British Journal of Pharmacology (1996) 117, 283–292

Participation of 5-HT_1-like and 5-HT_{2A} receptors in the contraction of human temporal artery by 5-hydroxytryptamine and related drugs

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1 We investigated the hypothesis that, as in some other large human arteries, 5-HT-induced contraction of the temporal artery is mediated through two co-existing receptor populations, 5-HT_1-like-and 5-HT_{2A}. Temporal arterial segments were obtained from patients undergoing brain surgery and rings prepared set up to contract with 5-HT and related agents. Fractions of maximal 5-HT responses mediated through 5-HT_1-like and 5-HT_{2A} receptors, f_1 and $f_2 = 1 - f_1$, were estimated by use of the 5-HT_{2A}-selective antagonist ketanserin.

2 In rings with intact endothelium 5-HT evoked contractions with a -log EC_{50}, M of 7.0. Ketanserin (10–1000 nM) antagonized part of the 5-HT-induced contractions. Ketanserin-resistant components of 5-HT-induced contractions were found with -log EC_{50}, M of 6.9 and f_1 of 0.17 (100 nM ketanserin) and -log EC_{50}, M of 6.4 and f_1 of 0.20 (1000 nM ketanserin).

3 In rings with endothelial function attenuated by enzymatic treatment, 5-HT caused contractions with a -log EC_{50}, M of 7.2 that were partially blocked by ketanserin. Ketanserin-resistant components of 5-HT-induced contractions were found with -log EC_{50}, M 7.4 and f_1 of 0.16 (100 nM ketanserin) and -log EC_{50}, M of 7.5 and f_1 of 0.14 (1000 nM ketanserin).

4 The ketanserin-resistant component of 5-HT-evoked contraction was blocked by methiothepin (100–1000 nM) consistent with mediation through 5-HT_1-like receptors.

5 In rings with intact endothelium the 5-HT_1-like-selective agonist, sumatriptan, caused small contractions with a -log EC_{50}, M of 6.5 and intrinsic activity of 0.21 with respect to 5-HT that were resistant to blockade by 1000 nM ketanserin but antagonized by 100 nM methiothepin.

6 In rings with intact endothelium the 5-HT_{2A} receptor partial agonist SK&F 103829 (2,3,4,5-tetrahydro-8[methyl sulphonyl]-1H3-benzazepin-7-ol methensulphonate) contracted rings with a -log EC_{50}, M of 5.0 and an intrinsic activity of 0.49 with respect to 5-HT; the effects were antagonized by ketanserin 1000 nM.

7 We conclude that 80–86% of the maximum 5-HT-evoked contraction of human temporal artery is mediated through 5-HT_{2A} receptors, the remainder through 5-HT_1-like-receptors, regardless of whether or not endothelium is functional. The 5-HT_1-like-receptors are more likely to be 5-$HT_{1D\beta}$ receptors than 5-$HT_{1D\alpha}$ receptors and sumatriptan is a full agonist for these receptors. As found in arteries of other species, SK&F 103829 is a partial agonist for 5-HT_{2A} receptors of human temporal artery.

Keywords: Contraction of human temporal artery; 5-HT_{2A} and 5-HT_1-like receptors; endothelium; sumatriptan; SK&F 103829; 5-hydroxytryptamine

Introduction

Dilatation of extracranial arteries contributes to the pain in migraine in approximately one-third of patients (Lance, 1992). A classical example of extracranial vascular dilatation during migraine is that of the temporal artery; constriction caused by ergotamine administration alleviates pain (Graham & Wolff, 1938). Intracarotid administration of 5-hydroxytryptamine (5-HT) also decreases pulse amplitude of the human temporal artery (Lance, 1992), apparently through 5-HT_2 receptors (now 5-HT_{2A}, see Hoyer *et al.*, 1994 for nomenclature) as proposed by Edvinsson *et al.* (Edvinsson *et al.*, 1992; Jansen *et al.*, 1993) from *in vitro* experiments. The involvement of 5-HT_{2A} receptors is supported by early evidence showing that constriction of isolated, perfused segments of human temporal artery is blocked by both pizotifen and cyproheptadine (Carroll *et al.*, 1974), antagonists that possess high affinity (nanomolar) for 5-HT_{2A} receptors of brain (Leysen *et al.*, 1982) and blood vessels (Mylechrane, 1990). However, one observation appears to be inconsistent with the 5-HT_{2A} nature of 5-HT receptors that mediate constriction in human temporal artery. Edvinsson *et al.* (1992) and Jansen *et al.* (1993) reported that the 5-HT_1-like receptor agonist, sumatriptan (Humphrey *et al.*, 1990) contracted segments of temporal artery with an intrinsic activity as high (0.7 with respect to 5-HT) as that of sumatriptan for cerebral (pial) arterial segments and with nearly the same potency. Even more puzzling was the observation of Edvinsson *et al.* (1992) and Jansen *et al.* (1993) that the contractile effects of sumatriptan were resistant to blockade by methiothepin, an antagonist that blocks both 5-HT_1-like receptors and 5-HT_{2A} receptors with nanomolar affinity (Bradley *et al.*, 1986). These observations led Edvinsson *et al.* (1992) to suggest that sumatriptan acted through a receptor type in human temporal artery that was not within the profile known for 5-HT_{2A} receptors and 5-HT_1-like receptors. Before, however, accepting the existence of an unusual 5-HT receptor subtype, implicit in the suggestion of Edvinsson *et al.* (1992), we decided to study the 5-HT receptors of human temporal artery with a different approach.

We considered the possibility that the 5-HT receptors of

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human temporal artery may be heterogenous, as is known for other large human arteries (Kaumann *et al.*, 1993). For example, in coronary arteries (Chester *et al.*, 1990; Kaumann *et al.*, 1994), and mesenteric artery (Kaumann *et al.*, 1993) 5-HT appears to interact with two distinct populations of receptors, 5-HT_1-like and 5-HT_{2A}, the first blocked by methiothepin, the latter blocked by both ketanserin and methiothepin. We therefore carried out experiments on isolated rings of human temporal artery and used ketanserin and methiothepin as antagonists of the contractile effects of 5-HT. The results were analysed by a simple procedure based on the assumption of the coexistence of 5-HT_1-like receptors and 5-HT_{2A} receptors and their quantitative participation in the contractile effects of 5-HT assessed as described for human coronary artery (Kaumann *et al.*, 1993; 1994). We found a small component of the 5-HT-evoked contractions mediated through 5-HT_1-like receptors and a large component mediated through 5-HT_{2A} receptors. Because the component of the 5-HT-evoked contraction mediated through 5-HT_1-like receptors was small we would expect only a small effect of sumatriptan. On the other hand, because we found that a large portion of the 5-HT-evoked contractions were mediated through 5-HT_{2A} receptors, we anticipated that the partial agonist for 5-HT_{2A} receptors, SK&F 103829, would cause more pronounced contractions than sumatriptan. We therefore also studied the effects of sumatriptan and SK&F 103829.

Mathiau *et al.* (1994) recently claimed that temporal artery rings prepared from patients suffering cluster headache exhibit spontaneous phasic contractions that are exaggerated by 5-HT. They proposed that both spontaneous phasic contractions and 5-HT-induced phasic contractions are phenomena specific for patients with cluster headache because they were not observed in patients operated for glioblastoma removal. We therefore searched systematically for a history of cluster headache and other cephalagias in our patients as well as for the phasic contractions occuring spontaneously and/or in the presence of 5-HT. To obtain insight into which 5-HT receptor population is involved in the mediation of phasic contractions we also investigated whether sumatriptan elicited phasic contractions.

Methods

Patients

Segments of human temporal artery were obtained from 37 patients (13 females, age 22–77 and 24 males, age 24–66). Preliminary experiments have shown that electrocoagulation of the temporal artery during surgery made it difficult to obtain reproducible results, presumably through variable arterial damage. The use of electrocoagulation was therefore avoided. Fifteeen patients were operated for brain tumours (2 glioblastomas, 9 meningiomas, 4 metastasis); 2 for seizures (amigdalohipocampectomy); 9 for cerebrovascular malformations; 7 for brain injury; 3 for intraorbital tumours; 1 for intraorbital pseudotumour. All patients were interrogated for the occurence of acute and/or chronic headache. Until and during surgery patients received some of the following medications: dexamethasone, clonidine, urapidil, nifedipine, nimodipine, phenobarbitone, carbamezepine, frusemide, famotidine, pirenzipine, flunitrazepam, dopamine, sorbitol, manitol and antibiotics. Premedication was with diazepam. Anaesthesia was with fentanyl midazolam, thiopentone, propofol and muscle relaxation with pancuronium or succinylcholine.

Isolated temporal artery preparation

All arteries were free of macroscopic atheroma. Arterial segments of 500–800 μm outer diameter, obtained during neurosurgery, were placed in oxygenated Ringer solution containing (mM): Na^+ 147, K^+ 4, Ca^{2+} 2.3, Cl^- 155.6, at room temperature and transferred immediately into the laboratory and dissected and set up in a physiological solution containing (mM): Na^+ 142, Cl^- 126, K^+ 5.84, HCO_3^- 25, Ca^{2+} 2.5, $H_2PO_4^-$ 1.175, Mg^{2+} 1.175, SO_4^{2-} 1.175, glucose 5.56. The arterial segments were cleaned of adhering fat and connective tissue and cut into up to 12 rings of 3–4 mm length. Each arterial ring was mounted on an L-shaped brace in an organ bath containing 10 ml of physiological solution. The solution was gassed with 20% O_2 in 75% N_2 and 5% CO_2 at 37°C. The rings were stretched once to 10 mN and left at that length thereafter. Changes in arterial tension were transformed by a mechanoelectrical transducer (TF6V5-Fleck, Mainz Germany) and recorded continuously on a multichannel recorder (Linearcorder Mark VII WR 3310-Graphtec, Tokyo, Japan). Tissues were allowed to equilibriate for at least 2 h.

Concentration-effect curves

Peak force values were taken, regardless of whether contractions were phasic or tonic. Cumulative concentration-effect curves for 5-HT, SK&F 103829 and sumatriptan were determined in 0.5 log unit concentration steps. Enough time was allowed to obtain equilibrium responses. To investigate reproducibility two successive concentration-effect curves to 5-HT were determined with a 1 h interval between them. Antagonists were incubated for at least 1 h before a concentration-effect curve for an agonist was begun. The effects of the agonists were expressed as percentages of the response to a maximally effective 5-HT concentration (3 μM) administered at the start of the experiment.

Assessment of endothelial function

After observing a maximal 5-HT-induced contraction the functional integrity of the endothelium was assessed with the relaxation caused by 3 μM acetylcholine. Endothelial function was reduced by treating arterial segments for 15 min with collagenase 0.2%, a procedure shown to be effective in removing endothelial cells (Furchgott & Zawadzki, 1980).

Data analysis and statistics

5-HT data from every experiment in the absence and presence of ketanserin were fitted to a model of two receptor populations by nonlinear regression using Excel and Sigma Plot for Windows. One of the receptor populations (5-HT_{2A}) was assumed to be competitively blocked by ketanserin. The equation used (Kaumann *et al.*, 1994) was:

$$\text{Effect} = f_1 \cdot [5\text{-HT}]/([5\text{-HT}] + K_1) + f_2 \cdot [5\text{-HT}]/[5\text{-HT}] + K_2(1 + [\text{ket}]/K_{\text{ket}}) \quad (1)$$

where K_1 is EC_{50} of 5-HT for 5-HT_1-like receptors, K_2 is EC_{50} of 5-HT for 5-HT_{2A} receptors, f_1 represents the maximal effect mediated through 5-HT_1-like receptors, f_2 the maximal effect mediated through 5-HT_{2A} receptors and K_{ket} is the dissociation equilibrium constant for ketanserin (ket).

EC_{50} values of 5-HT are reported in log form. A single overall fit of the concentration-effect curve for 5-HT was also carried out and the -log EC_{50} estimated. The evaluation of concentration-effect curves for both sumatriptan and SK&F 103829 were based on a model of interaction with a single receptor population. Data are expressed as mean ± s.e.mean. Significance of differences were assessed by use of the non-parametric Mann-Whitney U test. P levels < 0.05 were considered significant.

Drugs and materials

5-Hydroxytryptamine creatine sulphate (5-HT) was purchased from Aldrich (Steinheim, Germany). SK&F 103829 (2,3,4,5-

tetrahydra-8-[methylsulphonyl]-1H3-benzazepin-7-ol-methensulphonate) was a gift of SmithKline Beecham (The Frythe, Welwyn, UK). Sumatriptan was a gift of Glaxo (Ware, UK). Acetylcholine chloride was obtained from Merck (Darmstadt, Germany). Methiothepin was obtained from Paesel & Lorei (Frankfurt/Main, Germany). Ketanserin and collagenase type XI (2-5 FALGPA hydrolysis units per mg solid) were obtained from Sigma (Deisenhofen, FRG). The collagenase was dissolved in the physiological solution described above.

Results

Spontaneous fast phasic contractions

Arterial rings tended to be quiescent. However, some rings developed fast phasic contractions, with up to one contraction per minute. The incidence of fast phasic contractions was 17/108 rings with functional endothelium (from 32 patients) (Table 1). Collagenase-treated arterial segments did not show spontaneous fast phasic contractions (0/12 rings from 5 patients).

Effects of collagenase

Acetylcholine relaxed 5-HT-induced contractions (Figures 1 and 2, Table 2). Collagenase treatment of arterial segments marginally attenuated 100 mM KCl contractions, slightly reduced 5-HT-induced contractions and nearly abolished acetylcholine-evoked relaxations (Figure 3, Table 2). The marked reduction of acetylcholine-evoked relaxation of 5-HT-induced contractions is consistent with a lesion and/or reduction of endothelial cells caused by collagenase (Furchgott & Zadwadzki, 1980).

Table 1 Incidence of cephalalgia (headache), spontaneous fast phasic contractions and fast phasic contractions induced or facilitated by 5-HT and sumatriptan*

Patient No.	*Disease*	*Cephalalgia*	*Fast phasic contractions*		
			Spontaneous	*5-HT*	*Sumatriptan*
1 M 64y	Glioblastoma	+	0/4	2/4	
2 F 35y	Metastasis	+	1/3	3/6	
3 F 53y	Metastasis	+	1/3		2/3
4 F 33y	Meningioma and classical migraine	+	1/6	1/3	
5 F 35y	Arteriovenous malformation	+	2/9	2/9	0/9
6 M 46y	Pseudotumour orbitae	+	1/6	2/6	2/6
7 F 60y	Meningioma	–	3/3	1/3	1/3
8 M 31y	Cranial cerebral trauma	–	0/6	1/6	0/6
9 M 53y	Aneurysma	–	0/11	5/11	0/5
10 F 62y	Meningioma	–	0/6	1/6	
11 F 23y	Glioblastoma	–	0/3	2/3	
12 M 56y	Aneurysma	–	3/6	6/6	
13 M 54y	Aneurysma	–	4/4	4/4	
14 M 30y	Cranial cerebral trauma	–	1/6	2/6	
15 M 43y	Aneurysma	–	0/6	2/6	
16 M 50y	Cranial cerebral trauma	–	0/5	1/5	
17 M 32y	Cranial cerebral trauma	–	0/5	1/5	
18 F 52y	Aneurysma	–	0/6	3/6	
19 F 77y	Meningioma	–	0/6	2/6	
20 F 22y	Cranial cerebral trauma	–	0/6	4/6	

*Not included are results from patients whose rings did not exhibit any fast phasic contractions.

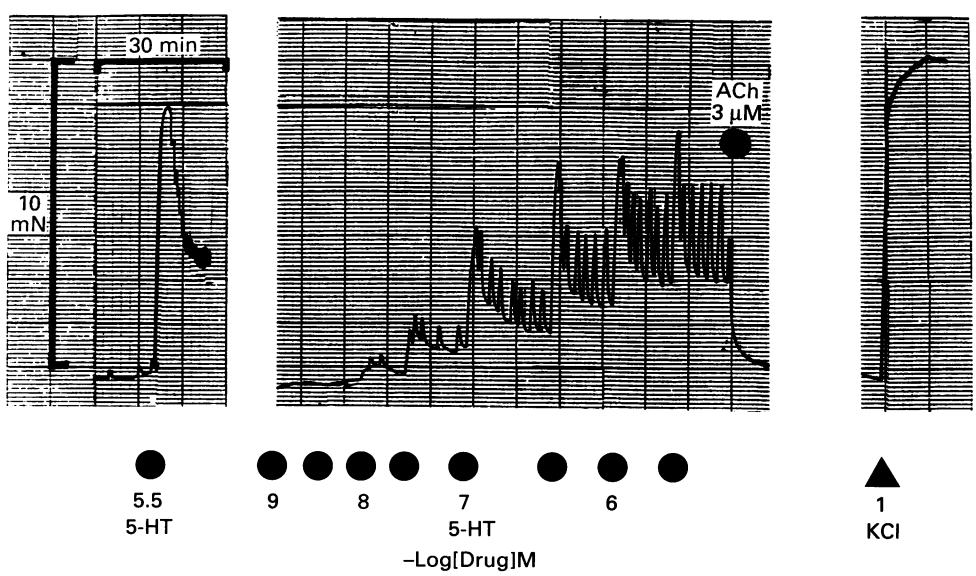

Figure 1 Fast phasic contractions caused by 5-HT; relaxation by acetylcholine (ACh). Arterial ring with intact endothelium from patient No. 12 of Table 1.

Effects of 5-HT

5-HT caused fast phasic contractions (for a representative experiment see Figure 1) in 45/107 rings (Table 1) and tonic contractions in all rings with intact endothelial function as assessed by the acetylcholine-evoked relaxation (Figures 1 and 2). 5-HT did not produce fast phasic contractions in 12 arterial rings from 5 patients with endothelial function blunted by incubation with collagenase as shown by the markedly reduced relaxant response to acetylcholine (Figure 3, Table 2). The relationship between the incidence of fast spontaneous contractions, 5-HT-induced fast phasic contractions and the incidence of headaches (cephalalgias) is shown in Table 1. None of the 37 patients suffered from cluster headache.

Two successive concentration-effect curves for 5-HT-induced contractions were reproducible and superimposable in rings both with intact or reduced endothelial function (Figure 4). Submaximal effects of 5-HT tended to relax partially with a time course considerably slower ('slow phasic contractions' half times of min) than that of the fast phasic contractions (half times of seconds); this pattern persisted partially in rings treated with collagenase (Figure 3). Patterns of fast and slow phasic contractions, as well as tonic contractions, were reproducible in several rings from the same artery (Figures 2 and 3). Ketanserin (10–1000 nM) blocked partially and surmountably contractions to the same extent in rings with both preserved or reduced endothelial function (Figures 2–4). The log (concentration-ratio of EC_{50}) values for 5-HT, determined

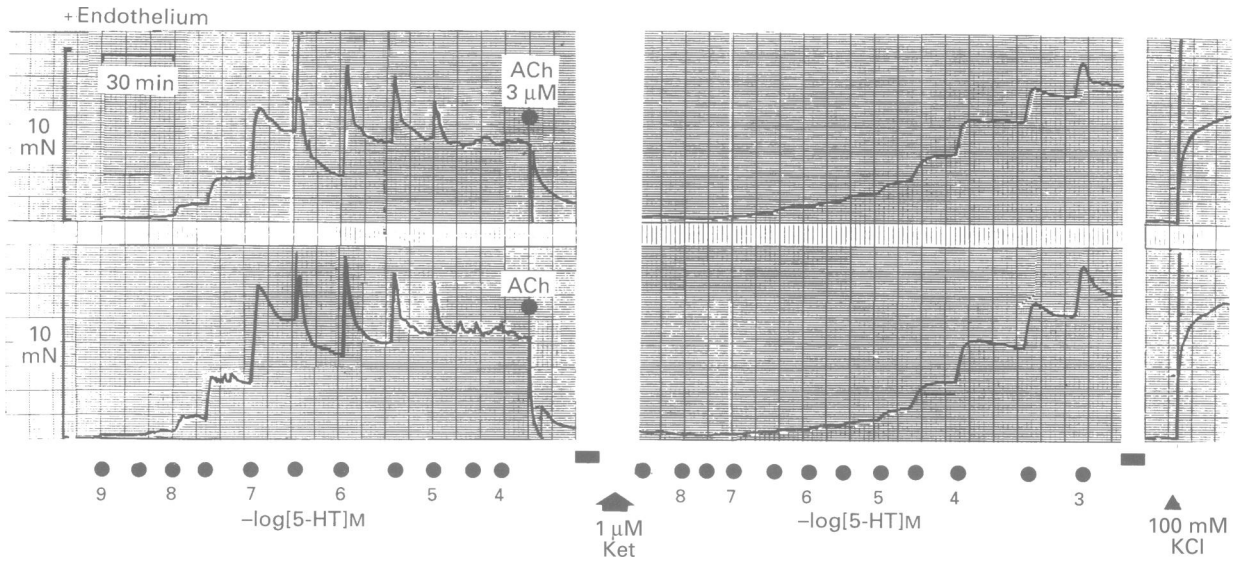

Figure 2 Reproducibility of responses to 5-HT and partial antagonism by ketanserin. Two arterial rings with intact endothelium from patient No. 18 of Table 1. After a first concentration-effect to 5-HT (left hand panels) the tissues were exposed to acetylcholine 3 μM (ACh) the drugs washed out, the tissues incubated with ketanserin (Ket) 1 μM and a second concentration-effect curve to 5-HT determined thereafter; 5-HT was washed out and the tissue exposed to 100 mM KCl. To demostrate the complete 5-HT response, the baseline was decreased after 300 nM 5-HT. To demonstrate the complete acetylcholine-evoked relaxation the baseline was lifted.

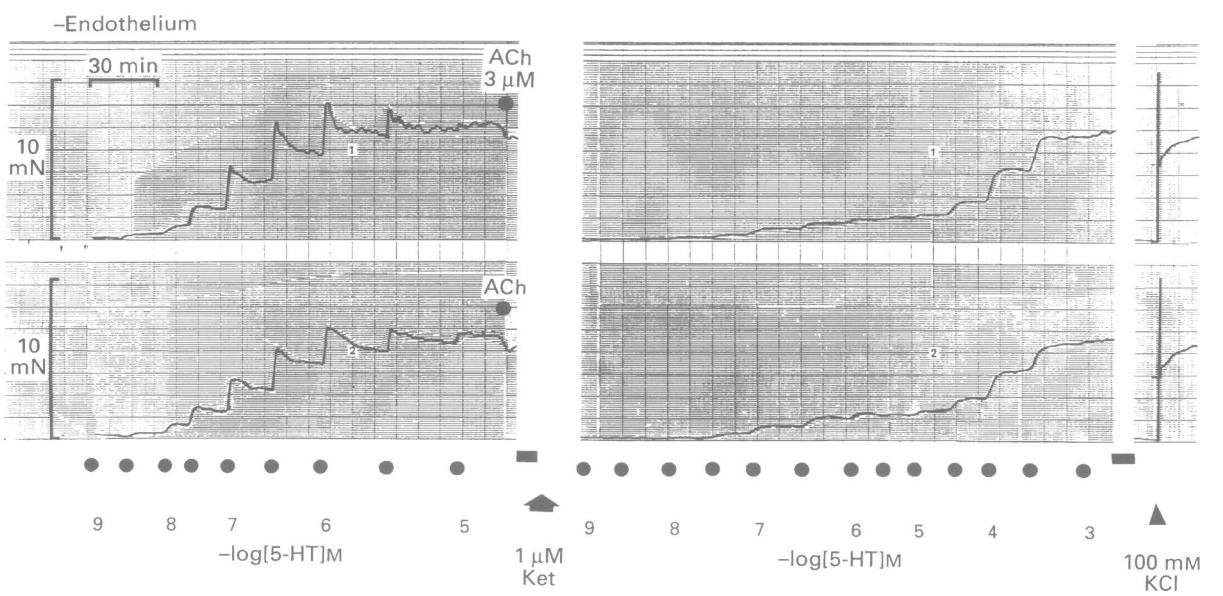

Figure 3 Reproducibility of responses of 5-HT and partial antagonism by ketanserin. Two rings from an artery treated with collagenase of a 27 y female patient with intraorbital arterio-venous malformation. Other details as in Figure 2.

Table 2 Effects of collagenase treatment on responses to KCl, 5-HT and acetylcholine

Condition	n	Force (mN) KCl (100 mM)	Force (mN) 5-HT (3 μM)	5-HT (% of KCl)	Acetylcholine % relaxation
Untreated	10	13.7±1.1	9.5±1.1	69	85±4
Treated	5	11.6±0.7	6.6±0.4	57	8±1
P-value		NS	NS		$P<0.01$

NS = non significant.

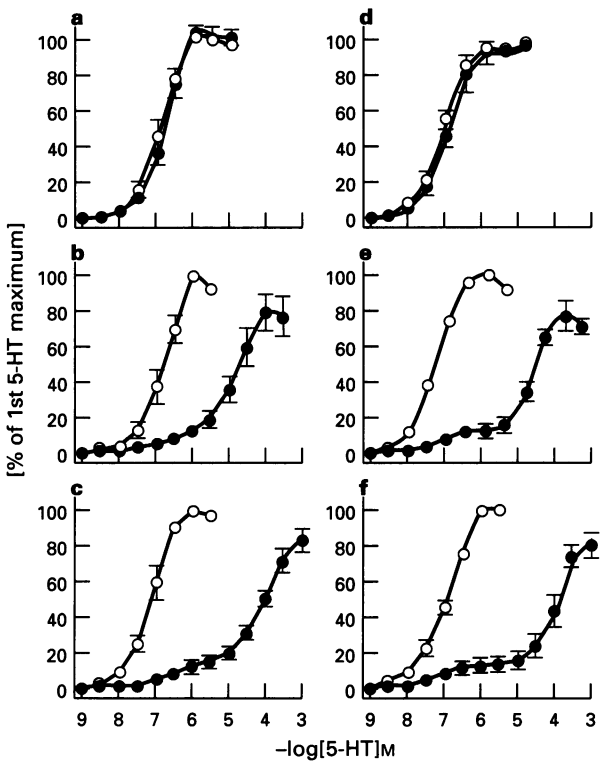

Figure 4 Comparison of the partial antagonism of 5-HT-induced contractions by ketanserin and reproducibility of concentration-effect curves to 5-HT in arterial rings not treated (a, b, c) and treated with collagenase (d, e, f). Two successive concentration-effect curves were determined, the first in the absence of ketanserin (○), the second (●) in the absence of ketanserin (a, $n=5$; d, $n=4$) or presence of 100 nM ketanserin (b, $n=5$; e, $n=6$) or 1 μM ketanserin (c, $n=5$; f, $n=4$). *n* refers to number of rings. Data without and with collagenase treatment were from 6 and 5 patients respectively.

from EC_{50} values caused by ketanserin 100 nM was 1.98±006 ($n=5$) and 2.37±0.14 ($n=5$) in rings with intact and reduced endothelial function respectively. The log (concentration-ratio) of 5-HT caused by 1000 nM ketanserin was 2.99±0.07 ($n=5$) and 2.93±0.04 ($n=5$) in rings with intact and reduced endothelial function respectively. Although 10 nM ketanserin shifted part of the concentration-effect curve of 5-HT to the right it did not affect phasic contractions (not shown). Higher concentrations of ketanserin also attenuated (100 nM) or abolished (1000 nM) both fast phasic contractions and slow phasic contractions (Figures 2 and 3).

A ketanserin-resistant component of 5-HT-induced contractions was uncovered in the presence of ketanserin (Figures 2–4). f_1 values were small (0.14–0.20) in rings with both intact or reduced endothelial function (Table 3); 5-HT was significantly more potent in eliciting ketanserin-resistant contractions in rings with reduced endothelial function than in rings with intact endothelial function (Figures 2–4, Table 3). Methiothepin (100–1000 nM) antagonized 5-HT-induced contractions completely in a concentration-dependent manner (Figure 5).

Effect of sumatriptan

Sumatriptan caused, on occasion (Table 1), fast phasic contractions (For a representative experiment see Figure 6). Sumatriptan more often caused tonic contractions with a slow phasic component at high concentrations (as illustrated in Figure 7) with a -log EC_{50} (M) of 6.46±0.09 and intrinsic activity of 0.21±0.04 with respect to 5-HT in rings with intact functional endothelium. The effects of sumatriptan were unaffected by 1 μM ketanserin but blocked surmountably by methiothepin 100 nM (Figures 7 and 8).

Effects of SK&F 103829

SK&F 103829 caused tonic contractions with some slow phasic components at high concentrations (For a representative ex-

Table 3 Analysis of the effects of 5-HT

		Ketanserin 100 nM) 5-HT$_1$ like	Ketanserin 100 nM) 5-HT$_{2A}$	Ketanserin 1000 nM 5-HT$_1$ like	Ketanserin 1000 nM 5HT2A
Endothelium functional (10 rings from 6 patients)					
$-\log EC_{50}$	7.01±0.05	6.89±0.18	4.95±0.03	6.44±0.10	4.07±0.05*
f_1		0.17±0.03		0.20±0.02	
Endothelium non-functional (10 rings from 5 patients)		7.37±0.08	4.99±0.14	7.46±0.16†	4.02±0.05*
$-\log EC_{50}$	7.15±0.05	0.16±0.06		0.14±0.04	
f_1					

†$P<0.02$, between $-\log EC_{50}$ values for 5-HT$_1$ like receptors (1000 nM ketanserin) with and without functional endothelium.
*$P<0.01$ between $-\log EC_{50}$ values with 100 nM and 1000 nM ketanserin

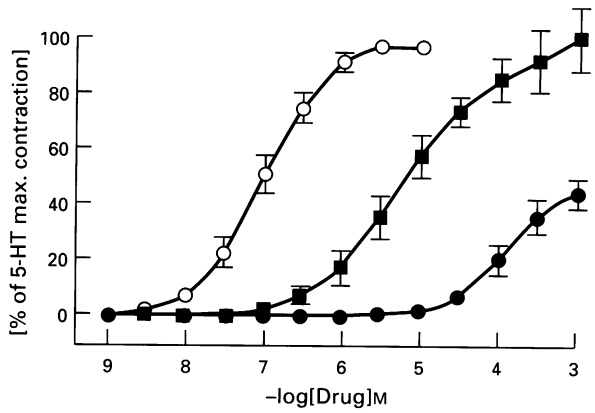

Figure 5 Antagonism by methothepin of 5-HT-induced contractions in arterial rings with intact endothelial function. Data from 7 patients: (○) represent data from a first concentration-effect curve to 5-HT ($n=7$ rings); (■) and (●) are data from a second concentration-effect curve to 5-HT in the presence of 100 nM methiothepin ($n=6$ rings) and 1 μM methiothepin ($n=6$ rings) respectively.

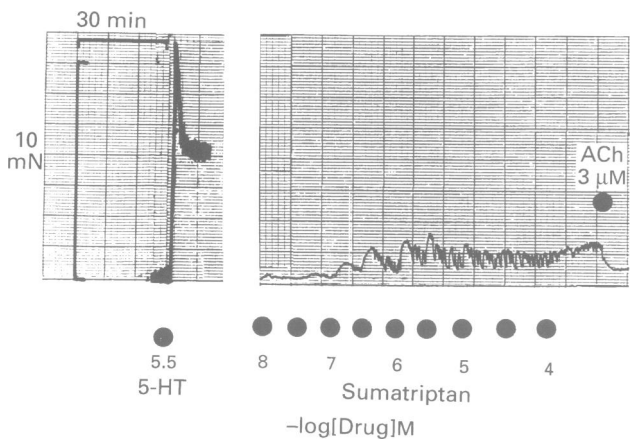

Figure 6 Tonic and fast phasic contractions elicited by sumatriptan and relaxation by acetylcholine in an arterial ring with intact endothelial function from patient No. 6 of Table 1.

periment see Figure 9) but no fast phasic components (Table 1). The SK&F 103829-induced contractions had a -log EC_{50} (M) of 4.95 ± 0.10 and intrinsic activity of 0.49 ± 0.08 with respect to 5-HT. The effects of SK&F 103829 were antagonized surmountably by ketanserin 1 μM (Figures 9 and 10).

Discussion

Coexistence of 5-HT_{2A} receptors and 5-HT_1-like receptors

Our analysis revealed 80–86% (i.e. $f_2 = 1 - f_1 = 0.80 - 0.86$) of the maximum 5-HT-induced tonic contractions are mediated through 5-HT_{2A} receptors, the remaining 16–20% through 5-HT_1-like receptors. The 2 and 3 log unit concentrations-ratios of 5-HT caused by 100 nM and 1000 nM ketanserin are consistent with the nanomolar affinity reported for cloned human 5-HT_{2A} receptors (Branchek *et al.*, 1990). The ketanserin-resistant component was blocked by methiothepin, as expected from its 5-HT_1-like nature. The low intrinsic activity of sumatriptan (0.2 with respect to 5-HT) did not differ significantly from the f_1 fractions (0.14–0.20) for the interaction of 5-HT with 5-HT_1-like receptors, suggesting that sumatriptan is a full agonist at these receptors. The relatively high intrinsic activity of SK&F 103829 (0.5 through 5-HT_{2A} receptors) and the blockade of its effects by ketanserin is consistent with similar observations in arterial 5-HT_{2A} receptors of rat and calf (Taylor & Kaumann, 1994).

Our results with both 5-HT and sumatriptan and our interpretation differ from those of Edvinsson *et al.* (1992) and Jansen *et al.* (1993) also obtained *in vitro* from human temporal arterial segments. Jansen *et al.* interpreted their results with 5-HT as evidence for mediation through 5-HT_{2A} receptors and did not consider the participation of 5-HT_1-like receptors. For sumatriptan, Jansen *et al.* (1993) reported results with a considerably greater intrinsic activity (0.7, as opposed to 0.2 from our present work). Furthermore, Jansen *et al.* (1993) claimed that the effects of sumatriptan were not blocked by methiothepin (although it is not clear which concentration was used) and concluded that the human temporal artery does not possess 5-HT_1-like receptors and that sumatriptan may act through a receptor that is neither 5-HT_2 or 5-HT_1-like (Edvinsson *et al.*, 1992). We were unable to confirm under our conditions the high efficacy of sumatriptan-induced contraction and its insensitivity to methiothepin. Reasons for this discrepancy are unknown to us but it cannot be attributed to a distorting factor of endothelial origin because both the results of Jansen *et al.* (1993) and our own results were obtained from rings with intact endothelial function, as assessed by acetylcholine-evoked relaxation. On the other hand, our results show that the low intrinsic activity of sumatriptan (0.2 with respect to 5-HT) is consistent with the small ketanserin-resistant f_1 fraction, and the blockade of both by methiothepin. The analysis of our data has thus yielded a picture of internal consistency best interpreted by the coexistence of a major component of the 5-HT-induced tonic contraction mediated through 5-HT_{2A} receptors and a minor component mediated through 5-HT_1-like receptors.

The characteristics of 5-HT_1-like receptors that mediate both the minor part of the tonic contractions caused by 5-HT and the sumatriptan-induced contractions suggests involvement of one of the two cloned 5-HT_{1D} receptors, 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$, which have a remarkedly similar pharmacology (relevance to human arteries reviewed by Kaumann *et al.*, 1993). Cloned and transfected 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors have different affinity for ketanserin, with K_D values around 100 nM for the former around 10 μM for the latter (Kaumann *et al.*, 1994). The effects of both sumatriptan and the 5-HT_1-like component of 5-HT-induced contraction (the latter in collagenase-treated arteries) were resistant to blockade by ketanserin 1 μM, making it unlikely that they are of 5-$HT_{1D\alpha}$ nature. By exclusion, the 5-HT_1-like receptors of human temporal artery that mediate 5-HT-induced contractions could be a 5-$HT_{1D\beta}$ population. A similar conclusion has been reached from equivalent studies of 5-HT-induced contractions of human coronary artery which also express a mixture of 5-HT_{2A} and 5-HT_1-like (possibly 5-$HT_{1D\beta}$) receptors (Kaumann *et al.*, 1993, 1994). mRNA for 5-$HT_{1D\beta}$ receptors has been detected in human cerebral arteries (Hamel *et al.*, 1993) consistent with the existence of these receptors and mediation of the contractile effects of 5-HT and sumatriptan. As discussed above, it is likely that the 5-HT_1-like receptors that mediate contraction in human temporal artery (this work) and coronary artery (Kaumann *et al.*, 1993; 1994) are also 5-$HT_{1D\beta}$, perhaps suggesting that these receptors may be functional in other large human arteries as well.

Nature of the phasic contractions of 5-HT and sumatriptan

Both 5-HT and sumatriptan but not SK&F 103829 caused, on occasion, fast phasic contractions, suggesting a more likely involvement of 5-HT_1-like receptors than 5-HT_{2A} receptors. Phasic contractions were also observed in rings of human coronary artery with both 5-HT and sumatriptan (Cocks *et al.*, 1993; Kaumann *et al.*, 1994) but not in the presence of nifedipine (Cocks *et al.*, 1993), suggesting an involvement of

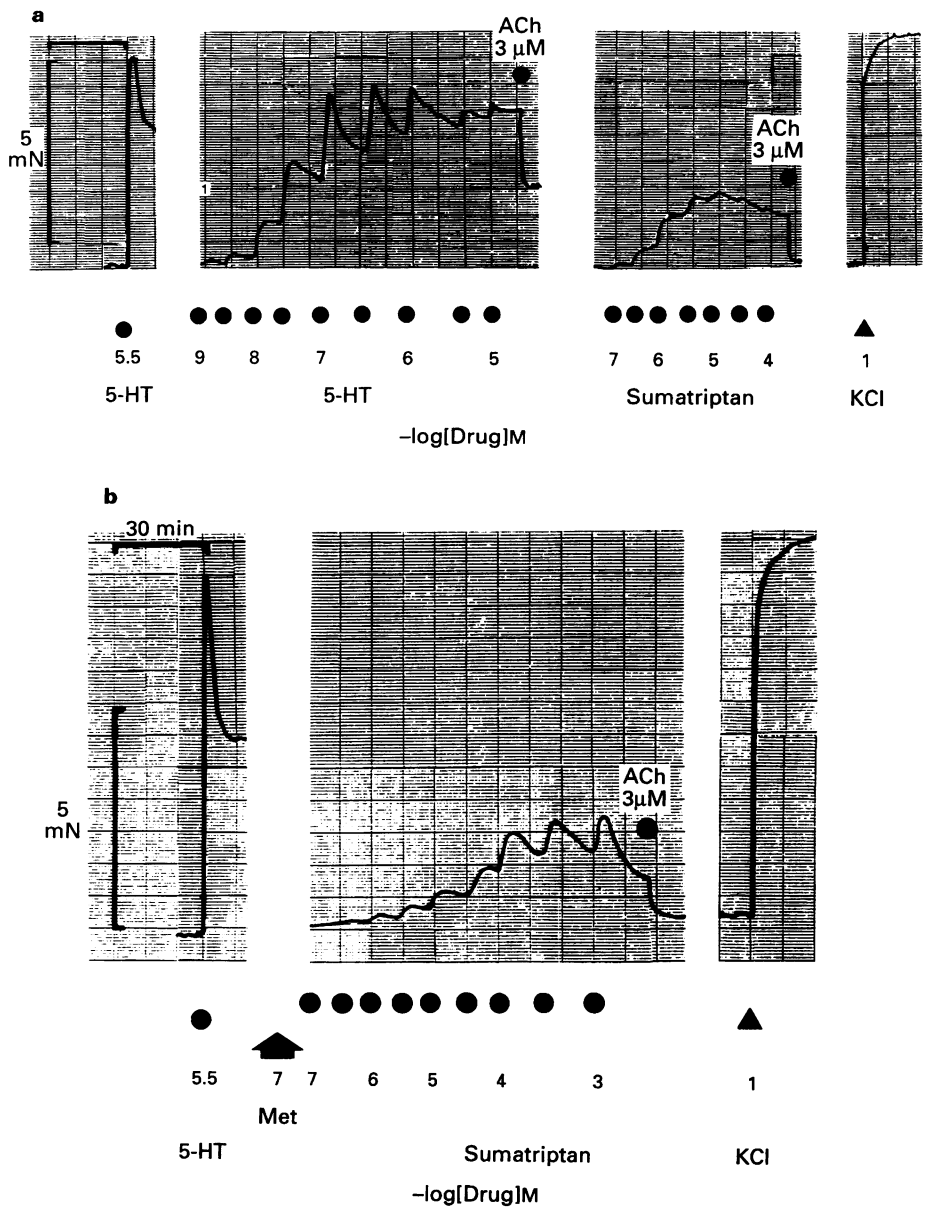

Figure 7 Comparison of the contractile effects of 5-HT and sumatriptan (a). Antagonism of the contractile effects of sumatriptan by methiothepin (Met) 100 nM (b). Arterial rings with intact endothelial function from a 28 y male patient with an intraorbital retention cyst.

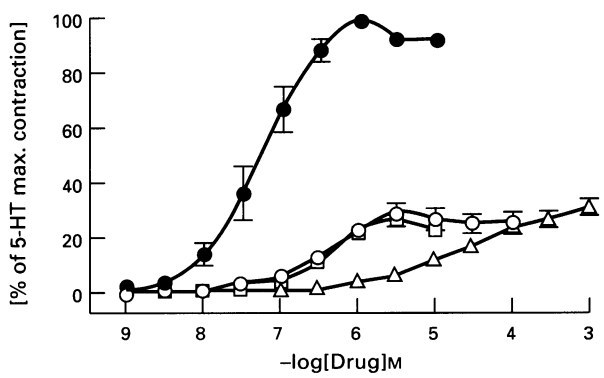

Figure 8 Comparison of the contractile effects of 5-HT (●, $n=11$ rings) and sumatriptan (all other symbols). Data from arteries with intact endothelial function of 8 patients. Effects of sumatriptan in the absence of antagonists (○, $n=8$ rings). Lack of blockade by ketanserin 1 μM (□, $n=5$ rings) and antagonism by methiothepin 100 nM (in the presence of ketanserin) (△, $n=6$ rings). All data are from a single concentration-effect curve determined on each ring.

Ca^{2+} entering through smooth muscle L Ca^{2+} channels; this may also be the mechanism of the generation of fast phasic contractions in human temporal artery. We did not observe spontaneous or 5-HT-induced fast phasic contractions (and relaxations) in temporal arteries treated with collagenase, suggesting participation of the endothelium.

Slow phasic contractions caused by relatively high 5-HT concentrations were reduced but not abolished in collagenase-treated arteries (Figure 3). Part of the slow phasic contractions caused by 5-HT in the presence of functional endothelium could be due to the release of an endothelium-dependent relaxing factor (Furchgott & Zawadzki, 1980), that would account in part for the slow relaxing phase of these contractions. Ketanserin reduced or abolished both 5-HT-evoked fast and slow phasic contractions in rings with preserved endothelial function but the concentrations were higher (100–1000 nM, Figure 2) than those already causing blockade of 5-HT_{2A} receptors (10 nM). Two endothelial 5-HT receptors are candidates for the mediation of endothelium-derived relaxation by 5-HT, 5-HT_{2B} and 5-HT_{2C} (see Fozard & Kalkman, 1994). Affinity estimates of ketanserin for the 5-HT_{2C} receptors are

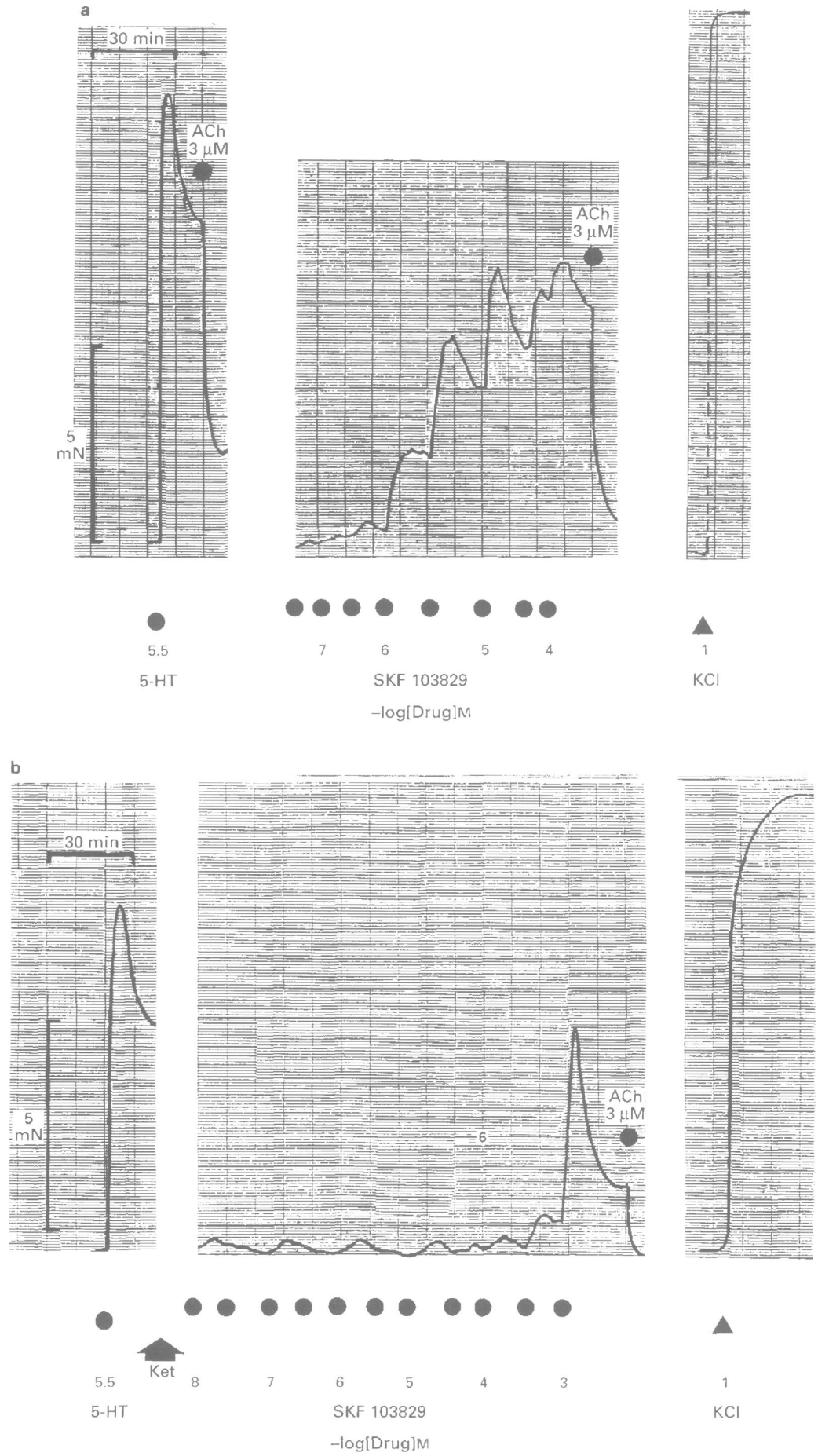

Figure 9 Comparison of the contractile effects of 5-HT and SK&F 103829 (a). Antagonism of the contractile effects of SK&F 103829 by ketanserin (Ket) 1 μM (b). Arterial rings with intact endothelial function from patient No. 14 Table 1.

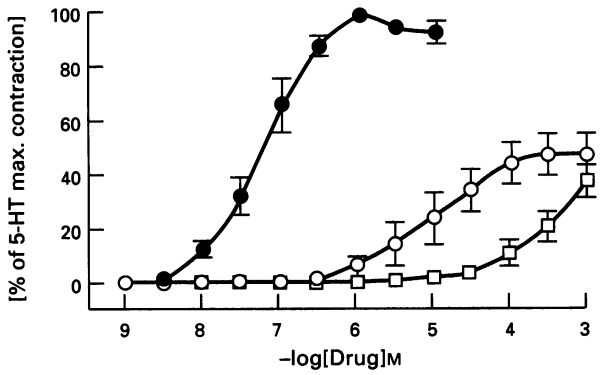

Figure 10 Comparison of the contractile effects of 5-HT (●, $n=13$ rings) and SK&F 103829 (○, $n=8$). Antagonism of the contractile effects of SK&F 103829 by ketanserin 1 μM (□, $n=6$). All data are from single concentration-effect curves obtained on arterial rings from 8 patients.

usually higher ($pK_D \sim 6.7-7.6$: Hoyer *et al.*, 1985; 1986; Herndon *et al.*, 1992; Bonhaus *et al.*, 1995) than for 5-HT_{2B} receptors ($pK_D \sim 5.5-6.7$: Loric *et al.*, 1992; Wainscott *et al.*, 1993; Bonhaus *et al.*, 1995) in a variety of species including man (Bonhaus *et al.*, 1995). In cell lines derived from mouse teratocarcinoma, 5-HT_{2B} receptors are expressed during differentiation that have even relatively high affinity for ketanserin ($pK_D \sim 7.2-7.4$, Loric *et al.*, 1995). The blocking potency of ketanserin (effective at 100 nM and higher concentrations) against the relaxant components of 5-HT-induced phasic contractions in the temporal artery is so far consistent with the interaction with 5-HT_{2C} receptors and/or 5-HT_{2B} receptors. It has previously been suggested that endothelial 5-HT_{2C} receptors mediate 5-HT-induced relaxation in porcine pulmonary artery (Glusa & Richter, 1993) and rat jugular vein (Bodelsson *et al.*, 1993), but ketanserin (1 μM) failed to cause antagonism, which is inconsistent with its submicromolar affinity for 5-HT_{2C} receptors and leaves open the possibility of mediation through 5-HT_{2B} receptors. Ellis *et al.* (1995) have indeed recently suggested that endothelium-dependent relaxation of rat jugular vein by 5-HT is mediated through 5-HT_{2B} receptors. Future work with an antagonist selective for 5-HT_{2B} receptors compared to 5-HT_{2C} and 5-HT_{2A} receptors (Baxter *et al.*, 1995) could clarify the role of endothelial 5-HT receptors (and their nature) in human temporal artery.

The residual slow phasic contractions caused by 5-HT in rings with endothelial function reduced by collagenase treatment could be due to the persistence of residual endothelial function or to desensitization of the contractile response. Either mechanism would be expected to be prevented by ketanserin but we still do not know which is relevant. Alternatively, the residual relaxation could be mediated through activation of 5-HT receptors located on the smooth muscle cells.

Clinical implications

Mathiau *et al.* (1994) reported that isolated temporal arteries of patients with cluster headache, but not with glial tumours, exhibited phasic contractions and that 5-HT caused rhythmical phasic contractions. Although we observed both spontaneous phasic contractions and 5-HT-induced phasic contractions in arterial rings from several patients, none of these patients nor the patients without phasicity had a history of cluster headache. The generalisation of Mathiau *et al.* (1994) is therefore not valid. Furthermore, although some patients had cephalagias, these appeared unrelated to the occurrence of spontaneous phasic contractions and 5-HT-induced phasic contractions.

Our evidence suggests that the human temporal artery with intact endothelium can be constricted mainly through 5-HT_{2A} receptors and to a minor extent through 5-HT_1-like (possibly 5-$HT_{1D\beta}$) receptors. Since dilatation of the temporal artery appears to contribute to pain in about one third of migraine patients (Lance, 1992) therapeutic constriction of the artery could in principle be more effectively achieved with an agonist selective for 5-HT_{2A} receptors than for 5-HT_1-like receptors. To avoid endothelium-dependent relaxation, a 5-HT_{2A}-selective agonist should also probably possess low affinity for and efficacy through both 5-HT_{2B} and 5-HT_{2C} receptors.

We are grateful to Dr A.M. Brown for reading the manuscript.

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(Received August 31, 1995
Accepted September 22, 1995)