

RNA Editing of Neurotransmitter Receptors in the Mammalian Brain

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(Published 21 May 2002)

The term "RNA editing" was first used to describe the insertion of single uridines in mitochondrial RNAs of trypanosomes and currently refers to a number of different mechanisms that result in single-nucleotide changes in various types of RNAs (1, 2). Many examples of pre-messenger RNA (pre-mRNA) editing have been described, and these posttranscriptional modifications result in protein diversity through alterations in start and stop codons, frame shifts, and single-amino acid changes at functionally important positions. Editing of pre-mRNA can also influence alternative splicing, and editing of transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs) can alter the efficiency and fidelity with which nuclear transcripts are translated. In mammals, pre-mRNA editing involves the deamination of cytidine (C) or adenosine (A) to yield uridine (U) and inosine (I), respectively. The pre-mRNA encoded by the gene encoding apolipoprotein B is subject to C-to-U editing, and A-to-I editing has effects on receptors for the important central nervous system (CNS) neurotransmitters glutamate and serotonin.

Mammalian A-to-I editing was first reported more than a decade ago (3). In certain α -amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)- and kainate-type glutamate receptors (GluRs), RNA editing results in a glutamine-to-arginine switch at a residue, the Q/R site, that is now known to lie within the pore of these tetrameric receptor channels. Four separate genes encode subunits that form AMPA-type receptors (GluRs 1 through 4, also called A through D), and five genes (GluRs 5 through 7 and KA1 and KA2) encode subunits that co-assemble to form kainate-type receptors (4). In the normal brain, virtually all (>99%) pre-mRNA encoding GluR2 is edited at the Q/R site, whereas GluRs 1, 3, and 4 are not edited at this site (3). The pre-mRNAs for GluR5 and GluR6 are edited at intermediate levels, and most neurons probably express both Q and R variants of these subunits (3). In each case, the Q-to-R switch reduces the permeability of the channels to calcium (5-7); decreases their susceptibility to block by internal polycations, such as spermine (8, 9); and lowers their unitary conductance (10, 11). A-to-I editing also produces isoleucine-to-valine and tyrosine-to-cysteine alterations at the so-called "I/V" and "Y/C" sites in the first transmembrane segment (TM1) of GluR6, as well as an arginine-to-glycine (R/G) change in GluRs 2, 3, and 4. The TM1 editing in GluR6 causes changes in calcium permeability that depend on the editing status of the Q/R site (12). Editing at the R/G site in the distal portion of the extracellular loop between the second and third transmembrane segments hastens the rate at which AMPA-type channels recover from desensitization (13).

In addition to the editing of cation channels, editing also occurs in a heterotrimeric GTP-binding protein (G protein)-coupled receptors. Five different editing events (A through E) have been identified for the serotonin (5-HT) 2C receptor. Here, A-to-I editing results in amino acid changes in the second intracellular loop of the protein, with resultant alterations in G protein coupling (Fig. 1).

Site-specific deamination of adenosines to inosine is critically dependent on the secondary structure of the substrate pre-mRNA (Fig. 2). All presently known neuronal pre-mRNA substrates for A-to-I editing contain an imperfect inverted repeat that allows hydrophobic base pair interactions between exonic and intronic sequences (14). The regions of partial base pair complementarity are located at variable distances from each other in the linear sequence. For example, in GluR2, the intronic editing site complementary sequence (ECS) is approximately 300 nucleotides (nt) downstream from the Q/R editing site, whereas about 1860 nt separate the Q/R site and ECS in the pre-mRNAs encoding GluR5 and GluR6 (15-17). The duplex pre-mRNA structure is interrupted by single-strand bulges and loops that are critical for editing site recognition. Therefore, secondary structure determines which adenosines are edited, and duplex imperfections are thought to target the enzyme, adenosine deaminase that acts on RNA (ADAR), to its editing site (2, 18, 19).

At present, three members of the ADAR family are known. These enzymes exhibit variable NH₂-terminal regions and have one or more double-stranded RNA binding domains that are followed by a COOH-terminal catalytic domain (2, 20). ADAR1 and ADAR2 are expressed in many tissues, but are most abundant in the brain. All known neuronal substrates for A-to-I editing are edited by ADAR1 and ADAR2. The third member of the family, ADAR3, is exclusively expressed in neuronal tissues, but is catalytically inactive toward both extended double-stranded RNAs and known editing substrates (2, 21). ADAR3 might compete with ADAR1 and ADAR2 for RNA substrates and hence function as a negative regulator of A-to-I editing (22). A further potential mechanism of regulating editing activity involves alternative splicing of ADAR2 pre-mRNA to generate four different protein isoforms. One alternative splicing event results in differential insertion of an Alu cassette into the catalytic domain of the enzyme. Inclusion of this Alu cassette causes a twofold decrease in editing activity (23). Moreover, the pre-mRNA encoding ADAR2 is itself a substrate for A-to-I editing, which generates a new 3' splice site. Cleavage at this new splice site changes the reading frame to encode a truncated protein that is either unstable or inefficiently translated. Thus, ADAR2 pre-mRNA editing appears to function as an autoinhibitory mechanism to regulate ADAR2 activity (24).

Studies with mice deficient for ADAR1 and ADAR2 provided further insight into their overlapping and distinct substrate

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specificities. Mice deficient for ADAR2 show reduced editing at 18 of 20 sites investigated (25). The Q/R site in GluR2 was affected most substantially, although decreased editing was also evident for the R/G sites in GluRs 2, 3, and 4; the Q/R sites of GluR5 and GluR6; and the A, C, and D sites of the 5-HT_{2C} receptor. In homozygous ADAR2 mutants, editing at the Q/R site in GluR2 was reduced so that the mutant mice had 10 times fewer arginine-containing receptors than did wild-type mice. The ADAR2 mutants displayed early-onset seizures and postnatal lethality, a phenotype that appears to be entirely due to reduced Q/R editing of GluR2 in the ADAR2 mutants (25, 26). Results from ADAR1 knockout mice confirmed that Q/R editing of GluR2 is principally mediated by ADAR2 (27). ADAR1 knockout mice show reduced editing of the A and B sites of 5-HT_{2C} pre-mRNA, as well as reduced editing of the R/G site in GluR2 and the Q/R site of GluR5 (27).

In addition to the effect of editing on ADAR2 splicing, other results point toward possible mechanistic links between editing and splicing. Reduced Q/R editing of GluR2 in knockout mice is accompanied by reduced splicing of GluR2 pre-mRNA, the nuclear accumulation of incompletely processed GluR2 transcripts, and a fivefold reduction in cytoplasmic GluR2 expression levels as compared with those in wild-type mice (25, 26). Although editing and splicing of the nascent transcript are thought to occur cotranscriptionally, they are unlikely to occur at the same time. Reenan *et al.* showed that editing of transcripts encoding the *Drosophila* sodium channel, *para*, precedes splicing and that the secondary structure required for editing needs to be resolved (by the double-stranded helicase *maleless*) to enable recognition of the downstream 5' splice site (28). Failure to resolve the secondary structure leads to an abnormal splicing pattern due to exon skipping ("splicing catastrophe"). It is also easy to imagine that lack of editing of adenosines that are located near the intronic 5' splice site could decrease the "strength" of the splice site and thus diminish the frequency of its cleavage. Moreover, editing events near exon-intron junctions can enhance cleavage at alternative 5' splice sites. For example, R/G editing appears to enhance selection of the alternative exon encoding the flip splice variant of GluR4 (13) (Fig. 2). Decreased ADAR2 activity in malignant gliomas leads not only to decreased editing of GluR2 pre-mRNA, but also to increased alternative splicing of 5-HT_{2C} pre-mRNA and expression of a truncated, nonfunctional receptor protein (29).

AMPA-type GluRs mediate most fast excitatory transmission in the mammalian brain, and the first structure-function data obtained for this class of receptor was that O/R switching altered calcium permeability and rectification. The pore region of all

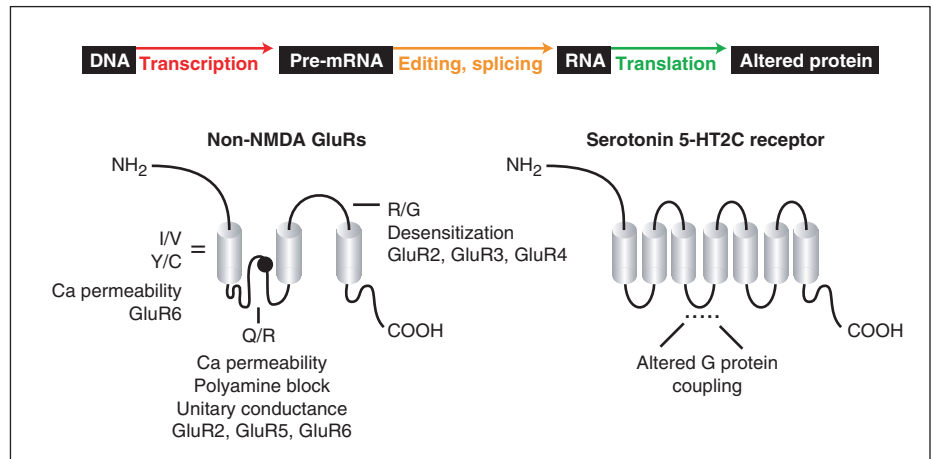


Fig. 1. Cartoons of the transmembrane topology of a GluR subunit (left) and the 5-HT_{2C} receptor (right). The approximate positions of amino acid residues altered by site-specific conversion of adenosine to inosine in the respective pre-mRNAs are indicated. The Q/R site edited in GluR2, GluR5, and GluR6 lies within the reentrant loop region that forms the channel pore. The I/V and Y/C sites are unique to GluR6 and occur within the first transmembrane helix. The R/G site is edited in GluRs 2 through 4 and immediately precedes the flip/flop cassette that is alternatively spliced in each AMPA-type subunit. This region is likely involved in intersubunit contacts within subunit dimers and may affect desensitization by influencing the extent of binding-domain closure upon agonist binding (75-78). The 5-HT_{2C} editing sites are closely spaced within the second intracellular loop of this seven transmembrane receptor and are named A, B, C'(E), C, and D (53, 54). Editing at these sites potentially alters three codons. Editing at the A and A/B site(s) converts an isoleucine to a valine (I/V), and editing at the B site generates a methionine at this site. Editing at the C site converts an asparagine to a serine (N/S), and editing of the C' site generates an aspartate. The combined editing at the C' and C sites generates a glycine (I/G). Editing at the D site converts the isoleucine to valine (I/V).

ionotropic GluRs is a reentrant p loop similar to that in certain voltage-gated channels. The structure of the bacterial potassium channel, KcsA, is a good model of the GluR pore (albeit rotated 180°), and the Q/R site in non-N-methyl-D-aspartate (non-NMDA) receptors maps to the extracellular portion of what is known to be the selectivity filter in KcsA (30-33). The corresponding residue in NMDA receptors controls calcium permeability and block by magnesium ions (34). Given the central role of calcium as an intracellular messenger and its known role in synaptic plasticity and neurodegenerative disorders, the role of editing in calcium-dependent phenomena has received a great deal of attention. Q/R editing also has profound effects on internal block by polyamines and channel conductance. The kinetics of polyamine block suggest that it could mediate frequency-dependent changes in synaptic strength during repetitive firing (35). In GluR2 editing-deficient mice, reductions in seizure threshold are accompanied by increases in both calcium permeability and current amplitude, suggesting that increased unitary conductance is at least partially responsible for the lethal phenotype (36).

The effects of R/G editing on native channel function are largely unknown, but R/G editing increases the rate at which recombinant channels recover from desensitization. Unlike Q/R editing of GluR2, R/G editing increases substantially during development (13). At some central synapses, desensitization contributes to shaping excitatory postsynaptic currents, and at these synapses, developmentally regulated alterations in R/G editing might be a major determinant of the fidelity of synaptic transmission, especially at high firing frequencies (37-39)

Compared with AMPA and NMDA receptors, the function of kainate receptors was slow to be revealed. In addition to activating the receptors that bear its name, kainate is a partial, incompletely desensitizing agonist at AMPA receptors. Progress was speeded greatly, however, by the development of subtype-selective ligands and genetically engineered mice. Postsynaptic kainate receptors have been identified throughout the neuroaxis, notably in the CA3 region of the hippocampus (40, 41), spinal neurons (42), and the retina (43). There is also substantial evidence that presynaptic kainate receptors regulate the release of both excitatory and inhibitory transmitters (44). Q/R editing of GluR5 and GluR6 (subunits of the kainate receptor) increases during development. In cerebellar granule cells, these increases are accompanied by reductions in the apparent unitary conductance of native kainate-type channels (45, 46), and kainate receptors in spinal dorsal root ganglion neurons show decreases in calcium permeability that parallel developmental increases in Q/R editing of GluR5 (47).

Surprisingly, GluR5 and GluR6 knockout mice show few phenotypic differences from wild-type animals, with the exception of a reduced susceptibility to kainate-induced seizures in the GluR6 mutants (48). Detailed electrophysiological studies with these mice confirmed earlier reports that kainate receptors can modulate synaptic plasticity in the hippocampus (49). At mossy fiber synapses on CA3 pyramidal neurons, this modulation of synaptic plasticity depends on the presence of GluR6 but not of GluR5 (50). The reduced paired-pulse and frequency-dependent facilitation in GluR6 knockouts was unexpected, given previous work showing that activation of presynaptic receptors on mossy fiber terminals inhibits glutamate release (51). However, the absence of GluR6 may change receptor properties, with consequent changes in calcium entry in response to action potential invasion. In addition, GluR6 knockout mice displayed significant NMDA receptor-independent long-term potentiation (LTP), a portion of which was independent of metabotropic GluR signaling. In editing-deficient GluR6 mutants, but not in wild-type mice, NMDA-independent LTP can also be evoked in the dentate gyrus by stimulation with high-frequency bursts (tetanic stimulation) delivered through the medial perforant pathway (52). Although not established with certainty, it seems likely that the LTP unmasked in the editing-deficient mutants reflects increased calcium flux through postsynaptic receptors.

Although GluR editing has received the bulk of the attention, the serotonin 5-HT_{2C} receptor is also modified by A-to-I editing. Five closely spaced adenosines located within sequences encoding the putative second intracellular domain of the receptor (a region thought to be of general importance for G protein coupling) can be converted to inosines by the editing enzymes ADAR1 and ADAR2. Theoretically, 32 different 5-HT_{2C} mRNAs that encode 24 different receptor isoforms could result, and a mixture of mRNA editing variants is expressed in both the rodent and human brain (53, 54). For example, 23 different 5-HT_{2C} mRNA variants are expressed in the human dorsal prefrontal cortex, and these variants encode 15 different receptor proteins (55).

Studies on transfected cells expressing individual 5-HT_{2C} receptor isoforms indicate that certain editing combinations silence the constitutive activity and decrease the efficiency of agonist-stimulated activation of G protein. The editing combinations ABCD, ABC'C, and ABC'CD give rise to receptors with

reduced function (the VSV, VGI, and VGV isoforms, respectively), with combined editing at the C and C' sites producing the most substantial down-regulation of receptor-mediated signaling (53, 54, 56, 57). Substantial changes in editing site preferences were found in the prefrontal cortex of depressed suicide victims that result in the predominant expression of the edited isoform that couples least efficiently to G protein. The changes

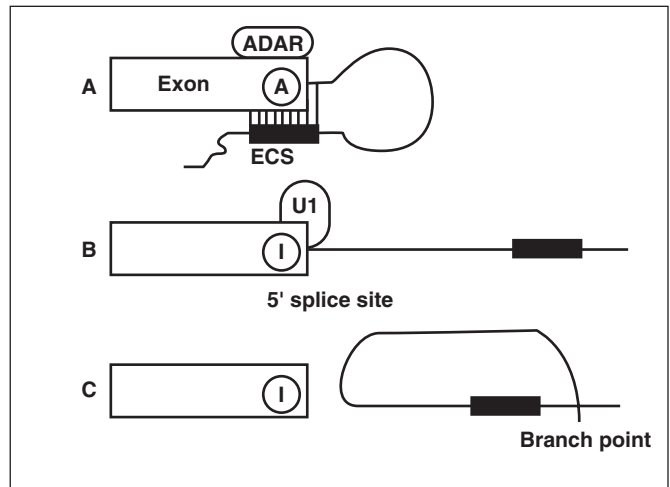


Fig. 2. Editing precedes splicing. An RNA double helix is required to target the ADAR to the adenosine that will be edited (A). The 5' splice site sequence serves as a component of the double-stranded RNA structure required for editing, as well as an essential cis-acting element of the splicing machinery. Resolution of the double-stranded RNA configuration is required for the first step of intron removal, namely, cleavage of the 5' splice site (B) and formation of an intron/exon lariat containing the 3' splice site (C). Because the strength of the splice site is determined by surrounding nucleotide sequences, editing of adenosines within this primary sequence could alter recognition of the 5' splice site and lead to skipping of the next exon. In GluRs 2, 3, and 4 pre-mRNA, the R/G site is directly adjacent to the 5' splice site (AG:GU). The two downstream exons, named flop and flip, are alternative exons that are mutually exclusive in the fully processed mRNA. In GluR4 pre-mRNA, editing increases during development and alters the adenosine adjacent to the 5' splice site (AG:GU). This appears to promote inclusion of the more proximal flop exon at the expense of the more distal flip exon (13).

in suicide victims were opposite to those observed in mice treated chronically with the serotonin-selective reuptake inhibitor fluoxetine (the widely prescribed antidepressant drug Prozac), suggesting that the synaptic concentration of serotonin can influence editing of 5-HT_{2C} pre-mRNA (55).

In principle, RNA editing provides an ideal mechanism for fine-tuning receptor phenotypes (14, 19), although at present this is an attractive but unproven hypothesis. It is still unclear whether complete Q/R editing of GluR2 is required for normal CNS function, and editing-deficient GluR5 mice failed to exhibit detectable phenotypes in tests expected to depend on the properties of GluR5-containing receptors (58). For the 5-HT_{2C} receptor, the multiplicity of receptor mRNAs in individual neurons is unknown, and the functional consequence of expressing multiple mRNA variants remains mysterious. The effect of GluR editing on receptor function in the intact nervous system

is similarly difficult to predict. The non-NMDA GluRs are not subject to strict constraints on subunit assembly, and although certain subunit combinations may be preferred (59, 60), the assembly of both AMPA and kainate receptors is probably largely combinatorial (61, 62). Central neurons express multiple subunits from each subtype family, and individual cells probably express receptors with many different subunit compositions and stoichiometries. For AMPA receptors, this view is supported by the marked heterogeneity of subunit-dependent properties seen for single native receptor molecules (63). The inclusion of a single edited subunit renders heteromeric channels calcium-impermeant (6, 12), but channels containing edited and unedited GluRs show polyamine block (64) and unitary conductances (10, 11, 65) that are intermediate between those of homomeric channels. The unknown dependence of these latter properties on subunit stoichiometry complicates forecasting the effect of alterations in editing, even in cell types where the relative abundance of related subunits is known. It is therefore highly likely that the functional consequences of GluR editing will depend on which related subunits are present in a given cell, as well as their relative abundance. This relative abundance can in turn be influenced by editing (25), and the fraction of individual GluRs that appear at the cell surface and their location are regulated by subunit-specific mechanisms (66-73).

Despite these complications, there seems no doubt that RNA editing is an important source of protein diversity, and the high inosine content of brain mRNA strongly suggests that many additional editing substrates remain to be identified (74). What percentage of these unidentified editing events will cause important functional changes remains to be seen, but their detection will certainly be aided by the rapid accumulation of genomic sequence data. Recent elegant studies in editing-deficient mice have begun to elucidate the effects of quantitative changes in GluR2 editing (36), the potential roles of kainate receptors in synaptic plasticity (52), and the *in vivo* substrate selectivity of ADAR1 (27) and ADAR2 (25). The extension of these transgenic strategies holds great promise and will likely reveal much about the role of RNA editing in shaping synaptic function in the intact brain.

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Citation: C. Schmauss, J. R. Howe, RNA editing of neurotransmitter receptors in the mammalian brain. *Science's STKE* (2002), http://www.stke.org/cgi/content/full/OC_sigtrans;2002/133/pe26.