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Messenger RNA Editing of the Human Serotonin 5-HT_{2C} Receptor

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RNA encoding the rat serotonin 5-HT_{2C} receptor undergoes editing whereby one to four adenosines are converted to inosines. This conversion can change up to three codons out of a stretch of five in the second intracellular loop of the receptor. RNA editing of the rat 5-HT_{2C} receptor that changes all three codons was shown previously to alter intracellular signaling by 5-HT without changing its receptor-binding affinity. We analyzed 5-HT_{2C} receptor editing in human brain and hypothalamic RNA samples and confirmed that all four adenosine editing sites observed in rat were also present in human samples. Additionally, we identified a novel editing site in the middle edited codon that extends the repertoire of 5-HT_{2C} receptors by six additional protein isoforms. We observed that editing reduces both the binding affinity and functional potency of agonists for recombinant human 5-HT_{2C} receptor isoforms. This effect on binding affinity was proportional to the agonist's intrinsic activity, with full agonists most affected, and antagonists showing no effect. These data suggest that RNA editing may alter coupling energetics within the ternary complex, thereby altering agonist binding affinities, G protein coupling, and functional responses. RNA editing may thus provide a novel mechanism for regulating 5-HT synaptic signaling and plasticity.

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In addition to RNA splicing, transcripts of diverse genes (mRNAs, tRNAs, rRNAs) can be altered co- or post-transcriptionally by nucleotide insertion, deletion, or modification. This process, termed RNA editing, has been documented in cells, organelles, and viruses of lower and higher eukaryotes (reviewed by Simpson and Emeson 1996; Smith et al. 1997). The most dramatic form of editing occurs in coding regions, where it may cause translational frameshifts, or generate stop or missense codons. The resulting proteins are truncated and/or contain amino acids not encoded by the original non-edited transcripts. Therefore, mRNA editing can be viewed as a protein diversity-generating mechanism that enables a single gene to produce multiple proteins with potentially distinct structures and functions.

Nucleotide deamination appears to mediate the majority of mRNA editing identified in mammalian cells. For example, a cytosine deaminase converts cytosine to uridine at position 6666 in apolipoprotein B (apoB) mRNA (Powell et al. 1987). This creates a stop codon and results in the synthesis of a truncated protein. Because the truncated apoB protein is missing the low-density lipoprotein receptor domain, only the long form of apoB can facilitate cholesterol uptake via the

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low-density lipoprotein receptor pathway (Hodges and Scott 1992). In another example of mRNA editing in mammals, an adenosine deaminase enzyme(s) converts several adenosines to inosines in mRNAs coding for several subunits of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate-sensitive glutamate receptor channel (GluRs). Because inosines in mRNA are recognized as guanosines by ribosomes, codons for glutamine, arginine, isoleucine and tyrosine are changed in several discrete positions to arginine, glycine, valine and cysteine, respectively (reviewed by Seeburg 1996). These single amino acid changes alter channel function by reducing calcium permeability (Jonas et al. 1994) and by enhancing recovery from desensitization (Lomeli et al. 1994).

The most extensive example of mRNA editing of a single gene transcript by adenosine deaminase, and the only reported case of RNA editing for a G protein-coupled receptor, is that of the rat serotonin 5-HT$_{2C}$ receptor in which 10 edited mRNA isoforms were identified (Burns et al. 1997). These isoforms are generated by combinatorial editing at up to four adenosine sites located in three nearby codons: ***A*UA** (sites A and B) for isoleucine (position 157), **AAU** (site C) for asparagine (position 159), and ***A*UU** (site D) for isoleucine (161) (see Table 1). Editing at sites A, AB, and D changes isoleucine (I) to valine (V) and at site C asparagine (N) to serine (S). Due to the codon degeneracy at the B site, seven 5-HT$_{2C}$ receptor protein isoforms (INI, INV, ISV, VNI, VNV, VSI, and VSV) are produced from one non-edited (INI) and 10 edited mRNA isoforms. Pharmacological characterization of INI and VSV isoforms transiently expressed in NIH3T3 cells, revealed that 5-HT was 10–15-fold less potent in eliciting intracellular signaling in the VSV isoform. Because the three edited amino acids are located in the second intracellular loop of the receptor, a region implicated in the coupling of receptors to G protein-mediated signaling cascades (e.g., Gomeza et al. 1996), editing may modulate 5-HT$_{2C}$ receptor-mediated signaling by interfering with receptor G–protein interactions (Burns et al. 1997).

The 5-HT$_{2C}$ receptor is one of 14 cloned 5-HT receptor subtypes (Hartig 1997). Because of the important role for this receptor in regulating mood, appetite, and sexual behavior (e.g., Roth et al. 1998), we sought to determine if RNA editing of this receptor occurred in the human brain, and whether this process modulated 5-HT-mediated signal transduction.

MATERIALS AND METHODS

Materials

[^{3}H]5-HT (98 Ci/mmol), [^{3}H]mesulergine (50 Ci/mmol), and myo-[2-^{3}H]inositol (15–20 Ci/mmol) with stabilizer were purchased from Pharmacia Amersham (Arlington Heights, IL). All other reagents were purchased from Research Biochemicals Inc. (Natick, MA), Sigma Chemical Co. (St. Louis, MO), or Gibco BRL (Grand Island, NY) unless otherwise noted.

Human RNA and cDNA Cloning of Edited Isoforms

Total human brain RNA derived from a 60-year-old male who died of cardiac arrest was purchased from Clontech (Palo Alto, CA; Cat. No. 64020-1, lot # 38134). Total hypothalamic RNA was derived from a 50-year-old male who died of an accident. Oligo dT primed first-strand cDNA was synthesized using 5 μg of RNA and Superscript Reverse Transcription kit (Gibco BRL) under the manufacturer's recommended conditions. The edited region of the human 5-HT$_{2C}$ receptor cDNA was amplified by the polymerase chain reaction (PCR) method using a Perkin-Elmer 9600 Thermal Cycler, Boehringer Manheim (Indianapolis, IN) PCR buffer and Expand High Fidelity polymerase (2.6 units). Oligonucleotide primers were designed from GenBank's human 5-HT$_{2C}$ receptor sequence #U49516: forward primer 5′ TGTCCCTAGCCATTGCTGATATG (position 1006–1029) and reverse primer 5′ TTTGCAGAGTTCTCTTCCTCGG (position 1542–1564). PCR conditions were: 0.5 μM each primer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$; initial denaturation at 94°C, 2 minutes; followed by 94°C, 30 seconds; 55°C, 30 seconds; 72°C, 1 minute for 30 cycles. Final extension was for 6 minutes at 72°C. PCR fragments were cloned into the TA vector (Invitrogen) and individual plasmid isolates were sequenced using the ABI Prism 377 DNA Sequencing System.

The full-length 5-HT$_{2C}$ receptor coding sequence was obtained by the PCR method using forward primer 5′ ACCG*CTCGAG*CCTAAGACTGAAGCAATCATGG (position 713–732) and reverse primer 5′ CTA*GTCTAG*AGACTGTGCTGTTCTTTCTCACACAC (position 2098–2124). The design of primers was also based on the human sequence #U49516, and for cloning purposes, XhoI and XbaI restriction sites were included in the forward and reverse primers, respectively. A human adult brain cDNA library from Gibco BRL was used as the template (Cat. No. 10418-010, lot # FCE 002, derived from a 36-year-old female). PCR conditions were the same as above with these exceptions: 0.3 μM of each primer, 1.0 mM $MgCl_2$; 10 cycles with annealing at 47°C, 40 seconds and extension at 72°C for 90 seconds followed by 20 cycles with annealing at 55°C, 40 seconds; 72°C, 90 seconds with 20-second extension per cycle. Final extension was 72°C for 7 minutes. PCR fragments were digested with XhoI and XbaI, and cloned into an EBV ori-P vector (Horlick et al. 1997). This plasmid has the cytomegalovirus (CMV) immediate early promoter to drive receptor expression, Epstein Barr virus origin of replication (EBV ori-P) for the maintenance as an episome in cells expressing EBV nuclear antigen 1 (EBNA1), and the *E. coli* hygromycin B resistance gene. Because this cloning ap-

proach yielded only the INV and VNV isoforms, Transformer Site-Directed Mutagenesis kit (Clonetech) was used to modify INV to INI, and VNV to VSV and VGV isoforms.

Stable Expression of 5-HT_{2C} Receptor Isoforms in HEK293E Cells

Stable cell lines were generated by transfecting human embryonal kidney (HEK) 293EBNA cells (Invitrogen, Carlsbad, CA) with plasmids containing cDNAs for the INI, VNV, VSV, or VGV edited isoforms of the 5-HT_{2C} receptor using the Calcium Phosphate Transfection System kit from Gibco BRL. Cells were maintained as adherent cultures at 37°C in a humid environment (5% CO_2) in Dulbecco's Modified Eagle medium (DMEM, Gibco BRL) containing dialyzed 10% fetal bovine serum, and 250 μg/ml each of hygromycin and G418 antibiotics. These cell lines were harvested between passages 3–6 for biochemical analyses.

5-HT_{2C} Receptor Agonist and Antagonist Radioligand Binding

On the day of assay, membranes containing the 5-HT_{2C} receptor were prepared from whole cells in 50 mM Tris HCl (pH 7.7) containing 1.0 mM EDTA (tissue buffer) as described previously (Fitzgerald et al. 1998). [^{3}H]mesulergine and [^{3}H]5-HT were used as the antagonist and agonist radioligands, respectively. For [^{3}H]5-HT experiments, assays were conducted in polypropylene 96-well plates (Costar Corp., Cambridge, MA) and were initiated by the addition of 5-HT_{2C} receptor membrane homogenate (30–80 μg/well) to assay buffer (50 mM Tris HCl, 0.5 mM EDTA, 10 mM pargyline, 10 mM $MgSO_4$, 0.05% ascorbic acid, pH 7.4) containing [^{3}H]5-HT with or without competing ligand. For the [^{3}H]mesulergine experiments, the assay buffer used was identical to that used in the [^{3}H]5-HT assay except for the substitution of 10 mM $CaCl_2$ for 10 mM $MgSO_4$. [^{3}H]5-HT and [^{3}H]mesulergine assays were incubated to equilibrium for 60 and 45 minutes at 37°C, respectively, and terminated by rapid filtration (Cell Harvestor, Inotech Biosystems Inc., Lansing, MI) with ice-cold 50 mM Tris HCl buffer (pH 7.5) over GFF glass-fiber filters that had been pre-soaked in 0.3% polyethyleneimine. Filters were dried and then counted by liquid scintillation spectroscopy. Saturation studies were performed using 16 concentrations of [^{3}H]5-HT or [^{3}H]mesulergine to determine equilibrium binding parameters. Nonspecific binding was determined in the presence of 10 μM mianserin. For competition experiments, binding resulting from a fixed concentration of [^{3}H]5-HT (2–10 nM) or [^{3}H]mesulergine (1–2 nM) was competed with 12 concentrations of ligand (10 pM to 10 μM).

Phosphoinositide Hydrolysis Studies

The ability of various ligands to stimulate phosphoinositide (PI) hydrolysis was monitored in whole cells using a variant (Egan et al. 1998) of a protocol described previously (Berridge et al. 1982). HEK293E (human embryonic kidney) cells expressing the human 5-HT_{2C} receptor were plated onto poly-D-lysine-coated 24-well plates (Biocoat, Becton Dickinson, Bedford, MA) in DMEM containing high glucose, 2mM glutamine, 10% dialyzed fetal calf serum, 250 μg/ml hygromycin B, and 250 μg/ml G418. Following a 24–48-hour period, the growth media was removed and replaced with DMEM without fetal calf serum and inositol (Gibco BRL). The cells were then incubated with DMEM (without serum and inositol) containing a final concentration of 0.5 μCi/well myo-[^{3}H]inositol for 16–18 hours. Following this incubation, the cells were washed with DMEM (without serum or inositol) containing 10 mM LiCl and 10 μM pargyline and then incubated for 30 minutes with the same media but now containing one of several test compounds. [^{3}H]phosphoinositides were extracted and separated by anion exchange chromatography as described previously (Egan et al. 1998).

Data Analyses

The equilibrium dissociation constants (K_D) and maximal number of binding sites (B_{max}) from the saturation experiments, and the apparent dissociation constants (K_is) from the competition experiments were calculated using an iterative nonlinear regression curve-fitting program (GraphPad Prism, San Diego, CA). For the PI hydrolysis experiments, EC_{50} values were calculated using a one-site "pseudo" Hill model: $y = [(R_{max} - R_{min})/(1 + R/EC_{50})^{nH})] + R_{max}$ where R = response (DeltaGraph, Monterey, CA). E_{max} (maximal response) was derived from the fitted curve maxima (net IP stimulation) for each compound. Intrinsic activity (IA) was determined by expressing the E_{max} of a compound as a percentage of the E_{max} of 5-HT (IA = 1.0). Statistical comparisons of group means were made using independent *t*-tests (GraphPad Prism).

RESULTS

Distribution of 5-HT_{2C} Receptor mRNA and Predicted Protein Isoforms in Human Brain and Hypothalamus

To assess the 5-HT_{2C} receptor isoform distribution we sequenced over 50 individual clones derived by the RT-PCR method from total human brain and from hypothalamic RNA. After eliminating clones containing the alternatively spliced version of 5-HT_{2C} receptor cDNA (Canton et al. 1996) and clones where sequences of both

strands were not identical, we were able to compare sequences of 44 individual clones from brain and 42 individual clones from hypothalamus. In both samples we detected editing of the same four adenosines in the three amino acid codons reported by Burns et al. (1997) for rat 5-HT$_{2C}$ receptor mRNA: I (amino acid position 156), N (position 158), and I (position 160). These amino acid positions differ between rat and human 5-HT$_{2C}$ receptors because only the former has a leucine at position 46. In addition, we detected a new adenosine editing site in the asparagine codon 158 (***A*AU**, site E) that is adjacent to the C site (**A*A*U**) of Burns et al. (1997) (see Table 1). Editing at the E site alone converts asparagine to aspartic acid, and in combination with editing at the C site, to glycine.

All mRNA isoforms and their predicted protein isoforms detected in the total brain and hypothalamic RNAs are listed in Table 1. In the brain and hypothalamus we detected 17 and 18 edited 5-HT$_{2C}$ receptor mRNA isoforms, respectively. Because editing at site A alone or in combination with site B converts I to V, results are expressed as the percentage of predicted protein isoforms in brain and hypothalamus rather than the percentage of individual mRNA isoforms. The nonedited isoform (INI) accounted for only 7% of the mRNA population in the whole brain and 12% in hypothalamus. The most prevalent isoform in the whole brain was VSV (at 32%) whereas IGI, IDI, and IDV were not detected. In hypothalamus the VNV isoform was most abundant (at 28%) whereas ISV and ISI were not detected. In neither whole brain nor hypothalamus did we detect editing at the B site alone, nor did we detect edited mRNAs capable of encoding the VDI and IGV isoforms. Editing at the novel human site E represented 15% and 25% of predicted protein isoforms in the whole brain and hypothalamus, respectively.

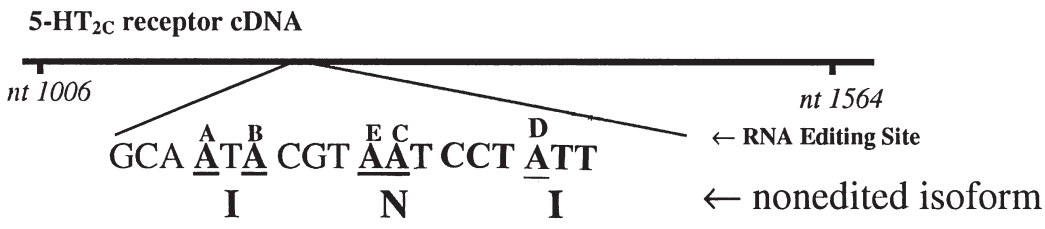
Radioligand-Binding Studies

Radioligand-binding experiments were conducted to evaluate the relative binding affinities of various agonists and antagonists for the non-edited (INI) and for three multiply edited (VNV, VSV, VGV) isoforms of the human 5-HT$_{2C}$ receptor. These four isoforms were stably expressed in HEK293E cells and grown as adherent cultures. They were harvested between passages 3 and 6 post-transfection to insure comparable receptor densities. Saturation isotherms generated using the agonist radioligand [^{3}H]5-HT revealed that editing reduced binding affinity in a graded fashion. The affinity (K_D) of 5-HT was decreased in the fully edited isoforms VSV and VGV, but to a lesser, but statistically nonsignificant (p = 0.13) extent in the partially edited isoform VNV (see Figure 1). In addition, the affinity of 5-HT for the VGV isoform was significantly less than its affinity for the VSV and VNV isoforms. In contrast, the affinity of the antagonist radioligand [^{3}H]mesulergine did not vary significantly among the four isoforms. Total receptor

Table 1. Distribution of Human 5-HT$_{2C}$ Receptor Isoforms

RNA Isoform (Sites Edited)	Total Brain Population	Hypothalamus Population	Predicted Protein Isoform
nonedited	7%	12%	INI
A, AB	2	12	VNI
AC, ABC	11	7	VSI
C	2	–	ISI
ABD, AD	16	28	VNV
ACD, ABCD	32	12	VSV
D	9	5	INV
CD	7	–	ISV
ABCDE, ACDE	5	7	VGV
CE	–	5	IGI
ABCE, ACE	5	7	VGI
E	–	2	IDI
DE	–	2	IDV
ABDE, ADE	5	2	VDV

Values represent percentage of predicted protein isoforms out of 42 hypothalamic and 44 brain RNA samples sequenced; (–) signifies none detected.



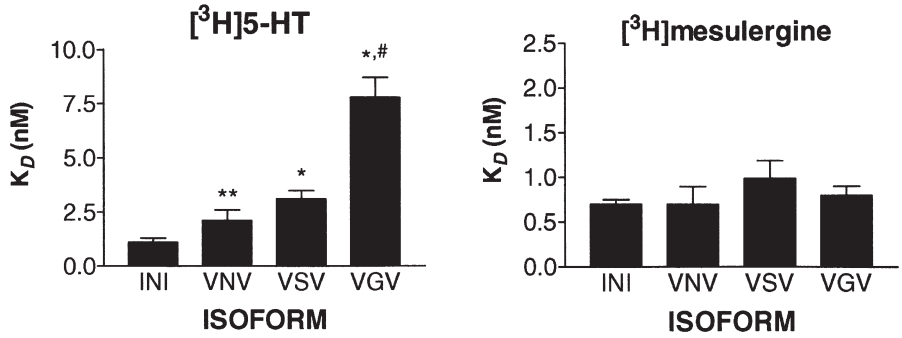


Figure 1. Saturation isotherm-derived binding affinities of [^{3}H]5-HT and [^{3}H]mesulergine for the non-edited (INI), partially edited (VNV), and fully edited (VSV, VGV) isoforms of the human 5-HT$_{2C}$ receptor expressed in HEK293E cells. [^{3}H]5-HT isotherms were best fit by a two-site/state model ($p < .05$, GraphPad Prism) for the INI, VNV, and VSV isoforms, whereas the isotherms for the VGV isoform were best fit to a one-site/state model. Only the K_D to the high-affinity agonist state is depicted. All antagonists isotherms for [^{3}H]mesulergine binding were best fit to a one-site/state model. Total receptor densities (B_{max}) as defined by [^{3}H]mesulergine ranged from 3.5 to 6 pmol/mg protein. (*Significantly different from INI value, **p = .013 vs INI, $^{\#}$significantly different from VSV value.)

densities for the four cell lines as defined by [^{3}H]mesulergine binding ranged between 3.5 and 6 pmol/mg protein.

We followed these observations by examining the apparent binding affinities of other ligands in competition for [^{3}H]5-HT binding sites (high-affinity agonist sites). In support of the saturation experiments, the competition experiments showed that 5-HT had the highest affinity (lowest apparent K_i) for the INI isoform of the receptor relative to the VGV, VSV, and VNV isoforms, and its affinity for the VGV isoform was significantly less than for the VSV and VNV isoforms (see Table 2). A similar observation was made for the full agonist α-methyl 5-HT. The impact of RNA editing on agonist binding was proportional to the intrinsic activity of the ligand (as defined by a low receptor reserve, partially edited VNV cell line), as shown in Figure 2. Full agonists were most affected by editing, antagonists were essentially unaffected, and partial agonists displayed an intermediate effect. The antagonists mianserin and clozapine did, however, display a significantly greater affinity for the VGV isoform, compared to the INI isoform of the receptor.

We also assessed whether use of the antagonist ligand [^{3}H]mesulergine would impair our ability to observe editing effects on the binding affinities of ago-

Table 2. Binding Affinities of Agonists and Antagonists at the Edited (VSV, VGV, VNV) and Unedited (INI) Isoforms of the Human 5-HT$_{2C}$ Receptor

	INI (nM)	VNV (nM)	VSV (nM)	VGV (nM)	VGV/INI (X)
Agonists					
5-HT	3.0 ± 2.2	4.3 ± 0.5	9.0 ± 2.7*	19.4 ± 2.2*, +,#	6.5
*m*CPP	15.3 ± 2.1	24.4 ± 1.1*	53.7 ± 2.7*,#	88.2 ± 14.7*,#	5.8
αm5-HT	5.0 ± 0.5	7.9 ± 0.7*	13.3 ± 1.6*	40.5 ± 5.4*,+,#	8.1
(–)DOI	4.5 ± 1.1	7.1 ± 0.3	11.9 ± 2.3*	20.2 ± 3.5*,#	4.5
Oxymetazoline	96.5 ± 7.8	ND	243.8 ± 22.7*	205 ± 19.0*	2.1
(+)LSD	5.7 ± 1.1	3.8 ± 0.4	8.4 ± 2.4	4.3 ± 0.4	0.8
(+)Lisuride	8.4 ±1.3	ND	9.5 ± 1.2	8.7 ± 0.5	1.0
Antagonists					
Fluoxetine	80.3 ± 9.5	114 ± 16	91.3 ± 13.4	101 ± 15	1.2
Ketanserin	73.1 ± 15.6	42.4 ± 4.5	54.7 ± 16.8	43.7 ± 5.6	0.6
Mianserin	4.4 ± 0.8	1.2 ± 0.2*	3.6 ± 0.8	2.0 ± 0.3*	0.5
Clozapine	16.7 ± 1.4	7.8 ± 0.3*	20.5 ± 6.3	7.7 ± 1.2	0.5
Spiperone	1627 ± 443	ND	1426 ± 226	856 ± 113	0.5

Competition experiments were conducted as described in the Materials and Methods.

Depicted are mean K_i values (± SEM) of competing ligands versus a fixed concentration (1–2×K_D) of [^{3}H]5-HT from 4–6 independent experiments.

Binding affinities (K_i) were calculated using the iterative nonlinear regression analysis (GraphPad Prism).

ND = not determined. Significantly different from *INI, $^+$VSV, and $^{\#}$VNV values, all p-values $< .05$.

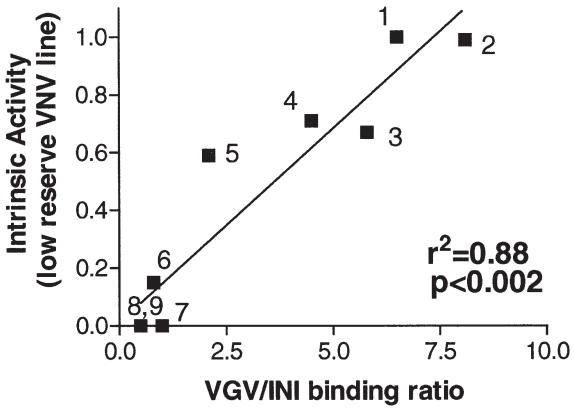


Figure 2. Effect of RNA editing on binding affinities is proportional to intrinsic activity. The ratios of agonist binding affinities from Table 2 (K_i at VGV isoform divided by K_i at INI isoform) for six agonists and two antagonists are plotted against intrinsic activities (maximal PI hydrolysis activities) measured with the partially edited VNV isoform. Intrinsic activities were measured in a low receptor density, low receptor reserve VNV line where intrinsic activities approximate intrinsic efficacies. This line is not the same as the higher density VNV line used for comparison with the other higher density-matched cell lines (INI, VSV, VGV). The R^2-value was determined by linear regression analysis (GraphPad Prism). (1 = 5-HT, 2 = α-methyl 5-HT, 3 = *m*CPP, 4 = (−)DOI, 5 = oxymetazoline, 6 = (+)LSD, 7 = (+)lisuride, 8 = clozapine, 9 = mianserin.)

nists. Indeed, the VGV/INI K*i* ratios for 5-HT, DOI 1-(2,5-dimethoxy-4-iodophenyl-2-aminopropane), *m*CPP (metachlorophenylpiperazine), and oxymetazoline (2.8-, 2.2-, 1.7-, and 1.9-fold, respectively) were attenuated using an antagonist radioligand compared to the ratios obtained with the agonist [^{3}H]5-HT (6.5-, 4.5-, 5.8-, 2.1-fold, respectively; see Table 2).

Phosphoinositide Hydrolysis Studies

Table 3 displays the abilities of 5-HT, *m*CPP, and (+)LSD (lysergic acid diethylamide) to stimulate phosphoinositide hydrolysis in HEK293E whole cell assays, using cells expressing the non-edited and edited isoforms of the human 5-HT$_{2C}$ receptor. The mean potencies (EC_{50} values) of 5-HT and *m*CPP decreased in a graded fashion with RNA editing just as seen with their binding affinities (Table 2). The only differences, however, were that significant potency differences for 5-HT or *m*CPP were not observed between the fully edited isoforms (VSV versus VGV), but were observed between the VNV and INI isoforms. RNA editing also altered the functional properties of the weak agonist LSD. The intrinsic activity of LSD markedly decreased with RNA editing to the extent that nearly full agonism was seen in the INI cell line, partial agonism with the VSV and VNV cell lines, and no significant stimulatory response could be observed in the VGV cell line. This was somewhat unexpected since RNA editing had no significant impact on the binding affinities of LSD for the three edited isoforms. Lastly, basal PI hydrolysis was not inhibited in any of the cell lines by the previously identified inverse agonists mianserin, clozapine, and methiothepin (n = 3, data not shown).

DISCUSSION

Our study demonstrates that a majority of human brain 5-HT$_{2C}$ receptor mRNA is modified, presumably due to an adenosine deaminase editing activity, that results in the creation of 20 novel mRNA and 13 predicted protein isoforms of the receptor. These findings are in good agreement with the original study by Burns et al. (1997) on 5-HT$_{2C}$ receptor editing in rat brain. However, there are at least three major observations that extend the work of the prior study. First, at least five editing sites are used in human brain while only four major editing sites were reported in the rat. Editing with this additional site (site E) extends the diversity of human 5-HT$_{2C}$ receptor mRNA and protein isoforms to 21 and 14, respectively (versus 11 and 7 in the rat). Second, full editing that results from the change of all three amino acids

Table 3. Functional Potencies of Agonists at Four Major Edited Isoforms of the Human 5-HT$_{2C}$ Receptor

	INI		VNV		VSV		VGV	
	EC_{50} (nM)	IA	EC_{50} (nM)	IA	EC_{50} (nM)	IA	EC_{50} (nM)	IA
5-HT	7.2 ± 2.0	1.0	31.5 ± 8.6*	1.0	51.4 ± 13.1*	1.0	66.5 ± 16.1*	1.0
*m*CPP	15.0 ± 3.2	1.0	41.5 ± 7.4*	0.9 ± 0.1	63.8 ± 12.9*	1.0	107 ± 25*,#	0.8 ± 0.1
LSD	6.2 ± 1.8	0.9 ± 0.1	9.3 ± 2.0	0.64 ± 0.1	14.8 ± 4.8**	0.4 ± 0.02	NA	NA

Phosphoinositide hydrolysis was measured in adherent cultures by anion exchange chromotography as described in the Materials and Methods.

Depicted are the mean (EC_{50}, nM) ± SEM potencies and intrinsic activities from 3–8 independent experiments. Intrinsic activities (IA) were calculated by referencing the net maximal stimulation of [^{3}H]IP release for each compound against the curve maxima for the full agonist 5-HT (IA = 1.0).

Maximal 5-HT stimulated DPMs did not substantially differ across the three edited isoforms, except that basal counts for the INI isoform were usually the highest.

NA = not active; *significantly different from INI values, $p < 0.05$, **$p = 0.07$ vs. INI; #significantly different vs. VNV.

(INI → VSV, VGV, or VDV) is more prevalent in human (45%) than in rat (11%) brain. Third, it was reported that RNA editing of the rat 5-HT_{2C} receptor reduced the functional potency of 5-HT without a commensurate change in ligand binding affinity. Using our procedures, we observed that RNA editing can profoundly alter both agonist high-affinity binding as well as intracellular signaling at the human receptor. These editing effects are proportional to the intrinsic activity of the ligand, with full agonists being most affected by editing and antagonists showing little effect.

The discovery of a novel editing site (E) extends the diversity of potential human 5-HT_{2C} receptors by an additional six protein isoforms. The presence of the VGV isoform was significant in whole brain and hypothalamus. However, it is important to emphasize that these measurements were derived from only two individuals that were unmatched with regard to age, race, and other characteristics. Future analysis with an extended population will be needed to confirm the exact frequency distribution of human 5-HT_{2C} receptor isoforms. The reason for more extensive editing at the C and E sites in humans compared to rats is not known but there are several possibilities. Editing in the INI motif of the rat 5-HT_{2C} receptor requires the formation of an imperfect stem-loop structure between exon 3 and intron 3 sequences of pre-mRNA (Burns et al. 1997). This region is the target for either the double stranded RNA-specific deaminase (DRADA) or double stranded-RNA specific editase 1 (RED 1). It may be that the stem-loop structure formed in the human pre-mRNA 5-HT_{2C} receptor transcripts is different and favors more editing at the C site and/or makes the E site more available for editing. This notion can be adequately explored once the sequence of human intron 3 is known. Another possibility may be that human cells express higher levels of DRADA or RED 1 enzyme. Indeed, it was shown that enhancing cytidine deaminase catalytic capacity by over-expression of the enzyme increases both site-specific and promiscuous editing of apoB mRNA (Sowden et al. 1996). Lastly, it is conceivable that human neuronal cells simply have an additional adenosine deaminase activity or regulatory factors that facilitate increased editing at the C and E sites.

A major observation in the present study is that RNA editing in the human receptor reduces both high-affinity agonist binding and functional potencies in a graded fashion (i.e., reductions were less apparent in the partially edited VNV line than the fully edited VSV and VGV lines). Moreover, these reductions were in proportion to the intrinsic activity of the tested ligand. This differs from the study by Burns et al. (1997), in which a reduction in the functional potency of 5-HT was not accompanied by any measurable changes in binding affinity. This disconnect between binding affinity and function in rat was somewhat surprising since it runs counter to most predictions made by modern models of receptor–G protein interaction (e.g., ternary complex, two-state models; e.g., Wreggett and De Lean 1984; Leff 1995). In our study, the negative impact of RNA editing on agonist binding depended on the intrinsic efficacy of the ligand. For example, 5-HT and α-methyl 5-HT demonstrate a significantly reduced affinity for the edited isoforms of the 5-HT_{2C} receptor (6–8-fold) whereas the partial agonists DOI was less affected by the editing. As one proceeds down in efficacy to weak partial agonists (e.g., LSD) or antagonists, there are virtually no differences in their affinities for the non-edited versus edited isoforms. With respect to function, reduction in potency with RNA editing also seems to depend on the intrinsic activity of the ligand. In addition, it is important to emphasize one unexpected observation in the case of LSD. Although there was no significant change in its binding affinity or functional potency with RNA editing, its efficacy (intrinsic activity) dramatically changed. It behaved as a full agonist at the INI isoform, a weak partial agonist at the VSV isoform, but an antagonist (or extremely weak partial agonist) at the VGV isoform of the human 5-HT_{2C} receptor. This is very intriguing since editing may produce a qualitative, agonist-specific shift toward another signaling pathway in the absence of any discernable change in ligand binding affinity. It would be worthwhile to examine whether, as a consequence of editing, LSD couples to a different G protein and subsequent activation of a distinct signaling cascade (e.g., from inositol phosphate hydrolysis to arachidonic acid production; see Berg et al. 1998).

There are several possible reasons why our ligand binding data differs from that reported previously (Burns et al. 1997). The failure to observe a significant change in agonist binding affinity with the rat 5-HT_{2C} VSV edited receptor may have resulted from species differences of the receptor (rat vs. human), from differences in the cells used for the assays (mouse 3T3 vs. human HEK293), from use of a transient expression system in the rat study, or from the use of an antagonist radioligand to assess agonist affinity. Use of an antagonist may be especially significant, since it may have masked the effect of editing on binding to the high-affinity (coupled) state of the receptor. We examined agonist affinities at the INI and VGV isoforms using the antagonist radioligand [^{3}H]mesulergine and found that isoform differences were noticeably attenuated relative to that observed with the agonist [^{3}H]5-HT. This may be due to the fact that a majority of the antagonist binding signal was derived from binding to a low-affinity state of the receptor, which may be less affected by editing.

Based on results from other gene systems it is evident that editing may be an important mechanism by which cellular and tissue functions can be modified in response to changing developmental, environmental, and pathological conditions. For example, shifts in lipid homeostasis caused by dietary changes or alcohol consumption

modulate profoundly apoB editing (Lau et al. 1995). Plasticity in editing changes have also been noted in GluR2, R5 and R6 during transient cerebral ischemia in the rat (Paschen et al. 1996). In the case of the 5-HT$_{2C}$ receptor, editing may be a novel way in which the post-receptor signaling pathways can be modified both qualitatively and quantitatively. First, a shift toward increased editing (e.g., away from INI) may afford the system a way in which to dampen (desensitize) in response to sustained agonist over-stimulation. Second, changes in cellular (or regional) editing patterns in response to developmental, environmental, or aging cues may shift receptor-mediated signal transduction away from one intracellular pathway toward an alternate signaling cascade (e.g., from inositol phosphate toward arachidonate pathways). Lastly, it would be very interesting to assess the possible role of editing in the pathophysiology of neuropsychiatric illness (either as a etiologic or compensative factor) and its response to psychoactive agents. For example, GluR2 editing appears to be compromised in a brain region-specific manner in schizophrenia, Alzheimer's, and Huntington's diseases (Akbarian et al. 1995).

While the exact physiological role for 5-HT$_{2C}$ receptor editing is not clearly defined, continued study of its pharmacological and regulatory properties will shed more light on its role in 5-HT-mediated synaptic signaling and plasticity. The present work extends the known functional roles of RNA editing of this receptor to include modulation of high-affinity agonist binding, selective changes in G protein coupling (for LSD), and discovery of an additional editing site (site E) in the human receptor. It remains to be determined whether RNA editing is unique to the 5-HT$_{2C}$ receptor or is prominent in other members of the 7TM super-family of receptors.

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