RNA Editing of the Human Serotonin 5-Hydroxytryptamine 2C Receptor Silences Constitutive Activity*

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** Abstract **

RNA transcripts encoding the serotonin 5-hydroxytryptamine 2C (5-HT$_2$C) receptor (5-HT$_2$C-R) undergo adenosine-to-inosine RNA editing events at up to five specific sites. Compared with rat brain, human brain samples expressed higher levels of RNA transcripts encoding the amino acids valine-serine-valine (5-HT$_2$C-VSV) and valine-glycine-valine (5-HT$_2$C-VGV) at positions 156, 158, and 160, respectively. Agonist stimulation of the nonedited human receptor (5-HT$_2$C-IN) and the 5-HT$_2$C-VSV and 5-HT$_2$C-VGV receptor variants stably expressed in NIH-3T3 fibroblasts demonstrated that serotonergic agonists were less potent at the edited receptors. Competition binding experiments revealed a guanine nucleotide-sensitive serotonin high affinity state only for the 5-HT$_2$C-VSV receptor; the loss of high affinity agonist binding to the edited receptor demonstrates that RNA editing generates unique 5-HT$_2$C-Rs that couple less efficiently to G proteins. This reduced G protein coupling for the edited isoforms is primarily due to silencing of the constitutive activity of the nonedited 5-HT$_2$C-R. The distinctions in agonist potency and constitutive activity suggest that differential edited 5-HT$_2$C-Rs exhibit distinct responses to serotonergic ligands and further imply that RNA editing represents a novel mechanism for controlling physiological signaling at serotonergic synapses.

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1 The abbreviations used are: 5-HT, 5-hydroxytryptamine; 5-HT$_2$C-R, 5-H$T_2$C receptor; PCR, polymerase chain reaction; DMEM, Dulbecco’s modified Eagle’s medium; DOI, (±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-amino propane; DMT, N,N-dimethyltryptamine; LSD, lysergic acid diethylamide.

RNA editing is a post-transcriptional modification resulting in an alteration of the primary nucleotide sequence of RNA transcripts by mechanisms other than splicing. The enzymatic conversion of adenosine to inosine by RNA editing has been identified within an increasing number of RNA transcripts, indicating that this modification represents an important mechanism for the generation of molecular diversity. Several of these editing events have been shown to have significant consequences for cellular function. Transcripts encoding the B-subunit (GluR-B) of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid subtype of glutamate receptor undergo RNA editing events that modulate both the ion permeation and electrophysiological characteristics of this glutamate-gated ion channel (1–3). Mice that are deficient in their ability to edit GluR-B transcripts die at 3 weeks of age due to epileptic seizures, suggesting that editing of GluR-B RNA is important in the modulation of normal glutamatergic neurotransmission (4). These results suggest that the consequences of editing events within other, diverse RNA molecules might also have important ramifications for cellular function.

The monoamine 5-hydroxytryptamine (serotonin; 5-HT) interacts with a large family of receptors to induce signal transduction events important in the modulation of neurotransmission (5). The 2C subtype of serotonin receptor (5-HT$_2$C-R) is a member of the G protein-coupled receptor superfamily and stimulates phospholipase C, resulting in the production of inositol phosphates and diacylglycerol (6). We have recently shown that RNA transcripts encoding the rat, mouse, and human 5-HT$_2$C-R undergo adenosine-to-inosine RNA editing events at five positions, termed A, B, C, D, and E (previously termed C’) (7, 8), resulting in an alteration of amino acid coding potential within the putative second intracellular loop of the encoded protein. Editing at the A site, or at the A and B sites concurrently, converts an isoleucine to a valine at amino acid 156 of the human receptor, while editing at the B position alone generates a methionine codon at this site (Fig. 1A). Editing at C converts asparagine to a valine at amino acid 158 of the human receptor, while editing at the B position alone generates a methionine codon at this site (Fig. 1A). Editing at C converts asparagine to a valine at amino acid 158 of the human receptor, while editing at the B position alone generates a methionine codon at this site (Fig. 1A). Editing at C converts asparagine to a valine at amino acid 158 of the human receptor, while editing at the B position alone generates a methionine codon at this site (Fig. 1A). Editing at C converts asparagine to a valine at amino acid 158 of the human receptor, while editing at the B position alone generates a methionine codon at this site (Fig. 1A). Editing at C converts asparagine to a valine at amino acid 158 of the human receptor, while editing at the B position alone generates a methionine codon at this site (Fig. 1A). Editing at C converts asparagine to a valine at amino acid 158 of the human receptor, while editing at the B position alone generates a methionine codon at this site (Fig. 1A).
play a role in the altered coupling properties of the fully edited receptor, suggesting that RNA editing of the 5-HT2C receptor presents a novel mechanism for regulating neuronal excitability by stabilizing receptor signaling and enhancing the signal: noise ratio at serotoninergic synapses.

**EXPERIMENTAL PROCEDURES**

**Preparation of RNA, Reverse Transcription-PCR, and Editing Efficiency Analyses**—Total RNA isolated from whole human brain was obtained from CLONTECH (Palo Alto, CA). Reverse transcription-PCR amplification of 5-HT2CR messenger mRNA was performed as described previously (7) with the antisense oligonucleotide 5′-GCAGTAACAT-CAAAACTTTGGTTGCTTAAGACTGAAGC-3′ (coordinates 723–747 relative to the translocation start site of the human 5-HT2CR cDNA; Ref. 17) employed for cDNA synthesis. The sense oligonucleotide primer 5′-CCAGGGATTCTCAAACTTTGGTTGCTTAAGACTGAAGC-3′ (coordinates –30 to –5; Ref. 18) and the original cDNA synthesis primer or, in some cases, 5′-ATTAGAACCTTGGTTGCTCCCGTCTGG-3′ (coordinates 372–398; Ref. 17) and the cDNA priming oligonucleotide (boldface sequence indicates introduced EcoRI restriction sites) were used for PCR amplification of human 5-HT2CR sequences. Amplification proceeded for 75 s at 94 °C, for 75 s at 50 °C, and for 150 s at 72 °C for 35 cycles. Products were purified on a 2% agarose gel, and primer extension analyses at the A and C/D sites were performed as described (7). For sequencing analysis, PCR fragments were digested with EcoRI and HindIII and unidirectionally subcloned into pKB2II (Stratagene). Individual cDNA isolates were sequenced using the fmole sequencing system (Promega) as described previously (7).

**Preparation of Human 5-HT2CR Isoform DNA and Expression in Cell Culture**—Human 5-HT2CR variants were prepared by oligonucleotide-directed mutagenesis of human INI receptor DNA (a gift of Dr. Alan Saltzman) as described previously (10, 19). These constructs were unidirectionally cloned into the eukaryotic expression vector pCMV (a gift of Dr. David Russell) at the EcoRI and XbaI sites. Transient experiments in NIH-3T3 cells were performed as described previously (7). For transient expression into the COS-7 cell line, cells were plated in 24-well plates at a density of 1 × 105 cells/well 24 h prior to transfection.

**Stable Transfectants**—Stable cell lines were generated by co-electroporation of human isoforms in pCMV2 (60 μg) and pRC/CMV (Invitrogen, Carlsbad, CA) (6 μg) using electroporation conditions described previously (7). Single clones were selected using 1 mg/ml G418 (Geneticin®) in DMEM containing 10% dialyzed horse serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc.) and 1 μg/ml 2-bromolysergic acid diethylamide.

**Phosphoinositide Hydrolysis Assay**—Transiently transfected cells were prepared for inositol monophosphate analysis as described previously (NIH-3T3 cells, 7; COS-7 cells, 21). For stable cell lines, cells were washed three or four times with Hanks’ buffer prior to plating in a 24-well plate (Falcon 3047, Becton-Dickinson Laboratories, Lincoln Park, NJ) in DMEM containing 10% dialyzed calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. 24 h later, cells were labeled for 16–20 h with 1 μCi of [3H]inositol (20–25 Ci/mmol, NEN Life Science Products) in serum-free DMEM containing 100 units/ml penicillin and 100 μg/ml streptomycin. For isolation of inositol monophosphates from both transient and stable transfecants, agonists were added in the presence of 10 mM lithium chloride and 10 μM pargyline. Incubations continued for 30 min unless otherwise noted. [3H]Inositol monophosphates were isolated as described previously (22).

**Radioligand Binding**—Radioligand binding assays were performed in transiently transfected cells as described previously (7, 21). Stable cell lines were prepared by washing three or four times in Hanks’ buffer, resuspending the cells in 0.5 ml of DMEM containing 10% dialyzed calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin for 24 h. Subsequently, cells were placed into serum-free DMEM containing 100 units/ml penicillin and 100 μg/ml streptomycin for 16–18 h prior to analyses. [3H]Mesulergine binding was assayed in crude membrane preparations in 50 mM Tris-HCl, 10 mM MgCl2, pH 7.5, as described previously (23). Competition binding was performed with 0.5 nM [3H]Mesulergine in the presence of increasing concentrations of nonlabeled competitor at 37 °C for 30 min. In some experiments, 100 μM guanosine 5′-β,γ-imido)triphosphate (Gpp(NH)p) was added during the incubation. IC50 values were determined by fitting data to a sigmoidal model with variable slope using GraphPad Prism® (GraphPad Software Inc., San Diego, CA). One-site and two-site binding curves were compared using the F ratio. IC50 values were converted to Kd using the transformation of Cheng-Prusoff (24).

**RESULTS**

The 5-HT2CR Editing Pattern in Human Brain Is Distinct from Rat Brain—To determine the editing pattern for 5-HT2CR transcripts in human brain, whole brain total RNA was amplified by reverse transcription-PCR. The extent of editing at specific sites and the editing patterns within individual RNA species were determined by primer extension analysis and by subcloning and sequencing individual cDNA isolates, respectively. These studies revealed that the extent of editing in human brain at the A and D positions was similar to the levels previously identified within rat brain RNA, while editing at position B was lower in human brain (Ref. 7; Fig. 1B). By contrast, the editing efficiency at the C position was much higher in 5-HT2CR transcripts isolated from human compared with rat brain (60 versus 35%). Editing at the E position (previously referred to as C') was barely detectable in rat brain mRNAs from a variety of brain regions (<5%; Ref. 7), but this position was edited with much higher efficiency in human brain transcripts (Fig. 1B).

Due to increased levels of editing at the C and E positions, the pattern of editing observed within individual human brain 5-HT2CR transcripts differed from that found in the rat (Fig. 1C). For example, the 5-HT2C-VST isoform, which comprised approximately 10% of the 5-HT2CR transcripts isolated from whole rat brain, represented almost 40% of the messages derived from whole human brain (Fig. 1C). The enhanced editing at the E position in humans was also reflected by the appearance of variant RNAs encoding the novel 5-HT2C-VGV, 5-HT2C-VGI, and 5-HT2C-VGI protein isoforms (Fig. 1C). RNA encoding the 5-HT2C-VSV variant, which represented approximately 50% of the transcripts isolated from rat brain (7), only comprised 8% of the total 5-HT2CR RNAs found in human brain (Fig. 1C). Together, these results indicated that editing of the 5-HT2CR RNA differs considerably between rodent and human species and further suggested that the isoforms specific to human brain may have distinct functional roles.

**Differential Signaling Profiles of the Human Edited Receptor Isoforms**—To assess the functional consequences of editing within human 5-HT2CR RNAs, we transiently expressed six of the major human isoforms in NIH-3T3 fibroblasts and examined the ability of these receptors to stimulate the phospholipase C signal transduction cascade by measuring the accumulation of [3H]inositol monophosphates. Results from these studies revealed that the human 5-HT2C-VST receptor exhibited a 5-fold shift in potency for 5-HT compared with the 5-HT2C-INI receptor (Fig. 2). The 5-HT2C-VSV receptor exhibited a more substantial rightward shift in the dose-response curve for 5-HT, with an EC50 value of 59 versus 2.3 nM for the 5-HT2C-INI receptor variant. The EC50 values for all other isoforms were not significantly different from the EC50 observed for the 5-HT2C-INI receptor.

To more carefully examine the properties of the human edited receptor isoforms, stable cell lines of the human 5-HT2C-INI, 5-HT2C-VSV, and 5-HT2C-VSV receptor isoforms were generated in NIH-3T3 cells. The density of receptors in these cell lines was 258 ± 87 for 5-HT2C-INI, 173 ± 28 for 5-HT2C-VSV, and 1054 ± 260 fmol/mg protein for the 5-HT2C-VSV isoform. Similar to the phenotype observed in the transient transfections, 5-HT exhibited a lower potency when interacting with either
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Fig. 1. 5-HT<sub>2C</sub>R editing efficiencies in human versus rat brain. A, the positions of the editing sites within human 5-HT<sub>2C</sub>R RNA are shown with the nonedited RNA sequence at the top and the fully edited sequence indicated at the bottom. The positions of the five editing sites, encompassing amino acids 156–160 of the human 5-HT<sub>2C</sub>R sequence are shown. B, the editing efficiency at each editing site for whole human (black bars) and whole rat (white bars) brain is shown. Editing efficiencies at the A, C, and D sites were determined by a combination of primer extension (n = 2 independent analyses) and sequencing analyses (n = 50 independent cDNA clones for human samples); values represent the mean ± S.E. Editing efficiencies at the B and E sites were determined by sequencing of 50 independent cDNA clones from human brain. C, the relative expression level, presented as a percentage of total isoform expression derived from a sequencing analysis of 50 (human) or 100 (rat) brain cDNA clones, is compared for 5-HT<sub>2C</sub>R RNA derived from human (black bars) versus rat (white bars) brain. Rat data are from Burns et al. (7).

the 5-HT<sub>2C</sub>SVS or 5-HT<sub>2C</sub>SVG isoforms as compared with the 5-HT<sub>2C</sub>INII receptor (Fig. 3A). The 5-HT<sub>2C</sub>SVG receptor-expressing cells, however, did not exhibit the substantial EC<sub>50</sub> shift seen in the transient experiments. It is possible that the higher receptor expression in the 5-HT<sub>2C</sub>SVG receptor line resulted in a significant receptor reserve for the 5-HT response, causing the EC<sub>50</sub> value to appear inappropriately low. To address this possibility, 5-HT<sub>2C</sub>SVG receptor cells were treated with the alkylating agent phenoxbenzamine (25). This treatment inactivated approximately 60% of the 5-HT<sub>2C</sub>SV<sub>S</sub> receptors, resulting in a statistically equivalent density between the three cell lines, thereby allowing a more direct comparison of EC<sub>50</sub> values. Subsequent stimulation of this partially inactivated population with 5-HT revealed that stably expressed 5-HT<sub>2C</sub>SVG receptors now exhibited a decreased maximum response (20% of untreated cells) and a 29-fold lower EC<sub>50</sub>, for 5-HT when compared with the 5-HT<sub>2C</sub>INV isoform (Fig. 3A). This potency shift agrees closely with the 26-fold difference determined in transient transfection analyses (Fig. 2).

Stimulation with other 5-HT<sub>2C</sub>R agonists revealed similar discrepancies in EC<sub>50</sub> values between different edited isoforms. (±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane (DOI), a hallucinogenic agonist with structural similarity to amphetamine, was 9- and 43-fold less potent at the 5-HT<sub>2C</sub>SVS and 5-HT<sub>2C</sub>SVG receptors, respectively, when compared with its 5-HT<sub>2C</sub>INII isoform (Fig. 3B). N,N-dimethyltryptamine (DMT), a hallucinogenic indoleamine, was 40-fold less potent at the 5-HT<sub>2C</sub>SVS receptor and 91-fold less potent at the 5-HT<sub>2C</sub>SVG variant compared with the response generated by interaction of this ligand with the 5-HT<sub>2C</sub>INII receptor (Fig. 3C).

Two Agonist Affinity States Are Observable Only for the 5-HT<sub>2C</sub>INII Isoform—According to current theory of receptor/G protein interaction, the coupling of receptor with G protein results in an increase in the affinity of agonists for the receptor. Competition binding analyses were performed to evaluate differences in agonist affinity states for the 5-HT<sub>2C</sub>INII and 5-HT<sub>2C</sub>SVS receptors (Fig. 4). Competition binding regularly revealed a low and high affinity state upon interaction of 5-HT with the 5-HT<sub>2C</sub>INII receptor (Fig. 4A); competition binding curves could never be fit by a one-site model (data not shown). The 5-HT high affinity population of 5-HT<sub>2C</sub>INII Receptors could be shifted to low affinity when binding analyses were performed in the presence of 100 µM Gpp(NH)p, while the curves for fully edited 5-HT<sub>2C</sub>SVG receptor remained unchanged. In the presence of Gpp(NH)p, significantly higher affinities were consistently observed for agonists when the 5-HT<sub>2C</sub>INII receptor was compared with the 5-HT<sub>2C</sub>SVG variant (Table I). This was not the case, however, when competition analyses were performed with mianserin, an inverse agonist at the 5-HT<sub>2C</sub>R and a ligand that is predicted to preferentially interact with uncoupled receptors.
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**DISCUSSION**

RNA editing is a post-transcriptional process that contributes to molecular diversity within cells. 5-HT_{2CR} RNA transcripts undergo adenosine-to-inosine RNA editing events resulting in the generation of distinct amino acids within the second intracellular loop of the protein (7), a region known to be important for G protein coupling (9–16). Editing within rat 5-HT_{2CR} RNA is mediated by the coordinated actions of a family of adenosine deaminases termed ADARs (adenosine deaminases that act on RNA; Refs. 7 and 27). These editing events are conserved among rodent and human species (8), suggesting that they serve an important role in receptor function.

Editing in the human brain creates a differential complement of receptor isoforms due to an increase in the percentage of isoforms edited at position 158, most notably the 5-HT_{2C-VSV} and 5-HT_{2C-VGV} isoforms. Transient transfection of the principal human isoforms revealed that 5-HT exhibited the lowest potency at the novel 5-HT_{2C-VGV} receptor. The 5-HT_{2C-VSV} receptor was the only other isoform to show a significant difference in 5-HT potency relative to the nonedited isoform. In cell lines stably expressing the 5-HT_{2C-VSV}, 5-HT_{2C-VGV}, or 5-HT_{2C-INI} isoforms, agonists exhibited a decreased potency when interacting with the edited receptor isoforms with the effect again being most dramatic upon interaction with the 5-HT_{2C-VGV} receptor. The potency difference between 5-HT_{2C-VSV} and 5-HT_{2C-VGV} receptors was augmented when a majority of the receptors were expressed at similar densities indicated that the 5-HT_{2C-VSV} isoform elicited 5-fold greater levels of basal [^{3}H]inositol monophosphate generation compared with the 5-HT_{2C-VGV} receptor (Fig. 5). The addition of 1 μM methysergide, a neutral antagonist, blocked 5-HT-stimulated inositol phosphate formation but had no effect on the basal activity of the 5-HT_{2C-INI} receptor (data not shown).

The disparity in basal activity mirrors the differences in “precoupling” ability reflected in the competition binding experiments, again suggesting that the 5-HT_{2C-INI} receptor interacts more efficiently with the G protein linked to phospholipase C. Importantly, these analyses also revealed that similar maximal responses were obtained for both receptor isoforms (Fig. 5), indicating that the primary effect of editing may be to alter agonist-independent activity rather than affecting the intrinsic ability of receptor isoforms to promote G protein coupling.

**Constitutive Activity Differences between Human 5-HT_{2CR} Isoforms**—Rat 5-HT_{2CR}Rs have been shown to exhibit constitutive activity (22, 23), defined as the ability of a receptor to stimulate downstream signaling events in the absence of agonist occupation (reviewed in Ref. 26). The binding studies described previously suggested that the edited receptors might exhibit differential abilities to stimulate basal levels of inositol phosphate hydrolysis. Studies of constitutive receptor activity were performed in COS-7 cells, where transient expression gave reproducibly high levels of receptor expression, which is needed to accurately evaluate agonist-independent activity. An analysis of transient transfection experiments in which the nonedited and edited receptors were expressed at similar densities indicated that the 5-HT_{2C-INI} isoform elicited 5-fold greater levels of basal [^{3}H]inositol monophosphate generation compared with the 5-HT_{2C-VGV} receptor (Fig. 5). The addition of 1 μM methysergide, a neutral antagonist, blocked 5-HT-stimulated inositol phosphate formation but had no effect on the basal activity of the 5-HT_{2C-INI} receptor (data not shown). The disparity in basal activity mirrors the differences in “precoupling” ability reflected in the competition binding experiments, again suggesting that the 5-HT_{2C-INI} receptor interacts more efficiently with the G protein linked to phospholipase C. Importantly, these analyses also revealed that similar maximal responses were obtained for both receptor isoforms (Fig. 5), indicating that the primary effect of editing may be to alter agonist-independent activity rather than affecting the intrinsic ability of receptor isoforms to promote G protein coupling.

**Fig. 3.** Differential responses of agonists to activate phosphoinositide hydrolysis in human edited 5-HT_{2CR} stable cell lines. The EC_{50} for activation of phosphoinositide hydrolysis by 5-HT (A), DOI (B), and DMT (C) in NIH-3T3 cells stably expressing 5-HT_{2C-INI}, 5-HT_{2C-VSV}, and 5-HT_{2C-VGV} receptors is shown. Values represent the mean ± S.E. of 3–16 independent determinations performed in triplicate for each drug. Receptor densities (B_{max}) were as follows: 5-HT_{2C-INI}, 258 ± 87 fmol/mg; 5-HT_{2C-VSV}, 173 ± 70 fmol/mg; and 5-HT_{2C-VGV}, 1054 ± 260 fmol/mg (n = 4–6). Inactivation of 5-HT_{2C-VGV} receptor-expressing cells with 3.2 μM phenytoinomazine resulted in a decrease in receptor density to 305 ± 141 fmol/mg (n = 3). A, *, these values were significantly different from 5-HT_{2C-VGV} alone (p < 0.0001). 5-HT_{2C-VSV} and 5-HT_{2C-VGV} were not significantly different from each other (p = 0.4550). B, *, a significant difference from 5-HT_{2C-VSV} (p = 0.0160); **, a significant difference from 5-HT_{2C-INI} (p = 0.0141) for 5-HT_{2C-VGV} p = .0059 for 5-HT_{2C-VGV}). **, this value was significantly different from 5-HT_{2C-VGV} alone (p < 0.0001). 5-HT_{2C-VSV} and 5-HT_{2C-VGV} were not significantly different from each other (p = 0.0476). C, *, a significant difference from 5-HT_{2C-VSV} (p = 0.0141) for 5-HT_{2C-VGV} p = .0059 for 5-HT_{2C-VGV}). **, a significant difference from 5-HT_{2C-VSV} (p = 0.0160); **, a significant difference from 5-HT_{2C-INI} (p = 0.0006) and 5-HT_{2C-VSV} (p = 0.0444). All statistical analyses were performed using individual unpaired Student’s t tests.

These results again support the hypothesis that the 5-HT_{2C-INI} receptor couples more efficiently to G proteins, even in situations where no agonist is present to induce the coupled state.
two-site competitive binding model. The high affinity state of 5-HT, representing approximately 50% of the binding, was eliminated by the addition of the GTP analogue, Gpp(NH)p. In contrast, the 5-HT competition binding curves with 5-HT$_{2C-VGV}$ receptors were best fit by a one-site competitive binding model, and the affinity approximated the low affinity site found with 5-HT$_{2C-VSV}$ and 1.09 ± 0.21 for 5-HT$_{2C-VGV}$. Data were statistically analyzed by unpaired Student’s $t$ test.

### Table 1

Relative affinities of agonists for 5-HT$_{2C-INI}$ and 5-HT$_{2C-VGV}$ receptors

| Agonist | Human INI | Human VGV | $p$ |
|---------|-----------|-----------|-----|
| 5-HT without Gpp(NH)p | | | |
| High | 0.68 ± 0.13 | 167 ± 16.1 | 0.0007 |
| Low | 85.7 ± 17.2 | 167 ± 16.1 | 0.0007 |
| DOI with Gpp(NH)p | 57.4 ± 12.0 | 302.4 ± 46.4 | 0.0118 |
| DMT with Gpp(NH)p | 41.0 ± 3.7 | 70.4 ± 9.0 | 0.0240 |
| LSD with Gpp(NH)p | 382.3 ± 47.0 | 1325 ± 122.2 | 0.0006 |
| Mianserin with Gpp(NH)p | 4.35 ± 0.94 | 12.9 ± 1.4 | 0.0028 |

The $K_i$ values for 5-HT, DOI, DMT, LSD, and mianserin were determined by competition for 1 nM [3H]mesulergine as described under “Experimental Procedures.” Data were fit to one- and two-site models using GraphPad Prism software. Values represent the mean ± S.E. of 3–6 independent analyses performed in duplicate. $K_i$ values were calculated using the method of Cheng and Prusoff (24) using the mesulergine $K_i$ values, determined in the presence of 100 μM Gpp(NH)p, of 0.99 ± 0.07 for 5-HT$_{2C-INI}$ and 1.09 ± 0.21 for 5-HT$_{2C-VGV}$. Data were statistically analyzed by unpaired Student’s $t$ test.

The ternary complex model of receptor/G protein interaction has recently undergone revision to accommodate the finding that some receptors have the ability to induce effective G protein coupling in the absence of an agonist, termed constitutive activity (28). The model predicts that receptors have the capacity to spontaneously isomerize from an inactive conformation, termed R, to an active state, R*, with the R* version of the receptor having the ability to interact with G proteins. In experimental paradigms studying the properties of constitutively active mutant receptors, it has been observed that these receptors exhibit both a higher level of basal activity and a greater potency for agonists (28, 29). Since the nonedited 5-HT$_{2C-R}$ exhibits constitutive activity (22, 30), we considered the possibility that the agonist potency differences generated by RNA editing may reflect differential abilities to isomerize to the R* state, with the 5-HT$_{2C-INI}$ receptor existing more readily in the R* form compared with the edited 5-HT$_{2C-VSV}$ and 5-HT$_{2C-VGV}$ receptor cell lines. The densities of the receptors, estimated using a single concentration of [3H]mesulergine (0.5 nM), were 2.7 ± 0.2 pmol/mg for 5-HT$_{2C-INI}$ and 2.9 ± 0.2 pmol/mg for 5-HT$_{2C-VGV}$. The constitutive activity of transiently expressed 5-HT$_{2C-INI}$ and 5-HT$_{2C-VGV}$ receptors. cDNAs for edited receptors were transiently expressed in COS-7 cells, and basal [3H]inositol monophosphate formation was measured after a 35-min incubation.

FIG. 4. 5-HT affinity for human edited 5-HT$_{2C}$Rs and modulation by the GTP analogue Gpp(NH)p. Competition binding analyses are shown for 5-HT$_{2C-INI}$ (A) and 5-HT$_{2C-VGV}$ receptor cell lines (B) in the presence (closed symbols) and absence (open symbols) of 100 μM Gpp(NH)p. Increasing concentrations of 5-HT were incubated with [3H]mesulergine as described under “Experimental Procedures.” Data were fitted to both one- and two-site models using GraphPad Prism software. The mean $K_i$ values for 5-HT, determined by the method of Cheng and Prusoff, for 5-HT$_{2C-INI}$ were 0.68 ± 0.13 nM (high affinity) and 85.7 ± 17.2 nM (low affinity) in the absence of Gpp(NH)p and 57.4 ± 12.0 nM in the presence of 100 μM Gpp(NH)p. For 5-HT$_{2C-VGV}$, the mean $K_i$ values for 5-HT were 167.0 ± 16.1 nM in the absence of Gpp(NH)p and 302.4 ± 46.4 nM in the presence of 100 μM Gpp(NH)p, respectively. $K_i$ values for [3H]mesulergine, determined in the presence of 100 μM Gpp(NH)p, were as follows: 0.99 ± 0.07 for 5-HT$_{2C-INI}$ and 1.09 ± 0.21 for 5-HT$_{2C-VGV}$ (p = .601). Data represent the mean ± S.E. of four or five independent determinations performed in duplicate.

FIG. 5. Constitutive activity of transiently expressed 5-HT$_{2C-INI}$ and 5-HT$_{2C-VGV}$ receptors. cDNAs for edited receptors were transiently expressed in COS-7 cells, and basal [3H]inositol monophosphate formation was measured after a 35-min incubation. #, p < 0.05 versus vector alone; *, p < 0.01 compared with vector and the 5-HT$_{2C-VGV}$ receptor cell line. The densities of the receptors, estimated using a single concentration of [3H]mesulergine (0.5 nM), were 2.7 ± 0.2 pmol/mg for 5-HT$_{2C-INI}$ and 2.9 ± 0.2 pmol/mg for 5-HT$_{2C-VGV}$.
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5-HT$_{2CR}$ Ile/Val156 is critical for forming a “cage” around the mion receptor has shown that the Ile corresponding to the pinging. Molecular modeling of the gonadotropin-releasing hormone receptors residing in the R* conformation might underlie the distinction in constitutive activities. An alternative explanation is that both receptor isoforms exhibit the same ability to convert to R*, but the combination of amino acids within the 5-HT$_{2C-INI}$ receptor promotes more efficient interaction with the G protein, due to either the favorable conformation of the second intracellular loop or actual contact of the nonedited amino acids with the G protein. In experiments designed to test the hypothesis, we found that 5-HT treatment elicited an increase in [H]inositol phosphate formation of the same magnitude in cells expressing equal densities of 5-HT$_{2C-INI}$ or 5-HT$_{2C-VGV}$ receptors, strongly suggesting that the two receptor isoforms have equivalent ability to couple to G proteins when bound by agonists. Thus, these results indicate that 5-HT$_{2C-INI}$ receptors have a greater propensity to spontaneously isomerize into or maintain the active R* conformation than 5-HT$_{2C-VGV}$ receptors. Thus, we propose that one consequence of RNA editing of the 5-HT$_{2C-R}$ is to silence its constitutive activity, thus increasing the signal:noise ratio at sites where editing efficiency is high.

The extended ternary complex model predicts that receptor isoforms with high levels of constitutive activity would exhibit higher affinity for agonists, even in the absence of G protein coupling (28, 29). As a first step at evaluating agonist affinities in the absence of G proteins, we compared the binding affinities in the presence of Gpp(NH)p, assuming that this reflects the affinity of the G protein-uncoupled receptor. The affinity of 5-HT for the 5-HT$_{2C-VGV}$ Receptor was 5-fold lower for the 5-HT$_{2C-INI}$ isomorph; significant differences were also found for the other agonists tested but not for the inverse agonist mianserin. These observations suggest that some structural perturbation allows the edited receptors to assume an inactive receptor conformation with lower affinity for agonists. Studies of agonist affinities using purified receptor protein are needed to definitively show that the agonist affinity differences do not depend on G protein interactions.

Recent structure-activity studies of other G protein-coupled receptors point to the importance of amino acid residues in positions analogous to the edited sites in the dynamics of receptor/G protein interactions. Amino acid residues that reside at positions analogous to Ile/Val$_{156}$ and Ile/Val$_{160}$ in other G protein-coupled receptors are important in terms of general G protein coupling ability and receptor regulation (10, 12, 16). For example, within the m1 and m3 muscarinic receptors and the $\beta_2$ adrenergic receptors, the amino acid at Ile$_{156}$ must be bulky and hydrophobic to induce a productive G protein interaction (10), consistent with our evidence that editing at position 160 contributes to a low efficiency of G protein coupling. Molecular modeling of the gonadotropin-releasing hormone receptor has shown that the Ile corresponding to the 5-HT$_{2CR}$ Ile/Val$_{156}$ is critical for forming a “cage” around the arginine of the highly conserved DRY sequence in the second intracellular loop (16). It was proposed that the “arginine cage” stabilizes interaction with the adjacent aspartate residue, thereby enabling the receptor to remain in an active state.

Alteration of the Ile$_{156}$/Val$_{156}$ position of the 5-HT$_{2C}$ receptor in the context of other edited amino acids might produce different conformations of this critical arginine, allowing some edited isoforms to couple more efficiently than others. The expression of more than 12 predominant isoforms in the human brain further suggests that unique degrees of coupling ability might be produced with each distinct edited receptor.

The consequences of RNA editing within 5-HT$_{2C-R}$ RNA are summarized in the model presented in Fig. 6, with A representing the 5-HT$_{2C-INI}$ receptor and B representing the 5-HT$_{2C-VGV}$ receptor isomorph. In this model, the 5-HT$_{2C-INI}$ receptor has a much greater capacity to isomerize to the active R* conformation (represented in boldface type and described by the constant $J$). The R* form of the receptor interacts efficiently with G proteins in the absence of agonist (H); upon agonist binding, the response is enhanced. For edited 5-HT$_{2C-VGV}$ receptors, however, the J constant is much smaller, reflecting a decreased spontaneous ability of these receptors to convert to the coupling-competent state. It is only upon binding of agonist that these receptors achieve the ability to significantly convert to a form able to elicit efficient G protein interaction. Our current working hypothesis is that the edited receptor has a lower value for the J constant, while the M constant, an index of the intrinsic affinity of an isoform to couple to G proteins, remains relatively constant.

In summary, the current results demonstrate that the 5-HT$_{2C-INI}$ receptor isoform exhibits a greater level of constitutive activity than does the edited 5-HT$_{2C-VGV}$ receptor isoform and therefore possesses a greater propensity to spontaneously isomerize to the R* state. The differential degrees of constitutive activity and the accompanying secondary alterations in agonist potency and G protein coupling could have important implications for the physiological effects of 5-HT.

Brain regions that contain the nonedited receptor would be more sensitive to 5-HT that may be tonically released at distinct sites, possibly generating considerable noise in the system. In addition, the magnitude of signal produced by firing of presynaptic serotonergic terminals may be reduced because of a high basal tone at the constitutively active 5-HT$_{2C-INI}$ receptor. Region-specific generation of edited isoforms, coupled with the possibility that individuals have different editing patterns, suggests that the repertoire of expressed edited 5-HT$_{2C-R}$s might determine the response to endogenous serotonin as well as control the signal:noise ratio at central serotonergic synapses.

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