Expression of serotonin receptor mRNAs in blood vessels

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Abstract **Using RT-PCR we distinguished mRNAs for all known G-protein coupled serotonin receptors expressed in various rat and porcine blood vessels. Nearly all vessels expressed $5HT1_{D\beta}$, 5-HT_{2A}, 5-HT_{2B}, 5-HT_4, and 5-HT_7 receptor mRNA to different extents. New splice variants of the porcine 5-HT_4 receptor were observed. Similar PCR assays were performed with endothelial and smooth muscle cells from human pulmonary artery, aorta, and with endothelial cells from human coronary artery and umbilical vein. All endothelial cells expressed 5-$HT_{1D\beta}$, 5-HT_{2B}, and 5-HT_4 receptor mRNA, whereas in smooth muscle cells 5-$HT_{1D\beta}$, 5-HT_{2A}, 5-HT_7, and in some experiments 5-HT_{2B} receptor mRNA were found. A model for the regulation of vascular tone by different 5-HT receptors is proposed.**

Key words: Endothelium; Nitric oxide; 5-HT receptor; 5-HT_4 receptor; Smooth muscle

1. Introduction

It has been established that serotonin stimulates both, contraction and relaxation of blood vessels [1,2,3]. Extensive efforts have been made to unravel the molecular identity of the serotonin receptors involved in the regulation of blood vessel tone and the biochemical mechanisms by which they exert their functions.

Thirteen different mammalian G-protein coupled 5-HT receptor types have been identified by molecular cloning, which have been grouped into seven families [4]. The five 5-HT_1 receptor subtypes, 5-HT_{1A} to 5-HT_{1F}, inhibit adenylate cyclase activity after expression in mammalian cell lines (the former 5-HT_{1C} receptor is now named 5-HT_{2C}, the 5-HT_{1B} receptor is the rodent analogue of the 5-$HT_{1D\beta}$ receptor found in other species). The three 5-HT_2 receptor subtypes, 5-HT_{2A} to 5-HT_{2C}, stimulate the hydrolysis of phosphatidylinositol. 5-HT_4, 5-HT_6, and 5-HT_7 receptors enhance adenylate cyclase activity. No functional coupling has yet been described for the 5-$HT_{5A/B}$ receptor subtypes [5]. 5-HT_3 receptors, which are receptor–channel complexes, may also represent a heterogenous group [6].

Using organ bath experiments, it has previously been demonstrated that 5-HT_{2C}-like and 5-HT_1-like receptors induce vessel relaxation in an endothelium-dependent fashion [7–11]. Other 5-HT_1-like receptors were reported to trigger relaxation of blood vessels independent of the presence of an intact endothelium [12,13]. Smooth muscle contraction is induced independently of the endothelium by activation of 5-HT_{2A} receptors [1,3]. In addition, 5-HT_1-like receptors with a pharmacological profile resembling that of 5-HT_{1B}/5-$HT_{1D\alpha}$/5-$HT_{1D\beta}$ receptors have been shown to induce smooth muscle contraction [14]. It is clear that serotonin regulates vasoconstriction and vasorelaxation in a complex way which involves the interaction of several serotonin receptor subtypes with conflicting functional effects.

The rapid cloning of new 5-HT receptors has not been matched by the discovery of subtype-selective ligands. Quite often, receptor ligands that were considered to be selective for a given subtype turned out to have high affinity for one of the more recently discovered 5-HT receptors. In addition, the characterization of the receptor subtypes involved was hampered by species dependent receptor pharmacology for the functional assays. In the absence of highly specific receptor ligands, it has become much more difficult to characterize pharmacological responses as being mediated by a certain 5-HT receptor subtype.

Using RT-PCR we have identified the 5-HT receptor mRNAs expressed in whole blood vessels and in various endothelial and smooth muscle cells by amplification and sequencing of their cDNAs. The function of most of these defined receptors in vascular cells is then discussed in the context of the numerous previous publications dealing with functional analyses.

2. Materials and methods

2.1. Oligonucleotides

The following oligonucleotides (ON) were used as primers for RT-PCR with the respective accession number and position of the PCR product in the coding sequence in brackets: rat 5-HT_{1A} (J05276, position 4–202) ON 1: GAT GTG TTC AGT TTT GGC CAG G, ON 2: GGA GCG CTC CAA GGC GAT GGC A; human 5-HT_{1A} (M83181, position (−133)–197) ON 3: CCT GCT TGG GTC TCT GCA TTC C, ON 4: GGA GCG CTC CAA GGG GAT GGC A; rat 5-HT_{1B} (M89954, position 98–325, also used for porcine tissue) ON 5: ACT ACA TTT ACC AGG ACT CCA T, ON 6: CAG TGA CCG TGT ACA TGG TGC; human 5-$HT_{1D\beta}$ (M75128, position 140–742) ON 7: CCT GGA AAG T(AC)C TGC TGG T, ON 8: CGG TC(CT) TGT TGG G(TC)G TCT GT; rat 5-HT_{1D} (M89953, position 262–702) ON 9: TCA TGC CCA TCA GCA T(CA)G CC, ON 10: CTT CCC (AG)TA GAG (TG)GA GGG TG; human 5-$HT_{1D\alpha}$ (M81589, position 272–711) ON 11: TCA TGC CCA TCA GCA T(AC)G CC, ON 12: CTT CCC (AG)TA GAG (TG)GA GGG TG; human 5-HT_{1E} (Z11166, position 134–629, also used for porcine tissue) ON 13: TGG CTA TTG GCA CCA CCA AGA A, ON 14: TTG GCC GCG TGG TAA ATC CG; rat/human 5-HT_{1F} (L05596, position 138–503) ON 15: TGC (AG)AT (TC)AT TGT GAC (CT)CG GA, ON 16: C(GT)G CTA (TG)TT CCT TGG TGC CTC; rat 5-HT_{2A} (M36966, position 336–600) ON 17: CAT CCT GTA TGG GTA CCG GT, ON 18: AAA GAC CTT CGA ATC ATC CTG; porcine 5-HT_{2A} (position 349–600 in rat sequence) ON 19: TAC CGG TGG CCT TTG CCT AG, ON 18; human 5-HT_{2A} (M36966, position 560–837) ON 20: ACT CCA GAA CTA AGG CAT TT, ON 21: AGC TAA TTT GGC CCG TGT GCC; rat 5-HT_{2B} (X66842, position 349–571, also used for porcine tissue) ON 22: AGG CTA CAT GGC CCC TCC CAC T, ON 23: TAG GGA CTG GGA TGG CGA TG; human 5-HT_{2B} (X77307, position 404–835) ON

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24: ACG TTC TCT TTT CAA CCG CA, ON 25: CCG GTG ACG AGC AAG GTG TT; rat 5-HT_{2C} (M21410, position 567–917) ON 26: TAT CCC TGT GAT TGG ACT GAG, ON 27: GTT GAT AGC (CT)TG CAT GGT GC; human 5-HT_{2C} (M81778, position 323–611) ON 28: CCC TGT CTC TCC TGG CAA TC, ON 29: TTG TTC ACG AAC ACC TTT TC; rat 5-HT_4 degenerate cloning oligonucleotides (position 244–827) ON 30: GT(GC) CAG GAC ATC TGG TTC TA(TC) GG(CT) GA(GA) ATG TT, ON 31: AA(AG) AA(GC) GGC GCC CAG CAG AAA CAG AAG CA(TG) CCC AT; rat 5-HT_4 specific primers (position 346–743) ON 32: CTG GAT AGG TAT TAT GCC ATC T, ON 33: GTG CTG TGC TGG TCA GCT GTC T; ON 32, ON 31 were used for porcine tissue and are positioned in the rat sequence 346–827; rat 5-HT_4 degenerate oligonucleotides for cloning the human 5-HT_4 receptor (position 304–827) ON 34: CTA CTC ACC AC(ACG) GC(AC) TC(ACG) ATT TT, ON 31; human 5-HT_4 specific primers (length of PCR-product 397 bp) ON 35: CTG GAT AGG TAT TAC GCC ATC T, ON 36: GTG CTG TGC TGG TCA GCT GTC T; rat 5-$HT_{5A/B}$ (L10072/L10073, position A306/B346-A662/B702) ON 37: CTG GGC G(CA)C G(CT) T GGC AGC T, ON 38: TCC AGT A(CG)A C(AG)A AGA GCA CCA C; human 5-HT_{5A} (X81482, position 306–662) ON 37, ON 38; rat 5-HT_6 (L19656, position 135–418) ON 39: GCT GCT GAT CGT GCT CAT TTG CA, ON 40: CTG TCA TGC GCA GCT TGT AGC GCA; human 5-HT_6 (Z49119, length of PCR-product 200bp) ON 41: CTG CCC GCA AGC AGG CCG TGC A, ON 42: GTC ACA AAG AAC ATG CCC AGC; rat 5-HT_7 (L19654, position 713–1044, also used for porcine tissue) ON 43: AGG ATT TTG GCT ACA CGA TC, ON 44: GAG GAA AAA CGG CAG CCA GCA; human 5-HT_7 (L21195, position 704–1035) ON 45: AGG ACT TTG GCT ATA CGA TT, ON 46: GAG GAA AAA TGG CAG CCA G; human von Willebrand factor (X04385, position 2522–2859) ON 47: AGT GAA GAT TGG CTG CAA CAC T, ON 48: CAA AGT GAG TCT CAT CCT TCA T; human endothelial NO-synthase (M93718, position 1424–1992) ON 49: TTC CAT CAG GAG ATG GTC AAC TA, ON 50: GAG CAA AGG CGC AGA AGT GGG GGT A. Oligonucleotides were synthesized on an Applied Biosystems 380A synthesizer and purified by gel filtration through Sephadex G-25 columns (Boehringer).

2.2. Tissues, cell culture, RNA-preparation and RT-PCR

RNA was isolated according to the guanidinium method [15]. Blood vessels were carefully isolated and cleaned of adhering parenchyma and connective tissue. The tissue was homogenized in RNA-buffer using a POLYTRON homogenizer. Human primary cells (pulmonary artery endothelial (HUPAEC) and smooth muscle cells (HUPASMC), aortic endothelial (HUAEC) and smooth muscle cells (HUASMC), coronary artery (HUCAEC) and umbilical vein endothelial cells (HUVEC)) and culture media were purchased from Clonetics (San Diego). Cells were grown according to the supplier's instructions and used for up to four passages. Cells (1×10^6) were washed twice with PBS, scraped off, spun for 15 s in a minifuge and dissolved in RNA-buffer. Residual DNA was digested with 10 U DNAse (Boehringer) per 10 μg RNA for 20 min at 37°C. For the RT-PCR, 1 μg of each RNA was reverse transcribed in a final volume of 20 μl using M-MLV reverse transcriptase (BRL) in PCR-Buffer (Boehringer), 200 μM dNTP (Cetus), 10 mM DTT, 5 μM hexanucleotides (Pharmacia), and 40 U RNasin (Promega) for 1 h at 37°C. 38 PCR cycles (55°C for 30 s, 72°C for 1 min, 94°C for 30 s) were performed in a final volume of 50 μl with 10% of the reverse transcription (RT) mixture, 10 pmol of each primer, 1 U Taq-DNA-polymerase (Boehringer), 250 μM dNTP and 2 μCi [α-^{32}P]dCTP. The ^{32}P-labeled PCR-products were separated on 4% agarose gels (NuSieve, FML). The gels were dried and exposed to X-ray films.

2.3. Direct PCR-sequencing

1 μl of all PCR-products was re-amplified in a second reaction, separated on a 4% agarose gel and the bands were extracted with the *QUIAEX* gel extraction kit (Quiagen). Approximately 100 ng DNA was used as template for the direct PCR-sequencing reaction. Dideoxy sequencing of both strands was performed using a Hot Tub DNA sequencing system (Amersham) or an automated sequencer (Applied Biosystems 373A). The DNASIS program (Hitachi) was used for sequence analyses.

2.4. 5-HT_4 receptor cloning

A set of degenerate primers was designed based on the published 5-HT_4 receptor amino acid sequence [16]. ON-30 (aa82–aa92) was located in transmembrane domain III, ON 31 in transmembrane region VI (aa265–aa275). PCR reactions were performed using rat colliculi cDNA under standard conditions except that the annealing temperature was lowered to 50°C. The PCR product was sequenced as described above. From the resulting nucleotide sequence, the specific primers ON 32 and ON 33 for the rat 5-HT_4 receptor were designed. Human and porcine 5-HT_4 receptor sequences were obtained by performing PCR with the primers ON 34/ON 31 and ON 32/ON 31, respectively, using human or porcine striatum cDNA as template. For the analysis of receptor expression, ON 32 and ON 31 were used for pig, ON 35 and ON 36 for human RNAs.

To clone the porcine 5-HT_4 splice variants, RT-PCR products from porcine coronary artery RNA were further amplified using kinased ON 32 and ON 31. Overhangs were filled with 2 U Klenow enzyme in 6 mM $MgCl_2$ for 20 min at 37°C. Products were isolated by gel electrophoresis, ligated into pBSKSII and sequenced using standard methods.

3. Results

3.1. Analysis of serotonin receptor expression in blood vessels by RT-PCR

Based on published serotonin receptor sequences we have designed PCR primers which distinguish all known G-protein coupled serotonin receptors. The functionality and specificity of the primer pairs were controlled by RT-PCRs with RNA from tissues known to express the receptors or, in cases where introns are absent, with genomic DNA (data not shown). PCR primer pairs were either species-specific or, where possible, selected such that they worked with cDNAs from several species (rat, mouse and human). All PCR products have been characterized by direct DNA sequencing.

3.2. Rat blood vessels

Using RT-PCR we analyzed mRNAs for the serotonin receptor subtypes expressed in various rat blood vessels (Fig. 1). Contamination with genomic DNA was excluded in all RNA preparations by performing the same RT-PCR procedure without adding reverse transcriptase (Figs. 1 and 3). Four different 5-HT receptor mRNAs, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_7 were found (Fig. 1A). The relative abundance of these mRNAs varied for different vessel types. None of the other 5-HT receptor types analyzed (5-HT_{1A}, 5-HT_{1D}, 5-HT_{1F}, 5-HT_{2C}, 5-HT_{5A},

Table 1
Percent homology of porcine 5-HT receptor partial nucleotide sequences with the rat and human homologs

Porcine	5-$HT_{1D\beta}$ (Z47984)[a]	5-HT_{1E} (Z48151)[a]	5-HT_{2A} (Z48152)[a]	5-HT_{2B} (Z48174)[a]	5-HT_4 (4A:Z48175)[a] (4B:Z48176)[a]	5-HT_7 (Z48177)[a]
Human	87.0 ($70.8_{1D\alpha}$)[b]	99.8	96.8	95.2	88.7 (Z48150)[a]	89.5
Rat	84.9 (71.2_{1D})[b]	–	88.0	87.1	85.4 (Z48153)[a]	88.4

[a] EMBL data library accession number.
[b] Sequence homology to the closely related 5-$HT_{1D\alpha/1D}$ receptor.

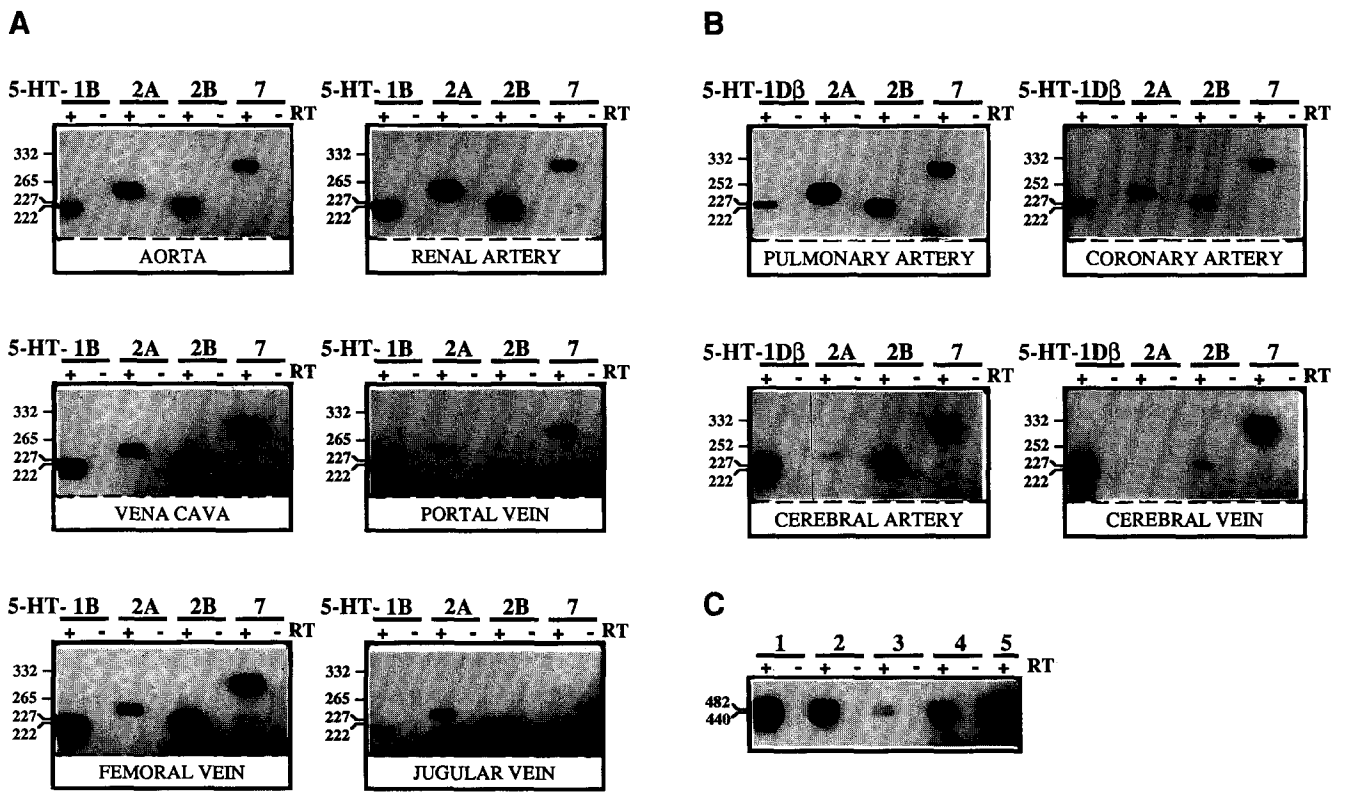

Fig. 1. Agarose gel electrophoresis of PCR-amplified products derived from cDNA of various rat (A) and porcine (B) blood vessels. As control for the absence of DNA contaminations, all RT-PCRs were performed with or without reverse transcriptase (+ or − RT) added to the cDNA synthesis reaction. (A) For rat tissues the following primers were used (size of the PCR-products in brackets): 5-HT_{1B}: ON5/ON6 (227 bp), 5-HT_{2A}: ON17/ON18 (265 bp), 5-HT_{2B}: ON22/ON23 (222 bp), 5-HT_7: ON43/ON44 (332 bp). (B) For porcine vessels: 5-HT_{1B}: ON5/ON6 (227 bp), 5-HT_{2A}: ON19/ON18 (252 bp), 5-HT_{2B}: ON22/23 (222 bp), 5-HT_7: ON43/44 (332 bp). (C) Porcine 5-HT_4 receptor primers ON32/ON31 gave rise to PCR-products of 440 bp and 482 bp, respectively. RNA was isolated from coronary artery (lane 1), cerebral artery (lane 2), pulmonary artery (lane 3), cerebral vein (lane 4). Lane 5 shows 5-HT_4 receptor mRNA expression in porcine colliculi as positive control.

5-HT_{5B}, and 5-HT_6) was expressed in vascular tissue (data not shown).

No nucleotide sequence had become available for the pharmacologically well-defined 5-HT_4 receptor. Using degenerate oligonucleotides which were based on the recently described rat protein sequence [16] we amplified and cloned a partial 5-HT_4 receptor cDNA sequence from rat colliculi. Subsequently, the corresponding cDNAs were also amplified from human and porcine striatum (Fig. 2, Table 1). In RT-PCR experiments no 5-HT_4 receptor mRNA was found in rat blood vessels (data not shown).

3.3. Porcine blood vessels

Multiple sets of PCR primers were designed based on the known mouse, rat and human sequences. Amplification of cDNAs from porcine pulmonary artery allowed the elucidation of partial sequences for the porcine homologues of those 5-HT receptors which we found to be expressed in rat blood vessels (5-$HT_{1D\beta}$, 5-HT_{2A}, 5-HT_{2B} and 5-HT_7). Since only human, no rodent, 5-HT_{1E} receptor sequences had been described, we also cloned and sequenced PCR products that were derived from porcine genomic DNA with primers specific for the human 5-HT_{1E} receptor. All porcine sequences showed very high homology to their rat and human equivalents (Table 1).

RT-PCRs with RNA isolated from various porcine blood vessels revealed that the same set of serotonin receptors that was observed in the rat was also present in the pig (Fig. 1B). No expression was detectable for the 5-HT_{1E} receptor mRNA (data not shown). The relative abundance of all mRNAs found was variable. While we did not find 5-HT_4 receptor mRNA in rat blood vessels, it was clearly expressed in vascular tissues from the pig (Fig. 1C). Moreover, all porcine blood vessels contained two splice variants of the 5-HT_4 receptor (called 5-HT_{4A} and 5-HT_{4B}). The larger 482 bp PCR product representing the 5-HT_{4B} splice form was not present in porcine colliculi (Fig. 1C, lane 5), human striatum or human endothelial cells (Fig. 3B). Subcloning, sequencing and alignment to the rat and human 5-HT_4 receptor sequences revealed that the 5-HT_{4B} isoform harbors an insertion of 14 amino acids compared to the porcine 5-HT_{4A} receptor. Fig. 2 shows an alignment of the partial porcine, human and rat 5-HT_4 receptor sequences including the new 5-HT_{4B} splice-variant found in porcine blood vessels.

3.4. 5-HT receptor expression in primary human endothelial and smooth muscle cells

To localize the cellular distribution of the 5-HT receptors found in whole blood vessels, we used primary cultures of human endothelial and smooth muscle cells from several different vessels. RT-PCRs were performed with RNAs isolated

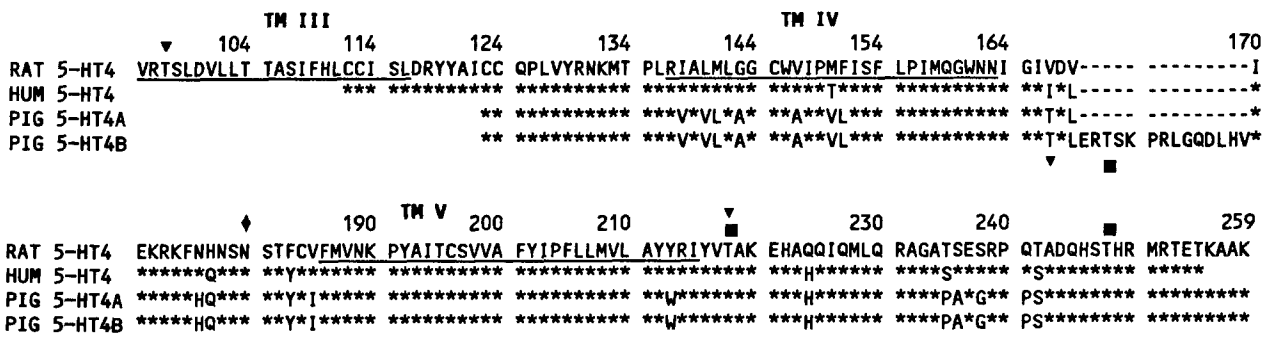

Fig. 2. Alignment of partial rat, human and porcine 5-HT_4 receptor sequences. Amino acid identities are illustrated by asterisks. Bars represent the putative membrane spanning regions. Recognition motifs for protein kinase C ■, casein kinase II ▼ and N-glycosylation ♦ are indicated. The nucleic acid sequences have been deposited at the EMBL data library. For accession numbers see Table 1.

from these cell cultures as described above. Primers were designed based on published human receptor sequences. We have deposited a partial human 5-HT_6 receptor sequence in the EMBO data base (accession number Z49119).

In cultured endothelial cells from pulmonary and coronary artery, umbilical vein and aorta, we found expression of 5-$HT_{1D\beta}$ and 5-HT_{2B} receptor mRNAs (Fig. 3A). Furthermore, there was weak expression of 5-HT_4 receptor mRNA in these cells (Fig. 3B). Although this expression was low compared to the abundance of the mRNA in human striatum (Fig. 3B, lane C), it was clearly detectable while it was totally missing in smooth muscle cells. In RNA isolated from one preparation of umbilical vein endothelial cells, minute amounts of 5-HT_7 receptor mRNA were also detected (Fig. 3A). Smooth muscle cells expressed a mixture of 5-$HT_{1D\beta}$, 5-HT_{2A}, 5-HT_7, and in some preparations small amounts of 5-HT_{2B} receptor mRNA (Fig. 3C). To ensure that the smooth muscle cultures were not cross-contaminated with endothelial cells, we performed RT-PCRs with primers for von Willebrand factor and endothelial nitric oxide synthase, specific markers for endothelial cells [17,18] (Fig. 3D). These cultures did not contain contaminating endothelial cells since both RNAs were not detectable. Conversely, the endothelial cell cultures should not be significantly contaminated with smooth muscle cells, since they do not contain 5-HT_{2A} receptor mRNA, which we found to be expressed in smooth muscle cells.

Table 2 represents a summary of all mRNA expression data.

4. Discussion

The molecular analysis of 5-HT receptor mRNA expression in vascular tissues revealed that only five of the 13 known G-protein coupled 5-HT receptor mRNAs are expressed in blood vessels (5-$HT_{1D\beta}$, 5-HT_{2A}, 5-HT_{2B}, 5-HT_4 and 5-HT_7). In agreement with this, 5-$HT_{1D\beta}$ mRNA had previously been found in bovine and human cerebral arteries [19]. 5-HT_{2A} receptor mRNA was shown to be expressed in rat aortic smooth muscle cells [20] and 5-HT_7 mRNA in human coronary artery [21]. No expression in blood vessels had before been shown for the 5-HT_{2B} and 5-HT_4 receptor mRNAs.

In addition, 5-HT receptor mRNA expression in primary human endothelial and smooth muscle cells was investigated. The same set of receptors that was observed in rat and porcine vessels was also found in these cells. The use of primary cells should minimize the risk that receptors expression changes as a consequence of maintaining cells in culture for extended periods of time. In whole human vessels, for instance the internal carotid and middle meningeal artery, we also found the same

Table 2
Serotonin receptor mRNAs expressed in rat and porcine blood vessels and human endothelial or smooth muscle cell cultures

Receptor type 5-HT		1A	1B/Dβ	1D/Dα	1E	1F	2A	2B	2C	4	5A/B	6	7
Rat	aorta	–	+	–	n.d.	–	+++	++	–	–	–	–	+
	renal artery	–	++	–	n.d.	–	+++	+++	–	–	–	–	+
	vena cava	–	+++	–	n.d.	–	++	++	–	–	–	–	+++
	portal vein	–	+++	–	n.d.	–	+	–	–	–	–	–	+
	femoral vein	–	+++	–	n.d.	–	+	++	–	–	–	–	++
	jugular vein	–	+	–	n.d.	–	++	+++	–	–	–	–	–
Pig	pulmonary artery	n.d.	+	n.d.	–	n.d.	+++	++	n.d.	+	n.d.	n.d.	++
	coronary artery	n.d.	++	n.d.	–	n.d.	+++	++	n.d.	+++	n.d.	n.d.	++
	cerebral artery	n.d.	+++	n.d.	–	n.d.	+	++	n.d.	++	n.d.	n.d.	++
	cerebral vein	n.d.	+++	n.d.	–	n.d.	–	+	n.d.	++	n.d.	n.d.	++
Human	HUVEC	–	+++	–	–	–	–	+++	–	+	–	–	+
	CAEC	–	+++	–	–	–	–	+	–	+	–	–	–
	PAEC	–	+++	–	–	–	–	+++	–	+	–	–	–
	AEC	–	+++	–	–	–	–	+++	–	+	–	–	–
	PASMC	–	++	–	–	–	+++	+	–	–	–	–	++
	ASMC	–	++	–	–	–	+++	+	–	–	–	–	+++

A semiquantitative evaluation of receptor mRNA levels (+, ++, +++) is based on a comparison of PCR signal densities. Receptors are marked – when no PCR signals were detected and n.d. when not determined.

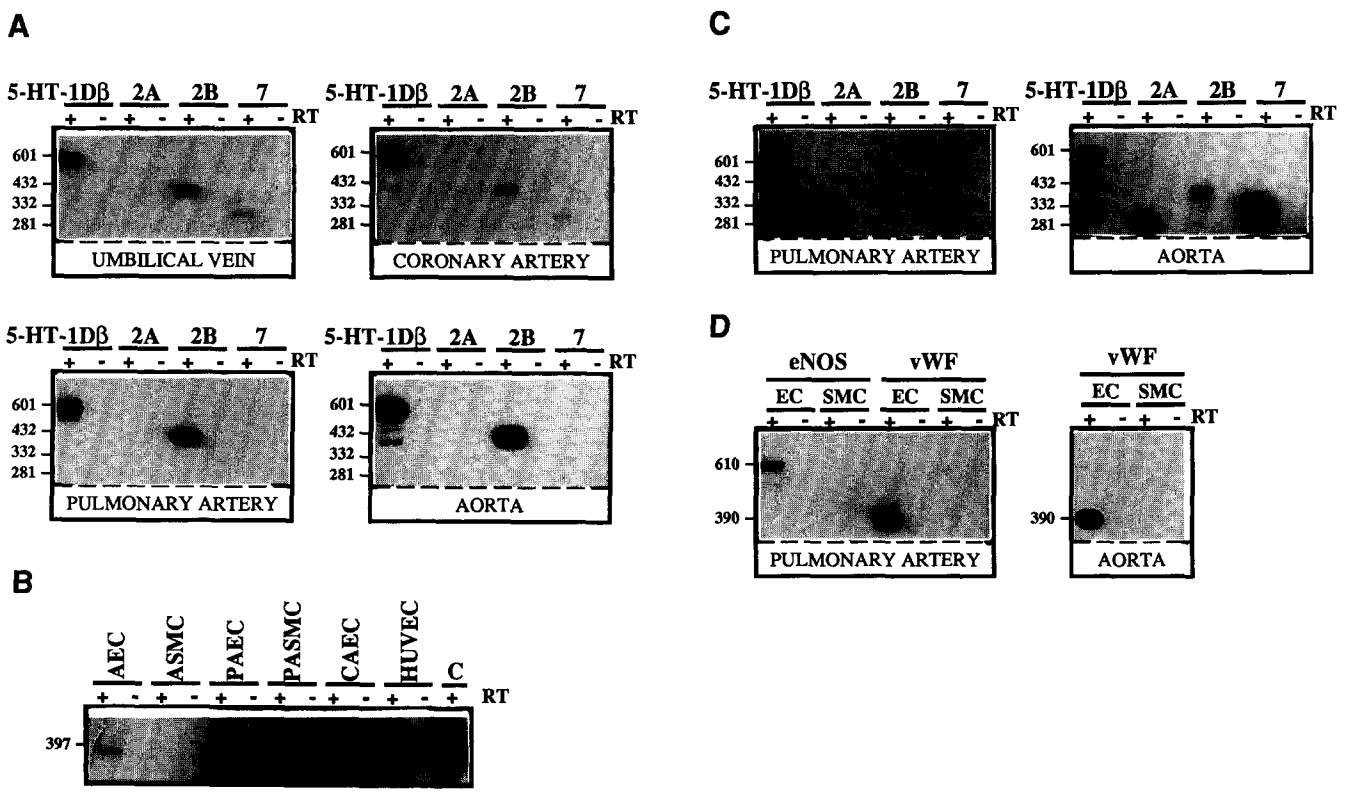

Fig. 3. Agarose gel electrophoresis of PCR-amplified products derived from cDNA of primary cultures of various human endothelial cells (HUVEC, human umbilical vein endothelial cells; CAEC, coronary artery endothelial cells; PAEC, pulmonary artery endothelial cells; AEC, aortic endothelial cells) and smooth muscle cells (ASMC, aortic smooth muscle cells; PASMC, pulmonary artery smooth muscle cells). As control for the absence of DNA contaminations, all RT-PCRs were performed with or without reverse transcriptase (+ or − RT) added to the cDNA synthesis reaction. (A) For human primary endothelial cells the following primers were used (respective size of the PCR-product in brackets): 5-$HT_{1D\beta}$ ON7/ON8 (601 bp), 5-HT_{2A} ON20/21 (281 bp), 5-HT_{2B} ON24/ON25 (432 bp), 5-HT_7 ON45/ON46 (332 bp). Occasionally, the 5-$HT_{1D\beta}$ primers used produced additional minor bands. DNA sequencing revealed that those were unrelated to the receptor, representing a type of artifact often seen in PCR experiments. (B) cDNA of human primary endothelial and smooth muscle cells was amplified with the human 5-HT_4 receptor primer-pair ON35/ON36 (397 bp). (C) For human primary smooth muscle cells the same human primers were used as in (A). (D) To test the purity of the cell cultures, cDNAs of human primary endothelial (EC) and smooth muscle cells (SMC) were analyzed for expression of the endothelial nitric oxide synthase (eNOS) using the primers ON49/ON50 (610 bp), and von Willebrand factor using the primers ON47/48 (390 bp).

receptor subtypes expressed, which is in agreement with the data obtained using cultured human cells (unpublished). The cellular distribution of receptor expression may be confirmed by in situ hybridization techniques.

Fig. 4 schematically summarizes the results of our study and combines them with previously published information about physiological responses and second messenger systems activated by 5-HT receptors [3]. It can be concluded that activation of 5-HT_{2B} receptors expressed on the endothelium relaxes the vessels. The pharmacological profile of the 5-HT_{2B} receptor fits best to the endothelial receptor, which has been classified as an 'atypical 5-HT' [3], 5-HT_{2C} [11], or 5-HT_{2C}-like receptor [10]. The latter publication shows that the endothelium-dependent relaxation of the pulmonary artery can be blocked by L-arginine, indicating that activation of the 5-HT_{2B} receptor stimulates nitric oxide release, which elicits a strong vasorelaxant activity [22] through activation of soluble guanylate cyclase [23]. Previously, we have shown that cloned 5-HT_{2B} receptors activate calcium-dependent chloride channels when expressed in Xenopus oocytes [24] and stimulate phosphoinositol hydrolysis in HEK 293 cells [25]. Therefore, we assume that NO is released from endothelial cells after the activation of the calcium-calmodulin-dependent endothelial NO synthase.

The other receptor mRNA found in relatively large quantities in endothelial cells encodes the 5-$HT_{1D\beta}$ receptor. 5-HT_1-like receptors mediating endothelium-dependent relaxation with pharmacological properties most closely related to those of 5-HT_{1D} receptors were first described in the coronary artery [26,8]. Although 5-$HT_{1D\beta}$ receptors are expressed in these cells, it should be noted, that the pharmacology of this relaxation was similar but not identical to that of the cloned 5-$HT_{1D\alpha}$ or 5-$HT_{1D\beta}$ receptors. Based on experiments performed with the cloned heterologously expressed 5-$HT_{1D\beta}$ receptors [27] and with human endothelial cells from the umbilical vein [28], the 5-$HT_{1D\beta}$ receptor inhibits cAMP formation. The endothelium dependent relaxation had previously been shown to be sensitive to pertussis toxin [29], which is in agreement with a G_i-protein mediated inhibition of adenylate cyclase. Therefore, it can be concluded that the 5-$HT_{1D\beta}$ receptor contributes to vessel relaxation. It remains to be determined whether an interaction between several 5-HT receptor subtypes (f.i. 5-$HT_{1D\beta}$ and 5-HT_{2B} receptors) is responsible for the somewhat unusual pharmacology of this relaxing response. The available pharmacological data do not exclude such an interaction since the pharmacology of 5-$HT_{1D\beta}$ and 5-HT_{2B} receptors is similar [30].

5-HT_4 receptors are potentially expressed on endothelial cells

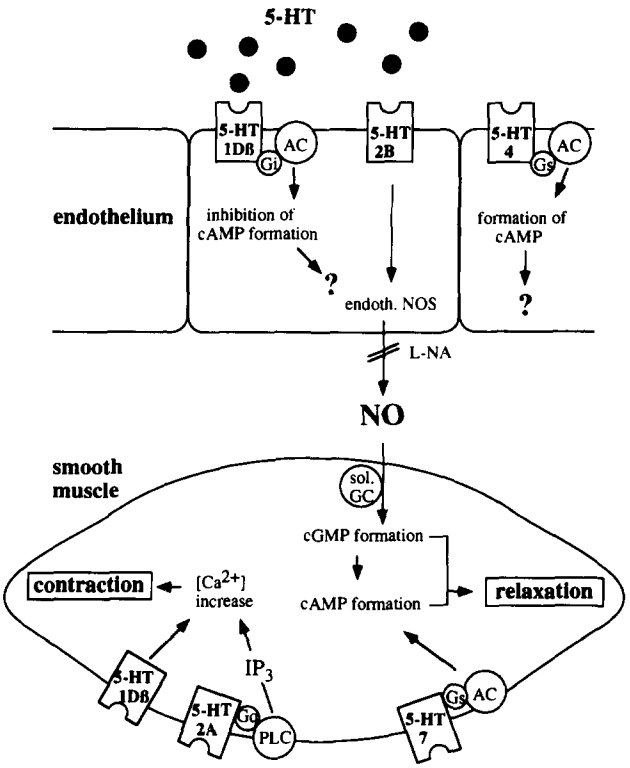

Fig. 4. Schematic model of vascular serotonin receptors.

based on the presence of small amounts of their mRNA. The positive coupling of this receptor type to adenylate cyclase observed in other tissues [31] renders it possible that it counteracts the effects of 5-$HT_{1D\beta}$ receptors in endothelial cells. Interesting are the striking differences in receptor mRNA levels and splice forms between rat, pig and human. Further research is required before the functional role of this receptor and its splice forms can be discussed.

A strong constrictor activity in blood vessels is exerted by 5-HT_{2A} receptors located on smooth muscle cells. Our PCR analysis is in agreement with multiple reports in the literature [3]. Activation of 5-HT_{2A} receptors in smooth muscle cells triggers intracellular calcium release after stimulating phosphatidylinositol hydrolysis [32]. The functional role of calcium in muscle contraction is well documented [33].

It has previously been assumed that an additional contractile response to serotonin may be mediated by the functional correlate of one of the central 5-HT_{1D} receptors [34,3]. Kaumann et al. [14] came to the conclusion that this endothelium independent response in porcine coronary arteries displays 5-$HT_{1D\beta}$ receptor pharmacology. This is confirmed by our finding that the receptor subtype is indeed expressed in vascular smooth muscle cells. In agreement with this, it was shown that these 5-HT_1-like receptors link to pertussis toxin-sensitive inhibition of cAMP accumulation and increases in intracellular Ca^{2+} in smooth muscle cells from bovine basilar artery [35].

Small amounts of 5-HT_{2B} mRNA were present in smooth muscle cells isolated from human pulmonary artery and aorta. This receptor elicits strong contractile responses of rat stomach fundus smooth muscle [36]. A role in vascular smooth muscle cells has not yet been found. We also detected mRNA for 5-HT_7 receptors in smooth muscle cells. 5-HT_1-like receptors mediating direct vasorelaxation in the absence of endothelium have been described in several blood vessels from various species [3]. The pharmacology of these responses is consistent with the conclusion that they are mediated by 5-HT_7 receptors. Heterologously expressed 5-HT_7 receptors were shown to stimulate adenylate cyclase activity [22]. An increase of cAMP in smooth muscle cells inhibits myosin light chain kinase, which would result in direct relaxation [37].

These data facilitate the interpretation of the many functional organ bath experiments described in the literature and provide a basis for the resolution of questions such as the unusual pharmacological properties of the endothelium dependent 5-$HT_{1D\beta}$ relaxation.

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