries, the vasocontractile response to 5-HT has been demonstrated to be primarily mediated by 5-HT_2 receptors (Chang & Owman, 1987; Chang *et al.*, 1988; Deckert & Angus, 1992). However, there is a report that both 5-HT_1 and 5-HT_2 receptors participate in the contractions elicited by 5-HT in rat basilar arteries (Descombes *et al.*, 1993). Therefore, an attempt to identify the 5-HT receptor(s) involved in the 5-HT-induced contractile response in the basilar arteries from WKY and SHRSP was performed by use of various 5-HT receptor agonists and antagonists.

In the present study, spiperone and ketanserin, which possess potent antagonistic activity of 5-HT_2 receptors, elicited the biphasic displacement of the concentration-response curves for 5-HT on basilar arteries from WKY and SHRSP, suggesting the involvement of at least two 5-HT receptor populations in 5-HT-induced contractions in these arteries. A similar pattern of 5-HT curves in the presence of 5-HT_2 receptor antagonist has been demonstrated in dog basilar (Frenken, 1989) and coronary arteries (Frenken & Kaumann, 1985), and human saphenous veins (Glusa & Müller-Schweinitzer, 1993). The apparent pA_2 values calculated from the spiperone- and ketanserin-sensitive component of 5-HT effect in rat basilar arteries amounted to 9.8 and 9.3 in WKY, and 9.8 and 9.4 in SHRSP, respectively. These values were similar to the affinity values from radioligand binding studies and pK_B or pA_2 values in second-messenger tests estimated at 5-HT_2 receptors in spiperone and ketanserin (Hoyer & Schoeffter, 1991; Hoyer *et al.*, 1994), indicating that the first part of the 5-HT-induced contraction in rat basilar arteries is mediated through 5-HT_2 receptors. Furthermore, methiothepin, a 5-HT_1 and 5-HT_2 receptor antagonist, potently antagonized 5-HT-induced contractions that were resistant to blockade by the 5-HT_2 receptor antagonist (spiperone) in WKY (pK_B value, 8.1) and SHRSP (8.1). In addition, during blockade of 5-HT_2 receptors, the contractile effects of 5-HT resembled those of 5-CT in WKY and SHRSP arteries. These findings suggest that the 5-HT_1-like

Table 3 The pD_2 values and maximal responses (E_{max}) for the concentration-response curves with several 5-HT receptor agonists in basilar arteries from WKY and SHRSP at 6 months of age

Agonists	*pD_2*		*E_{max} (mg)*	
	WKY	*SHRSP*	*WKY*	*SHRSP*
5-HT	7.19 ± 0.03	7.71 ± 0.04$^{\#\#}$	346 ± 20	321 ± 18
5-CT[a]	7.48 ± 0.06**	8.06 ± 0.09**$^{\#\#}$	84 ± 10**	150 ± 22**$^{\#}$
CGS 12066B	6.09 ± 0.05**	6.55 ± 0.07**$^{\#\#}$	58 ± 8**	129 ± 15**$^{\#\#}$
Pindolol	7.15 ± 0.08	7.68 ± 0.10$^{\#\#}$	39 ± 6**	82 ± 6**$^{\#\#}$
Propranolol	6.72 ± 0.05**	7.44 ± 0.15*$^{\#\#}$	39 ± 4**	94 ± 15**$^{\#\#}$
8-OH-DPAT	5.22 ± 0.04**	5.35 ± 0.07**	122 ± 24**	150 ± 21**
α-Methyl-5-HT	6.90 ± 0.03**	6.93 ± 0.04**	332 ± 19	311 ± 20
2-Methyl-5-HT	4.88 ± 0.04**	4.92 ± 0.10**	67 ± 13**	64 ± 15**
5-HT[b]	7.45 ± 0.04**	7.86 ± 0.05$^{\#\#}$	91 ± 9**	202 ± 14**$^{\#\#}$

All values are means ± s.e.mean, $n=5-13$. The pD_2 and E_{max} values used for 5-HT represent pooled data obtained with all agonist experiments. [a] Values for 5-CT were determined in the presence of ketanserin (100 nM). [b] Values for 5-HT were determined in the presence of spiperone (1 μM). $^{*}P<0.05$ and $^{**}P<0.01$, compared with 5-HT alone in respective group. $^{\#}P<0.05$ and $^{\#\#}P<0.01$, compared with the WKY.

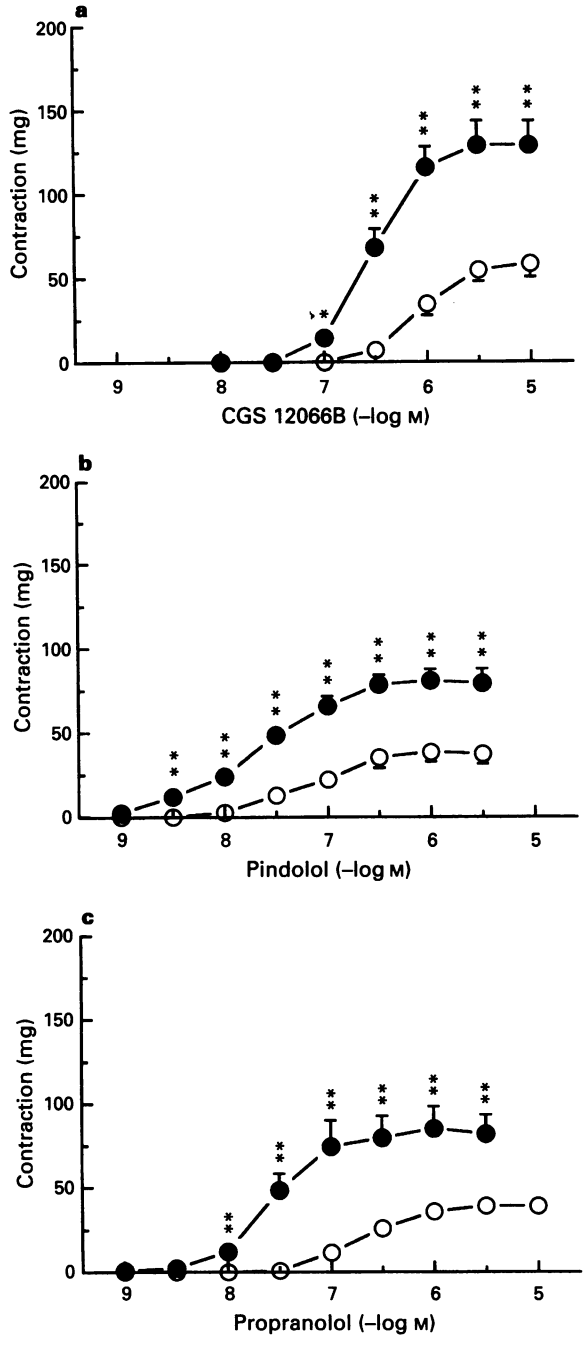

Figure 7 Contractile effects of CGS 12066B (a), pindolol (b) and propranolol (c) in basilar arteries from WKY (○) and SHRSP (●) at 6 months of age. Each point is the mean with s.e.mean, $n=6-8$. $^{*}P<0.05$ and $^{**}P<0.01$, compared with WKY.

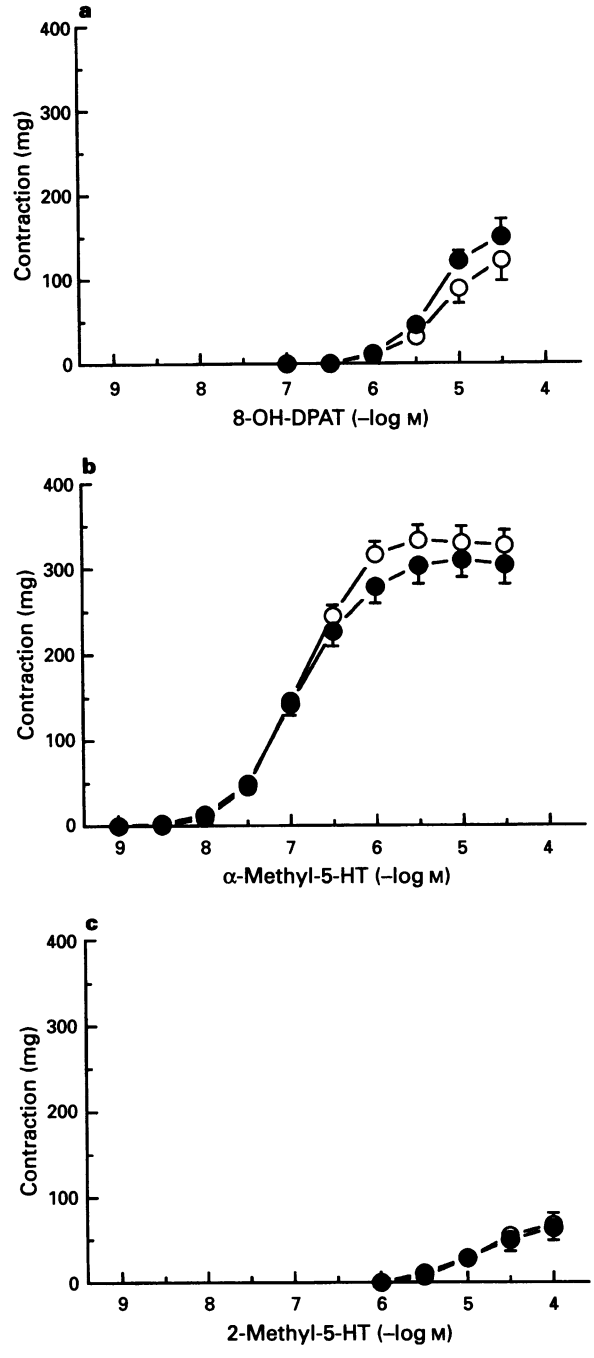

Figure 8 Contractile effects of 8-OH-DPAT (a), α-methyl-5-HT (b) and 2-methyl-5-HT (c) in basilar arteries from WKY (○) and SHRSP (●) at 6 months of age. Each point is the mean with s.e.mean, $n=5-9$.

receptors are also involved in 5-HT-induced contractions in WKY and SHRSP arteries, according to the criteria of classification established by Bradley *et al.* (1986). Thus, in rat basilar arteries, 5-HT may cause contractions by stimulation of a mixed receptor population of 5-HT_1-like and 5-HT_2 receptors.

On the other hand, from the observation that the weak agonist activity of 2-methyl-5-HT, a 5-HT_3 receptor agonist, and the lack of antagonist activity of MDL 72222, a 5-HT_3 receptor antagonist (Fozard, 1984), it would appear that in basilar arteries from WKY and SHRSP, the contractile responses induced by 5-HT may not be mediated by 5-HT_3 receptors. Similarly, the involvement of the 5-HT_4 receptor on 5-HT-induced contraction in WKY and SHRSP arteries can also be excluded, since in both arteries, cisapride, which possesses a 5-HT_4 receptor agonistic activity (Bockaert *et al.*, 1992), was devoid of agonist activity, and SDZ 205-557, a compound previously shown to be a potent selective 5-HT_4 receptor antagonist (Buchheit *et al.*, 1991), had no effect on the contractile response to 5-HT.

The present findings with agonists suggest that the 5-HT_{1B} receptor subtype is a candidate for the 5-HT_1-like receptor mediating contraction of the rat basilar artery. This contention is supported by several observations. 8-OH-DPAT is known to be a specific 5-HT_{1A} receptor agonist in the nanomolar range (Hoyer & Schoeffter, 1991; Hoyer *et al.*, 1994), whereas in basilar arteries from WKY and SHRSP, the high concentra-

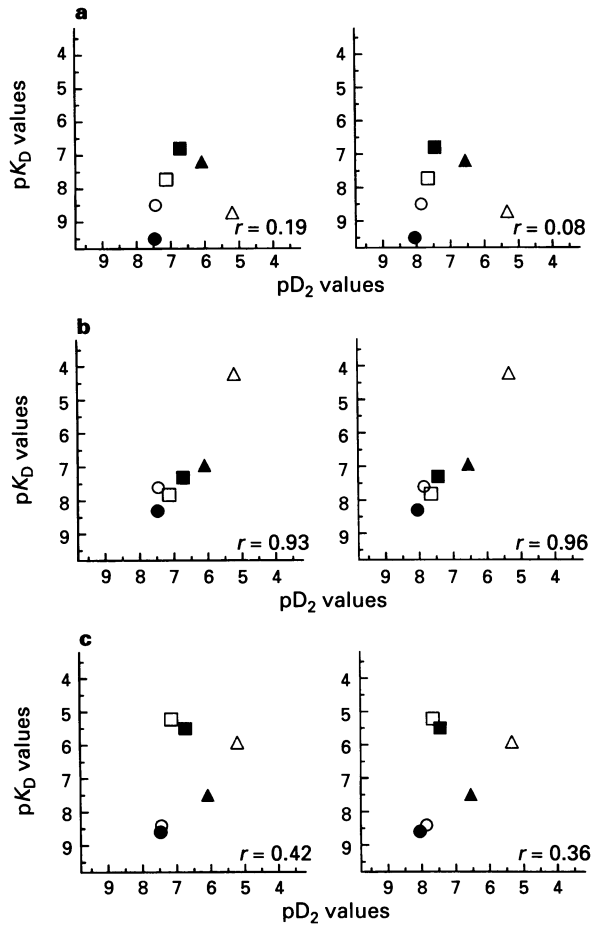

Figure 9 Correlation between the pD_2 values for the contractile effect of 5-HT receptor agonists in basilar arteries from WKY (left) and SHRSP (right) and their affinities for 5-HT_{1A} (a), 5-HT_{1B} (b) and 5-HT_{1D} (c) binding sites. The following agonists were used; 5-HT in the presence of 1 μM spiperone (○), 5-CT in the presence of 100 nM ketanserin (●), 8-OH-DPAT (△), CGS 12066B (▲), pindolol (□), propranolol (■). The pK_D values reported by Schoeffter & Hoyer, 1989a and Hoyer & Schoeffter, 1991 were used. Correlation coefficients in WKY and SHRSP are 0.19 and 0.08 for the 5-HT_{1A} subtype, 0.93 ($P<0.01$) and 0.96 ($P<0.01$) for the 5-HT_{1B} subtype, and 0.42 and 0.36 for the 5-HT_{1D} subtype.

tion needed to induce contractions and the pD_2 value was 5.2 for WKY and 5.4 for SHRSP. Thus, it is unlikely that the 5-HT_{1A} receptor is responsible for 5-HT-induced contractions in rat basilar arteries.

β-Adrenoceptor blocking agents such as pindolol and propranolol are widely used as 5-HT_{1A} and 5-HT_{1B} receptor antagonists in *in vitro* vessel studies. However, pindolol and propranolol have also been demonstrated to cause vasoconstrictions through activation of 5-HT_1-like receptors (Nakane *et al.*, 1993). In addition, pindolol and propranolol have been shown to behave as partial or full agonists at the 5-HT_{1B} receptors (Unsworth & Molinoff, 1992; Adham *et al.*, 1993), and to behave as partial agonists at 5-HT_{1D} receptors (Schoeffter & Hoyer, 1989b). In WKY and SHRSP basilar arteries, pindolol and propranolol elicited concentration-dependent contractions with pD_2 values of 7.2 and 6.7 for WKY and 7.7 and 7.4 for SHRSP, respectively, although the maximal responses produced by propranolol and pindolol were smaller than those produced by 5-HT. These agents have similar affinity values for 5-HT_{1B} receptors (pindolol, 7.8 and propranolol, 7.3) but are dissimilar to those for 5-HT_{1D} receptors (pindolol, 5.2 and propranolol, 5.5) as determined from radioligand binding studies (Hoyer & Schoeffter, 1991). Thus, pindolol and propranolol may contract the rat basilar artery through activation of 5-HT_{1B} receptors. In addition, analyses of the potencies of the 5-HT_1 receptor agonists, pindolol and propranolol in rat basilar arteries, and their reported affinity values from binding studies, reveal a highly significant correlation with 5-HT_{1B} receptors but not with 5-HT_{1A} and 5-HT_{1D} receptors. Recently, the 5-HT_{1B} receptor mediating vasoconstriction has been demonstrated in the rat caudal artery (Craig & Martin, 1993).

In SHRSP basilar arteries, contractile responses and pD_2 values of 5-HT and 5-CT during blockade of 5-HT_2 receptors were increased when compared to WKY basilar arteries. In addition, contractile responses and pD_2 values of CGS 12066B which has some 5-HT_{1B} receptor selectivity (Neale *et al.*, 1987; Hoyer *et al.*, 1994), of pindolol and of propranolol were greater in SHRSP arteries than in WKY arteries. These findings suggest that in SHRSP basilar arteries, contractions mediated by 5-HT_1-like receptors (similar to 5-HT_{1B} receptors) are enhanced, when compared to WKY basilar arteries. On the other hand, contractions in response to α-methyl-5-HT, a 5-HT_2 receptor agonist, were similar in basilar arteries from WKY and SHRSP, suggesting that the 5-HT_2 receptor mediating contractions in basilar arteries did not differ between WKY and SHRSP.

In the present study, the detailed mechanisms underlying the enhanced reactivities to 5-HT found in basilar arteries from SHRSP are not clear. Recently, thromboxane A_2 released from endothelial cells by the activation of endothelial 5-HT_1 receptors has been reported to participate in the contractions caused by 5-HT in the rat basilar artery (Descombes *et al.*, 1993). However, 5-HT-induced contraction in WKY and SHRSP basilar arteries was not affected by pretreatment with a cyclo-oxygenase inhibitor, indomethacin, and a thromboxane A_2 receptor antagonist, SQ 29,548 (Ogletree *et al.*, 1985). These findings suggest that a vasoconstrictor prostanoid is not responsible for the contractile response to 5-HT in basilar arteries from WKY and SHRSP.

In conclusion, the present study provides evidence that a mixed receptor population of 5-HT_1-like (similar to the 5-HT_{1B} receptor subtype) and 5-HT_2 receptors are responsible for 5-HT-induced contractions in rat basilar arteries, and that in SHRSP basilar arteries, the contractile response mediated by 5-HT_1-like receptors is greater than that in WKY basilar arteries. Recently, in cerebral arteries or arterioles from human subjects (Hamel & Bouchard, 1991; Hamel *et al.*, 1993a), cows (Hamel *et al.*, 1993a, b), and rabbits (Deckert *et al.*, 1994), postsynaptic 5-HT_{1D} or 5-$HT_{1D\beta}$ receptors have been shown to be involved in 5-HT-induced contractions. Thus, there may be regional and species differences in the heterogeneity of 5-HT receptors in the cerebrovasculature.

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