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Agonist-Promoted Down-Regulation and Functional Desensitization in Two Naturally Occurring Variants of the Human Serotonin$_{1A}$ Receptor

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We recently reported two naturally occurring polymorphisms of the human serotonin$_{1A}$ (5-HT$_{1A}$) receptor: glycine22→serine (Ser22) and isoleucine28→valine (Val28) in the putative amino-terminal domain of the receptor. To investigate the regulatory properties of these variants, the wild type (WT) and variant 5-HT$_{1A}$ receptors were stably expressed in CHO-K1 cells. WT, Ser22, and Val28 displayed similar high-affinity binding to [^{3}H]-8-OH-DPAT. Competition experiments with 5-HT$_{1A}$ agonists and antagonists demonstrated similar pharmacological profiles. Receptor agonist-promoted down-regulation was tested by exposure to 100 μmol/L 8-OH-DPAT. After 24-h exposure, WT and Val28 underwent 59.3 ± 3.9% and 59.5 ± 1.4% reduction in receptor density respectively, whereas the degree of down-regulation was significantly lower for Ser22 (21.4 ± 4.2%). Cell treatment for 24 h with 100 μmol/L 8-OH-DPAT reduced the 5-HT-induced inhibition of cAMP accumulation by 24.9 ± 5.1% for WT and 16.4 ± 0.8% for Val28, but only by 4.8 ± 3% for Ser22. We conclude that the Ser22 variant is capable of attenuating agonist-mediated receptor down-regulation and desensitization. ***[Neuropsychopharmacology 17:18–26, 1997]*** *© 1997 American College of Neuropsychopharmacology*

KEY WORDS: *Serotonin; 5-Hydroxytryptamine; 5-HT$_{1A}$ receptor, Down-regulation; Desensitization; Polymorphisms*

The human serotonin$_{1A}$ (5-HT$_{1A}$) receptor is a protein of 422 amino acids (Kobilka et al. 1987; Chanda et al. 1993) coded by an intronless gene located on chromosome 5 at 5q11.2-q13 (Kobilka et al. 1987). Like other members of the G-protein-coupled receptor family (Dohlman et al. 1991), the 5-HT$_{1A}$ receptor consists of seven transmembrane hydrophobic domains, with an extracellular aminoterminal domain and a cytoplasmic carboxyl-terminus. By coupling with the $G_{i/o}$ family of heterotrimeric G proteins, the 5-HT$_{1A}$ receptor has been shown to either inhibit or activate adenylate cyclase activity, open potassium channels, close calcium channels, and inhibit phosphatidylinositol turnover (reviewed in Boess and Martin 1994). The 5-HT$_{1A}$ receptor is expressed both presynaptically on the cell bodies and the dendrites of serotonergic neurons located in the raphe nuclei and postsynaptically with the highest density in the limbic system (Zifa and Fillon 1992; Burnet et al. 1995).

The 5-HT$_{1A}$ receptor is believed to play a role in a variety of behaviors, such as aggression, sexual behavior, and appetite control, and in several psychiatric disorders, such as mood disorders, anxiety disorders, anorexia ner-

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vosa, schizophrenia, and alcoholism (Zifa and Fillon 1992). Several lines of evidence indicate that 5-HT$_{1A}$ receptor agonists have anxiolytic, antiaggressive, and antidepressive properties (Newman et al. 1993; De Vry 1995). Furthermore, desensitization of the presynaptic 5-HT$_{1A}$ receptors has been reported after chronic administration of selective serotonin reuptake inhibitors (SSRIs), whereas long-term treatment with various classes of tricyclic antidepressant drugs (TCAs) or repeated electroconvulsive therapy have been shown to sensitize postsynaptic 5-HT$_{1A}$ receptors (Newman et al. 1993; Blier and de Montigny 1994). Thus, structural variants of the 5-HT$_{1A}$ receptor could alter its functional properties and lead to changes in behavior or drug responsiveness.

We recently reported two naturally occurring polymorphisms of the human 5-HT$_{1A}$ receptor gene, resulting in substitution of glycine to serine at amino acid 22 (Ser22) and isoleucine to valine at amino acid 28 (Val28) (Nakhai et al. 1995). Both the Gly22 and Ile28 residues are located in the putative amino-terminal extracellular domain of the receptor (Kobilka et al. 1987). The Ser22 variant was found only in a Finnish population sample at a frequency of 0.2%, whereas the Val28 variant was identified at a higher frequency (0.55%) and in different populations (Nakhai et al. 1995; Bergen et al. 1996). Erdmann et al. (1995) have also observed Val28 among patients affected by schizophrenia, bipolar affective disorder, and Tourette's syndrome, but found no difference in frequency between patients and controls. Interestingly, another naturally occurring polymorphism of the 5-HT$_{1A}$ receptor has been recently described in the putative amino-terminal extracellular domain of the receptor at amino acid 16 (proline to leucine) in a Japanese population sample (Harada et al. 1996).

It is possible that 5-HT$_{1A}$ receptor variants affect the pharmacological and regulatory properties of the 5-HT$_{1A}$ receptor. Brüss et al. (1995) recently showed no difference in the pharmacological profiles of the wild type (WT) 5-HT$_{1A}$ receptor and the Val28 variant transiently expressed in COS-7 cells. Nevertheless, naturally occurring variants in the extracellular amino-terminus of the human β_2-adrenergic receptor, which shares 43% amino acid identity with the 5-HT$_{1A}$ receptor (Kobilka et al. 1987), did not alter ligand affinity, but they altered long-term agonist-promoted down-regulation and functional desensitization (Green et al. 1994, 1995). Due to receptor down-regulation, the number of cellular receptors is decreased after prolonged exposure (hours) to agonist. This process plays an important role in cellular desensitization reducing physiological responses despite the continuous presence of the stimulus (Collins et al. 1992). Several lines of evidence indicate that 5-HT$_{1A}$ receptor down-regulation and desensitization are tightly related to the mechanism of antidepressant and anxiolytic drug action (Newman et al. 1993; Blier and de Montigny 1994). To investigate the functional and regulatory significance of the Ser22 and Val28 polymorphisms, these naturally occurring variants were stably expressed in Chinese Hamster Ovary (CHO-K1) cells. This study demonstrates that Ser22 is capable of attenuating agonist-mediated receptor down-regulation and desensitization.

MATERIALS AND METHODS

Construct, Transfection, and Cell Culture

DNA manipulations, plasmid construction and cloning were performed according to Sambrook et al. (1989). A λ clone containing the full length 5-HT$_{1A}$ receptor gene was isolated by plaque hybridization from a human genomic λ-DASH library (Stratagene, La Jolla, CA), using a probe derived from the human genomic clone G-21 (Kobilka et al. 1987) supplied by Dr. M.G. Caron. A 4200 bp *Bam*H I-*Bam*H I fragment containing the 5-HT$_{1A}$ receptor gene was isolated from the λ clone and inserted into *Bam*H I linearized pGEM-3Zf(+) to yield p5-HT$_{1A}$. A 1566 bp *Xba* I-*Bam*H I fragment of p5-HT$_{1A}$ containing the complete 5-HT$_{1A}$ receptor coding sequence was cloned into the *Bam*H I linearized eukaryotic expression vector pCDNA3 (Invitrogen, San Diego, CA) to yield p5-HT$_{1A}$WT. The fragment containing the 5-HT$_{1A}$ receptor coding sequence was inserted in the sense orientation as confirmed by dideoxy sequencing. Nucleotide substitutions at codons 64 and 82 of p5-HT$_{1A}$WT were generated by in vitro site-directed mutagenesis (Kunkel 1985) using oligonucleotides SER22, 5′-AGTGTTGCCGCTGGTCTCAAAGG-3′, and VAL28, 5′-CACGTCGGAGACACCAGTAGTGTT-3′, to form the plasmids p5-HT$_{1A}$SER22 and p5-HT$_{1A}$VAL28, respectively. The entire 5-HT$_{1A}$ coding and adjacent sequences of plasmids p5-HT$_{1A}$WT, p5-HT$_{1A}$SER22, and p5-HT$_{1A}$VAL28 were confirmed by dideoxy sequencing.

p5-HT$_{1A}$WT, p5-HT$_{1A}$SER22, and p5-HT$_{1A}$VAL28 were stably transfected into CHO-K1 cells (ATCC, Rockville, MD) by Lipofectamine (Life Technologies, Gaithesburg, MD) according to the manufacturer's protocol. Clones resistant to 500 μg/ml G-418 were assayed for expression of 5-HT$_{1A}$ receptor mRNA by slot blot hybridizaton (data not shown). Clones expressing high levels of 5-HT$_{1A}$ receptor mRNA were screened for binding of the high-affinity 5-HT$_{1A}$ agonist [^{3}H]-8-OH-DPAT [8-hydroxy-2-(di-n-propylamino)tetralin]. Cell lines expressing 400-1000 fmol 5-HT$_{1A}$ receptor/mg protein were selected for further studies.

Cells were grown in adherent culture at 37°C and 5% CO2 in Ham's F-12 media (Life Technologies, Gaithesburg, MD) supplemented with 10% fetal bovine serum (FBS), 146 μg/ml L-glutamine, 500 μg/ml G-418, 50 U/ml penicillin, and 50 μg/ml streptomycin. Then 24 h before harvesting the cells, the medium was replaced with

medium without antibiotics containing 10% charcoal-dextran-treated fetal bovine serum (CDFBS).

Radioligand Binding Studies

Nearly confluent CHO-K1 cells were washed with calcium and magnesium-free phosphate buffered saline (PBS), scraped in ice-cold PBS and centrifuged at 2000*g* for 10 min at 4°C. Cells were homogenized in 25 ml of 5 mmol/L Tris-HCl buffer (pH 7.4), containing 5 mmol/L EDTA and centrifuged at 48,000*g* for 20 min at 4°C. The resulting pellet was resuspended in 50 mmol/L Tris-HCl, pH 7.4, incubated at 37°C with constant agitation for 15 min to destroy any endogenous 5-HT, and centrifuged as above. This step was repeated. Finally, membrane pellets were stored at -80°C.

Equilibrium saturation experiments were performed as follows: membrane suspensions (approximately, 60 μg protein /ml) were incubated with 0.2–15 nmol/L [^{3}H]-8-OH-DPAT (specific activity 235 Ci/mmol; Amersham, Arlington Heights, IL) in a final volume of 1 ml of assay buffer (50 mM Tris-HCl, pH 7.4) for 20 min at 37°C. Then 10 μmol/L 5-HT was used to define nonspecific binding. Displacement studies were carried out at 1 nmol/L [^{3}H]-8-OH-DPAT in the presence of 12 concentrations of the unlabeled displacer drug. Membranes were incubated at 37°C for 20 min. The reaction was stopped by dilution in ice-cold assay buffer followed by rapid vacuum filtration through Whatman GF/C glass fiber filters. The filters were washed twice with 5 ml of ice-cold assay buffer. Radioactivity was measured in a Beckman LS 6000IC liquid scintillation counter at 55% efficiency.

Protein concentration was determined according to the method of Bradford (1976) using γ-globulin as the standard.

cAMP Assay

Samples were prepared according to Mak et al. (1996) with slight modifications. Briefly, cells were grown for 48 h in 96-well plates in Ham's F-12 medium containing 10% CDFBS. Cells were washed three times for 5 min with plain medium and incubated at 37°C with Earle's Balanced Salt Solution (EBSS) containing 0.1 mmol/L ascorbic acid, 100 μmol/L forskolin (FSK), and 5-HT ranging from 1 nmol/L to 3 μmol/L. After 30-min incubation, the medium was removed and 100 μl/well 3% perchloric acid was added. Cells were scraped from the wells and 40 μl/well 15% potassium bicarbonate was added to neutralize the samples. The 96-well plates were then centrifuged at 800*g* for 10 min at 4°C. Then 50 μl supernatant from each well were collected and assayed for cAMP accumulation using a radioassay kit (Diagnostic Products Corporation, Los Angeles, CA) according to the manufacturer's protocol. Results were expressed as pmol of cAMP accumulated/10^5 cells/min.

Receptor Down-regulation and Desensitization

Cells at 90% confluence expressing WT, Ser22, or Val28 5-HT$_{1A}$ receptors were washed twice with EBSS and incubated for the indicated times with Ham's F-12 medium containing 10% CDFBS, 0.1 mmol/L ascorbic acid and 8-OH-DPAT or (+)-WAY-100135 [(+)-N-tert-butyl-3,4-(2-methoxy phenyl) piperazin-1-yl-2-phenylpropanamide dihydro chloride; gift of Wyeth, Taplow, U.K.] at the indicated concentrations. Cells were then washed three times with fresh medium and [^{3}H]-8-OH-DPAT binding or cAMP assays were performed as above.

Data Analysis

Equilibrium-binding data, drug-competition binding data, cAMP dose-response studies were analyzed by the Prism computer program (GraphPad, San Diego, CA). Comparisons were by paired or unpaired Student's *t*-test as appropriate, with significance imparted at $p < .05$.

RESULTS

Pharmacological Characterization of WT, Ser22, and Val28 5-HT$_{1A}$ Receptors

Clones expressing 965±120 fmol WT 5-HT$_{1A}$ receptor/mg membrane protein, 433±87 fmol Ser22 5-HT$_{1A}$ receptor/mg membrane protein and 751±115 fmol Val28 5-HT$_{1A}$ receptor/mg membrane (mean ± SEM, $n = 6$) were selected for the study. [^{3}H]-8-OH-DPAT bound in a saturable fashion to the WT, Ser22, and Val28 5-HT$_{1A}$ receptors, fitting best to a single-site binding model in all cases (data not shown) with dissociation constants (K_D) of 2.94 ± 0.68 nM , 3.43 ± 0.76 nM and 1.51 ± 0.28 nM for the WT, Ser22, and Val28 respectively (mean ± SEM, $n = 6$), Pharmacological characterization of the expressed WT, Ser22, and Val28 5-HT$_{1A}$ receptors, assayed by [^{3}H]-8-OH-DPAT binding, was carried out using the selective agonist 8-OH-DPAT, the selective

Table 1. Affinities of Various Ligands for [3H]-8-OH-DPAT Binding to WT, Ser22, and Val28 5-HT$_{1A}$ Receptors Stably Expressed in CHO-K1 Cells

	5-HT1A Receptor		
Competing Ligand	WT pK_i	Ser22 pK_i	Val28 pK_i
8-OH-DPAT	8.91 ± 0.01	8.59 ± 0.13	9.03 ± 0.26
5-HT	8.7 ± 0.26	8.56 ± 0.09	8.62 ± 0.12
(+)-WAY-100135	8.33 ± 0.06	8.47 ± 0.06	8.25 ± 0.07

pK_i values (-log mol/l) were calculated as -log(IC_{50}/(1+C/K_D)), where IC_{50} is the concentration of the drug causing 50% inhibition of the specific radioligand binding, C is the radioligand concentration, and K_D is the equilibrium dissociation constant. Each value is the mean ± SEM of three independent determinations.

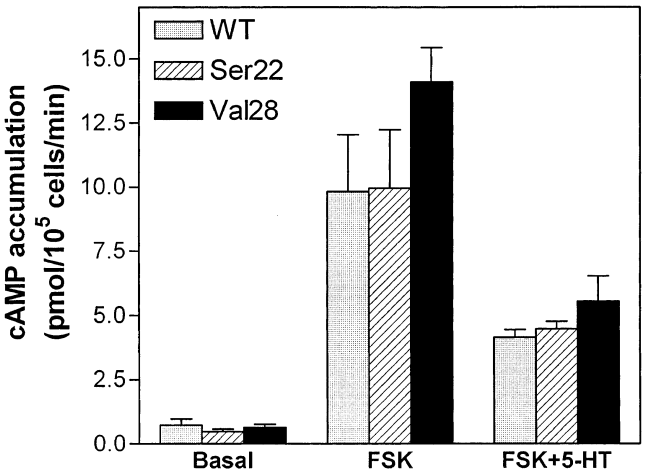

Figure 1. Basal, FSK-stimulated, and 5-HT-inhibited cAMP accumulation in CHO-K1 cells expressing the WT, Ser22, or Val28 5-HT$_{1A}$ receptors. cAMP assay was performed as described in Material and Methods. Concentrations of FSK and 5-HT were 100 μmol/L and 3 μmol/L respectively. Data represent the mean ± SEM of three independent determinations.

antagonist (+)-WAY-100135 and the endogenous ligand 5-HT. As shown in Table 1, WT, Ser22 and Val28 shared similar affinities for the compounds tested. The order of potency for [^{3}H]-8-OH-DPAT binding inhibition was 8-OH-DPAT>5-HT>(+)-WAY-100135 for the WT 5-HT$_{1A}$ receptor and the two variants. All the competing drugs gave monophasic inhibition curves, with Hill coefficients close to unity (data not shown).

Inhibition of cAMP Accumulation by WT, Ser22, and Val28 5-HT$_{1A}$ Receptors

5-HT$_{1A}$ receptor activation by agonists has been mostly associated with the inhibition of cAMP accumulation in hippocampal and cortical membranes and in several cell lines expressing cloned 5-HT$_{1A}$ receptor (Boess and Martin 1994). As shown in Figure 1, the basal cAMP accumulation in transfected CHO-K1 cells was enhanced 15 to 20-fold by exposure to 100 μmol/L (FSK). In cells expressing WT, Ser22, or Val28 5-HT$_{1A}$ receptors, 5-HT inhibited the FSK-stimulated cAMP accumulation with similar efficacy (Figure 1) and potency (EC_{50} values were: 42.94 ± 1.38 nmol/L, 37.8 ± 1.48 nmol/L and 40.27 ± 1.28 nmol/L for WT, Ser22, and Val28, respectively [mean ± SEM, n = 3]).

Agonist-Promoted Down-regulation

To study the long-term agonist-promoted down-regulation of the receptor, CHO-K1 cells expressing either WT, Ser22, or Val28 5-HT$_{1A}$ receptors were incubated for 24 h with various concentrations of the selective 5-HT$_{1A}$ agonist 8-OH-DPAT. The maximal reduction in the density of 5-HT$_{1A}$ receptors was observed at 100 μmol/L 8-OH-DPAT and the receptor down-regulation was abolished in presence of the selective 5-HT$_{1A}$ antagonist (+)-WAY-100135 (Figure 2). Therefore, 100 μmol/L 8-OH-DPAT was routinely used for the preincubation. Cells expressing either WT or Val28 and incubated for 24 h with 100 μmol/L 8-OH-DPAT showed a statistically significant decrease in 5-HT$_{1A}$ binding sites, whereas Ser22 displayed no significant down-regulation (Table 2). No change in K_D for both WT and variant 5-HT$_{1A}$ receptors was observed between controls and pretreated cells (Table 2). As shown in Figure 3, the degree of down-regulation after incubation of the cells with 100 μmol/L 8-OH-DPAT for 24 and 48 h was significantly lower for Ser22, as compared with the WT 5-HT$_{1A}$ receptor. To rule out the possibility that the lack of down-regulation in the cells expressing the Ser22 variant reflected some peculiarities of the individual clone studied, the long-term agonist-promoted down-

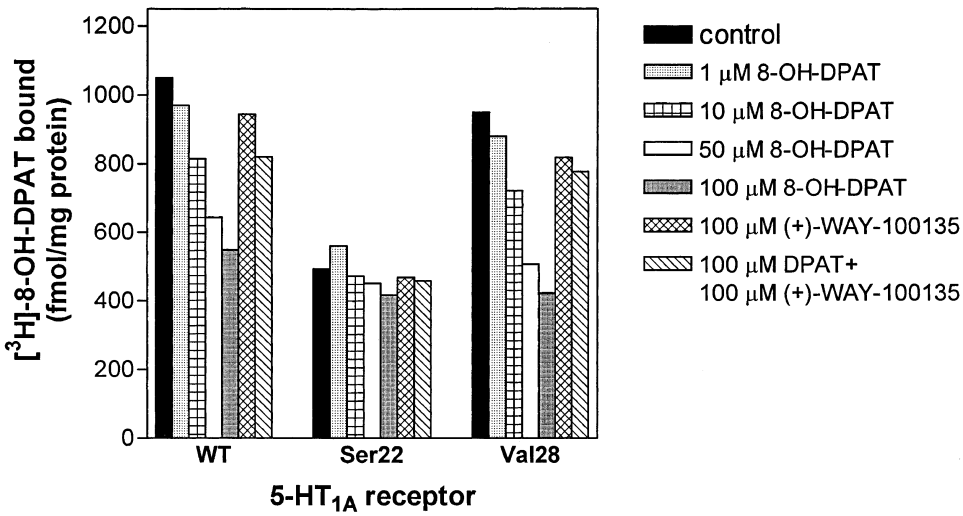

Figure 2. B_{max} values of [^{3}H]-8-OH-DPAT binding on WT, Ser22, or Val28 5-HT$_{1A}$ receptors stably expressed in CHO-K1 cells preincubated for 24 h with the indicated concentrations of unlabeled 8-OH-DPAT or (+)-WAY-100135. Data are from a representative experiment that was repeated two times with similar results.

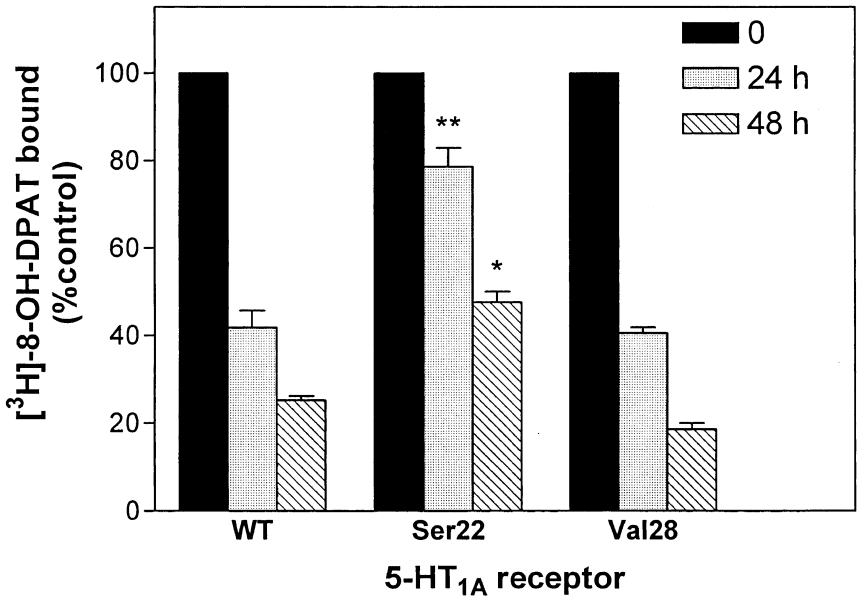

Figure 3. Time course of the agonist-promoted down-regulation of WT, Ser22, and Val28 5-HT_{1A} receptors stably expressed in CHO-K1 cells. Cells were incubated for the indicated times with 100 μmol/L 8-OH-DPAT, as described in Material and Methods. Bars show the maximal [^{3}H]-8-OH-DPAT binding (% of the control) at time 0 and after 24 and 48 h incubation. Values are the mean ± SEM of four independent determinations: * $p < .05$, ** $p < .005$ as compared with the WT 5-HT_{1A} receptor at the same incubation time.

regulation properties of a different Ser22 clone expressing ∽70 fmol receptor/mg protein was also studied. In agreement with the results presented above, this Ser22 clone did not undergo a statistically significant decrease in receptor density after 24 h preincubation with 100 μmol/L 8-OH-DPAT (Bmax control vs. Bmax pretreated cells were: 74 ± 6 fmol/mg protein vs. 68 ± 3 fmol/mg protein [mean ± SEM, $n = 2$]).

Agonist-Promoted Desensitization of the 5-HT_{1A} Receptor-Mediated Inhibition of cAMP Accumulation

Preincubation for 24 h with 100 μmol/L 8-OH-DPAT significantly decreased the maximal 5-HT-induced inhibition of FSK-stimulated cAMP accumulation in cells expressing either WT or Val28 5-HT_{1A} receptors, whereas no significant decrease of maximal inhibition of cAMP accumulation was observed in the cells expressing the Ser22 variant (Figure 4). Preincubation of cells expressing WT or variant 5-HT_{1A} receptors with 8-OH-DPAT did not lead to significant changes in the potency of 5-HT to inhibit cAMP accumulation (EC_{50} controls vs. EC_{50} pretreated cells values were: 42.94 ± 1.38 vs. 40.49 ± 0.46 nmol/L for the WT; 37.8 ± 1.48 vs. 47.25 ± 1.53 nmol/L for Ser22; 40.27 ± 1.28 vs. 45.87 ± 1.26 nmol/L for Val28 [mean ± SEM, $n = 3$]).

DISCUSSION

The aim of this study was to investigate the consequences of naturally occurring variants of the human 5-HT_{1A} receptor on its functional and regulatory properties. For this purpose, WT, Ser22, and Val28 5-HT_{1A} receptors were stably expressed in CHO-K1 cells. These cells do not ordinarily express any 5-HT receptor and, thus, are useful for pharmacological and functional studies of transfected 5-HT_{1A} receptors (Raymond 1991; Raymond et al. 1992; Newman-Tancredi et al. 1992).

Table 2. Agonist-Induced Down-regulation of WT, Ser22, and Val28 5-HT_{1A} Receptors Stably Expressed in CHO-K1 Cells

	Control		100 μmol/L 8-OH-DPAT for 24 h	
5-HT_{1A} receptor	Bmax (fmol/mg protein)	K_D (nmol/L)	Bmax (fmol/mg protein)	K_D (nmol/L)
WT	959 ± 73	2.18 ± 0.11	401 ± 59[a]	2.22 ± 0.1
Ser 22	515 ± 94	3.55 ± 0.94	404 ± 70	3.72 ± 0.57
Val28	798 ± 73	1.62 ± 0.26	324 ± 40[a]	1.9 ± 0.07

CHO-K1 cells stably expressing WT and variant 5-HT_{1A} receptors were incubated for 24 h with 100 μmol/L 8-OH-DPAT or vehicle, as described in Materials and Methods. Each value is the mean ± SEM of four independent determinations.

[a] $p < .005$ as compared to the control.

The expressed WT, Ser22, and Val28 5-HT$_{1A}$ receptors showed similar ligand-binding characteristics and coupling to adenylate cyclase. Furthermore, their pharmacological properties in this cellular expression system closely resembled the 5-HT$_{1A}$ receptor profile in brain (Hoyer et al. 1986; De Vivo and Maayani 1986; Dumuis et al. 1988; Marazziti et al. 1994). It was not surprising that WT, Ser22, and Val28 5-HT$_{1A}$ receptors displayed similar ligand-binding characteristics and showed no difference in coupling to the inhibition of adenylate cyclase activity. Indeed, the regions of the 5-HT$_{1A}$ receptor protein which are thought to be most important for these functions are the transmembrane hydrophobic domains and their connecting loops, rather than the putative amino-terminal extracellular domain where the Ser22 and Val28 amino acid substitutions are located (Dohlman et al. 1991; Peroutka 1994).

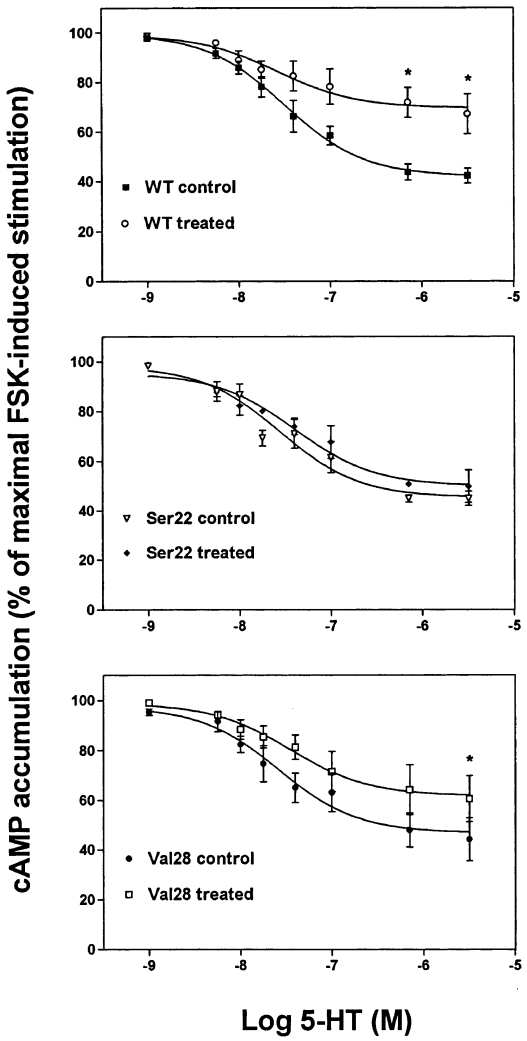

Figure 4. Agonist-promoted desensitization of 5-HT-induced inhibition of cAMP accumulation in CHO-K1 cells stably expressing the WT, Ser22, or Val28 5-HT$_{1A}$ receptors. Cells were incubated for 24 h with 100 μmol/L 8-OH-DPAT or vehicle and assayed for 5-HT-induced inhibition of FSK-stimulated cAMP accumulation as described in Material and Methods. The maximal FSK-stimulated cAMP accumulation was set equal to 100%. Each point represents the mean ± SEM of three independent determinations. * $p < .05$ as compared to the control.

Although the pharmacological and functional profiles of WT, Ser22, and Val28 receptors were similar, the long-term agonist-promoted down-regulation properties were remarkably different. The 24-h preincubation of the cells expressing the WT 5-HT$_{1A}$ receptor or the Val28 variant with increasing concentrations of 8-OH-DPAT led to a progressive decrease in receptor density with a maximal and statistically significant effect at a concentration of 100 μmol/L. Because there were no changes in receptor affinity (K_D) for the radioligand after the preincubation with the agonist, the observed receptor down-regulation was not due to retained agonist binding. Furthermore, the 5-HT$_{1A}$ receptors down-regulation was reversed by the addition of the selective 5-HT$_{1A}$ receptor antagonist (+)-WAY-100135, confirming that the decrease in receptor density was the result of a specific interaction of 8-OH-DPAT with 5-HT$_{1A}$ receptors and ruling out the possibility of irreversible agonist binding to the receptor. As compared with the WT and Val28 5-HT$_{1A}$ receptors, the Ser22 variant displayed a significantly lower degree of agonist-promoted down-regulation. Amino acid substitutions in the putative extracellular amino-terminal region of the β_2-adrenergic receptor can exert a similar effect on long-term agonist-promoted receptor down-regulation. For β_2-adrenergic receptors stably transfected in CHW-1102 fibroblasts, substitution of glutamine with glutamic acid at position 27 (Glu27) imparts nearly total resistance to agonist-promoted receptor down-regulation whereas substitution of arginine with glycine at amino acid 16 (Gly16) enhances receptor down-regulation after a 24-h treatment with 10 μmol/L isoproterenol (Green et al. 1994).

We also examined the effect of 5-HT$_{1A}$ agonist exposure on the ability of the WT 5-HT$_{1A}$ receptor and the Ser22 and Val28 variants to regulate intracellular cAMP. A 24-h incubation of cells expressing WT or Val28 with 100 μmol/L 8-OH-DPAT led to a statistically significant attenuation of the efficacy of 5-HT to inhibit cAMP accumulation. Essentially, no functional desensitization was observed in cells expressing the Ser22 variant. These results suggest that receptor down-regulation and functional desensitization are mechanistically linked in the expressed WT and variant 5-HT$_{1A}$ receptors. Interestingly, a reduced degree of both agonist-induced down-regulation and functional desensitization has also been reported for the previously mentioned Glu27 β_2-adrenergic receptor variant endogenously expressed in primary cultures of human airway smooth muscle cells (Green et al. 1995).

The observed alteration in long-term agonist-promoted down-regulation of the Ser22 variant is unlikely to be due to transcriptional differences among the transfected WT, Ser22, and Val28 5-HT_{1A} receptor genes. Indeed, the plasmids p5-HT_{1A}SER22 and p5-HT_{1A}VAL28 differed from the plasmid p5-HT_{1A}WT only at nucleotides 64 and 82 of the 5-HT_{1A} gene, respectively. Furthermore, transcription of the receptor genes in the expression vector pcDNA3 was under the control of a viral promoter (CMV) whose activity should not be influenced by agonist-promoted receptor activation. It is possible that the effect of amino-terminal variants on receptor down-regulation is a consequence of altered susceptibility to protein degradation. Cells expressing β_2-adrenergic receptors (a receptor structurally closely related to the 5-HT_{1A} receptor) engineered for deletions of amino acids 21-30 in the amino-terminal domain displayed normal ligand binding, but showed an increase in the level of immature receptor (Dixon et al. 1987). In agreement with these data, the β_2-adrenergic receptor variant Glu27 expressed in CHW-1102 fibroblasts, that did not undergo long-term agonist-promoted receptor down-regulation, exhibited an altered electrophoretic mobility as compared with the wild type (Green et al. 1994). This abnormal electrophoretic mobility may reflect an immature conformation of the Glu27 variant that can lead to an alteration of receptor degradation.

Behavioral, electrophysiological, receptor binding, and second messenger studies have provided evidence that down-regulation and/or desensitization of 5-HT_{1A} receptor occur in vivo as the consequence of prolonged treatment with several classes of antidepressant and anxiolytic drugs, such as SSRIs or 5-HT_{1A} agonists (Newman et al., 1993; Blier and de Montigny 1994; De Vry 1995; Albert et al. 1996). The main result of these adaptative changes is an enhancement of serotonergic neurotransmission after approximately 2 weeks. This effect accounts for the therapeutic action of SSRIs or 5-HT_{1A} agonists as well as the delayed onset of action of these drugs (Blier and de Montigny 1994). Clinical evidence indicates that blocking 5-HT_{1A} somatodendritic receptors with the β-adrenergic/5-HT_{1A} antagonist pindolol accelerates the antidepressant effect of SSRIs or monoamino oxidase (MAO) inhibitors and provides a full therapeutic response in patients resistant to antidepressant treatment (Artigas et al. 1994; Blier and Bergeron 1995). Thus, the resistence to receptor down-regulation and functional desensitization imparted by the Ser22 variant may impair the therapeutic action of widely used antidepressants and anxiolytics. The β_2-adrenergic receptor variant Gly16, which undergoes an enhanced long-term agonist-mediated down-regulation (Green et al. 1994), is associated with a requirement for continuous steroid and immuno therapy in asthmatic patients (Reihsaus et al. 1993). The enhanced agonist-mediated down-regulation of this β_2-adrenergic receptor variant may lead to a decreased efficacy of chronic β_2-adrenergicagonist therapy and explain the need for continuous corticosteroid treatment and immunotherapy (Reihsaus et al. 1993). Conversely, the Glu27 β_2-adrenergic receptor variant that imparts resistance to long-term agonist-mediated down-regulation (Green et al. 1994), is associated with lower airway reactivity in asthmatic patients and could have a protective effect against inflammation and bronchoconstriction (Hall et al. 1995).

When heterologous expression systems are used to gain insights into receptor regulation, the relevance to in vivo function can be questioned. Regional and cellular specificity of receptor regulation has been reported for the 5-HT_{1A} receptor expressed in different cerebral areas (Newman et al. 1993; Blier and de Montigny 1994) and in different cell lines (reviewed in Boess and Martin, 1994). Furthermore, promoter elements that regulate 5-HT_{1A} receptor expression in native cells are different from those of the viral promoter that controls the transcriptional activity of the 5-HT_{1A} receptor gene in the eukaryotic expression vector pcDNA3. Nevertheless, the potential usefulness of heterologous recombinant systems for identifying variants altering receptor regulation has been recently supported by the studies on naturally occurring variants in the extracellular amino-terminus of the human β_2-adrenergic receptor. When the β_2-adrenergic receptor variants Gly16 and Glu27 were expressed in CHW cells under the control of a viral promoter (Green et al. 1994), the functional differences observed were consistent with those measured in Gly16 and Glu27 variants endogenously expressed in primary cultures of human airway smooth muscle cells (Green et al. 1995).

In conclusion, two naturally occurring variants, Ser22 and Val28, in the putative amino-terminal region of the 5-HT_{1A} receptor do not alter receptor ligand binding and coupling to second messengers. However, Ser22 alters long-term agonist-promoted receptor down-regulation and functional desensitization. Taking into account the limitations that arise from the in vitro design of this study, we speculate that the relative resistence of the variant Ser22 5-HT_{1A} to receptor down-regulation and functional desensitization could modify the regulatory properties of the receptor in vivo and lead to altered sensitivity of the 5-HT_{1A} receptor to antidepressant and anxiolytic treatment in individuals who carry this rare 5-HT_{1A} receptor variant.

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