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REVIEW

Glutamate Receptor RNA Editing in Health and Disease

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Abstract—RNA editing is a post-transcriptional process with an important role in gene modification. This editing process involves site-selective deamination of adenosine into inosine in the pre-mRNA, leading to the alteration of translation codons and splicing sites in nuclear transcripts, thereby enabling functionally distinct proteins to arise from a single gene. One important instance is the neuron editing of the ionotropic glutamate receptors (iGluRs). GluRs play a key role in excitatory synaptic transmission and plasticity in the central nervous system (CNS); their channel properties are largely dictated by the subunit composition of the tetrameric receptors. AMPA/kainate channels are assembled from GluA1-4 AMPA or GluK1-5 kainate receptor subunits. In particular, three of the four AMPA and two of the five kainate receptor subunits are subject to RNA editing. The editing positions have been named on the basis of the amino acid substitutions, such as the Q/R site in AMPA GluA2; the Q/R site in GluK1 and GluK2; the R/G site in GluA2, GluA3, and GluA4; and the I/V and Y/C sites in GluK2. These amino acid changes lead to profound alterations of the channel properties. This paper reviews the most relevant data showing the importance of glutamate receptor RNA editing in finely tuning glutamatergic neurotransmission in the normal CNS and following alterations of the editing process in association with disease phenotypes. Overall, these data indicate that a highly regulated process of glutamate receptor editing is of key importance in the proper function of neuronal cells and in their ability to adapt and modulate synaptic function.

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The central nervous system (CNS) in higher eukaryotes is likely the most complex structure known in science. Recently, it has become increasingly clear that the simple transcription of the limited set of genes present in the genome is insufficient to explain the highly complex functions and physiology of the brain. Indeed, a series of other phenomena take place to expand the repertoire of neuronal transcripts and allow for such complex behavior. Among other important processes occurring at the RNA level [1], the post-transcriptional event of "RNA editing" has received increasing attention in recent years. The term "RNA editing" is normally used to identify any mechanism responsible for producing mRNA molecules with sequence information not specifically encoded in the DNA. The phenomenon of RNA editing was first described as the insertion of single uridines in the mitochondrial RNA of trypanosomes [2], but now it refers to any site-specific alteration in an RNA sequence (for review, see [3]). This post-transcriptional mechanism can result in protein diversity by altering start and stop codons, creating splicing sites, or single amino acid substitutions in functionally important positions.

Here, we focus on Adenosine to Inosine (A-I) RNA editing [4, 5], the most common form of editing in CNS, and on its potential role in regulating the properties of the ionotropic glutamate receptors (iGluRs), the main effectors of fast excitatory neurotransmission.

GLUTAMATE RECEPTOR STRUCTURE AND FUNCTION

Fast excitatory neurotransmission in the vertebrate CNS is mainly mediated by iGluRs. These receptors are ligand-gated ion channels permeable to Na^+, K^+ and Ca^{2+} that mediate the fast depolarization of the post-synaptic membrane after glutamatergic stimuli and induce post-synaptic action potentials to propagate neuronal information [6]. iGluRs are divided into AMPA (α-amino-3-hydroxy-5-methyl-4-isoxasolepropionic acid), kainate, and NMDA (N-methyl-D-aspartate) receptors on the

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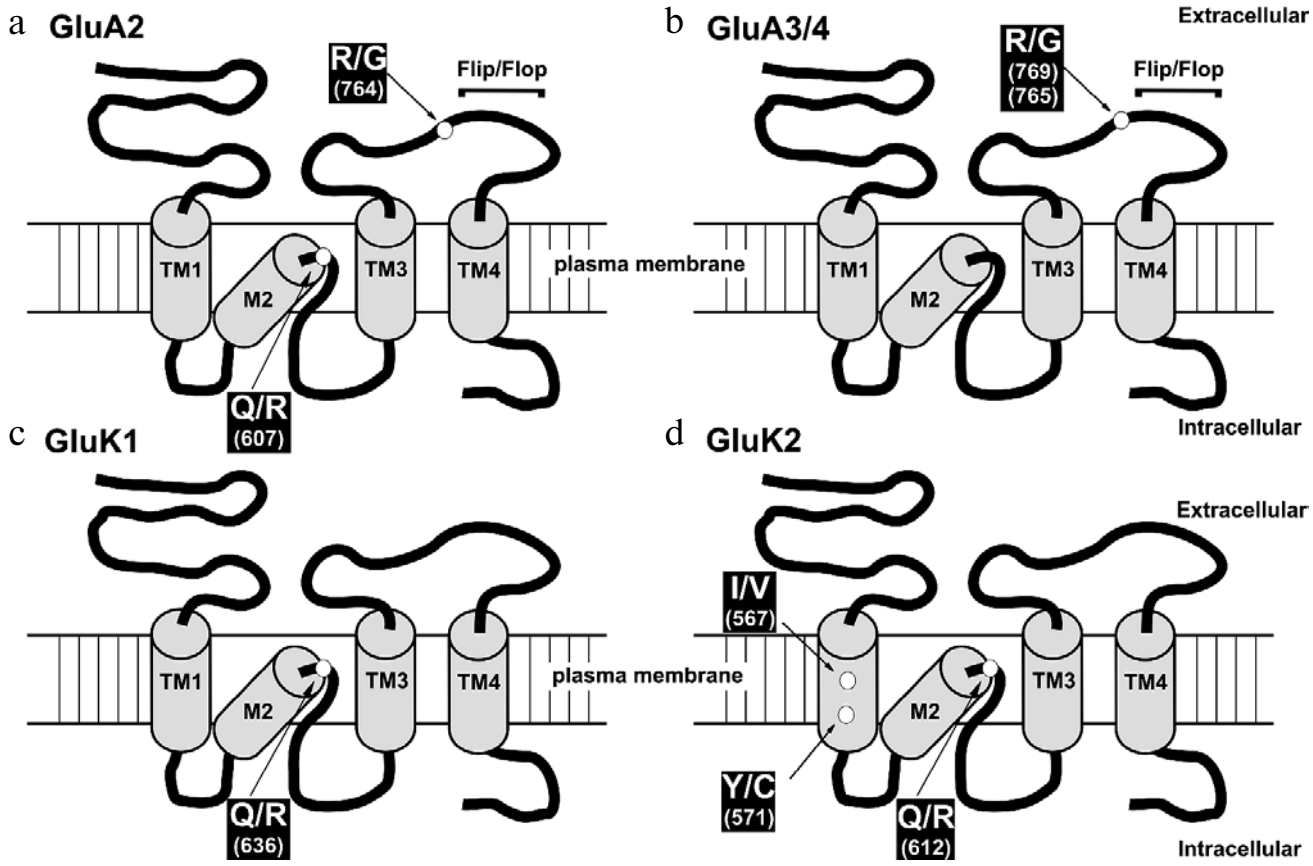
basis of their agonist selectivity, pharmacological characterization, and sequence similarity [6-8]. Glutamate receptors are tetrameric complexes, and functional receptors are formed exclusively by assemblies of subunits within the same functional receptor class. Mammals express four AMPA (GluA1-4), five kainate (GluK1-5), and seven NMDA (GluN1, GluN2A-GluN2D, GluN3A, and GluN3B) receptor subunits. In addition, two delta subunits (GluD1 and GluD2) have also been described (for the new IUPHAR nomenclature, see [9]).

AMPA receptors are hetero-oligomers consisting of combinations of four different subunits. In the adult hippocampus, GluA1, GluA2, and GluA3 combine to form two distinct populations, GluA1/GluA2 and GluA2/GluA3, which probably play different roles and are delivered to synapses by distinct mechanisms [10]. Each of the four AMPA receptor subunits is alternatively spliced in the extracellular region, generating the so-called "Flip" and "Flop" variants [11], which display different kinetic properties [12-14] increasing protein variability. The kainate receptor subunits GluK1, GluK2, and GluK3 also form both homo- and heteromeric channels, but GluK4 and GluK5 only form functional receptors when co-expressed with GluK1 to GluK3 [15]. Finally, functional NMDA receptors require the assembly of two GluN1 subunits together with either two GluN2 subunits or a combination of GluN2 and GluN3 subunits [16-18].

All ionotropic glutamate receptor subunits share a common basic structure. Each subunit possesses four transmembrane hydrophobic domains (TM1-4) within the central portion of the sequence. However, in contrast to other similar receptor subunits, the TM2 domain forms a re-entrant loop, giving these subunits an extracellular N-terminus and an intracellular C-terminus (figure).

RNA EDITING OF GLUTAMATE RECEPTORS

RNA editing may finely modulate the kinetic and ionic properties of glutamate receptors. At present, three



Schematic representation of glutamate receptor subunit structure. Each GluR subunit is composed of four transmembrane domains (TM1-TM4), an extracellular N-terminal region, and an intracellular C-terminal domain. a) GluA2 subunits have a Q/R editing site in the M2 domain. The R/G site and the Flip/Flop cassette subject to splicing are located between the TM3 and TM4 domains. b) GluA3 and GluA4 are edited at the R/G site and present the Flip/Flop cassette (a.a. 769 for GluA3 and a.a. 765 for GluA4). c) The GluK1 subunit is edited at the Q/R site. d) The GluK2 subunit is edited at the I/V and Y/C sites located in the TM1 domain and Q/R site. The amino acid position of each editing site is reported in brackets

enzymes that catalyze the A-I editing reaction have been characterized [19], all of which belong to the family of adenosine deaminases acting on RNA (ADAR). These enzymes present two or three RNA-binding domains at the N-terminal region and the catalytic domain at the C-terminal region. ADAR1 and ADAR2 are expressed in many tissues but especially in the brain; ADAR3 is only expressed in neuronal tissues, but its role in the editing reaction has not yet been clearly characterized. ADAR1 and ADAR2 are able to recognize specific double-stranded RNA structures generated by the hybridization of complementary exon and intron sequences in the pre-mRNA of specific transcripts, termed editing complementary sequences (ECSs). The enzymatic activity of ADARs leads to the modification of adenosines to inosines in specific coding regions; inosines are then recognized by the translational machinery as guanosines, altering the codon of the edited nucleotide and leading to an amino acid change in the expressed protein. The transcripts coding for five glutamate receptor subunits (AMPA GluA2, GluA3, GluA4 and kainate GluK1 and GluK2) are subject to RNA editing in different positions, giving rise to four different amino-acid changes, namely glutamine to arginine (Q/R), arginine to glycine (R/G), isoleucine to valine (I/V) and tyrosine to cysteine (Y/C) (Table 1).

In the case of the GluA2 subunit, enzymatic deamination results in the substitution of a codon for glutamine (CAG -Q-) with a codon for arginine (CIG -R-) in the second membrane domain of the receptor channel, at the so-called Q/R site. The presence of the positively charged amino acid arginine in the inner channel pore makes the receptor channel impermeable to Ca^{2+} [20], reduces its ion conductance [21] and alters its current/voltage relationship [22]. Under physiological conditions, virtually all pre-mRNAs encoding GluA2 are edited at the Q/R site, with the result that all GluA2-containing ion channels are impermeable to Ca^{2+}. The other AMPA receptor subunits do not present this editing site.

The relevance of editing at the Q/R site has been shown by the data obtained in knockout mice. Mutant mice, in which the GluA2 ECS sequence has been eliminated [23, 24] or the editing enzyme ADAR2 [25] suppressed, showed a sharp drop in Q/R editing levels and an

Table 1. Glutamate receptor editing sites and their functional implications

Receptor subunit	Editing site*	Functional changes	Modified in:
GluA2	**Q/R** $_{(607)}$	– Ca^{2+} impermeability [20]; – ER retention [29]; – correct mRNA maturation [25, 27]; – NPC inductor (unedited) [28]	– ALS [74]; – ischemia [61]; – glioblastoma progression [57, 58]; – epilepsy [23, 25]; – antidepressant treatments (*in vitro*) [73]
	R/G $_{(764)}$	– enhanced rate of recovery from desensitization [32]; – correlation with flip/flop splicing [35]	– SCI [77]; – epilepsy [66]; – murine model of schizophrenia [70]; – antidepressant treatments [72]
GluA3	**R/G** $_{(769)}$	– enhanced rate of recovery from desensitization [32]	– SCI [77]; – antidepressant treatments [72]; – murine model of schizophrenia [70]
GluA4	**R/G** $_{(765)}$	– enhanced rate of recovery from desensitization [32]	– antidepressant treatments [72]
GluK1	**Q/R** $_{(636)}$	– lower Ca^{2+} permeability [46]; – inhibition of receptor by membrane fatty acids [50]; – neuronal maturation in spinal horn (unedited) [49]	– epilepsy [64, 65]; – murine model of schizophrenia [70]
GluK2	**I/V** $_{(567)}$ **Y/C** $_{(571)}$	– finer modification of ionic properties [46]	
	Q/R $_{(621)}$	– ER retention [54]	– antidepressant treatments [72]; – murine model of schizophrenia [70]

* Editing site is named according to the amino acid modification produced. Amino acid position is reported in brackets.

altered ionic permeability. The mice were prone to epileptic seizures leading to death within a few weeks after birth. Moreover, these knockout mice displayed nuclear accumulation of incomplete spliced GluA2 pre-mRNA and decreased levels of GluA2 mRNA. The increase in unspliced GluA2 pre-mRNA was correlated with the reduction in the Q/R editing level, thus suggesting that editing might be a requirement for efficient splicing of this pre-mRNA [25], a result that has also been confirmed *in vitro* [26]; conversely, mutant mice with a constitutive arginine present at the GluA2 Q/R site show a vital phenotype [27].

A recent report [28] showed the presence of unedited GluA2 containing receptors in human neuronal progenitor cells (NPC), indicating that unedited GluA2 receptor is required during embryogenesis. Indeed, while NPCs differentiate into neurons, GluA2 subunits become fully edited as in the adult brain. The authors suggested that editing at the GluA2 Q/R site might be involved in the induction of neurogenesis. In addition to regulating the electrophysiology of the channel, Q/R editing determines the maturation and cellular trafficking of GluA2 [29]. The GluA2-R edited form is retained in the endoplasmic reticulum (ER) because the post-transcriptionally modified arginine located in the pore loop provides a retention signal favoring association with specific retention factors. Trafficking to the cell surface might only be allowed for the fully assembled, hetero-tetrameric receptors in which the retention signal is masked by the presence of other GluA subunits [30].

The AMPA receptor subunits GluA2, GluA3, and GluA4 are also edited at the R/G sites, where a codon for arginine (AGA -R-) is modified to a codon for glycine (IGA -G-). This site is located in the extracellular loop near the neurotransmitter binding site, at the mRNA level, just before the sequences involved in the splicing events forming the so-called Flip/Flop isoforms. Editing at these sites seems to affect, in coordination with the Flip/Flop cassette, the kinetic properties of the AMPA receptor channels [8, 31-34], especially the time of desensitization and the time of recovery from desensitization (Table 2). As a net effect, edited receptors have an enhanced rate of recovery from desensitization, generating ion channels that are able to respond more rapidly to a train of impulses [32]. As mentioned above, the adenosine subject to editing in the pre-mRNA is located in position –2 of the donor splice site involved in the splicing of the flip/flop exon. This localization led to the hypothesis of a direct correlation between editing and splicing. Editing events have been reported to precede splicing due to the interaction with the C-terminal domain of RNA polymerase II [35, 36], and editing at the R/G site was shown to lead to a reduction in splicing efficiency in the adjacent intron [37]. This phenomenon should be due to the inosine at the R/G site, but ADAR binding is not required to observe this effect [26]. However, no direct link between editing and the flip/flop exon selection has been clearly demonstrated [26, 37].

Finally, two kainate receptor subunits may also be subject to editing: whereas the kainate receptor GluK1 [38, 39] can be edited only at the Q/R site, the kainate GluK2 [40, 41] can be edited at two additional sites, the I/V and Y/C sites located in the transmembrane 1 domain (TM1), where an isoleucine (ATT) is modified to

Table 2. Recovery time from desensitization for the different R/G edited and flip/flop spliced AMPA receptor variants

	[33]	[8]	[34]	[32]
		τ_{rec}, msec		
GluA1 flip		147.0	178.0	
GluA1 flop		147.0	134.0	
GluA2 flip R			55.0	
GluA2 flip G	11.7		46.0	
GluA2 flop R			75.0	
GluA2 flop G	31.3		30.0	
GluA3 flip R		36.0	69.0	36.0
GluA3 flip G		15.0		15.0
GluA3 flop R			130.0	
GluA3 flop G				
GluA4 flip R		14.0	3.1	16.0
GluA4 flip G		6.0		6.0
GluA4 flop R		43.0	3.3	43.0
GluA4 flop G		13.0		31.0

a valine (ITT) and a tyrosine (TAC) to a cysteine (TIC), respectively.

In contrast to GluA2, editing of the Q/R site in GluK1 and GluK2 mRNAs (but not in GluK3) occurs at very low levels in the embryonic brain and increases to ~40% (GluK1) and ~80% (GluK2) of the mRNA transcripts within the first few days after birth in most regions of the brain [42-45]. This developmentally regulated Q/R editing reduces GluK Ca^{2+} permeability [20, 46] and channel conductance [21, 47] and alters the current–voltage relationship [22, 48] during cell maturation. As indicated by studies of the neurons of dorsal root ganglia, the unedited GluK1 subunit could play a developmental role in synapse formation in the spinal cord dorsal horn [49]. In later development, the Q/R site-edited channel may acquire other functions in which Ca^{2+} influx is not required. Moreover, RNA editing at the Q/R site determines GluK susceptibility to inhibition by *cis*-unsaturated fatty acids and blockage by cytoplasmic polyamines [50]. Channels formed by unedited subunits are strongly blocked by polyamines, but insensitive to fatty acids such as arachidonic acid (AA) and docosahexaenoic acid (DHA), whereas homomeric edited (R) channels are resistant to polyamine blockage but are inhibited by AA and DHA [50-52].

Knockout mice in which the GluK2 ECS [47] had been deleted indicate that unedited GluK2 may mediate synaptic plasticity and that the edited GluK2 receptors may reduce vulnerability to seizures. On the contrary, GluK1 mutant mice carrying a constitutive arginine (R) instead of the genomically encoded glutamine (Q) at the editing site do not exhibit developmental alteration or abnormal behavior [53]. Thus, the editing of this receptor seems to be unnecessary for survival, but it may be involved in higher-order mechanisms such as learning and memory.

In addition to the electrophysiologic modification induced by RNA editing in GluK receptors, a recent report [54] showed that edited and unedited GluK2 are differentially transported to the cell surface. The edited GluK2(R) is preferentially retained in intracellular compartments and accumulates predominantly as monomers/dimers in the ER. Conversely, the unedited GluK2(Q) exits the ER more efficiently and accumulates at the cell surface where it preferentially forms tetramers. Based on these data, the authors suggest that the assembly and intracellular distribution of GluKs are determined by RNA editing at the Q/R site. However, this conclusion is a subject of debate because another report [55] found no differences in the oligomerization and intracellular trafficking properties of unedited and edited GluK2 subunits.

The I/V and Y/C sites located in the first transmembrane domain seem to be involved, together with the Q/R site, in the fine regulation of ion permeability [41, 46], although no clear electrophysiologic data have been reported to support this conclusion until now.

With the exception of the GluA2 Q/R site, which is virtually fully edited in all brain areas, the editing levels of the other sites appear to be site-specifically regulated [44] in the adult CNS. Moreover, the glutamate receptor editing increases to a specific and fixed level during the development of the nervous system *in vivo* and during neuronal culture maturation and differentiation *in vitro* [44, 45, 56].

GLUTAMATE RECEPTOR RNA EDITING AND NEUROLOGICAL DISORDERS

The importance of glutamate receptors and of RNA editing as a modulator of their function in the nervous system raises the hypothesis that alterations in editing activity may be implicated in several neurological disorders.

A decrease in the RNA editing of the GluA2 Q/R site was shown in several forms of brain cancer [57, 58], and editing at this site was shown to be essential for suppressing the migration of glioblastoma cells *in vivo* [59]. In addition, ADAR deregulation has recently been suggested to play a role in cancer progression, leading to a modification of the editing site in coding but also in non-coding RNA, such as microRNA [60].

ADAR functions and in turn the modification of the GluA2 Q/R site have also been implicated in the susceptibility of neurons to ischemia [61]. Indeed, following transient ischemia, calcium permeability through AMPA receptors increases substantially in pyramidal neurons. Single-cell RT-PCR demonstrated that transient ischemia reduced GluA2 subunit mRNA editing and decreased the abundance of ADAR2 mRNA, whereas other neurons in the hippocampus that were not as sensitive to cell injury did not show any reduction in GluA2 editing or ADAR2 mRNA expression. Moreover, overexpression of ADAR2 in rat brain prevented the decrease in GluR2 editing, the increase in calcium permeability, and post-ischemic neuronal injury [61]. These data indicate a direct link between neuron vulnerability to ischemic insult and ADAR2 activity.

Furthermore, since RNA editing might be involved in the evolution of higher neurological functions such as learning, memory, and behavior, a great deal of attention has been given to its deregulation in complex diseases such as epilepsy, schizophrenia, and depression. While a link between epilepsy and editing at the GluA2 Q/R site has been clearly shown in knockout mice [23, 25], this association has not yet been clearly shown in humans [62, 63]. Two reports investigated the ratio of Q/R variants of GluA2, GluK1, and GluK2 subunits in the neocortex and hippocampus of epileptic patients [64, 65] and found no alteration in GluA2 editing, but they did, however, show an increase in the editing of GluK2 in hippocampal and neocortical tissue [64] and of GluK1 in neocortical tissue

[65]. Moreover, an increase in the editing of the GluA2 R/G site has been described in the hippocampus of temporal lobe epilepsy patients [66]. However, human data do not clarify whether the variation in editing levels is causative of or an adaptive reaction to the ongoing epileptic seizures.

Human genetic studies suggest the involvement of glutamate receptor subunit genes in the etiology of schizophrenia [67, 68]; in addition, the editing levels of the GluA2 Q/R site have been found to be slightly altered in the post-mortem cerebral cortex specimens from schizophrenia patients [69]. In a murine model of schizophrenia (phencyclidine (PCP)-treated rats), we found no alteration of the GluA2 Q/R site, but we did observe a decrease in the editing levels of the R/G sites of both GluA2 and GluA3 and a significant increase in the editing level of the GluK2 Q/R site. The variation in the editing level of the R/G sites suggested that chronic PCP treatment induced the formation of glutamate receptor subunits with slower resensitization kinetics and, with respect to kainate receptors, an increase in the Q/R editing level might generate receptor channels with a lower permeability to cations. PCP treatment induced a specific and site-selective reduction of glutamatergic neurotransmission [70] by modifying RNA editing levels.

Variation in the editing reaction has also been hypothesized to play a role in mood disorders; while convincing data are emerging linking RNA editing of serotonin receptor 2c [71] with mood disorder, no clear evidence has been provided to indicate modification of GluR RNA editing in human patients. However, we have reported that the extent of editing was altered at some of the editing positions in glutamate receptor mRNAs in the pre-frontal/frontal cortex and in the hippocampus of rats after a continuous 2-week treatment using various classical antidepressants [72] with different primary mechanisms of action. Further, the editing efficiency at the GluA2 Q/R site was significantly increased after treatment with seven antidepressants in a HeLa cell line that stably expresses half-edited GluA2 pre-mRNAs [73]. Taken together, these data indicate that RNA editing is a possible target for the molecular action of antidepressants and suggest a possible implication in the pathophysiology of mood disorders.

A clear correlation between RNA editing deregulation and human disease is evident in spinal cord pathologies. RNA editing has been reported to play a role in patients suffering from sporadic amyotrophic lateral sclerosis (ALS) [74, 75]. Glutamate excitotoxicity appears to contribute to the pathology of ALS, suggesting that the activation of glutamate receptors may lead to an excessive influx of Ca^{2+} in spinal neurons and consequently to cell death. In the spinal motor neurons of ALS patients, editing at the GluA2 Q/R position was severely decreased, leading the AMPA channels to be highly permeable to Ca^{2+} and in turn facilitating neuronal death. This phenomenon was not observed in the neurons of patients with other neurodegenerative disorders, including those with familial ALS. The authors suggest that ADAR2 deregulation might be the cause of under-editing, and indeed they found decreased ADAR2 expression levels in the spinal ventral gray matter of patients with sporadic ALS. Supporting this view, a recent report using a genetically modified mouse in which ADAR2 was conditionally inactivated showed that the modification induced a slow death of ADAR2 deficient neurons in the spinal cord [76].

The importance of GluR RNA editing in the proper function of spinal neurons is also supported by our recent report examining glutamate receptor expression and function after spinal cord injury (SCI) [77]. SCI induces a primary lesion in the spinal neurons due to the direct action of a mechanical force to the cord. In addition, a secondary lesion is achieved by a complex ischemic and inflammatory response that also involves excitatory neurotransmitter systems and intracellular signaling modifications [78], creating an unfavorable environment for synaptic survival and regeneration. Chief among these responses is the excitotoxicity induced by abnormal glutamate release that triggers the influx of Ca^{2+} [79, 80], leading to harmful events including cell death. We hypothesized that SCI might lead to a change in the RNA editing of AMPA receptors because they govern critical determinants of neuronal survival. The results demonstrate that SCI produces a decrease in AMPA receptor R/G editing. Further, these changes appear to be a specific regulatory adaptation rather than simply reflecting the immediate effects of the injury itself. Indeed, this modification extends outside the infarcted zone and includes areas of the spinal cord both caudal and rostral to the injury. The effects are selective for specific GluRs, and many of the changes persist long after the initial injury. However, the decrease in editing does not occur in parallel with a loss of ADAR expression, but it was associated with a partial deactivation of ADAR2 function as can be deduced by the observed down-regulation of ADAR2 self-editing. The reduced editing at the R/G site of AMPA receptor subunits is likely to reduce post-synaptic excitatory responses to glutamate, thus limiting the progression of cell death; however, prolonged suppression of GluR function in later stages might hinder synaptic plasticity and might inhibit proper rehabilitation. These observations provide direct evidence of the potential contribution of RNA editing to excitatory neural injury and recovery after SCI [77]. Moreover, this line of evidence indicates that drugs modulating ADAR2 activity are potential therapeutic tools for treating SCI.

This work reviewed the importance of RNA editing of glutamate receptors, which are the best-studied editing events in the mammalian brain, along with the editing of the 5-HT2c receptor. Glutamate receptor RNA editing

may be considered a molecular mechanism involved in the control of the response to excitatory stimuli. The fact that most neuronal editing sites are modified in variable proportion indicate that unedited and edited receptor subunits are present at the same time within the same cell, potentially generating heteromeric channels with distinct and peculiar functions. Moreover, the levels of editing in several sites may change during development and may be cell and tissue specific. Thus, variations in the extent of editing may be responsible for the change in the response of neuronal cells to glutamate due to the modification in the function of glutamate receptors. Furthermore, a growing list of other brain editing targets has been identified through bioinformatic and high throughput sequencing approaches [81, 82], indicating that RNA editing generates a diverse repertoire of proteins from a small fixed pool of genes; the modulation of this pool allows neuronal cells to dynamically react to rapid changes and favors the protection and fine tuning of the response of the nervous system. In line with this, some researchers have suggested that the editing of neuronal targets may be an indicator of higher order brain function [4]; changes in editing patterns might indicate modifications of brain functions that could specify neurological defects.

Furthermore, the RNA editing of the primate ALU sequence [83] and the correlation of RNA editing and microRNA biogenesis and function [84] are receiving increasing attention. These data indicate that RNA editing is deeply connected with proper cell function in multiple systems. However, the mechanisms underlying the regulation of ADAR function and RNA editing remain poorly understood. The use of high throughput approaches to identify new A-to-I editing targets might provide additional information on this particular phenomenon because it is clear that it contributes significantly to both the physiological and the pathophysiological conditions observed in the nervous system.

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