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SPECIAL REPORT

Functional, endogenously expressed 5-hydroxytryptamine 5-ht$_7$ receptors in human vascular smooth muscle cells

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Human uterine artery smooth muscle cells in culture were shown to express constitutively both 5-ht$_7$ receptor mRNA and 5-ht$_7$-like receptors functionally linked to cyclic AMP formation. 5-Carboxamidotryptamine (5-CT) and 5-HT enhanced forskolin-stimulated cyclic AMP accumulation in these cells, with pEC_{50} values of 7.12 and 6.25, sumatriptan being very weakly active. Both methiothepin (0.1 μM) and clozapine (1 μM), but not the 5-HT$_4$-receptor antagonist, SDZ 205-557 (10 μM) antagonized the effects of 5-CT. In reverse transcriptase-polymerase chain reaction analysis, the mRNA for 5-ht$_7$, but not for 5-HT$_4$ or 5-ht$_6$ receptors was found to be strongly expressed in the same cells. These findings represent a further step toward the recognition of 5-ht$_7$ receptors as real, functional receptors.

Keywords: 5-HT receptors; 5-ht$_7$ receptors; vascular smooth muscle cells; cyclic AMP

Introduction In the latest classification of receptors for 5-hydroxytryptamine (5-HT), 5-ht$_7$ receptors represent a structurally and pharmacologically distinct category. Transductionally, they share with 5-HT$_4$ and 5-ht$_6$ receptors the ability to stimulate cyclic AMP formation (see Hoyer *et al.*, 1994). However, as indicated by the lower case appellation, 5-ht$_7$ receptors still await full operational and transductional characterization in intact (i.e. not genetically engineered) tissues or cells. The cDNA encoding the 5-ht$_7$ receptor has been cloned in at least four species (see Hoyer *et al.*, 1994), including man (Bard *et al.*, 1993). High levels of 5-ht$_7$ receptor mRNA expression have been found in human brain and smooth muscles. It has been suggested that 5-ht$_7$ receptors might mediate relaxation in some smooth muscle preparations (Bard *et al.*, 1993; Martin & Wilson, 1995). We have recently shown that 5-ht$_7$ receptor mRNA is expressed in a variety of rat and human blood vessels and in human vascular smooth muscle cells (Ullmer *et al.*, 1995). We therefore set out to investigate whether 5-ht$_7$ receptors functionally coupled to cyclic AMP formation, are present in human vascular smooth muscle cells.

Methods Human uterine artery smooth muscle cells (HUASMC) were prepared and characterized as previously described (Fager *et al.*, 1989). They were propagated in Dulbecco's Modified Eagles Medium supplemented with 10% foetal calf serum, 100 iu ml^{-1} penicillin and 100 μg ml^{-1} streptomycin, split once weekly with trypsin/EDTA and used at passages 5 to 9. Subconfluent cells grown in 24-well plates were deprived of serum 24 h before the cyclic AMP measurements. Cyclic AMP accumulation was measured using the [^{3}H]-adenine pre-labelling technique and results were analyzed as previously described (Schoeffter *et al.*, 1995). Drugs were from Sandoz Pharma, Basel, Switzerland, or as mentioned in Schoeffter *et al.* (1995). Reverse transcriptase-polymerase chain reaction (RT-PCR) studies were performed using oligonucleotide primers specific for the various human 5-HT receptors, as recently described (Ullmer *et al.*, 1995). The ^{32}P-labelled PCR-products were separated on 4% agarose gels, which were subsequently dried and exposed to X-ray films.

Results In the presence of forskolin (10 μM), 5-carboxamidotryptamine (5-CT) and 5-HT induced further, con-

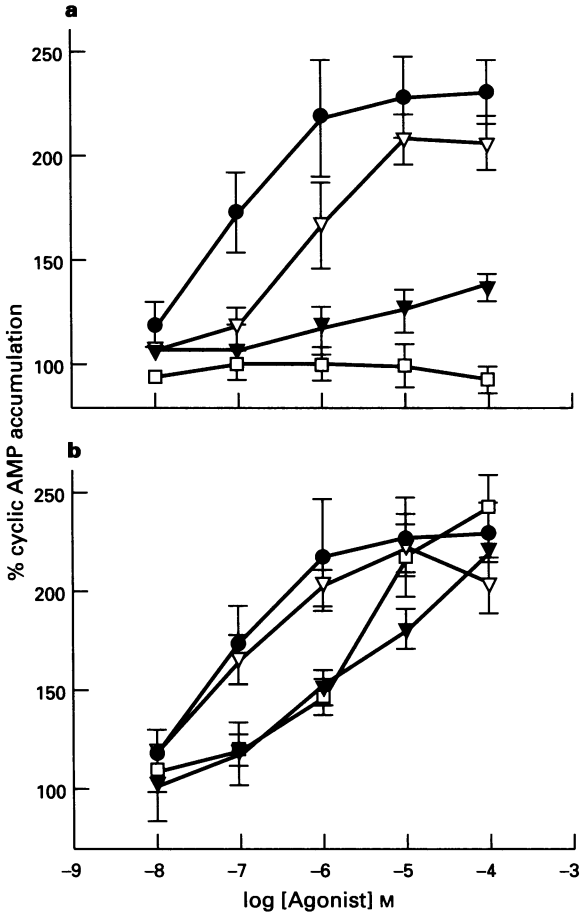

Figure 1 (a) Concentration-response curves of 5-CT (●), 5-HT (▽), sumatriptan (▼) and 8-OH-DPAT (□) for stimulation of cyclic AMP accumulation in HUASMC. (b) Concentration-response curves of 5-CT in the absence (●) and in the presence of methiothepin (0.1 μM; ▼), clozapine (1 μM; □) or SDZ 205-557 (10 μM; ▽). Means ±s.e.mean from 3 or 4 individual experiments. Results are expressed as percentage of forskolin-stimulated accumulation.

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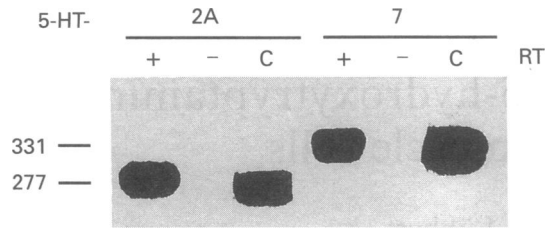

Figure 2 Agarose gel electrophoresis of PCR-amplified DNA from cDNA of HUASMC and of human total brain as positive control (lanes C). As control for the absence of genomic DNA contaminations, PCR's were performed with (lanes +) or without reverse transcriptase (lanes −). Primers specific for the human 5-HT_{2A} and 5-ht_7 receptor genes were used to amplify DNA fragments with the size of 277 and 331 base pairs, respectively, from cDNA samples, but not from samples generated without reverse transcriptase (for details see Ullmer *et al.*, 1995).

centration-dependent increases in cyclic AMP accumulation in HUASMC (Figure 1). Maximal effects amounted to 110–130% above forskolin-stimulated levels. 5-CT was at least as potent as 5-HT (pEC_{50} value 7.12 ± 0.22, $n=3$, versus 6.25 ± 0.27, $n=4$). Sumatriptan was weakly active, producing $37 \pm 6\%$ stimulation at 0.1 mM ($n=4$), whereas 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) was devoid of effect up to 0.1 mM (Figure 1). Both clozapine (1 μM) and methiothepin (0.1 μM) shifted the concentration-response curve of 5-CT to the right without significant depression of the maximal effect, whereas SDZ 205-557 (2-methoxy-4-amino-5-chlorobenzoic acid 2-(diethylamino) ethyl ester, 10 μM) did not significantly alter it (Figure 1). Estimated pK_B values were 7.54 ± 0.12 for clozapine and 8.30 ± 0.18 for methiothepin ($n=3$ for both). In RT-PCR studies, HUASMC expressed 5-ht_7 receptor mRNA almost as densely as 5-HT_{2A} receptor mRNA (Figure 2), which is predominantly expressed in vascular smooth muscle cells (Ullmer *et al.*, 1995). By contrast, no or very faint signals were found for 5-HT_4, 5-ht_{5A} and 5-ht_6 mRNA's (not shown).

Discussion Although the operational and transductional properties of human recombinant 5-ht_7 receptors have been studied in a number of expression systems, virtually nothing is known on the function of these receptors in their native state. Whereas 5-ht_7 binding sites have been recently identified in rat and guinea-pig brain (Sleight *et al.*, 1995; To *et al.*, 1995), it has been suggested that some peripheral vascular responses are mediated by receptors with the characteristics of recombinant 5-ht_7 receptors (Bard *et al.*, 1993; Martin & Wilson, 1995). Indeed, 5-ht_7 receptor mRNA is expressed in human vascular smooth muscle cells (Ullmer *et al.*, 1995; this study). We now show that receptors sharing the operational features of 5-ht_7 receptors mediate cyclic AMP increase in HUASMC. Three 5-HT receptor classes (5-HT_4, 5-ht_6 and 5-ht_7) are positively linked to adenylyl cyclase. The relatively high potency of 5-CT speaks for a 5-ht_7-like versus a 5-ht_6-like profile in HUASMC. The inability of SDZ 205-557, a potent and selective 5-HT_4 receptor antagonist (see Hoyer *et al.*, 1994) to displace the concentration-response curve of 5-CT virtually rules out the involvement of 5-HT_4 receptors. These pharmacological characteristics are strongly corroborated by RT-PCR studies, showing a substantial expression of 5-ht_7 receptor mRNA, but not of 5-HT_4 receptor or 5-ht_6 receptor mRNA, in the same cells. The relatively low pEC_{50} values of 5-CT and 5-HT found in the present model, compared to their affinities for the human recombinant 5-ht_7 receptor (pK_i 9 and 8.1; Bard *et al.*, 1993) suggest a limited number of spare receptors and/or a poor receptor-effector coupling efficiency. This could also provide an explanation for the lack of agonist effect of 8-OH-DPAT, which binds with low affinity to the human recombinant 5-ht_7 receptor (500 times less that 5-CT; Bard *et al.*, 1993). As a whole, the present findings represent a further step toward the recognition of 5-ht_7 receptors as real, functional receptors.

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