Naunyn-Schmiedeberg's Arch Pharmacol (1992) 345:500–508

Naunyn-Schmiedeberg's
Archives of
Pharmacology

5-Hydroxytryptamine-induced contractions of the human isolated saphenous vein: involvement of 5-HT_2 and 5-HT_{1D}-like receptors, and a comparison with grafted veins *

Willem A. Bax [1], Dicky Van Heuven-Nolsen [2], Egbert Bos [3], Maarten L. Simoons [3], and Pramod R. Saxena [1]

[1] Department of Pharmacology and [3] Thorax Centre, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, Post Box 1738, NL-3000 DR Rotterdam, The Netherlands, [2] Department of Pharmacology, Faculty of Pharmacy, University of Utrecht, Post Box 80.082, NL-3508 TB Utrecht, The Netherlands

Received December 16, 1991/Accepted February 5, 1992

Summary. The receptors mediating the contractile effect of 5-hydroxytryptamine (5-HT) on the human isolated saphenous vein, obtained from 42 patients undergoing coronary bypass surgery, have been further characterized using a number of 5-HT-related drugs. The rank order of agonist potency was 5-carboxamidotryptamine (5-CT) ≈ 5-HT > methysergide ≈ sumatriptan ≈ α-methyl-5-HT ≈ 5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indolesuccinate (RU 24969) ≈ 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) > 2-methyl-5-HT > 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT). Flesinoxan was inactive as an agonist. Ketanserin (1 μmol/l) hardly affected sumatriptan-induced contractions but it caused a rightward shift of the upper part of the concentration-response curve of 5-HT and 5-CT. The same concentration of ketanserin caused a parallel rightward shift of the concentration-response curves of α-methyl-5-HT and DOI with pK_B values of 7.1 and 7.1, respectively. The responses to sumatriptan were antagonized by methiothepin (0.1 μmol/l), metergoline (0.1 and 1 μmol/l), rauwolscine (1 μmol/l) and cyanopindolol (1 μmol/l); the calculated pK_B values were 7.3, 6.9, 7.3, 6.7 and 6.5, respectively. Contractions to 5-HT were antagonized by methysergide (1 μmol/l), methiothepin (0.1 μmol/l; $pK_B = 7.1$), ICS 205-930 (1 μmol/l; $pK_B = 5.9$) and flesinoxan (30 μmol/l; $pK_B = 5.3$). Remarkably, the contractions elicited by 2-methyl-5-HT were not attenuated by ICS 205-930, but were antagonized by methiothepin (0.1 μmol/l) and, more markedly, by ketanserin (1 μmol/l).

There was a high correlation between the functional pD_2 values of 5-HT_1-like receptor agonists (5-CT, 5-HT, methysergide, sumatriptan, RU 24969 and 8-OH-DPAT) and their reported binding affinities for the 5-HT_{1D} receptor in human or calf brain membranes. Such a correlation for the antagonism of sumatriptan-induced responses was less marked than for the agonists, but of the 5-HT_1-like receptor subtypes it was the highest for the 5-HT_{1D} receptor identified in human or calf brain membranes.

In 3 patients, undergoing heart transplantation, saphenous vein which had previously functioned as a graft for 6–11 years, was dissected out from the heart. Though the contractions to potassium were significantly smaller in the grafted veins, the pD_2 and E_{max} values (calculated as percentage of potassium-induced contractions) for 5-HT and sumatriptan were similar to those found in the veins obtained directly from the lower leg.

It is concluded that contractions in the human isolated saphenous vein induced by 5-HT are mediated by 5-HT_2 receptors as well as by a 5-HT_1-like receptor resembling the 5-HT_{1D} subtype found in brain membranes. It is also to be noted that 2-methyl-5-HT, considered selective for the 5-HT_3 receptor, contracts the saphenous vein mainly via 5-HT_2 receptors.

Key words: Bypass – Coronary bypass – 5-HT receptors – 5-HT_{1D} receptor – 5-HT_2 receptor – 5-Hydroxytryptamine – Human saphenous vein – Sumatriptan

Introduction

Saphenous vein and internal mammary artery are frequently used as a graft in coronary bypass operations. Several differences between these two vessels have been found that could possibly account for the superior patency of mammary artery grafts (Loop et al. 1986; Lüscher et al. 1988). Recently, Yang et al. (1991) reported that, when precontracted with noradrenaline, the human isolated saphenous vein contracts but the human isolated internal mammary artery relaxes to platelets. Platelet-derived ADP caused the mammary artery to relax while contractions in the saphenous vein were shown to be mediated by thromboxane A_2 and 5-HT. The 5-HT-induced saphenous vein contractions are sensitive to ketanserin and spiperone, both 5-HT_2 receptor antago-

* This study was supported by the Netherlands Heart Foundation, grant 89.252
Send offprint requests to W. A. Bax at the above address

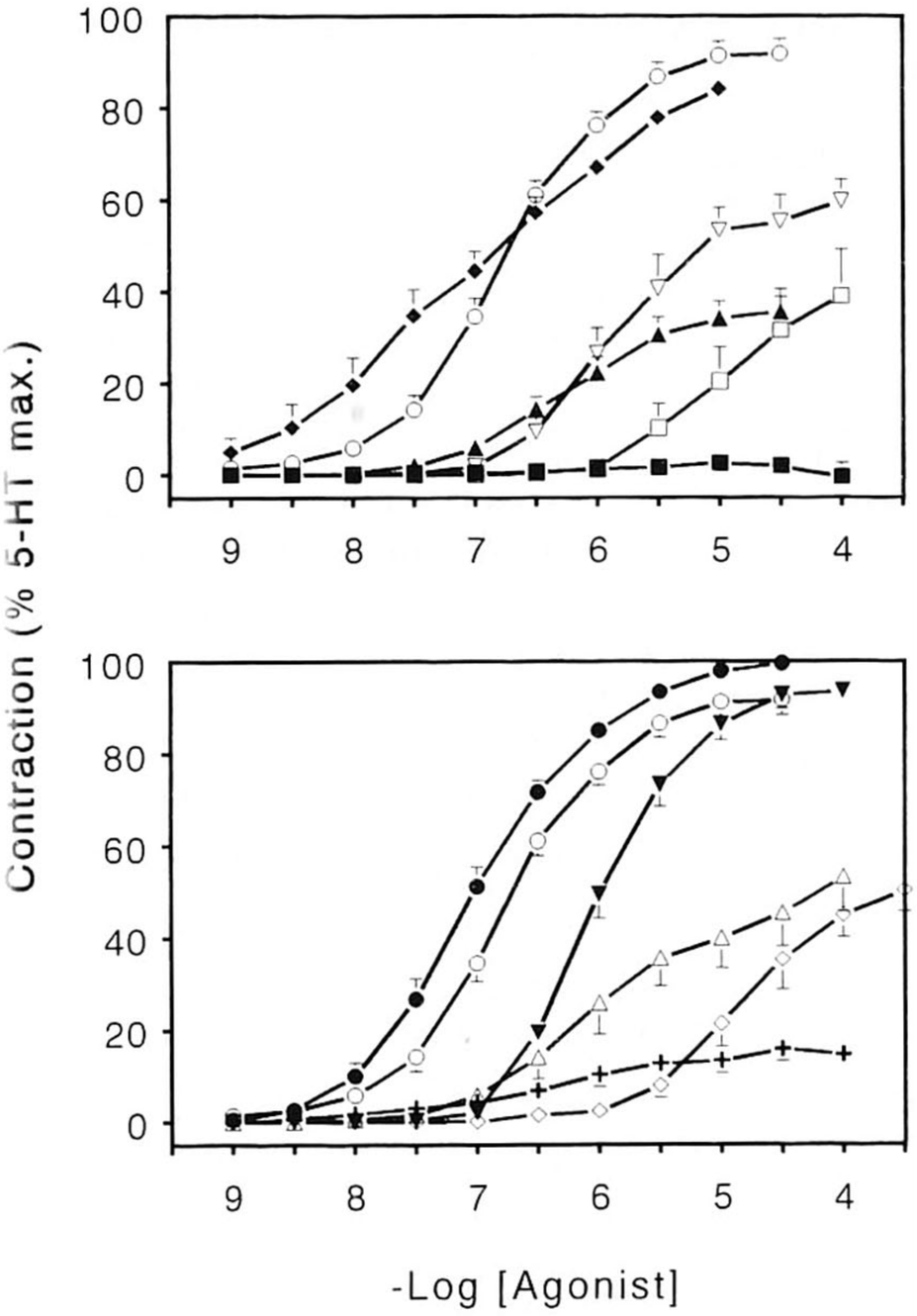

Fig. 1. Contractions of the human isolated saphenous vein to 5-HT receptor agonists. *Upper graph:* ○, 5-HT (*2nd curve*); ◆, 5-CT; ▽, RU 24969; ▲, sumatriptan; □, 8-OH-DPAT and ■, flesinoxan. *Lower graph:* ●, 5-HT (*1st curve*); ○, 5-HT (*2nd curve*); ▼, α-methyl-5-HT; △, DOI; ◇, 2-methyl-5-HT and +, methysergide. Agonist concentration refers to mol/l. E_{max} and pD_2 as well as the number of segments tested are mentioned in Table 1

nists. This ketanserin-sensitive receptor is activated mainly at high concentrations of 5-HT, while lower concentrations seem to activate a yohimbine- and methiothepin-sensitive 5-HT receptor (Docherty and Hyland 1986; Victorzon et al. 1986; Chester et al. 1990a; Docherty and Borton 1991). The present study was undertaken to further characterize the 5-HT receptors mediating contractions in the human saphenous vein. In addition, we also had the opportunity to compare the responses mediated by 5-HT receptors in the vessels which had functioned as coronary bypass grafts for a number of years. A part of the results of this investigation was presented to the British Pharmacological Society (Bax et al. 1992).

Methods

Tissue preparation. Leftover human saphenous vein was obtained intra-operatively during bypass surgery from 42 patients (29 males and 13 females; age: 44 to 77 years). The tissue was immediately placed in a cold, oxygenated Krebs' bicarbonate solution of the following composition (mmol/l): sodium chloride, 118; potassium chloride, 4.7; calcium chloride, 2.5; magnesium sulphate, 1.2; potassium dihydrogen phosphate, 1.2; sodium bicarbonate, 25; and glucose, 8.3; pH 7.4. The vein was brought to the laboratory within 30 min of removal and cleaned of adhesive fat and connective tissue.

Table 1. Agonist potency and maximal effect in the human isolated saphenous vein. pD_2 is expressed as $-\log (EC_{50})$ and E_{max} is expressed as a percentage of the maximal 5-HT contraction in the first concentration-response curve

Agonist	*n*	pD_2 (± SEM)	E_{max} (± SEM)
5-CT	5	7.1 (0.2)	89 (4)
5-HT, first	18	7.0 (0.1)	100 (0)
5-HT, second	18	6.8 (0.1)	93 (3)
Methysergide	4	6.3 (0.4)	16 (3)
Sumatriptan	18	6.1 (0.1)	37 (4)
α-Methyl-5-HT	3	6.0 (0.1)	94 (3)
RU 24969	5	5.8 (0.2)	62 (6)
DOI	8	5.7 (0.3)	57 (7)
2-Methyl-5-HT	7	4.8 (0.2)	49 (5)
8-OH-DPAT[a]	8	<4.6 (0.2)	>52 (10)
Flesinoxan	3	inactive	inactive

[a] The concentration-response curve did not achieve a plateau even at the highest concentrations studied (10^{-4} mol/l)
n, Number of observations

In addition, saphenous vein could also be obtained from three male patients (aged 46, 48 and 61 years) with progressive ischemic heart disease, undergoing cardiac transplantation. These patients had previously undergone coronary bypass surgery where saphenous vein had been used as a graft for a period of 6, 7 and 11 years, respectively, prior to transplantation. The vein was collected from these hearts within 45 min of cardiectomy.

The vessels were cut into rings of approximately 4–5 mm of length and suspended on stainless steel hooks in 8 ml organ baths containing the Krebs' bicarbonate solution, aerated with 95% O_2 and 5% CO_2 and maintained at 37° C. The tissues were allowed to equilibrate for at least 30 min and washed every 10 min. Changes in isometric tension were recorded with a Harvard isometric transducer. Preparations were then stretched to the optimum point of the length-tension relation, as determined by tension developed to 30 mmol/l K^+. The rings were left to equilibrate for another 30 min after which the actual experiment began. Endothelial integrity was not assessed in these experiments, since the presence of endothelium does not influence 5-HT-induced contractions in this vessel (Yang et al. 1989).

Determination of agonist potency. A cumulative concentration-response curve to 5-HT was obtained on each preparation until a maximum response (E_{max}) was obtained. After frequent washing the tissue was left to equilibrate for at least 30 min. When the baseline tone was reached, the tissue was exposed to either 5-HT again or to another test agonist. In each vessel segment, the second 5-HT concentration-response curve or the only concentration-response curve to the other agonists was expressed as percentage of the E_{max} of the first 5-HT curve.

To determine agonist potency in saphenous vein grafts that had been in situ for some years, a single concentration effect curve was obtained with either 5-HT or sumatriptan. These responses were expressed as a percentage of potassium (30 mmol/l)-induced contractions in the grafted veins. The response to potassium (30 mmol/l), and the E_{max} and pD_2 values (see below) of 5-HT and sumatriptan were compared to the respective contraction or curves in the ungrafted saphenous veins.

Determination of antagonist potency. A concentration-response curve to 5-HT was made and the preparation was washed as described above. About 30 min later, a second cumulative concentration-response curve was obtained with either 5-HT or another agonist, without or after incubation (30 min) with a certain concentration of an antagonist. The second curve without antagonist, mostly obtained in parallel, served as a control for the curve in the presence of antagonists.

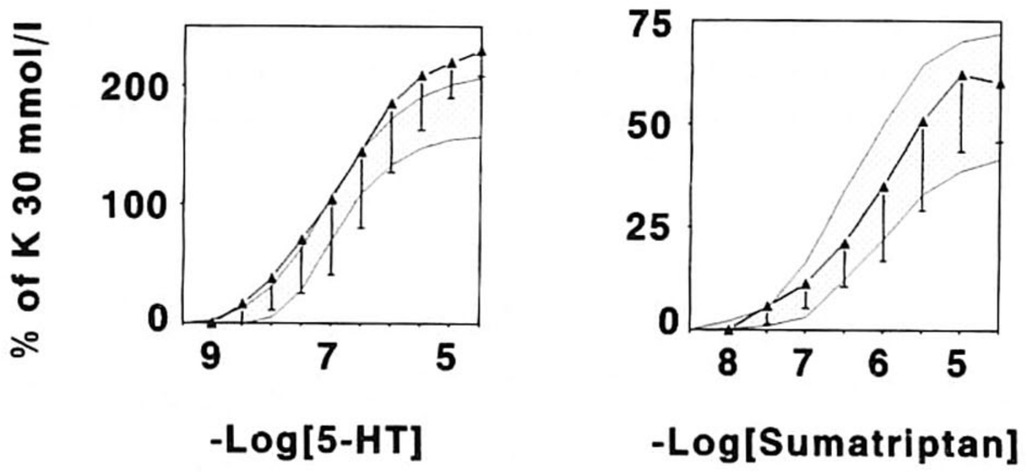

Fig. 2. Contractions to 5-HT and sumatriptan in saphenous veins which had been functioning for some years as a coronary bypass graft ($n = 3$) against a shaded area of the 95% confidence interval of data obtained in saphenous veins obtained directly from the lower leg ($n = 18$). Agonist concentration refers to mol/l. Contractions expressed as a percentage of potassium (30 mmol/l)-induced contractions. E_{max} and pD_2 as well as the number of segments tested are mentioned in Table 2

Table 2. Comparison of the effect (developed tension in g) of potassium (30 mmol/l), and the pD_2 (expressed as $-\text{Log}\,[EC_{50}]$) and E_{max} (expressed as a percentage of the potassium-induced contraction) values of 5-HT and sumatriptan in saphenous veins obtained directly from the lower leg ($n = 18$) and in those which had been used as a coronary bypass graft ($n = 3$)

	Ungrafted vein (± SEM)	Bypass graft (± SEM)
Effect 30 mmol/l potassium	5.5 (0.8)	0.5 (0.9)*
pD_2 5-HT	7.0 (0.1)	6.7 (0.5)
E_{max} 5-HT	181 (13)	242 (23)
pD_2 sumatriptan	6.1 (0.1)	5.8 (0.4)
E_{max} sumatriptan	59 (8)	70 (15)

* $p < 0.05$, when compared to data in fresh vein

Analysis of data. All curves were analyzed by means of a computerized curve fitting technique (De Lean et al. 1978) to obtain E_{max} (maximal response) and pD_2 ($-\text{Log}$ of the molar concentration of an agonist eliciting half maximal effect) values, which were averaged for the respective agonists. For antagonists approximate pK_B values were calculated, assuming the nature of antagonism to be competitive, by the equation described by Furchgott (1972):

$$pK_B = -\text{Log}[B] + \text{Log}\{([A_2]/[A_1]) - 1\},$$

where [B] is the antagonist concentration and $[A_1]$ and $[A_2]$ are, respectively, the equi-effective molar concentrations of the agonist needed to elicit half maximal effect in the absence and presence of [B]. For calculation of pK_B values only paired experiments were taken into account, unless mentioned otherwise.

All data are presented as mean ± SEM. For correlation the Pearson correlation coefficient was calculated. To test the hypothesis of a straight line underlying the points in Fig. 5, lack of fit statistics (Kleinbaum et al. 1988) was performed using $\alpha = 0.05$ as a critical value. Student's *t*-test for unpaired data was used for comparison of mean values. A *p*-value of ≤ 0.05 was assumed to denote a significant difference.

Compounds. The following drugs were used in this study: 5-carboxamidotryptamine maleate (5-CT; gift: Glaxo, Ware, UK), cyanopindolol (gift: Sandoz AG, Basel, Switzerland), 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI; Research Biochemicals Inc., Natic, Ma, USA), flesinoxan hydrochloride (gift: Duphar B. V., Weesp, The Netherlands), 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT; gift: Merrel Dow Research Institute, Strassbourg, France), 5-hydroxytryptamine creatinine sulphate (5-HT; Sigma Chemical Co., St. Louis, Mo., USA), 3α-tropanyl-1H-indole-3-carboxylate (ICS 205-930; gift: Sandoz AG, Basel, Switzerland), ketanserin tartrate (gift: Janssen Pharmaceutica, Beerse, Belgium), metergoline (gift: Farmitalia, Milan, Italy), methiothepin maleate (gift: Hoffmann-La Roche B. V., Mijdrecht, The Netherlands), 5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole succinate (RU 24969; gift: Roussel Laboratories, Hoevelaken, The Netherlands), 2-methyl-5-hydroxytryptamine maleate (2-methyl-5-HT; gift: Glaxo, Ware, UK), α-methyl-5-hydroxytryptamine maleate (α-methyl-5-HT; gift: Glaxo, Ware, UK), rauwolscine hydrochloride (Fluka AG, Buchs, Switzerland) and sumatriptan (gift: Glaxo, Ware, UK). We appreciate the generosity of the companies in providing the compounds.

Table 3. Approximate pK_B values of various antagonists against the agonists indicated

Antagonist	Concentration (μmol/l)	*n*	Agonist	pK_B (± SEM)[a]
Ketanserin	1	5	5-HT	Not calculated[b]
	1	5	5-CT	Not calculated[b]
	1	3	α-Methyl-5-HT	7.1 (0.1)
	1	5	DOI	7.1 (0.3)
	1	5	2-Methyl-5-HT	Not calculated[c]
	1	8	Sumatriptan	No antagonism
Methiothepin	0.1	5*	5-HT	7.1 (0.4)
	0.1	6	5-CT	Not calculated[b]
	0.1	3	RU 24969	7.9 (0.8)
	0.1	5	2-Methyl-5-HT	7.3 (0.1)
	0.1	4	Sumatriptan	7.3 (0.1)
Methysergide	1	3	5-HT	Not calculated[c]
Rauwolscine	1	6	Sumatriptan	6.7 (0.2)
Metergoline	0.1	4	Sumatriptan	6.9 (0.3)
	1	4	Sumatriptan	7.3 (0.3)
Cyanopindolol	1	4	Sumatriptan	6.5 (0.3)
Flesinoxan	30	3	5-HT	5.3 (0.2)
ICS 205-930	1	3	5-HT	5.9 (0.1)
	1	5	2-Methyl-5-HT	No antagonism

[a] For calculation of pK_B only paired experiments were taken into account unless marked by *
[b] Not calculated due to biphasic nature of antagonism
[c] Not calculated because concentration-response curve did not reach a plateau
n, Number of observations

Results

Effect of agonists

The concentration-response curves obtained on the human isolated saphenous vein with the different agonists and the derived values of pD_2 and E_{max} are shown in Fig. 1 and Table 1, respectively. It is to be noted that contractions in the second 5-HT concentration-response curve were only slightly less than in the first 5-HT curve. All other agonists, except flesinoxan, were found to contract the saphenous vein in a concentration dependent manner, but only 5-CT and α-methyl-5-HT acted as a full

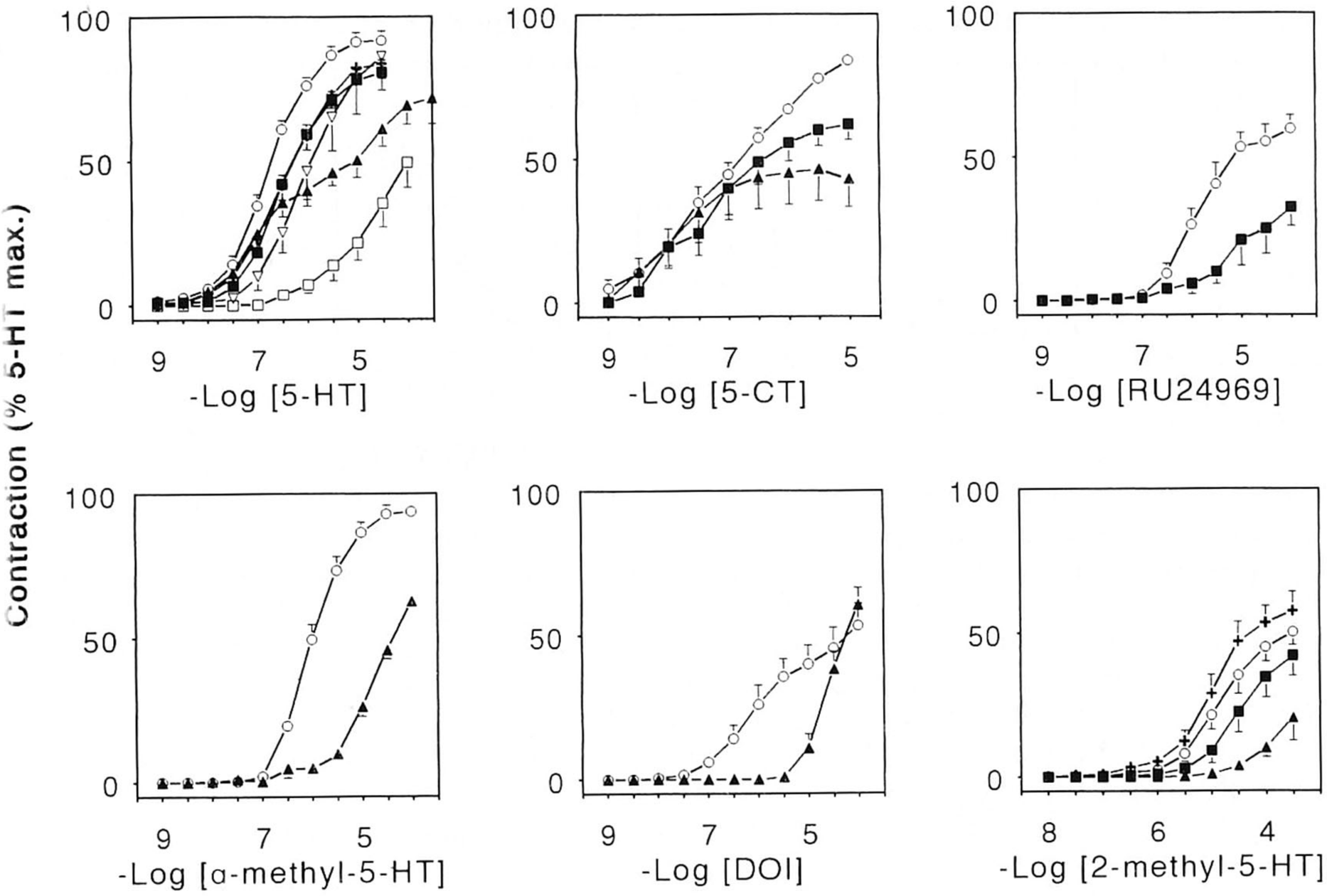

Fig. 3. Contractions of the human isolated saphenous vein to several 5-HT receptor agonists in the absence (control, ○) or presence of ketanserin (1 μmol/l, ▲), ICS 205-930 (1 μmol/l, +; hidden in part behind methiothepin curve), methiothepin (0.1 μmol/l, ■), methysergide (1 μmol/l, □) and/or flesinoxan (30 μmol/l, ▽). Agonist concentration refers to mol/l. pK_B values are mentioned in Table 3. Number of segments tested: 5-HT (control, 18; ICS 205-930, 5; flesinoxan, 4; ketanserin, 5; methiothepin, 5 and methysergide, 3), 5-CT control, 5; ketanserin, 5 and methiothepin, 6), RU 24969 (control, 5 and methiothepin, 5), α-methyl-5-HT (control, 3 and ketanserin, 3), DOI (control, 7 and ketanserin, 5) and 2-methyl-5-HT (control, 7; ICS 205-930, 5; ketanserin, 5 and methiothepin, 5). In cases where the number of experiments mentioned here does not correspond to the number mentioned in Table 3, unpaired experiments are displayed also

agonist. The rank order of potency was: 5-CT ≈ 5-HT > methysergide ≈ sumatriptan ≈ α-methyl-5-HT ≈ RU 24969 ≈ DOI > 2-methyl-5-HT > 8-OH-DPAT.

As shown in Table 2, the exposure to potassium (30 mmol/l) caused contractions of the saphenous veins that had been used as a graft, but the tension generated in these vessels was only about 10% of that observed in the non-grafted veins. Though the responses to both 5-HT and sumatriptan in the grafted vessels were also diminished, the respective E_{max} values, when expressed as a percentage of potassium (30 mmol/l)-induced contraction, as well as the pD_2 values were similar to those in the non-grafted veins (Table 2; Fig. 2).

Effect of antagonists

The effects of ketanserin (1 μmol/l), methiothepin (0.1 μmol/l), methysergide (1 μmol/l), flesinoxan (30 μmol/l) and ICS 205-930 (1 μmol/l) were studied against the responses to at least one of the following agonists: 5-HT, 5-CT, RU 24969, α-methyl-5-HT, DOI and 2-methyl-5-HT (Fig. 3). The calculated approximate pK_B values are shown in Table 3. The responses to 5-HT were antagonized by methysergide, ketanserin and methiothepin ($pK_B = 7.1 \pm 0.4$), but also to some extent by ICS 205-930 ($pK_B = 5.9 \pm 0.1$) and flesinoxan ($pK_B = 5.3 \pm 0.2$). The pK_B values for methysergide (concentration-response curve did not reach plateau) and ketanserin (biphasic concentration-response curve) were not calculated (see Table 3). The antagonism by ketanserin was more marked against contractions due to higher concentrations of 5-HT. The effects of low concentrations of 5-CT were little affected by ketanserin (1 μmol/l) or methiothepin (0.1 μmol/l), but those of high concentrations (≥0.1 μmol/l) were attenuated by both methiothepin and ketanserin. The contractile effects of RU 24969 were antagonized by methiothepin (0.1 μmol/l). Contractions to α-methyl-5-HT and DOI were attenuated by ketanserin (1 μmol/l), while those to 2-methyl-5-HT, being unaffected by ICS 205-930, were antagonized by methiothepin (0.1 μmol/l) and, even more potently, by ketanserin (1 μmol/l).

Figure 4 shows the effects of methiothepin (0.1 μmol/l), metergoline (0.1 and 1 μmol/l), ketanserin (1 μmol/l), cyanopindolol (1 μmol/l) and rauwolscine (1 μmol/l) on the responses to sumatriptan. The sumatriptan-induced contractions remained unaffected by ketanserin, but methiothepin, metergoline, cyanopindolol and rauwolscine behaved as antagonists (Fig. 4; Table 3).

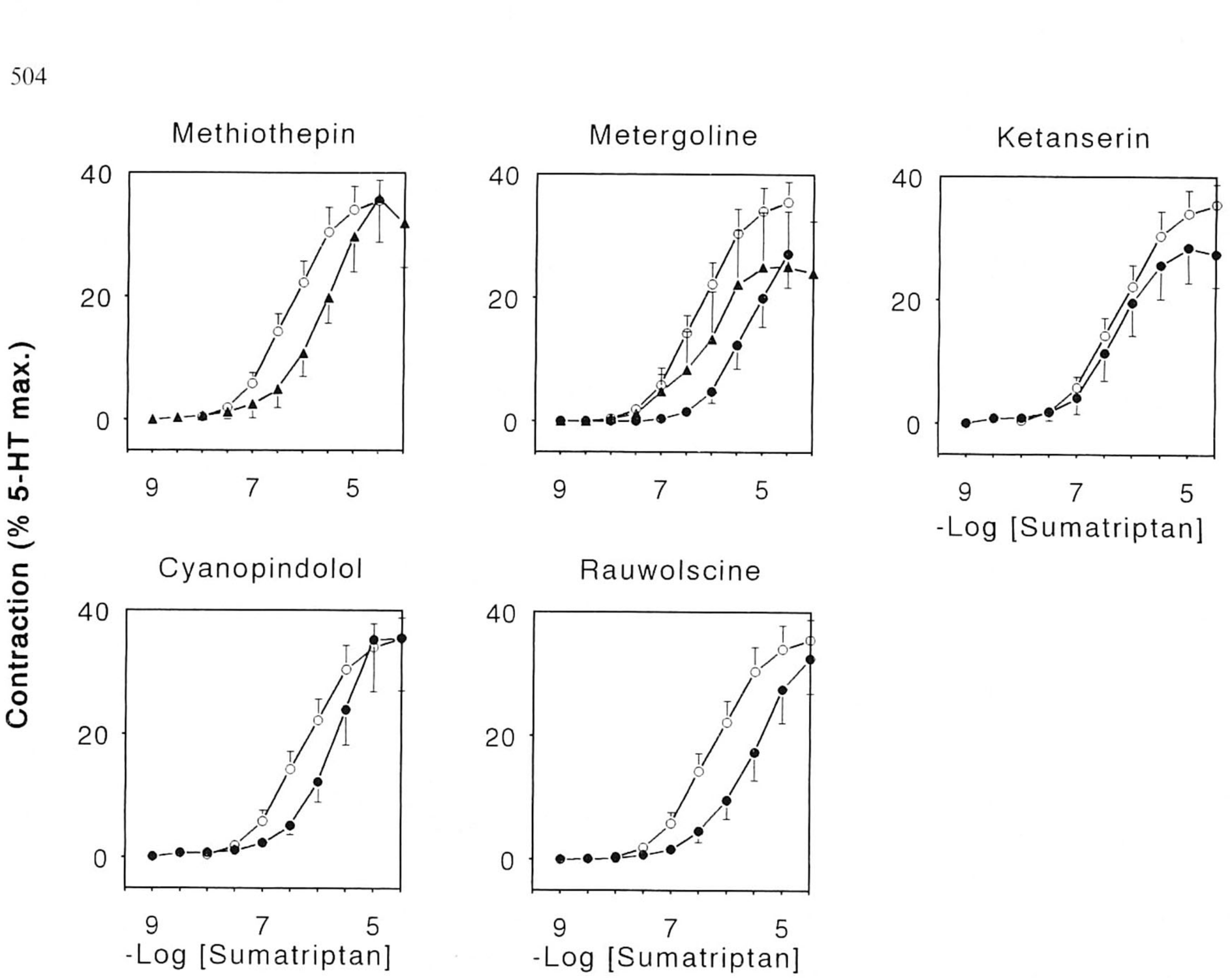

Fig. 4. Contractions of the human isolated saphenous vein to sumatriptan in the absence (control, ○) or presence of some antagonists (0.1 μmol/l, ▲ and 1 μmol/l, ●). Agonist concentration refers to mol/l. Number of segments tested: control, 18; methiothepin, 8; ketanserin, 8; metergoline (1 μmol/l), 7; metergoline (0.1 μmol/l), 6; cyanopindolol, 6 and rauwolscine, 8. pK_B values, calculated from paired experiments only, can be found in Table 3. In cases where the number of experiments mentioned here does not correspond to the number mentioned in Table 3, unpaired experiments are displayed also

Discussion

Previous studies (Docherty and Hyland 1986; Victorzon et al. 1986; Borton and Docherty 1988; Chester et al. 1990a; Docherty and Borton 1991) in the human isolated saphenous vein have revealed that 5-HT-induced contractions were: (i) partially antagonized by spiperone and ketanserin, both of which preferentially affected the contractions following high concentrations of 5-HT; and (ii) mimicked by 5-CT, sumatriptan, RU 24969, α-methyl-5-HT, 5-methoxytryptamine and 8-OH-DPAT. Since contractions to α-methyl-5-HT and high concentrations of 5-HT were antagonized by ketanserin and since the effect of sumatriptan was antagonized by methiothepin but not markedly by ketanserin, both 5-HT_1-like and 5-HT_2 receptors seem to be present in the human saphenous vein (Docherty and Hyland 1986; Borton and Docherty 1988; Chester et al. 1990a).

The frame-work of 5-HT receptor classification, originally proposed by Bradley et al. (1986), has now been expanded (Dumuis et al. 1988) and currently four main types of 5-HT receptors (5-HT_1-like, 5-HT_2, 5-HT_3 and 5-HT_4) are recognized (see Saxena and Villalón 1990). It is well established that the 5-HT_1-like receptor category is heterogeneous in nature (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D}) and even this subdivision seemingly does not completely correspond to the functional responses observed (Saxena and Ferrari 1989; Saxena and Villalón 1990; Van Heuven-Nolsen et al. 1991). It is with this background that we have attempted to further characterize the human saphenous vein 5-HT receptors by using a large number of 5-HT receptor agonists and antagonists.

Identification of the 5-HT receptors

The presence of a contractile 5-HT_1-like receptor in the human saphenous vein is revealed by the fact that: (i) 5-CT, sumatriptan, RU 24969 and 8-OH-DPAT, all of which have more or less selective agonist action at 5-HT_1-like receptors, elicited saphenous vein contractions; (ii) the contractions elicited by sumatriptan were resistant to blockade by ketanserin, but were antagonized by methiothepin and other compounds with affinities for 5-HT_1 binding sites (metergoline, cyanopindolol and rauwolscine); and (iii) methiothepin clearly antagonized the responses to RU 24969. It should, however, be noted that the putative 5-HT_{1A} receptor agonist flesinoxan (Wouters et al. 1988; Dreteler et al. 1990) did not behave as an agonist, but rather as a weak antagonist of the responses to 5-HT; such an antagonist effect of flesinoxan has been described at the 5-HT_1-like receptor in the rabbit saphenous vein (Martin and MacLennan 1990). Lastly, despite the above evidence for the presence of 5-HT_1-like

receptors in the saphenous vein, we have no adequate explanation why methiothepin, in contrast to its interaction with sumatriptan and RU 24969, did not cause a parallel shift of the lower part of the concentration-response curve of 5-CT. Possibly 5-CT acts as an agonist on other receptors than the 5-HT_1-like receptor present in this vessel; the antagonism by ketanserin of the upper part of the 5-CT-curve indicates that 5-CT activates 5-T_2 receptors, especially at high concentrations. This phenomenon has been observed before in the human coronary artery (Toda and Okamura 1990) and the rabbit aorta (Feniuk et al. 1985).

Our experiments also provide evidence in favour of 5-HT_2 receptor-mediated contractions of the human saphenous vein. Thus, the contractile effects of 5-HT were antagonized by ketanserin in a clearly biphasic manner, mainly affecting the higher concentrations of 5-HT. Moreover, the relatively selective 5-HT_2 receptor agonist DOI, as well as α-methyl-5-HT, caused concentration-dependent saphenous vein contractions that were also antagonized by ketanserin (Fig. 3). The calculated pK_B values (7.1 against both 5-HT_2 receptor agonists) are admittedly somewhat low compared to those in other tissues (Conti et al. 1990; Mylecharane 1991). It is, however, possible that the blocking activity of ketanserin is being underestimated because, certainly, α-methyl-5-HT has appreciable binding as well as functional affinity for 5-HT_1 receptor subtypes (Humphrey et al. 1990; Ismaiel et al. 1990; Mylecharane 1991). The presence of both 5-HT_1-like and 5-HT_2 receptors in the saphenous vein is further strengthened by the nature of the effects of methiothepin and methysergide; these mixed 5-HT_1-like and 5-HT_2 receptor antagonists shifted the concentration-response curve of 5-HT in a near parallel manner.

We would like to draw attention to three other findings in our present investigation. Firstly, as found earlier, particularly at 5-HT_1-like receptors (Saxena 1974; Saxena and Villalón 1990), methysergide had a partial agonist action weakly contracting the saphenous vein. Secondly, 2-methyl-5-HT contracted the human saphenous vein (Fig. 3). Since this compound is generally considered selective for the 5-HT_3 receptor (Bradley et al. 1986; Saxena and Villalón 1990), it may be argued that 5-HT_3 receptors also mediate contraction in this tissue. However, as also recently found in the rabbit isolated renal artery (Tadipatri and Saxena 1992), the contractions elicited by 2-methyl-5-HT were not attenuated (perhaps somewhat potentiated) by ICS 205-930. The low pD_2 value of 2-methyl-5-HT (4.8 ± 0.2) and the antagonism of its effects by methiothepin and, particularly, ketanserin strongly suggests that 2-methyl-5-HT contracted the human saphenous vein via 5-HT_2 receptors. However, in view of the tenfold higher affinity of 2-methyl-5-HT for the 5-HT_1 receptor subtypes than for the 5-HT_2 receptor (Hoyer 1989; Ismaiel et al. 1990), an additional involvement of 5-HT_1-like receptors can not be excluded. Thirdly, in the absence of 5-HT_3 receptors, the ability of ICS 205-930 (1 μmol/l) to weakly attenuate 5-HT-induced contractions may point to the existence of 5-HT_4 receptors in the human saphenous vein. On the other hand, though present in the brain (Dumuis et al. 1988), heart (Duncker et al. 1985; Kaumann et al. 1990; Villalón et al. 1990) and gastro-intestinal system (Clarke et al. 1989), the 5-HT_4 receptors have not yet been located in blood vessels.

Is the 5-HT_1-like receptor related to 5-HT_1 binding site subtypes?

Since receptor binding experiments clearly distinguish at least four 5-HT_1 binding site subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D}) (see Waeber et al. 1988; Hoyer 1989; Schoeffter and Hoyer 1989), we have compared agonist pD_2 and antagonist pK_B values obtained in the present experiments with the binding affinities at the above 5-HT_1 binding subtypes (Fig. 5). Admittedly, such a comparison is slightly hampered by the fact that we deal with two different receptor populations (5-HT_1-like and 5-HT_2) in the saphenous vein. In order to counter this potential disadvantage, we chose agonists (including 5-HT) with a relatively high affinity for 5-HT_1-like receptors and the pK_B values of antagonists against the selective 5-HT_1-like receptor agonist sumatriptan. The above comparison yielded the following correlation coefficients (r): 0.12, 0.74, 0.40, 0.97 and 0.98 for the agonists; and 0.65, 0.45, 0.21, 0.77 and 0.89 for the antagonists at, respectively, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, (calf caudate) and 5-HT_{1D} (human caudate) receptors. The correlation at the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} was not only lower than that at the 5-HT_{1D} receptors (both in the human and calf caudate membranes), but also a theoretical straight line connecting the correlation points (see Fig. 5) for these three 5-HT_1 binding subtypes (but not for agonists at 5-HT_{1D}) could be rejected ($\alpha = 0.05$). Therefore, the 5-HT_1-like receptor mediating contraction of the human isolated saphenous vein resembles the 5-HT_{1D} receptor subtype, a conclusion that is also supported by data reported earlier (Müller-Schweinitzer 1984; Docherty and Borton 1991). However, this receptor may not be entirely indentical to the 5-HT_{1D} receptor identified in the calf or human caudate membranes, since the order of binding affinity at the 5-HT_{1D} receptor site of methiothepin and metergoline is contradictory to the potency order of functional antagonism against sumatriptan. An analogue observation was made by Hamel and Bouchard (1991) for the 5-HT_{1D} receptor contracting the human isolated pial arteries. Indeed, Sumner and Humphrey (1989) have already proposed a subdivision for 5-HT_{1D} receptors, and the primary structure and function of the cloned 5-HT_{1D} receptor (Hamblin and Metcalf 1991) shows several marked discrepancies with the published 5-HT_{1D} receptor affinities (Waeber et al. 1988).

Comparison between and grafted saphenous veins

Apart from a recent solitary report on a 12-year old saphenous vein graft (Steen et al. 1991), we are not aware of any other pharmacologic investigations comparing such vessels. As also observed by us in the microscopic

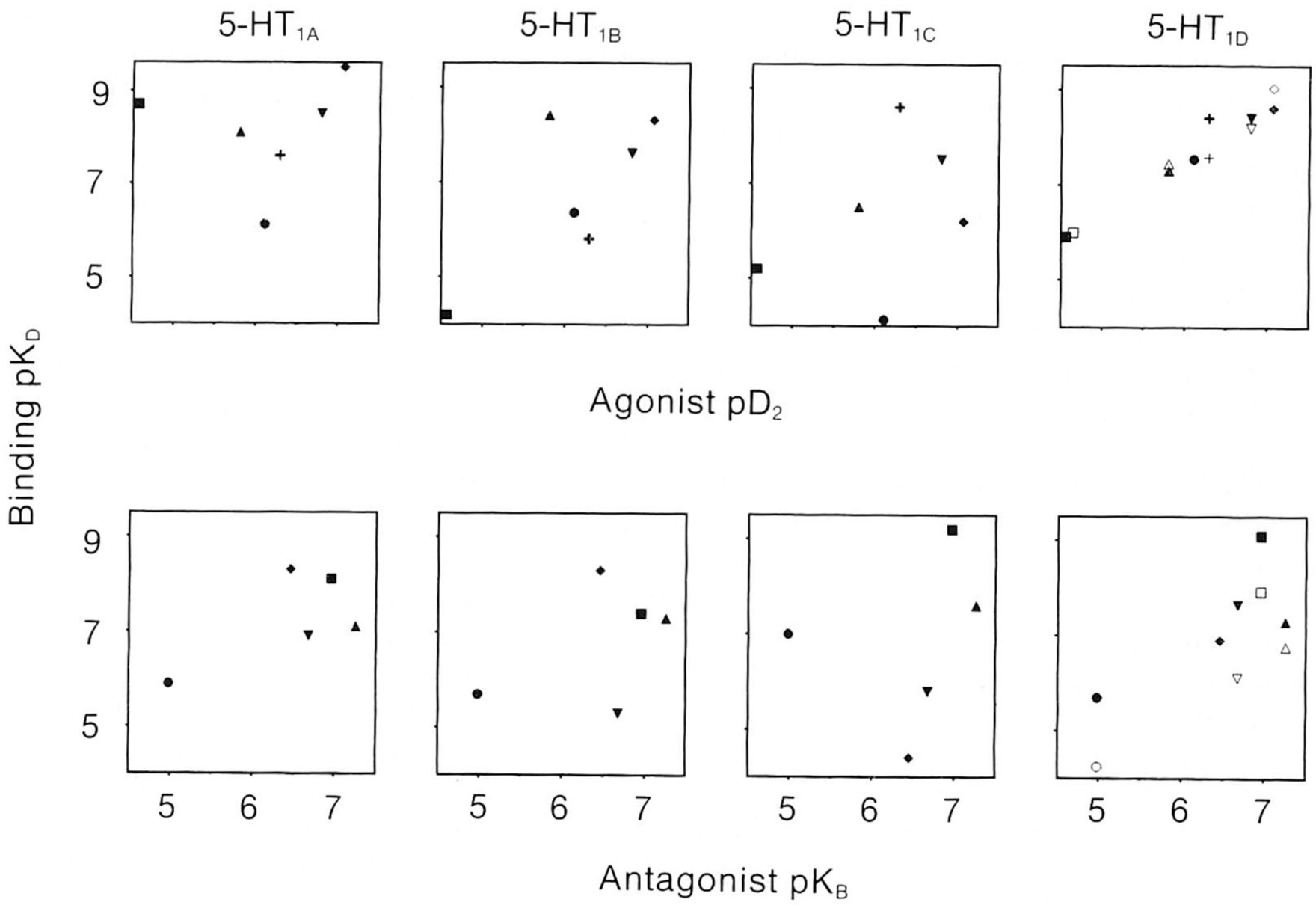

Fig. 5. Correlation between agonist pD_2 or antagonist pK_B (against sumatriptan) values obtained in our functional tests (Tables 1 and 3) and pK_D (expressed as $-$Log mol/l) values for 5-HT_1 binding site subtypes. All binding data are from Hoyer (1989), except those for sumatriptan (Schoeffter and Hoyer 1989) and for 5-HT_{1D} binding in the human caudate membrane (Waeber et al. 1988). Receptor binding was studied by the quoted authors in the following tissues: 5-HT_{1A}, pig frontal cortex; 5-HT_{1B}, rat frontal cortex; 5-HT_{1C}, pig choroid plexus; and 5-HT_{1D}, calf (*filled symbols*) or human (*open symbols*) caudate membranes. For ketanserin a pK_B value of 5.0 was calculated from four paired experiments. For metergoline a pA_2 of 7.0 was calculated using multiple regression analysis. Symbols for agonists: ▼, 5-HT; ◆, 5-CT; +, methysergide; ●, sumatriptan; ▲, RU 24969 and ■, 8-OH-DPAT. Symbols for antagonists; ■, metergoline; ▲, methiothepin; ▼, rauwolscine; ◆, cyanopindolol; ●, ketanserin. For correlation coefficients, see text

sections (results not shown), Steen et al. (1991) found a marked proliferation of intimal layers. Their pharmacological findings, restricted to α-adrenoceptors, indicated that such receptors mediating contraction of this grafted venous segment were 'atypical' in nature. However, with regard to 5-HT receptors, we observed no obvious change in the three grafted vessel segments studied by us; the pD_2 values as well as the maximal effects of 5-HT and sumatriptan, expressed as a percentage of potassium-induced contractions, were not different from those in the ungrafted saphenous veins. But, it appears that the general ability to contract, as indicated by the contractions to potassium (Table 2), was substantially less in the grafted vessels. In contrast, in animal experimental venous grafts, others have reported hyper-reactivity to 5-HT, which correlated with intimal thickening (Radic et al. 1991; Fann et al. 1990). However, it has to be kept in mind that we have only had the opportunity to study grafted saphenous veins from three patients, and that patients undergoing heart transplantation have apparently a more extensive pathological lesion than those undergoing coronary bypass surgery.

Comparison with 5-HT receptors in other human vessels

Extensive studies characterizing 5-HT receptors in the human basilar (Parsons et al. 1989) and pial (Hamel and Bouchard 1991) arteries have been performed. Some important differences between the results obtained here and those reported earlier may be pointed out. Firstly, in the basilar and pial arteries 5-HT-induced contractions were unaffected by ketanserin, indicating the absence of 5-HT_2 receptors. Secondly, unlike our experiments, 2-methyl-5-HT was ineffective in the basilar artery; however, despite the fact that contractile 5-HT_3 and 5-HT_2 receptors are absent, pial arteries did contract to 2-methyl-5-HT, presumably via a 5-HT_1-like mechanism. Thirdly, (±)-cyanopindolol did not affect sumatriptan-induced contractions in the basilar artery, but the concentrations used were ten times lower (0.1 μmol/l) than in our experiments. Lastly, the affinity for methiothepin was higher in the basilar (pK_B: 8.8) or pial (pA_2: 8.55) arteries than in our experiments (pK_B: approximately 7.1). It is possible that the 5-HT_1-like receptor subtype present in the saphenous vein may not be identical with that present in the basilar arteries. However, as in the present experiments, a high correlation was found between the functional pD_2 values in the pial arteries and the pK_D values for the 5-HT_{1D} binding sites in the human caudate membranes (Hamel and Bouchard 1991).

Like in the saphenous vein, both 5-HT_1-like and 5-HT_2 receptors have been reported in the human coronary artery and similar pD_2 values for sumatriptan, 5-HT and 5-CT have been found (Connor et al. 1989; Chester et al. 1990b; Toda and Okamura 1990). However, at present

not enough experimental data in human isolated coronary artery is available to make a valid comparison between the 5-HT_1-like receptor subtype contracting coronary artery and saphenous vein.

In conclusion, a mixed population of 5-HT_1-like and 5-HT_2 receptors is present in the human isolated saphenous vein. Low concentrations of 5-HT contract the vessel mainly via the 5-HT_1-like receptor, but at higher concentrations 5-HT_2 receptors are also recruited. The 5-HT_1-like receptor in the saphenous vein closely resembles the 5-HT_{1D} subtype. These results imply that, should 5-HT be involved in the pathophysiology, 5-HT_2 receptor antagonists, such as ketanserin, would not be entirely effective in preventing peri- or postoperative spasm of the saphenous vein graft. Since the human coronary vessels also contain 5-HT_1-like and 5-HT_2 receptors, the use of a mixed 5-HT_1-like/5-HT_2 receptor antagonist may well be more efficacious in ameliorating coronary artery or saphenous vein graft spasm.

Acknowledgements. This study was supported by the Netherlands Heart Foundation, grant 89.252. We gratefully acknowledge the inspiring cooperation with staff of the Thorax Centre in providing the tissue. We are also pleased to express our sincere thanks to Dr. W. Feniuk (Glaxo Group Research, Ware, UK) for critically reading the manuscript.

References

Bax WA, Van Heuven-Nolsen D, Bos E, Simoons ML, Saxena PR (1992) 5-HT_2 receptors and a receptor resembling the 5-HT_{1D} receptor subtype mediate contractions in human saphenous vein. Br J Pharmacol (in press)

Borton M, Docherty JR (1988) Actions of agonists at 5-hydroxytryptamine receptors in the human saphenous vein. Br J Pharmacol 93:266P

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

Chester AH, Bodelsson M, Arneklo-Nobin B, Tadjkarimi S, Tornebrandt K, Yacoub MH (1990a) Characterization of 5-HT receptors in the human saphenous vein, implications for the patency of bypass grafts. J Appl Cardiol 5:51–59

Chester AH, Martin GR, Bodelsson M, Arneklo-Nobin B, Tadjkarimi S, Tornebrandt K, Yacoub MH (1990b) 5-Hydroxytryptamine receptor profile in healthy and diseased human epicardial coronary arteries. Cardiovasc Res 24:932–937

Clarke DE, Craig DA, Fozard JR (1989) The 5-HT_4 receptor: Naughty but nice. Trends Pharmacol Sci 10:385–386

Connor HE, Feniuk W, Humphrey PPA (1989) 5-Hydroxytryptamine contracts human coronary artery predominantly via 5-HT_2 receptor activation. Eur J Pharmacol 161:91–94

Conti A, Monopoli A, Forlani A, Ongini E, Antona C, Biglioli P (1990) Role of 5-HT_2 receptors in serotonin-induced contraction in the human mammary artery. Eur J Pharmacol 176:207–212

De Lean A, Munson PJ, Rodbard D (1978) Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and physiological dose-response curves. Am J Physiol 235:E97–E102

Docherty JR, Borton M (1991) Further investigations of contractile responses to 5-HT in the human saphenous vein. Br J Pharmacol 104:106P

Docherty JR, Hyland L (1986) An examination of 5-hydroxytryptamine receptors in human saphenous vein. Br J Pharmacol 89:77–81

Dreteler GH, Wouters W, Saxena PR (1990) Comparison of the cardiovascular effects of the 5-HT_{1A} receptor agonist flesinoxan with that of 8-OH-DPAT in the rat. Eur J Pharmacol 180:339–349

Dumuis A, Bouhelal R, Sebben M, Bockaert J (1988) A 5-HT receptor in the central nervous system, positively coupled with adenyl cyclase, is antagonized by ICS 205-930. Eur J Pharmacol 146:187–188

Duncker DJ, Saxena PR, Verdouw PD (1985) 5-Hydroxytryptamine causes tachycardia in pigs by acting on receptors unrelated to 5-HT_1, 5-HT_2 or M type. Br J Pharmacol 86:596P

Fann JI, Sokoloff MH, Sarris GE, Yun KL, Kosek JC, Miller DC (1990) The reversibility of canine vein-graft arterialization. Circulation 82 [Suppl IV]: IV-9–IV-18

Feniuk W, Humphrey PPA, Perren MJ, Watts AD (1985) A comparison of 5-hydroxytryptamine receptors mediating contraction in rabbit aorta and dog saphenous vein: evidence for different receptor types obtained by use of selective agonists and antagonists. Br J Pharmacol 86:697–704

Furchgott RF (1972) The classification of adrenoceptors. An evaluation from the standpoint of receptor theory. In: Blaschko H, Muscholl E (eds) Handbook of experimental pharmacology, vol 33. Catecholamines. Springer, Berlin Heidelberg New York, pp 283–335

Hamblin MW, Metcalf MA (1991) Primary structure and function characterization of a human 5-HT_{1D}-type serotonin receptor. Mol Pharmacol 40:143–148

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

Hoyer D (1989) 5-hydroxytryptamine receptors and effector coupling mechanisms in peripheral tissues. In: Fozard JR (ed) The peripheral actions of 5-hydroxytryptamine. Oxford University Press, Oxford, pp 73–99

Humphrey PPA, Feniuk W, Perren MJ (1990) 5-HT in migraine: evidence from 5-HT_1-like receptor agonists for a vascular aetiology. In: Sandler M, Collins G (eds) Migraine: a spectrum of ideas. Oxford Medical Publications, Oxford, pp 147–168

Ismaiel AM, Titeler M, Miller KJ, Smiths TS, Glennon RA (1990) 5-HT_1 and 5-HT_2 binding pofiles of the serotonergic agents α-methyl-serotinin and 2-methyl-serotonin. J Med Chem 33: 755–758

Kaumann AJ, Sanders L, Brown AM, Murray KJ, Brown MJ (1990) A 5-hydroxytryptamine receptor in human atrium. Br J Pharmacol 100:879–885

Kleinbaum DG, Kupper LL, Muller KE (1988) Applied regression analysis and other multivariable methods. PWS-Kent, Boston, pp 237–240

Loop FD, Lytle BW, Cosgrove DM, Stewart RW, Goormastic M, Williams GW, Golding LAR, Gill CC, Taylor PC, Sheldon WC, Proudfit WL (1986) Influence of the internal-mammary-artery graft on 10-year survival and other cardiac events. N Engl J Med 314:1–6

Lüscher TF, Diederich D, Siebenmann R, Lehmann K, Stulz P, von Segesser L, Yang Z, Turina M, Grädel E, Weber E, Bühler FR (1988) Difference between endothelium-dependent relaxation in arterial and venous coronary bypass grafts. N Engl J Med 319:462–467

Martin GR, MacLennan SJ (1990) Analysis of the 5-HT receptor in rabbit saphenous vein exemplifies the problem of using exclusion criteria for receptor classification. Naunyn-Schmiedeberg's Arch Pharmacol 342:111–119

Müller-Schweinitzer E (1984) Alpha-adrenoceptors, 5-hydroxytryptamine receptors and the action of dihydroergotamine in human venous preparations obtained during saphenectomy procedure for varicose veins. Naunyn-Schmiedeberg's Arch Pharmacol 327:299–303

Mylecharane EJ (1991) 5-HT_2 receptor antagonists and migraine therapy. J Neurol 238:S45–S52

Parsons AA, Whalley ET, Feniuk W, Connor HE, Humphrey PPA (1989) 5-HT_1-like receptors mediate 5-hydroxytryptamine-induced contraction of human isolated basilar artery. Br J Pharmacol 96:434–440

Radic ZS, O'Donohoe MK, Schwartz LB, Stein AD, Mikat EM, McCann RL, Hagen PO (1991) Alterations in serotonergic receptor expression in experimental vein grafts. J Vasc Surg 14:40–47

Saxena PR (1974) Selective vasoconstriction in carotid vascular bed by methysergide: Possible relevance to its antimigraine action. Eur J Pharmacol 27:99–105

Saxena PR, Ferrari MD (1989) 5-HT_1-like receptor agonists and the pathophysiology of migraine. Trends Pharmacol Sci 10:200–204

Saxena PR, Villalón CM (1990) Cardiovascular effects of serotonin agonists and antagonists. J Cardiovasc Pharmacol 15 [Suppl 7]: S17–S34

Schoeffter P, Hoyer D (1989) How selective is GR 43175? Interactions with functional 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptors. Naunyn-Schmiedeberg's Arch Pharmacol 340:135–138

Steen S, Willén R, Sjöberg T, Carlén B (1991) Contractile and morphologic properties of a saphenous vein after 12 years as an aortocoronary bypass graft. Blood Vessels 28:349–353

Sumner MJ, Humphrey PPA (1989) Heterogeneous 5-HT_{1D} binding sites in porcine brain can be differentiated by GR 43175. Br J Pharmacol 98:29–31

Tadipatri S, Saxena PR (1992) Is 2-methyl-5-HT a selective 5-HT_3 receptor agonist? Br J Pharmacol (in press)

Toda N, Okamura T (1990) Comparison of the response to 5-carboxamidotryptamine and serotonin in isolated human, monkey and dog coronary arteries. J Pharmacol Exp Ther 253:676–682

Van Heuven-Nolsen D, Villalón CM, Den Boer MO, Saxena PR (1991) 5-HT_1-like receptors unrelated to the known binding sites? In: Fozard JR, Saxena PR (eds) Serotonin: Molecular biology, receptors and functional effects. Birkhäuser, Basel, pp 192–200

Victorzon M, Tapparelli C; Müller-Schweinitzer E (1986) Comparison of the actions of serotonergic agents on human saphenous vein and platelets. Eur J Pharmacol 124:107–111

Villalón CM, Den Boer MO, Heiligers JPC, Saxena PR (1990) Mediation of 5-hydroxytryptamine-induced tachycardia in the pig by the putative 5-HT_4 receptor. Br J Pharmacol 100:665–668

Waeber C, Schoeffter P, Palacios JM, Hoyer D (1988) Molecular pharmacology of 5-HT_{1D} recognition sites: radioligand binding studies in human, pig and calf membranes. Naunyn-Schmiedeberg's Arch Pharmacol 337:595–601

Wouters W, Hartog J, Bevan P (1988) Flesinoxan. Cardiovasc Drug Rev 6:71–83

Yang Z, Stulz P, Von Segesser L, Bauer E, Turina M, Lüscher TF (1991) Different interactions of platelets with arterial and venous coronary bypass vessels. Lancet 337:939–943

Yang Z, Diederich D, Schneider K, Siebenmann R, Stulz P, Von Segesser L, Turina M, Bühler FR, Lüscher TF (1989) Endothelium-derived relaxing factor and protection against contractions induced by histamine and serotonin in the human internal mammary artery and in the saphenous vein. Circulation 80:1041–1048