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Characterisation of 5-HT receptors in human coronary arteries by molecular and pharmacological techniques

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Abstract

5-Hydroxytryptamine (5-HT) can produce both vasoconstrictor and vasorelaxant effects in human coronary arteries and the response to 5-HT can be influenced by the presence of disease. The aim of the present study was to elucidate the 5-HT receptor subtypes responsible for mediating 5-HT-evoked contraction of human coronary arteries using pharmacological, molecular and immunocytochemical approaches. Normal human coronary arteries, with intact endothelium, were mounted in tissue baths, and the vascular responses to 5-HT and 5-HT receptor agonists were studied. The effects of 5-HT_1 and 5-HT_2 receptor antagonists on these responses were also studied. Expression of messenger ribonucleic acid (mRNA) encoding different 5-HT receptors in human coronary arteries, atrium, ventricle wall and epicardium was determined using reverse transcriptase-polymerase chain reaction (RT-PCR) and Southern blot analysis. The expression of 5-HT_{1B} or 5-HT_{1D} receptor protein was studied using subtype selective antibodies and standard immunocytochemical techniques. The rank order of 5-HT receptor agonist potency in causing vasoconstriction was 5-carboxamido tryptamine, (5-CT) > zolmitriptan = BW183C91 (N^{10}-desmethyl zolmitriptan) = α-methyl-5-hydroxytryptamine (α-CH_3-5-HT) = 5-HT = sumatriptan > 2-methyl-5-hydroxytryptamine (2-CH_3-5-HT) = 8-hydroxy-DPAT (8-OH-DPAT). α-CH_3-5-HT, 5-CT, 5-HT, zolmitriptan and BW 183C91 were significantly more potent (approximately 3-fold) than sumatriptan and 2-CH_3-5-HT, which in turn were more potent than 8-OH-DPAT. Ketanserin and methiothepin (5-HT_2 and 5-HT_1 receptor antagonists, respectively) caused parallel rightward shifts of the concentration–effect curves to α-CH_3-5-HT or 5-CT, respectively, without changing the maximum contractile response. In human coronary arteries, atrium, ventricle and epicardium. RT-PCR products corresponding to the human 5-HT_{2A}, 5-HT_{1B} and 5-HT_{1F} receptors were expressed in high levels, mRNAs coding for 5-HT_7, 5-HT_{1A} and 5-HT_{1D} receptors were only weakly expressed. No 5-HT_{1E} receptor mRNA was detected. In coronary arteries there was a differential expression of 5-HT_{1B} versus 5-HT_{1D} receptor mRNAs, with 5-HT_{1B} mRNAs being found in greater abundance. Dense 5-HT_{1B}-immunoreactivity was detected on smooth muscle layer within coronary artery, however, 5-HT_{1D}-immunoreactivity was not detected. It is concluded that 5-HT-evoked contraction of human coronary arteries is most probably mediated via the activation of both 5-HT_{1B} and 5-HT_{2A} receptors.

Keywords: Coronary artery, human; Pharmacology, in vitro; RT-PCR; Immunocytochemistry; 5-HT_{1B} receptor; 5-HT_{2A} receptor

1. Introduction

Migraine is a common and debilitating condition which takes the form of a severe, episodic and often throbbing headache. The development of sumatriptan for the acute treatment of migraine has emphasised the importance of 5-HT receptors as targets for novel therapeutic agents. Some clinical trials involving the assessment of sumatriptan as an anti-migraine agent have reported that 'chest symptoms' including chest heaviness, tightness or pressure may be experienced by some patients (Brown et al., 1991; Willet et al., 1992; Ploskler and McTavish, 1994). These side-effects are probably related to extracardiac mechanisms such as increased oesophageal contractions (Houg-

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ton et al., 1994). However, intravenous administration of sumatriptan may produce coronary artery constriction in patients undergoing coronary angiography (MacIntyre et al., 1992). Furthermore, there are a few reports where sumatriptan has been reported to cause cardiac ischemia and one where sumatriptan caused myocardial infarction (Willet et al., 1992; Lloyd and Simmons, 1993; Ottervanger et al., 1993). Although coronary side-effects are rare, an antimigraine drug with less cardiovascular side-effects would be of benefit. To achieve this it is important to characterise the 5-HT receptors responsible for the 5-HT-induced contraction in human coronary arteries.

An almost exponential development in the number of novel 5-HT receptor subtypes has occurred in recent years, much due to the molecular cloning techniques. Today seven different 5-HT receptor families (5-HT_1–5-HT_7) are thought to exist, some of them are heterogenous and contain several subtypes (Hoyer et al., 1994). Evidence is accumulating for the participation of either the 5-HT_1 or the 5-HT_2 receptors or both in the 5-HT-induced contraction of human coronary arteries (Connor et al., 1989; Bax et al., 1993; Kaumann et al., 1994; Ferro et al., 1995).

In the present study we established the 5-HT receptor subtypes responsible for mediating 5-HT-induced contraction in normal human coronary arteries using pharmacological and molecular approaches. Firstly we examined the in vitro effects of 5-HT receptor agonists (including the antimigraine drugs sumatriptan, zolmitriptan and its active metabolite BW183C91) (Dixon and Warrander, 1997) and 5-HT receptor antagonists on vasomotor tone; Secondly in coronary artery and in cardiac tissues we examined the expression of mRNAs corresponding to different 5-HT receptors using reverse transcriptase-polymerase chain reaction (RT-PCR) and, in addition, the expression of 5-HT_{1B}- or 5-HT_{1D}-receptor proteins in coronary artery were also examined using subtype selective antibodies (Longmore et al., 1997).

2. Methods

2.1. In vitro pharmacology

Coronary arteries (epicardial) were obtained from patients undergoing surgery for valvular diseases or removed at autopsy within 12 h after death. The vessels were macroscopically normal. All vessels were placed in buffer solution aerated with 5% CO_2 in O_2 and immediately transported to the laboratory for investigation. The study was approved by the Human Ethics Committee of Lund University.

The arteries were dissected free of connective tissues under a microscope and cut into cylindrical segments (1–2 mm long), outer diameter between 0.5–1.5 mm. The segments were mounted on two metal prongs, one of which was connected to a force displacement- transducer (FT03C) and attached to a Macintosh Plus computer, and the other to a displacement device. The position of the holder could be changed by means of a movable unit allowing fine adjustments of vascular tension by varying the distance between the metal prongs. The experiments were continuously recorded by the Macintosh software program Chart™. The mounted specimens were immersed in temperature-controlled tissue baths (37°C) containing a buffer solution of the following composition (mM): NaCl 119; $NaHCO_2$ 15; KCl 4.6; $MgCl_2$ 1.2; NaH_2PO_4 1.2; $CaCl_2$ 1.5; glucose 5.5. The buffer solution was continuously gassed with 5% CO_2 in O_2, giving a pH of 7.4. The segments were given an initial resting tension of 4 mN and were allowed to stabilise at this level of tension for 1 h. The contractile capacity of each vessel segment was examined by exposure to a K^+-rich (60 mM) buffer solution which had the same composition as the standard buffer except that some of the NaCl was exchanged for an equimolar concentration of KCl. These contractions served as the reference response and were set as 100%. When two reproducible contractions had been achieved (variation less then 10%), the vessels were used for further studies.

Vascular effects of the agonists were examined by cumulative application of increasing concentrations of the drugs. Each segment was exposed to a single agonist. For experiments with antagonists, a matched pairs protocol was used where one segment acted as control (no antagonist present) and for another segment from the same artery the agonist response was assessed following equilibration with the antagonist. Contractile responses were expressed relative to the K^+ reference response (= 100%). The maximum contractile response (E_{max}) and the potency of the agonists (pEC_{50}, negative logarithm of the molar concentration of agonist inducing half maximum response) were calculated. Where appropriate concentration–effect curves were fitted to the data using weighted least squares non-linear regression analysis (Prism, Version 2.0b, Graphpad Software). Statistical significant differences were determined with Mann–Whitney *U* using StatView II on a Macintosh IIcx. $P < 0.05$ was considered significant.

2.2. Drugs

The following drugs were used in the in vitro experiments: 5-hydroxytryptamine hydrochloride, 5-HT) from Sigma (USA); 5-carboxamidotryptamine (5-CT), 8-hydroxy-DPAT hydrobromide (8-OH-DPAT), 2-methyl-5-hydroxytryptamine (2-CH_3-5-HT), α-methyl-5-hydroxytryptamine hydrochloride (α-CH_3-5-HT), methiothepin and ketanserin were obtained from RBI (USA) and LY 334370 (own synthesis). Sumatriptan and GR 55562 were generous gifts from Dr. Helen Connor (Glaxo Wellcome, UK), and zolmitriptan and BW 183C91 (N^{10}-desmethyl metabolite of zolmitriptan) were generous gifts from Dr. Grame Martin (Wellcome, UK).

2.3. Reverse transcriptase-polymerase chain reaction and Southern blot analysis

Human tissues (atrium, ventricle wall, epicardium and coronary arteries), obtained from explanted hearts from patients undergoing cardiac transplantation or removed at autopsy within 12 h after death, were homogenised and total RNA extracted using guanidine isothiocyanate/CsCL cushion method. RNA was further treated with DNAase to remove any contaminating genomic DNA. cDNA was prepared from total RNA with random hexanucleotide primers using reverse transcriptase (Superscript II; BRL). An aliquot of the first strand cDNA (corresponding to 250 ng of total RNA) was amplified in a 50 μl PCR reaction mixture (200 μM dNTPs final concentration) containing 1.2 U of Taq polymerase in the buffer supplied by the manufacturer (Perkin-Elmer), and 1 μM of primers, using a program consisting of 30 cycles of 94°C/2′, 68°C/2′, and 72°C/3′, with a pre- and post-incubation of 95°C/5′ and 72°C/10′, respectively. PCR primers for the human serotonin receptors are shown in Table 1.

The PCR products were run on a 1.5% agarose gel and transferred to charged nylon membranes using [^{32}P]ATP labelled probes, which were internal probes to the PCR primers of the corresponding gene (see Table 1). Filters were washed under high stringency and exposed at −70°C to Kodak XAR film in the presence of an intensifying screen. Similar PCR and Southern blot analysis were conducted with primers and probe directed to the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (Clontech), except 22 cycles were used. This demonstrated that equal amounts of cDNA from the different tissues were being assayed for the individual serotonin receptor mRNA expression. In order to control for the amplification of contaminating genomic DNA (carried over during the RNA extraction), control PCR reactions were run in parallel with RNA which had not been converted to cDNA (i.e., minus reverse transcriptase).

2.4. Immunohistochemistry

Human coronary arteries were obtained post-mortem as routine histopathological samples from the University Hospital, Lund, Sweden. Specimens were collected in accordance with Swedish legislation (Transplantation act). The arteries were prepared for immunohistochemical studies as previously described (Longmore et al., 1997). Briefly, after fixation (4% formal saline, 4–7 days), the tissues were processed to wax and sectioned (7 μm). The sections were incubated overnight with the primary antibodies (i.e., selective for either human 5-HT$_{1B}$ or 5-HT$_{1D}$ receptors) and α-actin and Ulex europeas (smooth muscle and endothelial cell markers, respectively). The sections were then exposed to the secondary antibody (biotinylated goat anti rabbit IgG, Vectastain Elite Kit) and the immunoreactivity was visualised using diamino benzidine (0.0025% DAB in PBS with 0.03% H_2O_2) as the chromagen. Light microscopy was performed on a Leica (DMRB) microscope using normal Kohler illumination.

3. Result

3.1. Contractile effects

In human coronary arteries the rank order of agonist potency order was 5-CT > zolmitriptan = BW183C91 = α-CH$_3$-5-HT = 5-HT = sumatriptan > 2-CH$_3$-5-HT = 8-OH-DPAT (Table 2). α-CH$_3$-5-HT, 5-CT, 5-HT, zolmitriptan and BW183C91 evoked equally strong contractions which were significantly stronger and more potent than sumatriptan and 2-CH$_3$-5-HT, which in turn were

Table 1
Amino acid sequences of primers used for PCR studies (for DNA sequences, see Branchek, 1993; Bard et al., 1993). The final column shows the region of the receptors targetted with these primers (IC = intracellular loop 2 or 3)

Receptor subtype	Sense primer	Antisense primer	Targetted region	^{32}P Probe
5-HT$_{1A}$	5′GCTTCCGCATCC-GCAAGACGGT 3′	5′CTTCGCCTCGGC-GTTGCGCTC 3′;	IC$_3$, IC$_3$	5′AGGCAAGTGCTCTTTGG-CACTCGGTGCACCTCGAT
5-HT$_{1D}$	5′GCAATCACAGATG-CCCTGGAATACA 3′	5′AGGTGGAGTAGATG-GTGTAGGAGAT 3′	IC$_2$, IC$_2$	5′GAGAGGTGTTCACCAGA-ACATCTCCTCCTGGGCCTT
5-HT$_{1B}$	5′ACGCCGTGGAGTA-CTCAGCTAAAAG 3′	5′GGAGTAGACCGTGT-AGAGGATGTGG 3′;	IC$_2$, IC$_3$	5′TTCACCACGCATTCCGA-TCTTCGGCCTTAGCCTGAC
5-HT$_{1E}$	5′TACCACGCGGCCA-AGAGCCTTTACCA 3′	5′TGGTGCTAGAGAT-CTGCTGACGTTC 3′;	IC$_3$, IC$_3$	5′GAGAAGTCAGACACACAC-TGTGTAAGTTTACAACTT
5-HT$_{1F}$	5′GCTATAGCTTTGG-ATCGGTATCGAG 3′	5′CAATCCTACTTGC-TTGTCTCTTGTG 3′;	IC$_2$, IC$_3$	5′AATGTGGTCGTGCTTGA-TTCATCATCTCTGCTAGTT
5-HT$_{2A}$	5′TCGCCATCCAGA-ATCCCATCCACC 3′	′GATGGACTGCATA-GTCCTCCTGCC 3′;	IC$_2$, IC$_3$	5′CCGCTGGAAGAGCTTTTC-AAAGAACTCTGAGGGAG
5-HT$_7$	5′GGAACAGATCAA-CTACGGCAGAGT 3′	5′GGTGGTGGCTGCTTT-CTGTTCTCGCTTAAA 3′.	amino-terminus, IC$_3$	5′CGTCATCGCCCTGAATG-GAAGCTCCAGAAGGAGGT

Table 2
Contractile responses to 5-hydroxytryptamine (5-HT), 5-carboxytryptamine (5-CT), sumatriptan, 8-hydroxy DPAT (8-OH-DPAT), 2-methyl-5-hydroxytryptamine (2-CH_3-5-HT), α-methyl-5-HT (α-CH_3-5-HT), zolmitriptan and BW183C91 on human coronary arteries

	pEC_{50}	E_{max}	Relative potency	n
5-HT	6.2 ± 0.1	75 ± 25	1	6
5-CT	7.4 ± 0.3	87 ± 19	15.8	6
Sumatriptan	6.0 ± 0.2	41 ± 7	0.63	9
8-OH-DPAT	5.0 ± 0.1	8 ± 4	0.06	7
2-CH_3-5-HT	5.3 ± 0.3	37 ± 11	0.13	6
α-CH_3-5-HT	6.3 ± 0.3	126 ± 37	1.26	5
Zolmitriptan	6.6 ± 0.5	71 ± 7	2.51	5
BW183C91	6.4 ± 0.5	61 ± 11	1.58	5

pEC_{50} = negative logarithm of the molar concentration of agonist inducing half maximum contraction), E_{max} = maximum contraction (as percent of potassium induced contraction), n = number of patients from whom the vessels were collected. The data are expressed as mean values ± S.E.M.

stronger and more potent than 8-OH-DPAT (Table 2). The 5-HT_{1F} agonist LY 334370 (10^{-12} to 10^{-5} M) did not produce contraction of the human coronary artery segments (data not shown).

3.2. Antagonistic effects

The selective 5-HT_2 receptor antagonist ketanserin (0.1 μM) caused a parallel shift to the right of the concentration–effect curve to α-CH_3-5-HT (a 5-HT_2-receptor agonist) without changing the maximum contractile response. The pEC_{50} value was significantly reduced from 6.3 ± 0.3 without ketanserin to 5.3 ± 0.2 with 0.1 μM ketanserin present (pA_2 value = 8.0, see Fig. 1). The 5-HT_1-receptor antagonist methiothepin (0.1 μM) caused a parallel shift to the right of the concentration–effect curve to 5-CT (a selective 5-HT_1-receptor agonist) without changing the maximum contractile response. The pEC_{50} value for 5-CT was significantly reduced from 7.4 ± 0.3 without methiothepin to 6.1 ± 0.3 with 0.1 μM methiothepin present (pA_2 value = 8.3, see Fig. 2). Experiments on segments from 1 subject with GR 55562 (10^{-6} M) caused a parallel shift to the right of 5-CT induced contraction (data not shown).

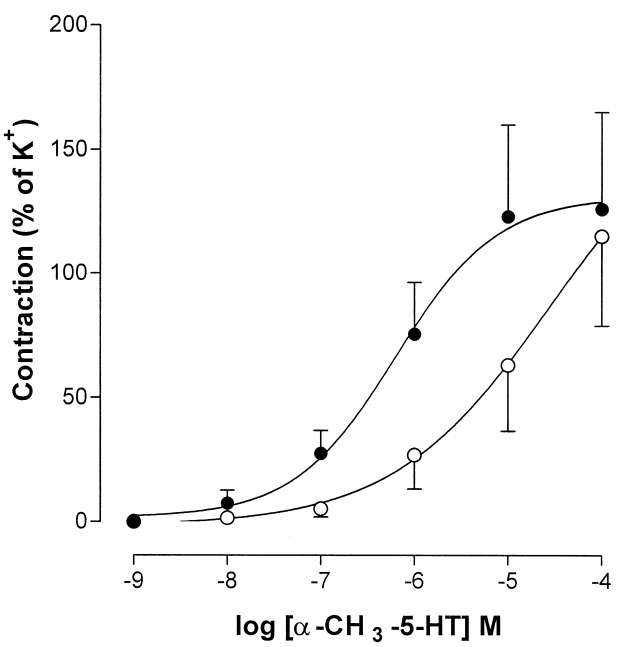

Fig. 1. The contractile response to α-CH_3-5-HT without (●) and with ketanserin 0.1 μM (○). The results are expressed as percentage of the potassium-induced contraction, each point representing mean ± S.E.M. *$P < 0.05$ (Mann–Whitney U).

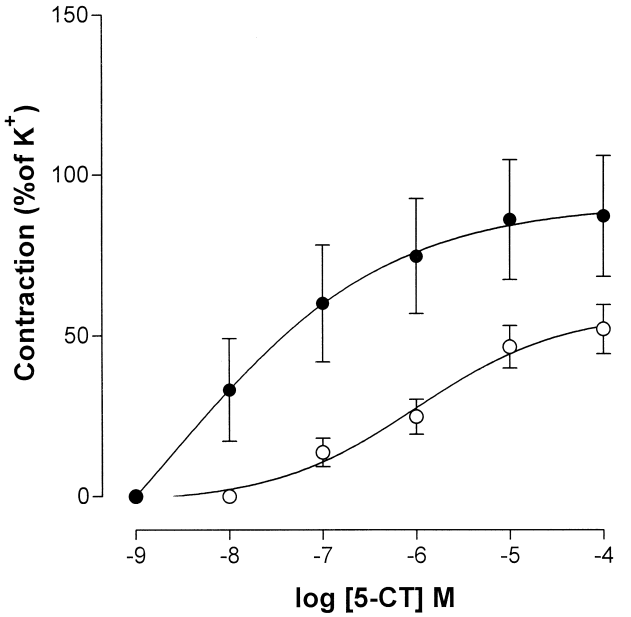

Fig. 2. The contractile response to 5-CT without (●) and with methiothepin 0.1 μM (○). The results are expressed as percentage of the potassium-induced contraction, each point representing mean ± S.E.M. *$P < 0.05$ (Mann–Whitney U).

3.3. Reverse transcriptase-polymerase chain reaction

In human coronary arteries, atrium, ventricle and epicardium, RT-PCR products corresponding to the human 5-HT_{2A}, 5-HT_{1B} and 5-HT_{1F} receptors were expressed at

Table 3
The expression of mRNA encoding different 5-hydroxytryptamine receptors in human cardiovascular tissues

Tissue	5-HT_{1D}	5-HT_{1B}	5-HT_{1E}	5-HT_{1F}	5-HT_{1A}	5-HT_{2A}	5-HT_7
Atrium	+	++	−	++1/2	++	+++	+
Ventricle wall	+	+	−	+++	1/2+	++	++
Epicardium	+	+	−	++1/2	1/2+	++	1/2+
Coronary artery	+	+++	−	++1/2	++	+++	+

Arbitrary scale: − = absence, + = faint, ++ = moderate, +++ = intense, relative to staining with the housekeeping gene (see Section 2).

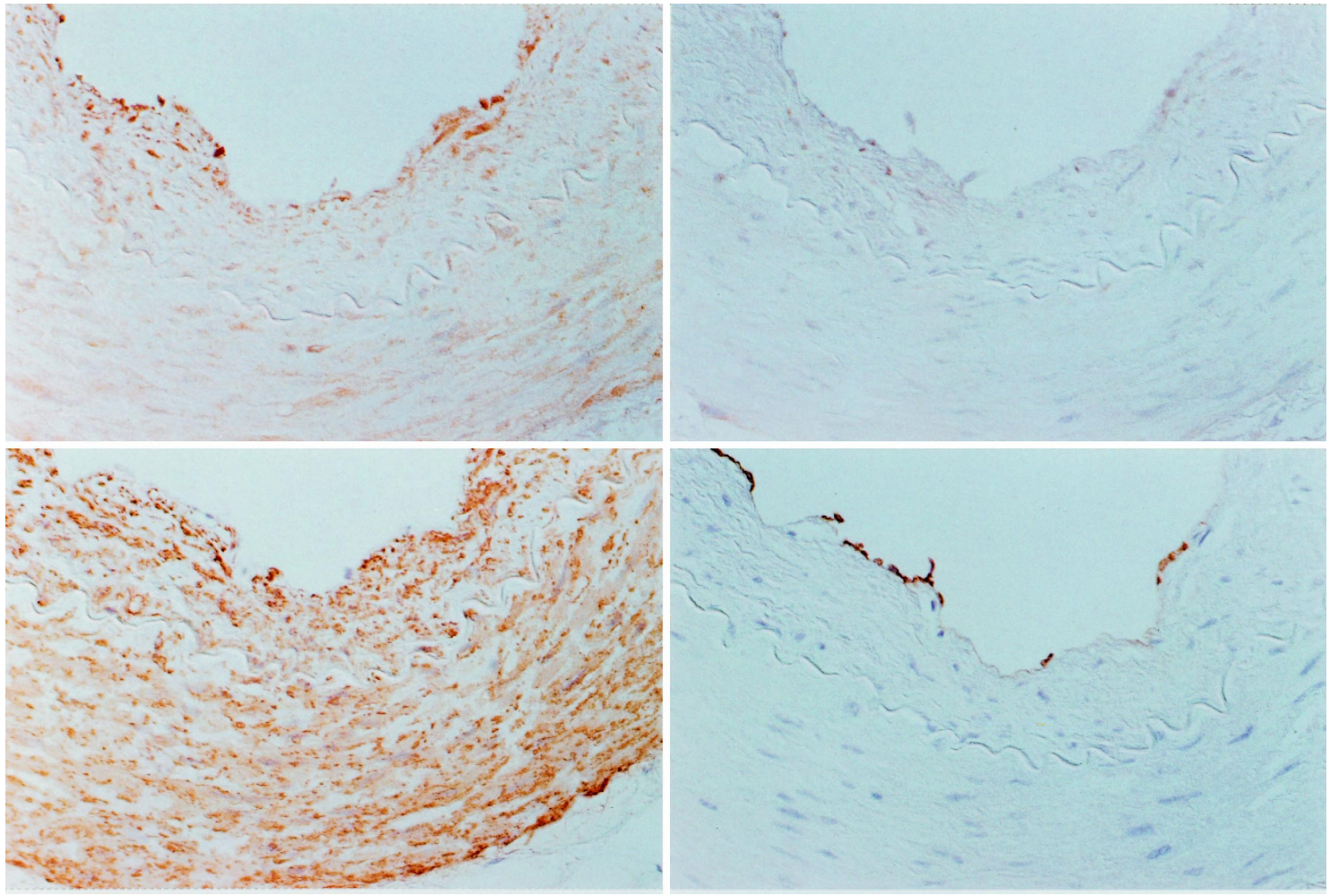

Fig. 3. Representative immunohistochemical findings in human coronary artery. Immunoreactivity was visualised using diamino benzidine as the chromagen (orange/brown staining) and haematoxylin was used as a counterstain to detect cell nuclei (blue/purple stain). Thin (7 μm) sections of the artery were obtained and high power micrographs are shown. Panel upper right shows 5-HT_{1D}-immunoreactivity was not detected on the vessel wall. Panel upper left shows that dense 5-HT_{1B}-immunoreactivity was detected on smooth muscle cells (defined using anti α-actin immunostaining in Panel lower left) and faint 5-HT_{1B}-immunoreactivity was detected on endothelial cells in upper left (defined using Ulex europeas immunostaining in Panel lower right).

high levels, 5-HT_7, 5-HT_{1A} and 5-HT_{1D} receptor mRNAs were only poorly expressed and no mRNA encoding the 5-HT_{1E} receptor was detected (Table 3). In coronary artery there was a differential expression between 5-HT_{1B} versus 5-HT_{1D} receptor mRNAs, where 5-HT_{1B} was found in greater abundance.

3.4. Immunohistochemistry studies

Coronary arteries were obtained from two donors and representative immunohistochemical findings are shown in Fig. 3. The arteries had a well developed smooth muscle layer (defined by anti α-actin immunostaining) and a well preserved endothelial layer (defined by immunostaining to Ulex europeas). Specific 5-HT_{1B}-receptor like immunoreactivity was seen in the smooth muscle cells (Fig. 3, upper left). Faint 5-HT_{1B}-immunoreactivity was seen on the endothelium. 5-HT_{1D}-immunoreactivity was not detected within the vessel wall (Fig. 3, upper right).

4. Discussion

In the present study the 5-HT_2 receptor agonist, α-methyl-5-HT, evoked a concentration-dependent contraction in human coronary arteries. The contraction was potently antagonised by the selective 5-HT_2 receptor antagonist, ketanserin ($pA_2 = 8.0$), without change of maximal contractile response. This fact together with the results from the RT-PCR where we found an intense expression of mRNA encoding the 5-HT_{2A} receptor in human coronary arteries strongly support the view that activation of 5-HT_2 receptors contributes to the 5-HT-evoked contraction in human coronary arteries (Connor et al., 1989). α-CH_3-5-HT has been shown to mediate effects not only on the 5-HT_2 receptors but also on 5-$HT_{1B/1D}$ receptors at higher concentrations which might explain the finding that the contraction induced by α-CH_3-5-HT is significantly stronger than that which the 5-$HT_{1B/1D}$ receptor agonists elicit (Fenuik et al., 1985).

The involvement of 5-HT_1 receptors in mediating the contractile response to 5-HT in human coronary arteries is further confirmed, since (a) sumatriptan and zolmitriptan, selective 5-$HT_{1B/1D}$ receptor agonists devoid of affinity at 5-HT_2 receptors, produced a vasoconstrictor response; (b) the 5-HT_1-receptor antagonist methiothepin inhibited 5-CT-induced contraction which agrees well with the antagonistic effect of GR 55562 on the 5-CT-induced contraction (Connor and Beattie, 1996), and (c) the rank order of agonist potency reflects the rank order of 5-HT_1-receptor affinity. In order to identify the 5-HT_1 receptor subtype involved in mediating contraction, in particular whether the vasoconstrictor 5-HT_1-like receptors are 5-HT_{1D} (previous 5-$HT_{1D\alpha}$, Hartig et al., 1996) or 5-HT_{1B} receptors (previous 5-$HT_{1D\beta}$) we used RT-PCR and found and intense expression of mRNA encoding the 5-HT_{1B} receptor while the expression of mRNA encoding the 5-HT_{1D} receptor was relatively faint.

This was confirmed using immunohistochemical studies which showed expression of 5-HT_{1B}-immunoreactivity (but not 5-HT_{1D}-immunoreactivity) within the smooth muscle wall of the arteries. These observations indicate that the vasoconstrictor effects to sumatriptan and zolmitriptan are mediated through activation of 5-HT_{1B} receptors and not 5-HT_{1D} receptors. This is in concert with the report by Kaumann et al. (1994) who, based on pharmacological analysis, reported that in human coronary arteries contraction mediated by the 5-HT_1-like receptor resembled more the 5-HT_{1B} than the 5-HT_{1D} receptor.

The novel and selective 5-$HT_{1B/1D}$ receptor agonist zolmitriptan has proven highly effective in the treatment of migraine (Visser et al., 1996). Zolmitriptan has the same pharmacological profile as sumatriptan but is less hydrophilic and has, therefore, access to central nervous system sites more easily (Goadsby and Edvinsson, 1994). Zolmitriptan undergoes hepatic metabolism and has three major metabolites, one of which is BW183C91 which differs from the parent molecule only in lacking a methyl group (N^{10}-desmethyl zolmitriptan) (Dixon and Warrander, 1997). It has 5-$HT_{1B/1D}$ agonist activity and may contribute to the anti-migraine action of zolmitriptan (Saeber et al., 1996). In clinical trials to date zolmitriptan has been shown to possess a favourable cardiovascular safety profile with no episodes of cardiac ischemia or ECG abnormalities and only mild and infrequent chest symptoms (Giorgi et al., 1996). In the present study, zolmitriptan and its metabolite (BW183C91) were significantly more potent (approximately 3-fold) in causing contraction of human isolated coronary artery compared to sumatriptan and this is consistent with the differences in binding affinity of these drugs at 5-HT_{1B}-receptors (Martin, 1997). The variation in maximum effect between the present study and that of previous studies (Martin et al., 1997; Maassen Van Den Brink et al., 1998) may be due to variation in clinical material examined.

Using RT-PCR we found moderate to intense expression of mRNA encoding the 5-HT_{1F} receptor in coronary artery. The possibility cannot be excluded that the 5-HT_{1F} receptor subtype (Adham et al., 1993) may contribute to some degree to the 5-HT-induced vasoconstriction of human coronary arteries. However, it has been reported that LY334370 (a selective 5-HT_{1F} receptor agonist) is devoid of vasoactive properties in rabbit saphenous vein (Johnson et al., 1997) which agrees well with the present data, i.e., lack of contractile effect on human coronary arteries. Furthermore, it has been shown previously that for a series of 5-HT receptor agonists that there is no relationship between 5-HT_{1F} receptor affinity and vasoconstrictor potency in human isolated cranial arteries (Razzaque et al., 1999). Most of the triptans have affinity for 5-HT_{1F} receptors (a receptor unknown at the time of the development of sumatriptan) and there is current debate whether 5-HT_{1F}

receptor activation contributes to the clinical effects of the triptans which are used as anti-migraine therapies. It is possible that a selective 5-HT_{1F} receptor agonists may be a clinically effective anti-migraine drug with reduced vasoconstrictor liability.

The role of 5-HT_{1A} receptors has been viewed from neuropsychiatry—the drugs designed would hopefully not be direct vascular constrictors and thus have limited coronary side effects. 5-HT_{1A} receptors do not seem to be involved in the 5-HT induced contraction in coronary artery, even if mRNA encoding the receptor was moderately expressed. 8-OH-DPAT only weakly contracted human coronary arteries (8 ± 4% of potassium-induced contraction) and it is likely that this response was mediated through activation of other 5-HT receptors since at high concentrations the selectivity of 8-OH-DPAT as a 5-HT_{1A} receptor agonist is lost. Similarly, the weak potency of 2-CH_3-5-HT also eliminates the involvement of 5-HT_3 receptors. The involvement of 5-HT_3 receptors mediating contraction in human coronary arteries seems unlikely since 2-CH_3-5-HT evoked only weak contraction.

RT-PCR studies showed expression of 5-HT receptor mRNAs in human cardiac tissues. With the exception of the well documented inotropic effects of 5-HT_3 and 5-HT_4 receptor activation, the role of the other 5-HT receptors in the heart is poorly understood (Saxena and Villalon, 1991). Recent studies have shown that tachycardia can be 5-HT_7 receptor mediated (Villalon et al., 1997). In experimental animals sumatriptan has been shown to have no effect on cardiac function (Villalon et al., 1997).

In conclusion, we have demonstrated that the 5-HT-induced contraction in human coronary arteries is mediated by the 5-HT_{2A} and 5-HT_{1B} receptors and that the new anti-migraine drug, zolmitriptan, has similar effects to that of sumatriptan.

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