# Characterization of 5-Hydroxytryptamine Receptors on the Isolated Pig Basilar Artery by Functional and Radioligand Binding Studies

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ABSTRACT—5-Hydroxytryptamine (5-HT)-receptor subtypes on pig basilar arteries were investigated by measuring the contractile responses to 5-HT agonists, the effects of antagonists on the responses and by carrying out a radioligand binding assay with [$^3$H]5-HT. The rank order of contractile agonist potency (according to the $pEC_{50}$ values) was 5-carboxamidotryptamine $\geqq$ 5-HT > $\alpha$-methyl-5-HT > (±)-8-hydroxy-dipropylaminotetralin. The contractile responses were not affected by endothelial denudation, and the 5-HT-induced contractions were antagonized competitively by ketanserin. Methiothepin shifted the 5-HT concentration-response curves to the right and downwards in a concentration-dependent manner. In the presence of ketanserin ($10^{-6}$ M), however, methiothepin antagonized the 5-HT-induced contractions competitively. Specific [$^3$H]5-HT binding to 5-HT receptors was saturable, reversible and showed high ($K_d$, 2.5 nM) and low ($K_d$, 710 nM) affinities, with respective $B_{max}$ values of 29.5 and 1950 fmol/mg protein. These results indicate that both 5-$HT_1$ and 5-$HT_2$ receptors are present on pig basilar arterial smooth muscle cells, and their stimulation results in contraction.

*Keywords*: Basilar artery (pig), Contraction, 5-$HT_1$ receptor, 5-$HT_2$ receptor, [$^3$H]5-Hydroxytryptamine

It is well known that serotonin (5-hydroxytryptamine; 5-HT) is a highly potent constrictor of basilar arteries. However, the nature of the 5-HT-receptor subtypes in various species has been reported to differ. The 5-HT-induced contractions of the basilar artery are mediated via stimulation of 5-$HT_1$ receptors in guinea pigs (1, 2), rabbits (3) and humans (4); 5-$HT_2$ receptors in rats (1, 2, 5) and monkeys (*Cercopithecus aethiops*) (6); and both in dogs (7, 8), sheep (9) and monkeys (*Macaca fascicularis*) (8). Shimokawa et al. (10) reported that contraction of the pig basilar artery is mediated via stimulation of 5-$HT_1$ receptors. Recently, we found that 5-HT-induced contractions of pig basilar arteries were inhibited by the 5-$HT_2$-receptor antagonist, ketanserin.

Therefore, the aim of this study was to clarify the distribution of 5-HT-receptor subtypes and their functional roles in the pig basilar artery by measuring the effects of 5-HT-receptor agonists and antagonists on this tissue in vitro and specific binding of [$^3$H]5-HT to membrane fractions.

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## MATERIALS AND METHODS

Basilar arteries from freshly slaughtered pigs were obtained at a local slaughterhouse and transferred to our laboratory immersed in ice-cold physiological salt solution (119 mM NaCl, 4.7 mM KCl, 1.6 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$, 1.2 mM $KH_2PO_4$ and 10 mM glucose) aerated with a mixture of 95% $O_2$ and 5% $CO_2$. Each basilar artery was dissected free and cleaned of adhering tissues, and two rings (outer diameter: 0.5-0.9 mm), about 4 mm in length, were cut from it. One ring was mounted vertically between two L-shaped stainless steel holders, fixing the upper region to an isometric force transducer (TB-611T; Nihon Kohden Kogyo, Tokyo) and suspended in a 5-ml water-jacketed organ bath containing oxygenated salt solution at 37℃ (pH 7.4). The other ring was subjected to endothelial denudation by gentle rubbing of the intimal surface with a stainless steel rod having a diameter equivalent to the lumen of the artery, and then it was mounted as above. The presence or absence of endothelial cells was determined by testing the relaxant response to bradykinin ($10^{-8}$-$10^{-6}$ M), which is abolished by endothelial denudation (10), and morphologically by scanning and transmission electron microscopy

after the experiments. Rings mounted in the organ bath were left to equilibrate for at least 120 min under a resting tension of 7.5 mN, which was optimal for inducing the maximal contraction. KCl (60 mM) solution was applied every 30 min until the amplitude of the contraction reached a constant value. Changes in KCl concentration in the physiological salt solution were compensated for by an equimolar adjustment of the NaCl concentration. The isometric tension development was displayed on an ink-writing recorder (WI-641G, Nihon Kohden Kogyo).

The cumulative concentration-response curve for each 5-HT-receptor agonist was obtained by adding solutions of each agonist directly to the bathing media. In tests with antagonists, the maximum response obtained with 5-HT alone was set as 100%, and subsequent concentration-response curves in the presence of increasing concentrations of antagonists were expressed as a percentage of the maximum in the control curve. After two reproducible control curves had been obtained, pretreatment with an antagonist was performed for 30 min before responses to 5-HT were examined. The log concentration-ratio of $EC_{50}$ values (i.e., the concentration producing a half-maximum response) in the absence or presence of antagonist was calculated and plotted against the logarithm of antagonist concentration to obtain the $pA_2$ value (11).

Isolated pig basilar arteries for the radioligand binding assay were cut longitudinally, and the endothelial cells were removed by gentle rubbing with a cotton rod, followed by rinsing with physiological salt solution. The rinsed basilar arteries were minced with scissors and then homogenized in 8 volumes of 50 mM Tris-HCl buffer (pH 7.4) using a Polytron homogenizer at a setting of 8 for 8 periods of 15 sec with 45-sec intervals in an ice-bath. The membrane fraction of basilar arteries was prepared as described previously (12, 13). Briefly, the homogenate was centrifuged at $500 \times g$ for 15 min. Then the supernatant was centrifuged at $100{,}000 \times g$ for 30 min. The pellet was resuspended in Tris-HCl buffer solution containing 10 $\mu$M pargyline, 4 mM calcium chloride and 0.1% ascorbate (14), and the suspension was used for the binding assay as a crude membrane fraction. These procedures were all performed at a temperature 4℃. The protein concentration of the final suspension was measured by the method of Lowry et al. (15) with bovine serum albumin as a standard.

Aliquots (approximately 300 $\mu$g of protein) of the membrane fraction were incubated with various concentrations of [$^3$H]5-HT in the presence or absence of 300 $\mu$M unlabeled 5-HT. After a 60-min incubation at 25℃, membrane-bound ligand was separated from unbound ligand by rapid filtration through a glass fiber filter (GF/C; Whatman, Maidstone, UK), which had been presoaked in 0.3% polyethylenimine solution to eliminate nonspecific binding to the filter (16). The filters were immediately washed 3 times with 5 ml of ice-cold buffer. Tissue-bound radioactivity was extracted from the filters in scintillation fluid (12), and radioactivity was counted by a liquid scintillation counter (LSC-3050; Aloka Co., Tokyo). Specific binding of [$^3$H]5-HT was defined as the difference between the binding in the absence and presence of 300 $\mu$M unlabeled 5-HT. In the competition experiment, aliquots of the membrane fraction were incubated in the presence of various concentrations of 5-HT-receptor antagonist together with 10 nM [$^3$H]5-HT. The value of the dissociation constant ($K_d$ or $K_i$), the maximum binding capacity ($B_{max}$) and Hill coefficient were calculated by the EBDA or LIGAND computer program (17), which analyzed Scatchard and Hill plots obtained from saturation and competition experiments.

Drugs used were as follows: 5-hydroxytryptamine (5-HT) (Merck, Darmstadt, FRG); 5-carboxamidotryptamine (5-CT), $\alpha$-methyl-5-hydroxytryptamine ($\alpha$-Me-5-HT), (±)-8-hydroxy-dipropylaminotetralin (8-OH-DPAT), 1$\alpha H$,3$\alpha$,5$\alpha H$-tropan-3-yl-3,5-dichlorobenzoate (MDL 72222) (Research Biochemicals, Inc., Natick, MA, USA); methiothepin maleate (Nippon Roche, Tokyo); ketanserin tartrate (Kyowa Hakko Kogyo, Tokyo); cyanopindolol (Sandoz Pharma Ag, Basel, Switzerland); prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Ono, Osaka); bradykinin acetate (Sigma Chemical, St. Louis, MO, USA); and 5-hydroxy [G-$^3$H]tryptamine creatinine sulfate ([$^3$H]5-HT) (Amersham, Buckinghamshire, UK; Specific activity: 396 GBq/mmol).

The results shown in the text, table and figures are expressed as mean values±S.E.M. Statistical analyses were performed by Student's paired *t*-test or Tukey's test after one-way analysis of variance. Significance was established when the probability level was equal to or less than 5%.

## RESULTS

### *Responsiveness to 5-HT-receptor agonists*

5-HT, 5-CT (a 5-$HT_1$-receptor agonist), 8-OH-DPAT (a 5-$HT_{1A}$-receptor agonist) and $\alpha$-Me-5-HT (a 5-$HT_2$-receptor agonist) evoked concentration-dependent contractions of pig basilar arteries with endothelium. The $pEC_{50}$ value (negative logarithm of the $EC_{50}$) for and maximum response ($E_{max}$) to each agonist are shown in Table 1. The rank order of agonist potency ($pEC_{50}$) was 5-CT $\geqq$ 5-HT > $\alpha$-Me-5-HT > 8-OH-DPAT. The $pEC_{50}$ of $\alpha$-Me-5-HT was significantly different from those of 5-HT and 8-OH-DPAT, but the $pEC_{50}$ of 5-CT was not significantly different from that of 5-HT. Neither the $pEC_{50}$ nor $E_{max}$ values were affected significantly by endothelial denudation. No relaxation was observed after application of these 5-HT-receptor agonists to pig basilar arteries

**Table 1.** Contractions of isolated pig basilar arteries with or without endothelium induced by some 5-HT-receptor agonists

| Agonist | Concentrations | With (+) or without (−) endothelium | $pEC_{50}$ | $E_{max}$ [1] |
|---|---|---|---|---|
| 5-HT | $10^{-10}-10^{-5}$ M | + | 7.70±0.10 | 100% |
| 〃 | 〃 | − | 7.88±0.12 | 98±3% |
| 5-CT | $10^{-10}-10^{-5}$ M | + | 8.00±0.33 | 32±4% |
| 〃 | 〃 | − | 8.06±0.23 | 40±9% |
| α-Me-5-HT | $10^{-10}-10^{-5}$ M | + | 6.57±0.15 | 58±7% |
| 〃 | 〃 | − | 6.66±0.11 | 55±9% |
| 8-OH-DPAT | $10^{-10}-10^{-4}$ M | + | 5.12±0.15 | 61±8% |
| 〃 | 〃 | − | 5.23±0.13 | 57±5% |

[1] The maximum contraction ($E_{max}$) induced by 5-HT ($10^{-5}$ M) in the endothelium-intact arteries was taken as 100%; the mean absolute value was 6.60±0.97 mN, which was not significantly different from the value (6.45±0.89 mN) obtained from endothelium-denuded arteries. The $pEC_{50}$ and $E_{max}$ values are means±S.E.M. of arteries from seven different animals.

precontracted with $PGF_{2\alpha}$ ($10^{-7}$ M) (data not shown). Therefore, the following experiments were carried out using endothelium-denuded arteries.

*5-HT-receptor antagonists*

Figure 1 shows the effect of ketanserin, a 5-$HT_2$-receptor antagonist, on the 5-HT-induced contractions of endothelium-denuded pig basilar arteries. Ketanserin ($10^{-8}-10^{-6}$ M) shifted the 5-HT concentration-response curves to the right in a concentration-dependent manner. The slope of the Schild plot was 0.81±0.14, which did not differ significantly from unity, and the calculated ketanserin $pA_2$ value was 9.58±0.13. Figure 2 shows the effect of methiothepin, a 5-$HT_1$- and 5-$HT_2$-receptor antagonist, on the 5-HT-induced contractions. Methiothepin shifted the 5-HT concentration-response curves to the right and downwards.

To determine whether 5-$HT_1$ receptors participated in the 5-HT-evoked contractions, the effect of ketanserin on such contractions in the presence of methiothepin ($10^{-8}$ M) was investigated. Ketanserin had no significant effect on the contractions under these conditions (Fig. 3). Figure 4 shows the effect of methiothepin on the 5-HT-induced contractions in the presence of ketanserin ($10^{-6}$

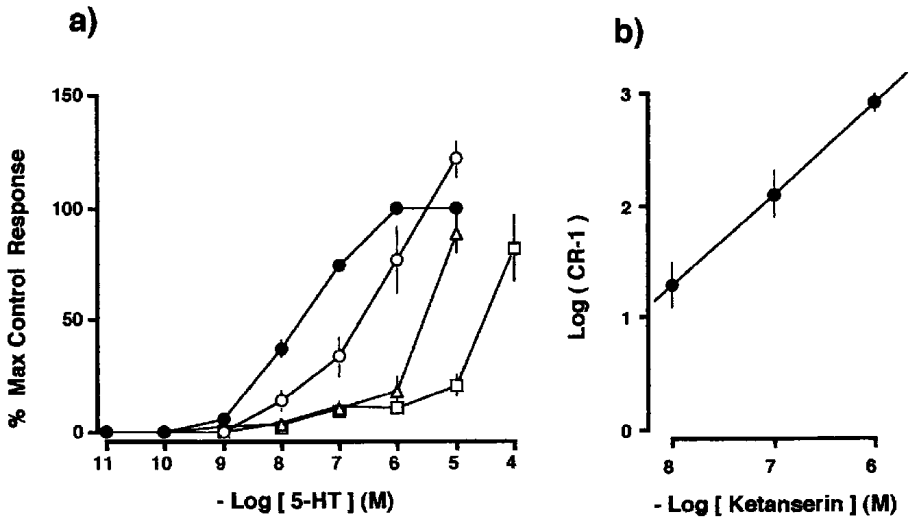


**Fig. 1.** Effect of ketanserin (○: $10^{-8}$ M, △: $10^{-7}$ M, □: $10^{-6}$ M) on the 5-HT-induced contractions (●) (a) of and the Schild plot (b) for endothelium-denuded pig basilar arteries. The maximum contraction induced by 5-HT in the absence of ketanserin was taken as 100%; the mean absolute value was 7.66±0.90 mN. Each point represents the mean±S.E.M. of arteries from seven different animals. Where error bars are not apparent, they are contained within the symbol. CR: An equieffective concentration-ratio of 5-HT, i.e., the ratio of the concentration of agonist producing 50% maximal response ($EC_{50}$) in the presence of ketanserin to $EC_{50}$ in the absence of antagonist.

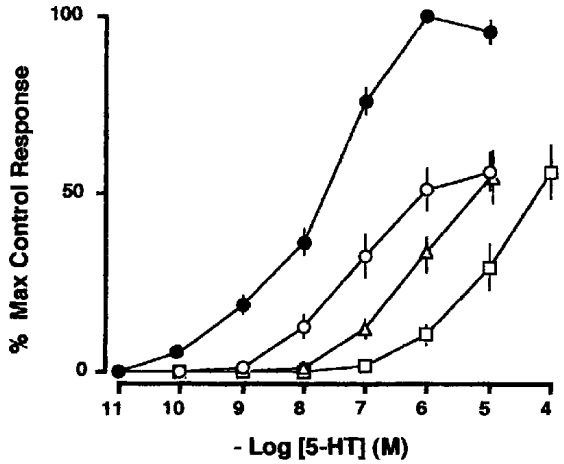


**Fig. 2.** Effect of methiothepin (○: $10^{-8}$ M, △: $10^{-7}$ M, □: $10^{-6}$ M) on the 5-HT-induced contractions (●) of endothelium-denuded pig basilar arteries. The maximum contraction induced by 5-HT in the absence of methiothepin was taken as 100%; the mean absolute value was 7.29±0.90 mN. Each point represents the mean±S.E.M. of arteries from seven different animals. Where error bars are not apparent, they are contained within the symbol.

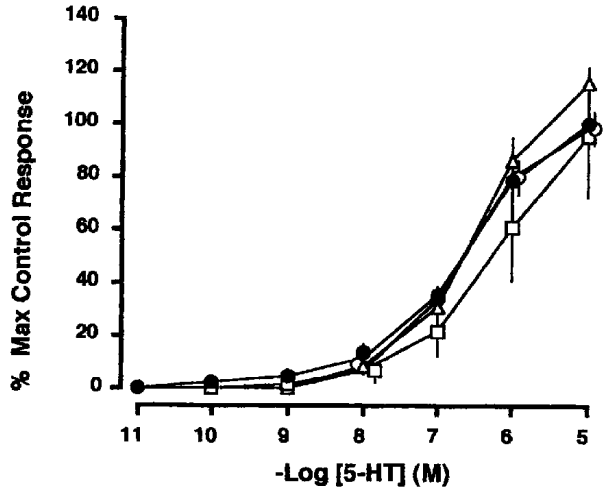


**Fig. 3.** Effect of ketanserin (○: $10^{-8}$ M, △: $10^{-7}$ M, □: $10^{-6}$ M) in the presence of methiothepin ($10^{-8}$ M) on the 5-HT-induced contractions (●) of endothelium-denuded pig basilar arteries. The maximum contraction induced by 5-HT in the absence of ketanserin was taken as 100%; the mean absolute value was 2.89±0.26 mN. Each point represents the mean±S.E.M. of arteries from six different animals. Where error bars are not apparent, they are contained within the symbol.

M). Under these conditions, methiothepin shifted the 5-HT concentration-response curves to the right in a concentration-dependent manner. The slope of the Schild plot was 0.91±0.25, which did not differ significantly from unity, and the calculated methiothepin $pA_2$ value was 8.92±0.23.

Figure 5 shows the effect of MDL 72222, a 5-$HT_3$-receptor antagonist, on 5-HT-induced contractions; it had no significant effect.

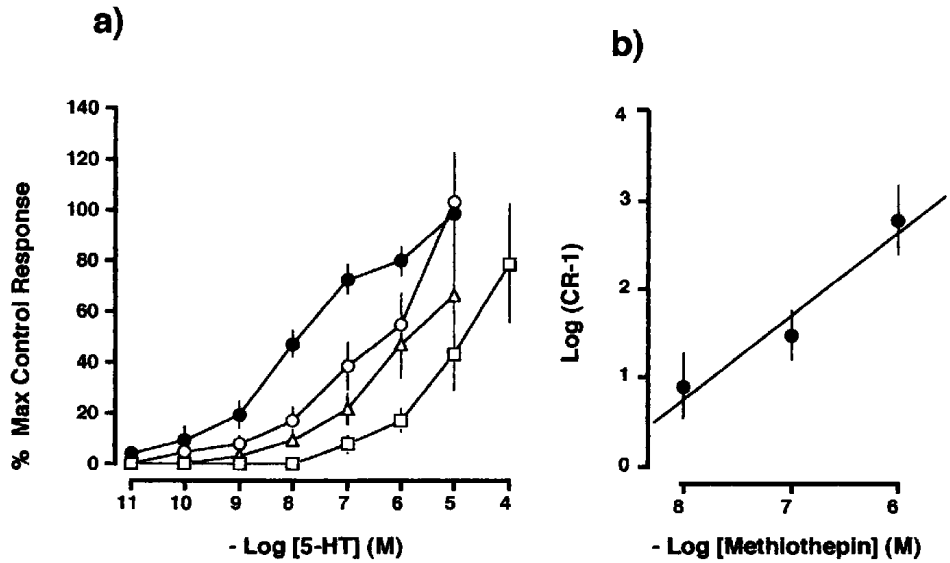


**Fig. 4.** Effect of methiothepin (○: $10^{-8}$ M, △: $10^{-7}$ M, □: $10^{-6}$ M) in the presence of ketanserin ($10^{-6}$ M) on the 5-HT-induced contractions (●) (a) of and the Schild plot (b) for endothelium-denuded pig basilar arteries. The maximum contraction induced by 5-HT in the absence of methiothepin was taken as 100%; the mean absolute value was 3.56±0.75 mN. Each point represents the mean±S.E.M. of arteries from six different animals. Where error bars are not apparent, they are contained within the symbol. CR: An equieffective concentration-ratio of 5-HT, i.e., the ratio of the concentration of agonist producing 50% maximal response ($EC_{50}$) in the presence of ketanserin to $EC_{50}$ in the absence of methiothepin.

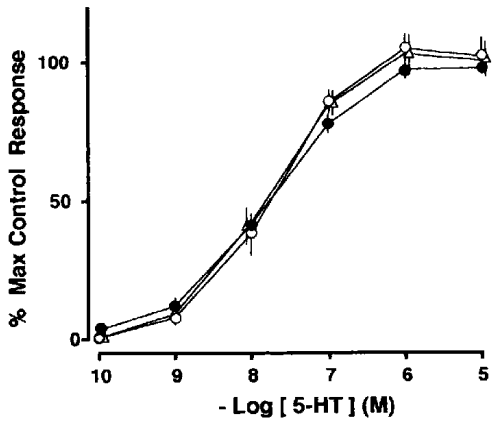


**Fig. 5.** Effect of MDL 72222 (○: $10^{-7}$ M, △: $10^{-6}$ M) on the 5-HT-induced contractions (●) of endothelium-denuded pig basilar arteries. The maximum contraction induced by 5-HT in the absence of MDL 72222 was taken as 100%; the mean absolute value was 7.18±0.91 mN. Each point represents the mean±S.E.M. of arteries from six different animals. Where error bars are not apparent, they are contained within the symbol.

*Binding of [$^3$H]5-HT to the membrane fraction from pig basilar arteries*

Figure 6 shows the specific binding of [$^3$H]5-HT to membrane fractions from pig basilar arteries and the Scatchard plot. The specific binding was saturable at high and low concentrations of [$^3$H]5-HT. The Scatchard plot also indicated the presence of high and low affinity binding sites. From the results of three experiments, the respective $K_d$ and $B_{max}$ values calculated for the high affinity site were 2.5±0.9 nM and 29.5±3.4 fmol/mg protein, whereas those for the low affinity site were 710±99 nM and 1950±420 fmol/mg protein.

Figure 7 shows the Scatchard plots for the specific binding of [$^3$H]5-HT in the presence and absence of ketanserin (1 $\mu$M), which inhibited the binding to the low affinity, but not the high affinity, site significantly. The $K_d$ and $B_{max}$ values for the high affinity site were 1.6±0.6 nM and 22.9±2.8 fmol/mg protein respectively, and did not

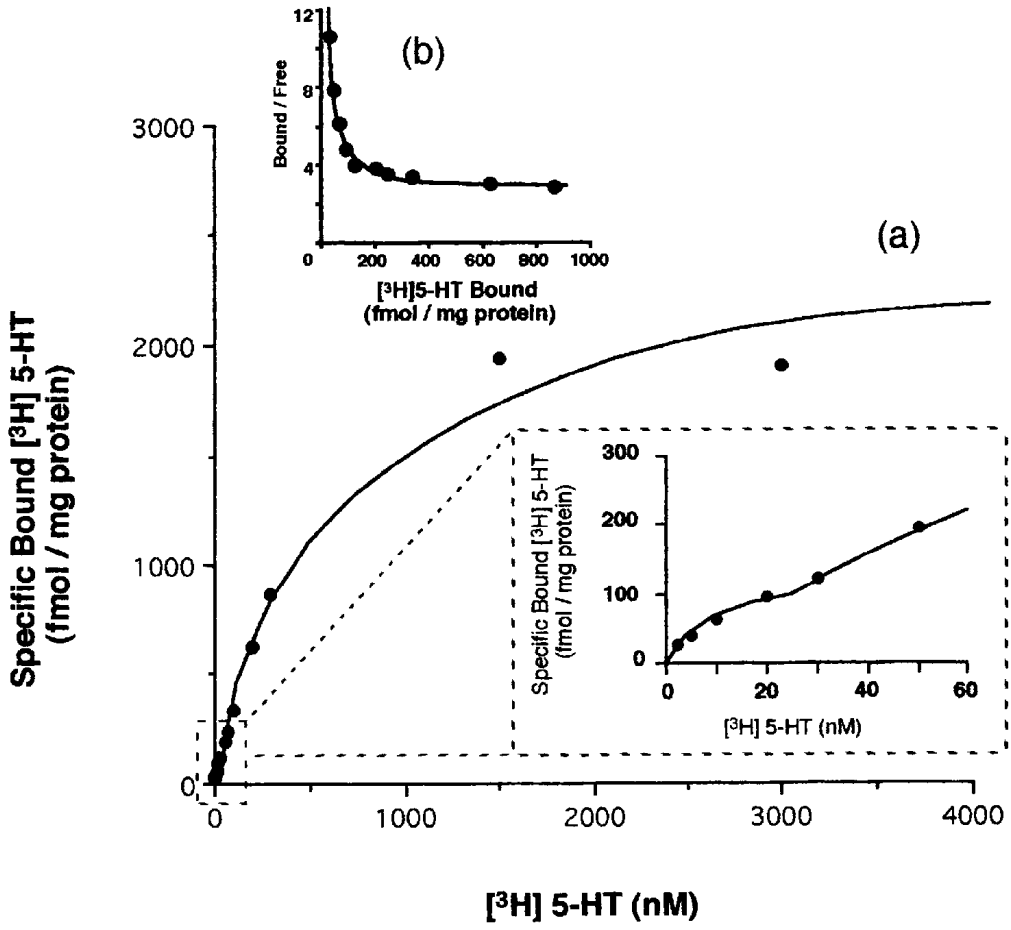


**Fig. 6.** Specific [$^3$H]5-HT binding to the membrane fraction from endothelium-denuded pig basilar arteries (a) and the Scatchard plot (b). Membrane fractions were incubated with increasing concentrations of [$^3$H]5-HT (0.25–3000 nM) in the absence (total) or presence (non-specific) of excess non-labeled 5-HT (300 $\mu$M), and the specific binding was calculated as the difference between the total and non-specific binding. The values are expressed as the means of three independent duplicate experiments. The insert shows part of the specific binding curve on an enlarged scale.

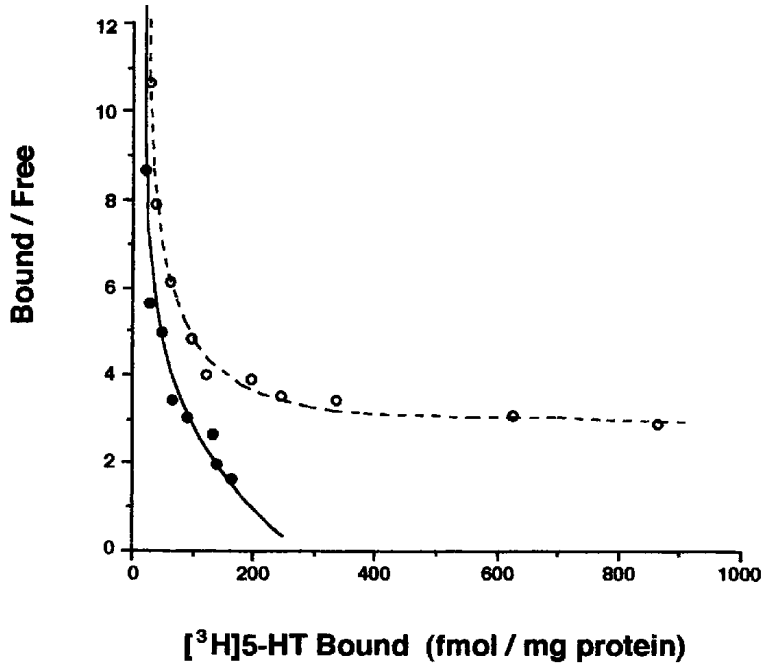


**Fig. 7.** Scatchard plots from the saturation study of [$^3$H]5-HT binding to the membrane fraction from endothelium-denuded pig basilar arteries in the presence (●) and absence (○) of ketanserin (1 $\mu$M). Each point represents the mean of three independent duplicate experiments.

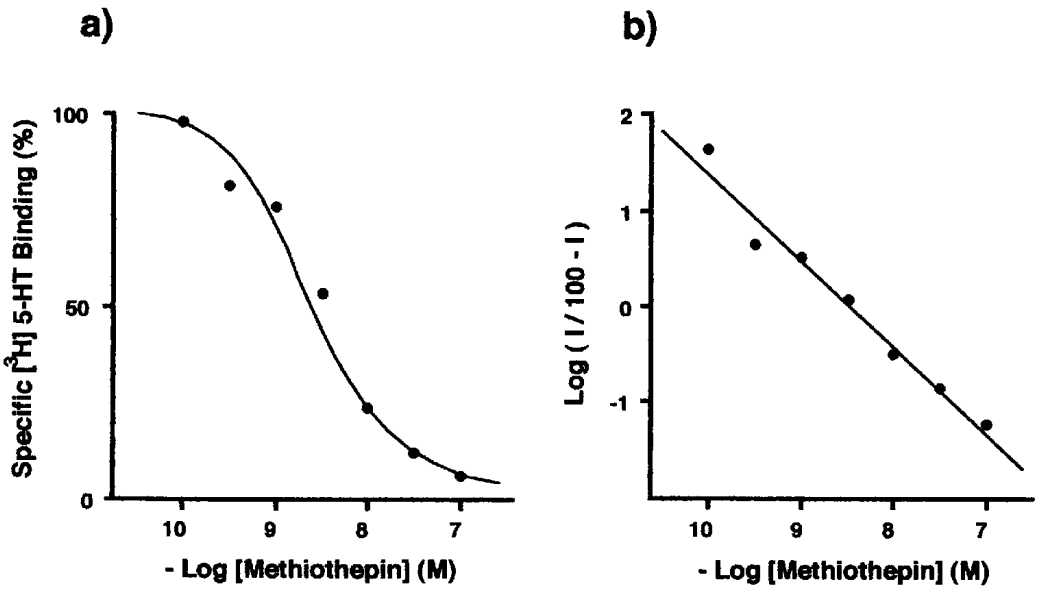


**Fig. 8.** Inhibition of specific [$^3$H]5-HT binding to the membrane fraction from endothelium-denuded pig basilar arteries by methiothepin in the presence of ketanserin (1 $\mu$M). The [$^3$H]5-HT binding expressed as a percentage of the specific [$^3$H]5-HT binding is shown on the ordinate (a). Hill plot for the inhibition of [$^3$H]5-HT specific binding by methiothepin (b). Each point represents the mean of two duplicate experiments with 10 nM [$^3$H]5-HT.

differ significantly from those of this site in the absence of ketanserin.

Figure 8 shows the displacement curves and Hill plot calculated from the competition experiments with methiothepin against [$^3$H]5-HT specific binding in the presence of ketanserin (1 $\mu$M). The Hill plot for methiothepin was a single straight line; the Hill coefficient was $0.94 \pm 0.12$, which did not differ significantly from

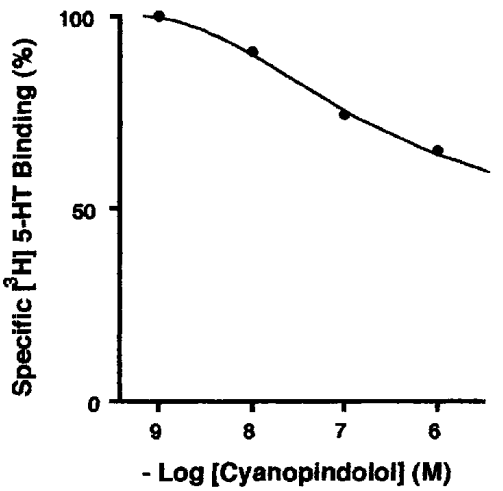


**Fig. 9.** Inhibition of [$^3$H]5-HT specific binding to the membrane fraction from endothelium-denuded pig basilar arteries by cyanopindolol in the presence of ketanserin (1 $\mu$M). The [$^3$H]5-HT binding expressed as a percentage of the specific [$^3$H]5-HT binding is shown on the ordinate. Each point represents the mean of two duplicate experiments with 10 nM [$^3$H]5-HT.

unity, and the calculated $K_i$ value was $0.5 \pm 0.1$ nM ($pK_i$, $9.3 \pm 0.1$).

Figure 9 shows the displacement curves obtained from the competition experiments with cyanopindolol, a 5-$HT_{1A}$- and 5-$HT_{1B}$-receptor antagonist, against [$^3$H]5-HT specific binding in the presence of ketanserin (1 $\mu$M). The $K_i$ value for cyanopindolol was greater than 126 nM ($pK_i$, $<6.9$)

## DISCUSSION

5-HT and some 5-HT-receptor agonists evoked concentration-dependent contractions of pig isolated basilar arteries with a rank order of potency ($pEC_{50}$) of 5-CT $\geqq$ 5-HT > $\alpha$-Me-5-HT > 8-OH-DPAT (Table 1). The $pEC_{50}$ value ($8.00 \pm 0.33$) of 5-CT (a 5-$HT_1$-receptor agonist) on the pig basilar artery was similar to those on human (mediated via the activation of 5-$HT_1$ receptors) (4) and dog (mediated via the activation of 5-$HT_1$ and 5-$HT_2$ receptors) (8) basilar arteries, but differed from that on the rat basilar artery (mediated via the activation of 5-$HT_2$ receptors) (5). These results suggest that 5-$HT_1$ receptors are present on pig basilar arterial smooth muscle cells and their stimulation results in contraction. The $pEC_{50}$ ($6.57 \pm 0.15$) of $\alpha$-Me-5-HT (a 5-$HT_2$-receptor agonist) on the pig basilar artery was similar to those on rat (5) and dog (8) basilar arteries, which suggests that 5-$HT_2$ receptors also are present on pig basilar arterial smooth muscle cells, and their stimulation results in contraction. 8-OH-DPAT has a high affinity for 5-$HT_{1A}$ receptors (18), but its $pEC_{50}$ ($5.12 \pm 0.15$) on the pig basilar artery was lower than its published affinity ($pK_d$, 8.7) (18), which suggests that this receptor is not involved in the 5-HT-induced contractions of pig basilar arteries.

Ketanserin, a 5-$HT_2$-receptor antagonist, inhibited the 5-HT-induced contractions competitively (Fig. 1). The $pA_2$ value for ketanserin ($9.58 \pm 0.13$) was similar to that (9.35) observed for the rat basilar artery (1). Methiothepin, a 5-$HT_1$- and 5-$HT_2$-receptor antagonist, shifted the 5-HT concentration-response curves to the right and downwards (Fig. 2). These data indicate that 5-HT-induced contractions of pig basilar arteries involve at least two 5-HT-receptor subtypes. Therefore, the $pA_2$ value of methiothepin in the presence of ketanserin ($10^{-6}$ M) was calculated; methiothepin inhibited the 5-HT-induced contractions competitively (Fig. 4), and its $pA_2$ value ($8.92 \pm 0.23$) was similar to that (8.8) for the human basilar artery (4). In the presence of methiothepin ($10^{-8}$ M), the 5-HT-induced contractions were not affected by ketanserin (Fig. 3). These findings imply that both 5-$HT_1$ and 5-$HT_2$ receptors are present on the pig basilar artery and agree well with those obtained with the 5-HT-receptor agonists. The 5-$HT_3$-receptor antagonist, MDL 72222, had no effect on the 5-HT-induced contractions (Fig. 5), which indicates that 5-$HT_3$ receptors are not involved in the 5-HT-induced contractions of pig basilar arteries.

A radioligand binding assay was carried out to quantify the distribution of the 5-$HT_1$ and 5-$HT_2$ receptors. Specific binding of [$^3$H]5-HT to the membrane fraction from endothelium-denuded pig basilar arteries was saturable at high and low concentrations of [$^3$H]5-HT (Fig. 6). The Scatchard plot showed high and low affinity sites. The $K_d$ value (2.5 nM) of the high affinity site was similar to the $K_i$ value (2.9 nM) of 5-HT for 5-$HT_1$ receptors (19), and the $K_d$ value (710 nM) of the low affinity site was similar to its $K_i$ value (928 nM) for 5-$HT_2$ receptors (20). Ketanserin (1 $\mu$M) did not inhibit binding to the high affinity site, but did inhibit that to the low affinity site (Fig. 7). These results suggest that the high and low affinity sites correspond to 5-$HT_1$ and 5-$HT_2$ receptors respectively. With respect to 5-$HT_2$ receptors, however, further studies with [$^3$H]spiperone or [$^3$H]ketanserin are required. In the presence of ketanserin (1 $\mu$M), the Hill plot obtained from the competition experiments with methiothepin against [$^3$H]5-HT specific binding was a single straight line; the Hill coefficient did not differ significantly from unity (Fig. 8). The $pK_i$ value of methiothepin was $9.3 \pm 0.1$, which did not differ significantly from its $pA_2$ value ($8.92 \pm 0.23$) obtained in the contractile experiments. The ratio of the high to low affinity site was approximately 1 : 65, which may reflect the ratio of 5-$HT_1$ to 5-$HT_2$ receptors present on pig basilar arterial smooth muscle.

Currently, 5-$HT_1$ receptors have been classified into at

least four subtypes, 5-$HT_{1A}$, 5-$HT_{1B}$ (21), 5-$HT_{1C}$ (22) and 5-$HT_{1D}$ (23), in the light of brain tissue binding assay results; and a 5-$HT_1$-like receptor has been demonstrated in a functional study (24). The $pK_i$ value of cyanopindolol, a 5-$HT_{1A}$- and 5-$HT_{1B}$-receptor antagonist, was lower than 6.9 (Fig. 9), which is much lower than its affinity for 5-$HT_{1A}$ ($pK_d$, 8.3) and 5-$HT_{1B}$ ($pK_d$, 8.3) receptors (18). These results suggest that 5-$HT_{1A}$ and 5-$HT_{1B}$ receptors are not involved in the 5-HT-induced contractions of pig basilar arteries. Our results indicate that the pig basilar arterial 5-$HT_1$ receptor characterized by functional and binding studies is likely to belong to the 5-$HT_{1D}$- (25, 26) or 5-$HT_1$-like-receptor subtype (27–29). So further studies are required to clarify this point.

In conclusion, our findings indicate that both 5-$HT_1$ and 5-$HT_2$ receptors are present on pig basilar arterial smooth muscle cells, and their stimulation results in contraction. The ratio of the high to low affinity binding site was approximately 1 : 65, and it may reflect the ratio of the 5-$HT_1$ to 5-$HT_2$ receptors present on this vascular tissue. The response to low concentrations of 5-HT might be predominantly mediated via the activation of 5-$HT_1$ receptors. So it is possible to speculate that 5-$HT_1$ receptors might play more an important role than 5-$HT_2$ receptors in the contraction of pig basilar arteries.

## REFERENCES


1 Chang J-Y, Hardebo JE and Owman Ch: Differential vasomotor action of noradrenaline, serotonin, and histamine in isolated basilar artery from rat and guinea-pig. Acta Physiol Scand **132**, 91–102 (1988)

2 Chang J-Y and Owman Ch: Cerebrovascular serotonergic receptors mediating vasoconstriction: further evidence for the existence of 5-$HT_2$ receptors in rat and 5-$HT_1$-like receptors in guinea-pig basilar arteries. Acta Physiol Scand **136**, 59–67 (1989)

3 Seager JM, Clark AH and Garland CJ: Endothelium-dependent contractile responses to 5-hydroxytryptamine in the rabbit basilar artery. Br J Pharmacol **105**, 424–428 (1992)

4 Parsons AA, Whalley ET, Feniuk W, Connor HE and Humphrey PPA: 5-$HT_1$-like receptors mediate 5-hydroxytryptamine-induced contraction of human isolated basilar artery. Br J Pharmacol **96**, 434–449 (1989)

5 Deckert V and Angus JA: Evidence that 5-$HT_2$ receptors predominantly mediate the contraction of the rat basilar artery to 5-hydroxytryptamine. Eur J Pharmacol **221**, 17–25 (1992)

6 Chang J-Y, Hardebo JE, Owman Ch, Sahlin Ch and Svendgaard N-Aa: Nerves containing serotonin, its interaction with noradrenaline, and characterization of serotonin receptors in cerebral arteries of monkey. J Auton Pharmacol **7**, 317–329 (1987)

7 Frenken M: Evidence for two populations of 5-hydroxytryptamine receptors in dog basilar artery. J Pharmacol Exp Ther **250**, 379–387 (1989)

8 Connor HE, Feniuk W and Humphrey PPA: Characterization of 5-HT receptors mediating contraction of canine and primate basilar artery by use of GR43175, a selective 5-$HT_1$-like receptor agonist. Br J Pharmacol **96**, 379–387 (1989)

9 Gaw AJ, Wadsworth RM and Humphrey PPA: Pharmacological characterization of postjunctional 5-HT receptors in cerebral arteries from the sheep. Eur J Pharmacol **179**, 35–44 (1990)

10 Shimokawa H, Kim P and Vanhoutte PM: Endothelium-dependent relaxation to aggregating platelets in isolated basilar arteries of control and hypercholesterolemic pigs. Circ Res **63**, 604–612 (1988)

11 Arunlakshana O and Schild HO: Some quantitative uses of drug antagonists. Br J Pharmacol **14**, 48–58 (1959)

12 Miyamoto A, Ito K and Nishio A: Characterization of $\beta$-adrenoceptors in pig basilar artery from functional and radioligand binding studies. Jpn J Pharmacol **61**, 93–99 (1993)

13 Miyamoto A and Nishio A: Characterization of histamine receptors in isolated pig basilar artery by functional and radioligand binding studies. Life Sci **53**, 1259–1266 (1993)

14 Peroutka SJ, Ison PJ, Liu DU and Barrett RW: Artifactual high-affinity and saturable binding of [$^3$H]5-hydroxytryptamine induced by radioligand oxidation. J Neurochem **47**, 38–45 (1986)

15 Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem **193**, 265–275 (1951)

16 Bruns RF, Lawson-Wendling K and Pugsley TA: A rapid filtration assay for soluble receptors using polyethylenimine-treated filters. Anal Biochem **132**, 74–81 (1983)

17 Munson PJ and Rodbard D: LIGAND: a versatile computerized approach for characterization of ligand-binding systems. Anal Biochem **107**, 220–239 (1980)

18 Hoyer D: Functional correlates of serotonin 5-$HT_1$ recognition sites. J Recept Res **8**, 59–81 (1988)

19 Peroutka SJ: Vascular serotonin receptors: correlation with 5-$HT_1$ and 5-$HT_2$ binding sites. Biochem Pharmacol **33**, 2349–2353 (1984)

20 Lyon RA and Titeler M: Pharmacology and biochemistry of the 5-$HT_2$ receptor. *In* The Serotonin Receptors, Edited by Sanders-Bush E, pp 59–88, The Humana Press Inc., Clifton (NJ) (1988)

21 Pedigo NW, Yamamura HI and Nelson DL: Discrimination of multiple [$^3$H]5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. J Neurochem **36**, 220–226 (1981)

22 Pazos A, Hoyer D and Palacios JM: The binding of serotonergic ligands to the porcine choroid plexus: characterization of a new type of serotonin recognition site. Eur J Pharmacol **106**, 539–546 (1985)

23 Heuring RE and Peroutka SJ: Characterization of a novel $^3$H-5-hydroxytryptamine binding site subtype in bovine brain membranes. J Neurosci **7**, 894–903 (1987)

24 Bradley PB, Engel G, Feniuk W, Fozard JR, Humphrey PPA, Middlemiss DN, Mylecharane EJ, Richardson BP and Saxena PR: Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology **25**, 563–576 (1986)

25 Hamel E and Bouchard D: Contractile 5-$HT_1$ receptors in human isolated pial arterioles: correlation with 5-$HT_{1D}$ binding sites. Br J Pharmacol **102**, 227–233 (1991)

26 Hamel E, Grégoire L and Lau B: 5-$HT_1$ receptors mediating contraction in bovine cerebral arteries: a model for human

cerebrovascular '5-$HT_{1D\delta}$' receptors. Eur J Pharmacol **242**, 75–82 (1993)

27 Ebersole BJ, Diglio CA, Kaufman DW and Berg KA: 5-Hydroxytryptamine$_1$-like receptors linked to increases in intracellular calcium concentration and inhibition of cyclic AMP accumulation in cultured vascular smooth muscle cells derived from bovine basilar artery. J Pharmacol Exp Ther **266**, 692–699 (1993)

28 Perren MJ, Feniuk W and Humphrey PPA: Vascular 5-$HT_1$-like receptors that mediate contraction of the dog isolated saphenous vein and carotid arterial vasoconstriction in anaesthetized dogs are not of the 5-$HT_{1A}$ or 5-$HT_{1D}$ subtype. Br J Pharmacol **102**, 191–197 (1991)

29 Schoeffter P, Waeber C, Palacios JM and Hoyer D: The 5-hydroxytryptamine 5-$HT_{1D}$ receptor subtype is negatively coupled to adenylate cyclase in calf substantia nigra. Naunyn Schmiedebergs Arch Pharmacol **337**, 602–608 (1988)