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*

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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 ≈ 2-methyl-5-HT ≈ 5-methoxy-3-(1,2,3,6-tetrahydroprydin-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 2-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 cyano-pindolol (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_{1D}-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 blood membranes. Such a correlation for the antagonism of sumatriptan-induced responses was less marked than for the agonists, but of the 5-HT_{1D}-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_{1D}-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 preconditioned 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-

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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. pD₂ is expressed as −log (EC₅₀) and E_max is expressed as a percentage of the maximal 5-HT contraction in the first concentration-response curve

<table>
<thead>
<tr>
<th>Agonist</th>
<th>n</th>
<th>pD₂ (± SEM)</th>
<th>E_max (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-CT</td>
<td>5</td>
<td>7.1 (0.2)</td>
<td>89 (4)</td>
</tr>
<tr>
<td>5-HT, first</td>
<td>18</td>
<td>7.0 (0.1)</td>
<td>100 (0)</td>
</tr>
<tr>
<td>5-HT, second</td>
<td>18</td>
<td>6.8 (0.1)</td>
<td>93 (3)</td>
</tr>
<tr>
<td>Methysergide</td>
<td>4</td>
<td>6.3 (0.4)</td>
<td>16 (3)</td>
</tr>
<tr>
<td>Sumatriptan</td>
<td>18</td>
<td>6.1 (0.1)</td>
<td>37 (4)</td>
</tr>
<tr>
<td>α-Methyl 5-HT</td>
<td>3</td>
<td>6.0 (0.1)</td>
<td>94 (3)</td>
</tr>
<tr>
<td>RU 24969</td>
<td>5</td>
<td>5.8 (0.2)</td>
<td>62 (6)</td>
</tr>
<tr>
<td>DOI</td>
<td>8</td>
<td>5.7 (0.3)</td>
<td>57 (7)</td>
</tr>
<tr>
<td>2-Methyl 5-HT</td>
<td>7</td>
<td>4.8 (0.2)</td>
<td>49 (5)</td>
</tr>
<tr>
<td>8-OH-DPAT*</td>
<td>8</td>
<td>&lt;4.6 (0.2)</td>
<td>&gt;52 (10)</td>
</tr>
<tr>
<td>Flesinoxan</td>
<td>3</td>
<td>inactive</td>
<td>inactive</td>
</tr>
</tbody>
</table>

* 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% O₂ and 5% CO₂ 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 agonist 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 pD₂ 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.
**Table 2.** Comparison of the effect (developed tension in g) of potassium (30 mmol/l), and the pD₂ (expressed as $-\log [E_{50}]$) and $E_{\text{max}}$ (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 ($n = 3$)

<table>
<thead>
<tr>
<th>Ungrafted vein (± SEM)</th>
<th>Bypass graft (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effect 30 mmol/l potassium</strong></td>
<td></td>
</tr>
<tr>
<td>pD₂ 5-HT</td>
<td>7.0 (0.1)</td>
</tr>
<tr>
<td>$E_{\text{max}}$ 5-HT</td>
<td>181 (13)</td>
</tr>
<tr>
<td>pD₂ sumatriptan</td>
<td>6.1 (0.1)</td>
</tr>
<tr>
<td>$E_{\text{max}}$ sumatriptan</td>
<td>59 (8)</td>
</tr>
</tbody>
</table>

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

**Analysis of data.** All curves were analyzed by means of a computerized curve fitting technique (De Lain et al. 1978) to obtain $E_{\text{max}}$ (maximal response) and pD₂ ($-\log$ of the molar concentration of an agonist eliciting half maximal effect values), which were averaged for the respective agonists. For antagonists approximate pKₐ values were calculated, assuming the nature of antagonism to be competitive, by the equation described by Furchgott (1972):

$$pK_a = -\log [B] + \log (|A_2|/|A_1|)-1,$$

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 pKₐ 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 means values. A $p$-value of $\leq 0.05$ 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), cyanoindolol (gift: Sandoz AG, Basel, Switzerland), 1-(2,5-dimethoxy-4-iodophenyl)-2-amino propane 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), 3,4-tropanyl-1H-indole-3-carboxylate (ICS 205-930; gift: Sandoz AG, Basel, Switzerland), ketanserin tartrate (gift: Janssen Pharmaceutica, Beerse, Belgium), metergoline (gift: Farmalat, Milan, Italy), methiothepin maleate (gift: Hoffmann-La Roche B. V., Mijdrecht, The Netherlands), 5-methoxy-3-(1,2,3,6-tetrahydro pyridin-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), 8-methyl-5-hydroxytryptamine maleate (8-methyl-5-HT; gift: Glaxo, Ware, UK), flesinoxan 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 pKₐ values of various antagonists against the agonists indicated

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Concentration (µmol/l)</th>
<th>n</th>
<th>Agonist</th>
<th>pKₐ (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketanserin</td>
<td>1</td>
<td>5</td>
<td>5-HT</td>
<td>Not calculated$^b$</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5</td>
<td>5-CT</td>
<td>Not calculated$^b$</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>2-Methyl-5-HT</td>
<td>7.1 (0.1)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5</td>
<td>DOI</td>
<td>7.1 (0.3)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5</td>
<td>2-Methyl-5-HT</td>
<td>Not calculated$^c$</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8</td>
<td>Sumatriptan</td>
<td>No antagonism</td>
</tr>
<tr>
<td>Methiothepin</td>
<td>0.1</td>
<td>5*</td>
<td>5-HT</td>
<td>7.1 (0.4)</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>6</td>
<td>5-CT</td>
<td>Not calculated$^b$</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>3</td>
<td>RU 24969</td>
<td>7.9 (0.8)</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>5</td>
<td>2-Methyl-5-HT</td>
<td>7.3 (0.1)</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>4</td>
<td>Sumatriptan</td>
<td>7.3 (0.1)</td>
</tr>
<tr>
<td>Methysergide</td>
<td>1</td>
<td>3</td>
<td>5-HT</td>
<td>Not calculated$^c$</td>
</tr>
<tr>
<td>Rauwolscine</td>
<td>1</td>
<td>6</td>
<td>Sumatriptan</td>
<td>6.7 (0.2)</td>
</tr>
<tr>
<td>Metergoline</td>
<td>0.1</td>
<td>4</td>
<td>Sumatriptan</td>
<td>6.9 (0.3)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4</td>
<td>Sumatriptan</td>
<td>7.3 (0.3)</td>
</tr>
<tr>
<td>Cyanopindolol</td>
<td>1</td>
<td>4</td>
<td>Sumatriptan</td>
<td>6.5 (0.3)</td>
</tr>
<tr>
<td>Flesinoxan</td>
<td>30</td>
<td>3</td>
<td>5-HT</td>
<td>5.3 (0.2)</td>
</tr>
<tr>
<td>ICS 205-930</td>
<td>1</td>
<td>3</td>
<td>5-HT</td>
<td>5.9 (0.1)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5</td>
<td>2-Methyl-5-HT</td>
<td>No antagonism</td>
</tr>
</tbody>
</table>

$^a$ For calculation of pKₐ 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 pD₂ and $E_{\text{max}}$ 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 2-methyl-5-HT acted as a full
agonist. The rank order of potency was: 5-CT ≈ 5-HT > methysergide ≈ sumatriptan ≈ z-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 pD2 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, z-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 ± 0.4), but also to some extent by ICS 205-930 (pK_B = 5.9 ± 0.1) and flesinoxan (pK_B = 5.3 ± 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 z-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).
**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 spiropene and ketanserin, both of which preferentially affected the contractions following high concentrations of 5-HT; and (ii) mimicked by 5-CT, sumatriptan, RU 24969, \( \alpha \)-methyl-5-HT, 5-methoxytryptamine and 8-OH-DPAT. Since contractions to \( \alpha \)-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 (methiothepin, 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-HT₁₄-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-T₃ 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-HT₃ 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-HT₂ 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 pKᵦ values (7.1 against both 5-HT₂ 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-HT₁ receptor subtypes (Humphrey et al. 1990; Ismaiel et al. 1990; Mylecharane 1991). The presence of both 5-HT₁-like and 5-HT₂ receptors in the saphenous vein is further strengthened by the nature of the effects of methiothepin and methysergide; these mixed 5-HT₁-like and 5-HT₂ 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-HT₁-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-HT₃ receptor (Bradley et al. 1986; Saxena and Villalón 1990), it may be argued that 5-HT₃ 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 pD₂ 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-HT₃ receptors. However, in view of the tenfold higher affinity of 2-methyl-5-HT for the 5-HT₁ receptor subtypes than for the 5-HT₃ receptor (Hoyer 1989; Ismaiel et al. 1990), an additional involvement of 5-HT₁-like receptors can not be excluded. Thirdly, in the absence of 5-HT₁ receptors, the ability of ICS 205-930 (1 μmol/l) to weakly attenuate 5-HT-induced contractions may point to the existence of 5-HT₄ 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-HT₄ receptors have not yet been located in blood vessels.

Is the 5-HT₁-like receptor related to 5-HT₁ binding site subtypes?

Since receptor binding experiments clearly distinguish at least four 5-HT₁ binding site subtypes (5-HT₁A, 5-HT₁B, 5-HT₁C and 5-HT₁D) (see Waebier et al. 1988; Hoyer 1989; Schoeffter and Hoyer 1989), we have compared agonist pD₂ and antagonist pKᵦ values obtained in the present experiments with the binding affinities at the above 5-HT₁ binding subtypes (Fig. 5). Admittedly, such a comparison is slightly hampered by the fact that we deal with two different receptor populations (5-HT₁-like and 5-HT₂), with the saphenous vein. In order to counter this potential disadvantage, we chose agonists (including 5-HT) with a relatively high affinity for 5-HT₁-like receptors and the pKᵦ values of antagonists against the selective 5-HT₁-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-HT₁A, 5-HT₁B, 5-HT₁C, 5-HT₁D (calf cua date) and 5-HT₁D (human cua date) receptors. The correlation at the 5-HT₁A, 5-HT₁B and 5-HT₁C was not only lower than that at the 5-HT₁D receptors (both in the human and calf cua date membranes), but also a theoretical straight line connecting the correlation points (see Fig. 5) for these three 5-HT₁ binding subtypes (but not for agonists at 5-HT₁D) could be rejected (z = 0.05). Therefore, the 5-HT₁-like receptor mediating contraction of the human isolated saphenous vein resembles the 5-HT₁D 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 identical to the 5-HT₁D receptor identified in the calf or human cua date membranes, since the order of binding affinity at the 5-HT₁D 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-HT₁D receptor contracting the human isolated pial arteries. Indeed, Sumner and Humphrey (1989) have already proposed a subdivision for 5-HT₁D receptors, and the primary structure and function of the cloned 5-HT₁D receptor (Hamblin and Metcalf 1991) shows several marked discrepancies with the published 5-HT₁D receptor affinities (Waebier 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
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 pD₂ 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-HT₂ receptors. Secondly, unlike our experiments, 2-methyl-5-HT was ineffective in the basilar artery; however, despite the fact that contractile 5-HT₁ and 5-HT₃ receptors are absent, pial arteries did contract to 2-methyl-5-HT, presumably via a 5-HT₁-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 (pKᵦ: 8.8) or pial (pA₂: 8.55) arteries than in our experiments (pKᵦ: approximately 7.1). It is possible that the 5-HT₁-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 pD₂ values in the pial arteries and the pKᵦ values for the 5-HT₁D binding sites in the human caudate membranes (Hamel and Bouchard 1991).

Like in the saphenous vein, both 5-HT₁-like and 5-HT₃ receptors have been reported in the human coronary artery and similar pD₂ 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-HT₄-like receptor subtype contracting coronary artery and saphenous vein.

In conclusion, a mixed population of 5-HT₄-like and 5-HT₂ receptors is present in the human isolated saphenous vein. Low concentrations of 5-HT contract the vessel mainly via the 5-HT₁-like receptor, but at higher concentrations 5-HT₂ receptors are also recruited. The 5-HT₁-like receptor in the saphenous vein closely resembles the 5-HT₁₃ subtype. These results imply that, should 5-HT be involved in the pathophysiology, 5-HT₂ 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-HT₁-like and 5-HT₂ receptors, the use of a mixed 5-HT₁-like/5-HT₂ receptor antagonist may well be more efficacious in ameliorating coronary artery or saphenous vein graft spasm.

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References


Duncker DJ, Saxena PR, Verdouw PD (1985) 5-Hydroxytryptamine causes tachycardia in pigs by acting on receptors unrelated to 5-HT₁, 5-HT₅ or M type. Br J Pharmacol 86:596P


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
