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REVIEW

# A-to-I RNA Editing Modulates the Pharmacology of Neuronal Ion Channels and Receptors

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**Abstract**—The regulation of neuronal excitability is complex, as ion channels and neurotransmitter receptors are underlying a large variety of modulating effects. Alterations in the expression patterns of receptors or channel subunits as well as differential splicing contribute to the regulation of neuronal excitability. RNA editing is another and more recently explored mechanism to increase protein diversity, as the genomic recoding leads to new gene products with novel functional and pharmacological properties. In humans A-to-I RNA editing targets several neuronal receptors and channels, including GluR2/5/6 subunits, the Kv1.1 channel, and the 5-$HT_{2C}$ receptor. Our review summarizes that RNA editing of these proteins does not only change protein function, but also the pharmacology and presumably the drug therapy in human diseases.

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The enzymatic deamination of adenosine in double-stranded or structured RNAs is mediated by ADARs (Adenosine Deaminases Acting on RNA). This site-specific reaction leads to the conversion of adenosine (A) to inosine (I) in mRNA; the translational machinery however interprets inosine as guanosine (G). Thus, the A-to-I conversion functionally leads to an A-to-G nucleotide exchange. The majority of A-to-I editing sites lie in non-coding regions [1]. However, some editing sites are exonic, and nucleotide exchanges by enzymatic deamination also lead to changes in the protein sequence. These RNA editing-generated changes in the amino acid sequence have been reported to cause drastic changes in protein function and pharmacology. The expression of ADARs is particularly high in the nervous system, and so are the A-to-I editing levels [2]. Accordingly, many proteins of the nervous system like ionotropic receptors, G protein-coupled receptors (GPCRs), and ion channels are amongst the neuronal targets of RNA editing. Most of these conversions in the RNA sequence are mediated by ADAR2. In the following we review the neuronal RNA editing targets GluR2/5/6, 5-$HT_{2C}$, and Kv1.1 which all show altered pharmacology upon editing in humans.

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## GluR SUBUNIT EDITING AT THE Q/R SITE DETERMINES BLOCK BY SPIDER TOXINS AND BLOCK BY ENDOGENOUS LIPIDS

**Composition of glutamate receptors, ion selectivity, and the Q/R editing site.** L-Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. Three different types of ionotropic glutamate receptors are known: NMDA, AMPA, and kainate receptors. The functional glutamate receptors are tetramers, which differ in their subunit compositions. AMPA receptors are formed by GluR1 to GluR4 subunits (also GluR-A to GluR-D) and GluR2 subunits are most often part of the functional receptors. The repertoire of kainate receptor subunits comprises GluR5 to GluR7, KA-1, and KA-2, while NMDA receptors are formed by NR1, NR2A to NR2D, NR3A, and NR3B subunits [3, 4]. The subunit composition of glutamate receptors defines the receptor subtype and the selectivity of the ionotropic receptor pore. All three glutamate receptor types conduct $Na^+$ ions. However, under normal conditions the NMDA receptor is the only $Ca^{2+}$ permeable glutamate receptor. The GluR2 subunit in AMPA receptors and the GluR5 and GluR6 subunits in kainate receptors are responsible for the $Ca^{2+}$ impermeability of these glutamate receptors [5]. The GluR subunits that regulate $Ca^{2+}$ permeability of

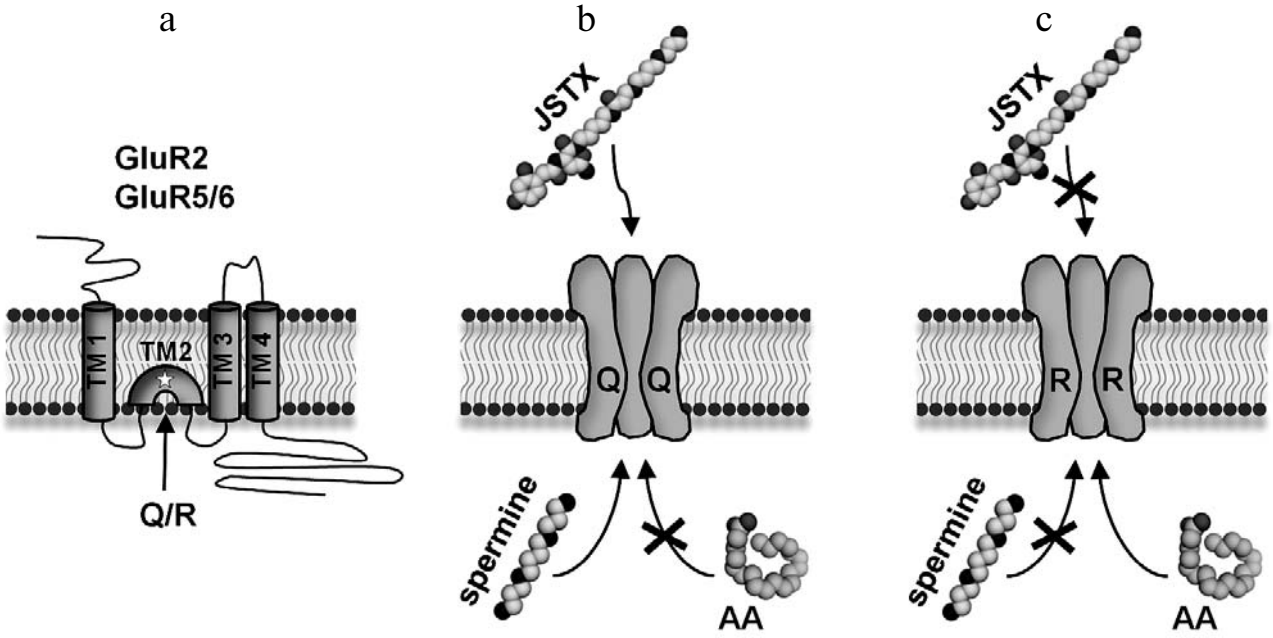


**Fig. 1.** Localization of the Q/R editing sites in GluR subunits. a) The GluR subunits GluR2, GluR5, and GluR6 undergo RNA editing leading to a Q-to-R exchange (see asterisk) in the pore-forming transmembrane segment 2 (TM2). b) Receptors formed by unedited subunits have a glutamine (Q) at the Q/R site. They are sensitive to intracellular block by polyamines (like spermine and spermidine) and extracellular block by Joro spider toxin (JSTX) but insensitive to block by lipids like arachidonic acid (AA). c) Receptors formed by edited GluR subunits have an arginine (R) at the Q/R site and are sensitive to block by arachidonic acid (AA) but insensitive to block by polyamines and JSTX.

AMPA and kainate receptors are undergoing an RNA editing in the pore forming region of the second transmembrane segment (TM2). This recoding of the genomic information leads to a glutamine (Q) to arginine (R) exchange (Fig. 1a) in the pore, preventing a $Ca^{2+}$ flux through these subunits. Most importantly, the arginine (R) residue at the Q/R editing site regulates $Ca^{2+}$ permeability of AMPA and kainate receptors in a dominant negative manner [6]. Thus, the incorporation of edited GluR2/5/6 subunits into the receptor tetramers leads to $Ca^{2+}$ impermeable pores.

The NMDA receptor subunits have an asparagine (N) residue at the respective position of the Q/R editing site in TM2 [7], which is therefore also called Q/R/N site. The asparagine does not impede the $Ca^{2+}$ permeability of glutamate receptors. Therefore, the different types of NMDA receptors are all permeable to $Ca^{2+}$.

In contrast, the ion selectivity of AMPA receptors is determined by different levels of GluR2 subunit expression and the editing at the Q/R site [8]. The GluR2 expression level is a dynamic parameter which is changed during development [9], by the influence of seizures [10], ischemic insults, and different drugs [11]. The extent of RNA editing of GluR2 at the Q/R site is physiologically changed during neuronal development, starting with low editing ratios in neuronal progenitor cells [9] and ending up with nearly complete editing of the GluR2 subunits in the mature brain [12]. Accordingly, $Ca^{2+}$ conducting AMPA receptors can arise either from a low abundance of GluR2 subunits [9, 13] or an under-editing of GluR2 subunits [14].

**Editing at the Q/R site reduces the sensitivity of glutamate receptors to spider toxins.** One of the physiological roles of editing at the Q/R site is to regulate the $Ca^{2+}$ permeability of glutamate receptors. However, the different extents of Q/R editing entail also pharmacological consequences for glutamate receptors. Glutamate receptors are, like other ion channels, targets for different drugs or toxins. Spider venom toxins are known to block NMDA, AMPA, and kainate receptors. The Joro spider toxin (JSTX), which was isolated from *Nephila clavata*, is one of the most potent blockers of glutamatergic receptors [15]. JSTX blocks NMDA receptors, "artificial" kainate receptors consisting of heterologously expressed homo-tetrameric unedited GluR6 subunits [16], and those native AMPA receptors that conduct $Ca^{2+}$ currents [16, 17]. Summarizing, JSTX only blocks unedited glutamate receptors, which do not have arginine residues at the Q/R site (Fig. 1, b and c). Thus, the potency of JSTX strongly depends on one single amino acid position, the Q/R editing site. This implies that differential changes in the editing ratio at the Q/R site are associated with changes in JSTX sensitivity of native glutamate receptors.

Argiotoxin (ATX) is another spider toxin known to block glutamate receptors. The parent compound of ATX

was isolated from *Argiope lobata*. Argiotoxin blocks glutamate receptors in the open, ligand-bound state [18]. The sensitivity of glutamate receptors to a synthetically produced analog of ATX ($ATX_{636}$) was shown to strongly depend on the amino acid of the Q/R editing site [18]. ATX blocks NMDA receptors with asparagine (N) located at the Q/R/N site and those AMPA and kainate receptors that do not contain any Q-to-R edited subunits [18]. Thus, similarly as for JSTX, the sensitivity of $ATX_{636}$ is restricted to native glutamate receptors that are permeable to $Ca^{2+}$ [19].

**Editing at the Q/R site increases the sensitivity of glutamate receptors to block by lipids and decreases block by spider toxins and polyamines.** Endogenous, physiologically occurring, *cis*-unsaturated fatty acids like arachidonic acid (AA) and docosahexaenoic acid (DHA) block AMPA [20] as well as kainate receptors [21]. RNA editing at the Q/R site of GluR subunits determines the block of glutamate receptors by AA and DHA. The block of kainate receptors by fatty acids is strongly dependent on the editing status of the receptor subunits [22]. Fully Q-to-R edited kainate receptors are already blocked by low concentrations of fatty acids, while the presence of unedited subunits leads to a massive loss of fatty acid sensitivity (Fig. 1, b and c) [22].

The inward rectification of AMPA and kainate receptors is mediated by the selective block of outward currents by intracellular polyamines [23]. This block is impeded by RNA editing at the Q/R site [24]. Bowie et al. showed that glutamate receptors formed exclusively by edited GluR6 subunits (R) did not develop inward rectification in the presence of spermine, while those homomeric for unedited GluR6 subunits (Q) were sensitive to spermine (Fig. 1, b and c) [23]. These results were supported by the finding that the physiologically occurring differences in editing at the Q/R site of the GluR6 mRNA in different neurons correlate with the rectification of the native kainate receptors [25]. Thus, while an arginine at the Q/R site favors block by lipids, this edited form reduces block by spider toxins and polyamines (Fig. 1, b and c).

**Clinical relevance of editing at the Q/R site.** The efficiency of RNA editing at the GluR2 Q/R site is altered under different pathophysiological conditions. Decreased editing of the GluR2 Q/R site has been reported for vitamin B1 deficiency (*in vitro*) [26] and for patients with sporadic amyotrophic lateral sclerosis (ALS) [27], while increased editing at the Q/R sites of GluR5 and GluR6 (but not GluR2) was detected in temporal lobe epilepsy (TLE) [28].

From a mechanistic point of view, the increased editing of GluR5/6 in TLE leads to a prevention of $Ca^{2+}$ currents through kainate receptors, while $Na^+$ currents are not affected. The increased editing ratio in epileptic patients is presumably of compensatory nature, as was suggested before [28]. The above-mentioned toxin JSTX has the same effect as an increased RNA editing at the Q/R site, as both reduce $Ca^{2+}$ currents through the glutamate receptors. This mechanistic notion is supported by the fact, that an analog of JSTX, 1-napthyl-acetyl-spermine (NASPM), has anticonvulsant effects *in vivo*. The seizure stage of amygdaloid kindled rats was significantly reduced by NASPM administration [29]. Thus, the block of $Ca^{2+}$ currents through glutamate receptors reduces the epileptic symptoms. Salamoni et al. have shown that JSTX can also reduce epileptiform activity, induced by lack of extracellular $Mg^{2+}$ or by application of NMDA, in hippocampal slices of human patients with temporal lobe epilepsy [30].

Kawahara et al. have isolated RNA from motor neurons of patients with sporadic amyotrophic lateral sclerosis (ALS) and of healthy control subjects to analyze GluR2 RNA editing [27]. In this study patients with sporadic ALS had a significantly reduced ratio of editing at the Q/R site of GluR2, which was specific for motor neurons [27]. This might lead to an increased $Ca^{2+}$ permeability of AMPA receptors in ALS and the resulting intracellular $Ca^{2+}$ overload might trigger neuronal death [27, 31]. The observation that certain antidepressants can increase the Q/R site editing of GluR2 led to the theory that those drugs could be useful to treat patients suffering from sporadic ALS [32].

## 5-$HT_{2C}$ RECEPTOR EDITING INFLUENCES $G_q$ PROTEIN-COUPLING

**RNA editing affects three amino acid positions in a critical region for the receptor coupling to G proteins.** The 5-$HT_{2C}$ receptor belongs to the superfamily of G protein-coupled receptors (GPCRs), which are monomeric receptors with seven transmembrane-spanning helices. The first GPCR that was identified as a target of mRNA editing was the 5-$HT_{2C}$ receptor. Five different positions (A-E) in the mRNA of this receptor are subject to RNA editing (Fig. 2a) [33]. The editing positions are distributed on three codons, thus editing (of some or all) of these sites can lead to amino acid changes in positions I156, N158, and I160 ($\mathbf{I_{156}}$-R-$\mathbf{N_{158}}$-P-$\mathbf{I_{160}}$) in the resulting receptor protein (Fig. 2a). This leads to the generation of different receptor isoforms [34]. While I160 can be edited to only one other amino acid (valine), I156 and N158 can be edited to two or three different amino acids, respectively (Fig. 2a). Consequently, 24 receptor isoforms can arise from the different combinations of possible amino acids on these three positions. So far, 20 subtypes have been detected in the human [33, 35, 36] and mouse brain [37], with different abundances strongly depending on the brain region. The frequency of the various isoforms is significantly different between species (human, rat, mouse) and even between different inbred mouse strains [37]. In general, the nomenclature of the different iso-

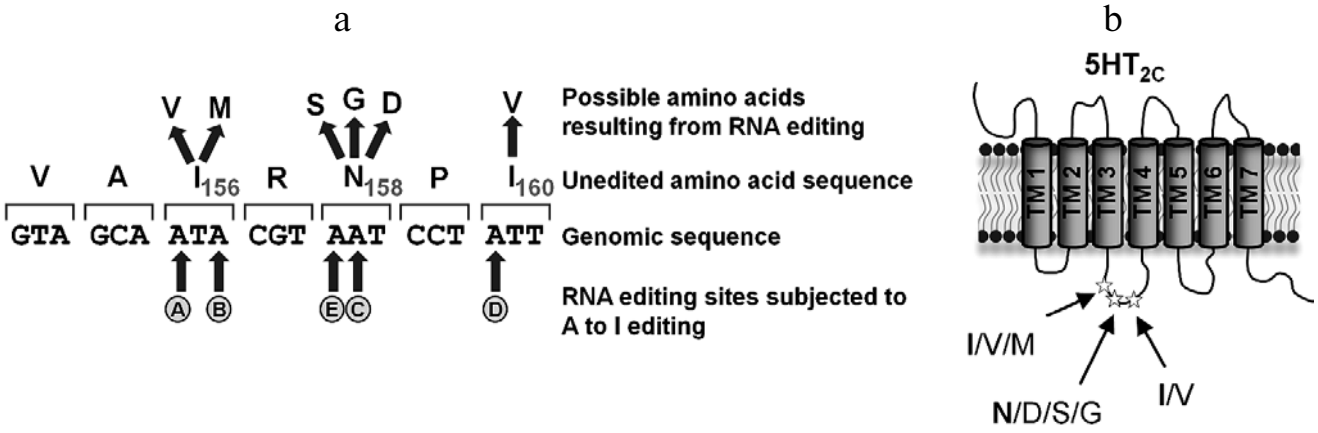


**Fig. 2.** RNA editing of the 5-$HT_{2C}$ receptor changes the amino acid sequence at three positions in the second intracellular loop. a) Five different adenosines in the RNA of the 5-$HT_{2C}$ receptor are target of RNA editing by ADARs (see A-E, bottom). The combination of edited and unedited sites determines the type of amino acid located at positions 156, 158, and 160. The unedited receptor isoform exhibits the amino acid residues INI at positions 156, 158, and 160, respectively. The position 156 can be taken by isoleucine (I), valine (V), or methionine (M), position 158 by asparagine (N), aspartate (D), serine (S), or glycine (G), and position 160 by isoleucine (I) or valine (V) (top). b) The amino acid positions that are target of RNA editing are located in the second intracellular loop of the 5-$HT_{2C}$ receptor (see asterisks), which is part of the G protein binding site.

forms refers to the amino acids at the positions 156, 158, and 160. Accordingly, the unedited receptor type is named INI and the fully edited isoform in humans VGV [39]. The rat 5-$HT_{2C}$ receptor appears to have only four editing sites (A to D), which is due to a negligible editing of the E site. Thus, the VSV isoform is often referred to as the fully edited isoform in rats [34, 38]. The three edited amino acids (I156, N158, and I160) are located in the second intracellular loop of the receptor (Fig. 2, a and b), a region supposed to be part of the G protein interaction site in many GPCRs [39].

**Editing status regulates receptor basal activity and agonist potency.** Burns et al. tested the functional consequences of RNA editing at the four editing sites in rat 5-$HT_{2C}$ receptors. In a fibroblast cell line transfected with the receptors, the potency of 5-HT was 10- to 15-fold reduced in the fully edited (5-$HT_{2C\text{-}VSV}$) compared to the unedited receptor (5-$HT_{2C\text{-}INI}$). Different mechanisms seem to contribute to the decreased agonist potency in intensely edited 5-$HT_{2C}$ receptor isoforms: an impaired coupling between 5-$HT_{2C}$ receptors and the $G_q$ protein [33, 34] and decreased agonist affinities of the receptor [33, 40, 41].

Also the ligand-independent intrinsic basal activity of the 5-$HT_{2C}$ receptor is determined by the editing status [40]. The unedited receptor isoform 5-$HT_{2C\text{-}INI}$ has the highest basal activity (measured as inositol-phosphate (IP) production), while it is decreased in edited receptor isoforms. The lowest degree of basal activity was observed for the human fully edited isoform 5-$HT_{2C\text{-}VGV}$ [40]. In accordance with this, the receptor activation induced by the agonist 5-HT is most prominent in the 5-$HT_{2C\text{-}VGV}$ isoform, where the IP production is increased 7.7-fold compared to the basal activity, and less effective in the unedited 5-$HT_{2C\text{-}INI}$ isoform, where 5-HT increased the receptor activity only 2-fold. The partially edited isoforms tested so far have receptor responses in between these two extremes. The maximum level of IP production, induced by very high concentrations of the agonist 5-HT, was similar for all 5-$HT_{2C}$ editing isoforms, showing that the agonist has still the ability to promote G protein-coupling [40].

A plausible explanation, which was examined by several groups, is that the agonist affinity of the 5-$HT_{2C}$ receptor is proportional to the intrinsic receptor activity, and that both features are decreased in edited receptors [33, 40, 41]. Presumably, the receptor can adopt an inactive conformation and an active conformation. The active conformation promotes G protein-coupling and agonist binding. The unedited 5-$HT_{2C\text{-}INI}$ favors the activated receptor conformation, resulting in a high degree of basal activity as well as high agonist affinity and potency. The fully edited 5-$HT_{2C\text{-}VGV}$ isoform preferably adopts the inactive receptor conformation, exhibiting reduced G protein-coupling and agonist binding [40]. The different 5-$HT_{2C}$ editing isoforms exhibit different tendencies to adopt receptor conformations that impair G protein-coupling, lower agonist-affinity, and also weaken the potency of the agonist [33, 40].

**Editing status can determine ligand affinities for the 5-$HT_{2C}$ receptor.** As mentioned above, the agonist affinity for 5-$HT_{2C}$ receptors is massively decreased in highly edited isoforms (e.g. 5-$HT_{2C\text{-}VSV}$ and 5-$HT_{2C\text{-}VGV}$) [33]. However, this does not apply to all 5-$HT_{2C}$ receptor agonists, as for example the binding affinity for LSD (lysergic acid diethylamide) is not affected by RNA editing. Upon LSD application a robust PI hydrolysis response can be measured in cells expressing the non-edited recep-

tor (5-$HT_{2C-INI}$). In contrast, no stable response of the fully edited receptor (5-$HT_{2C-VGV}$) was detected, while the affinity of the receptor for LSD was not altered by the editing status [33, 40]. LSD even had an antagonistic effect on fully edited receptors as preincubation of 5-$HT_{2C-VGV}$ expressing cells with LSD prevented the agonistic effect of 5-HT [36]. This example highlights that RNA editing can change not only the ligand potency but also the quality of the receptor action.

**Drugs and editing status determine the activated signal transduction pathway.** It has been known for a long time that 5-$HT_{2C}$ receptors can activate different signal

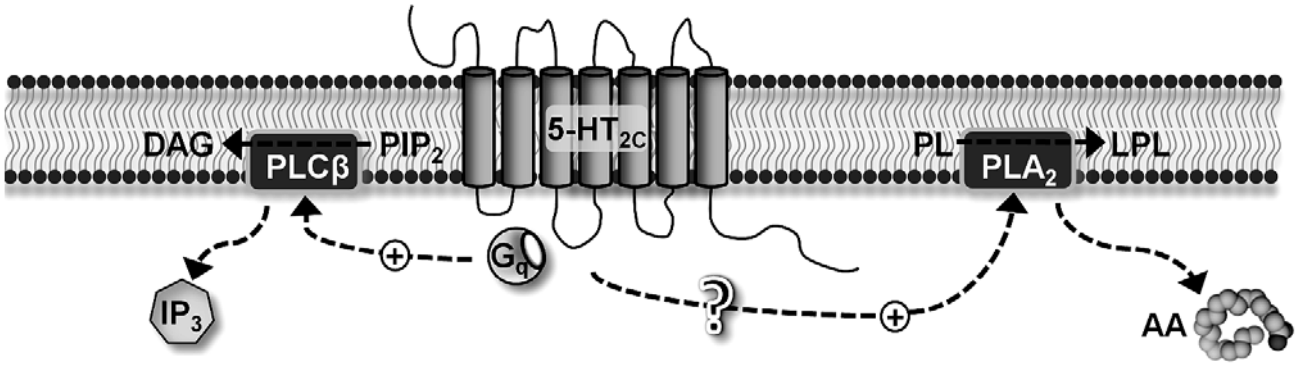


**Fig. 3.** The 5-$HT_{2C}$ receptor couples to different pathways. The 5-$HT_{2C}$ receptor can activate phospholipase C-β (PLC-β) via $G_q$ proteins and also phospholipase $A_2$ ($PLA_2$) via an unknown signal cascade. Activation of the $G_q$-PLC pathway leads to the production of diacylglycerol (DAG) and inositol trisphosphate ($IP_3$), while activated $PLA_2$ cleaves phospholipids (PL) to lysophospholipids (LPL), resulting in a release of arachidonic acid (AA).

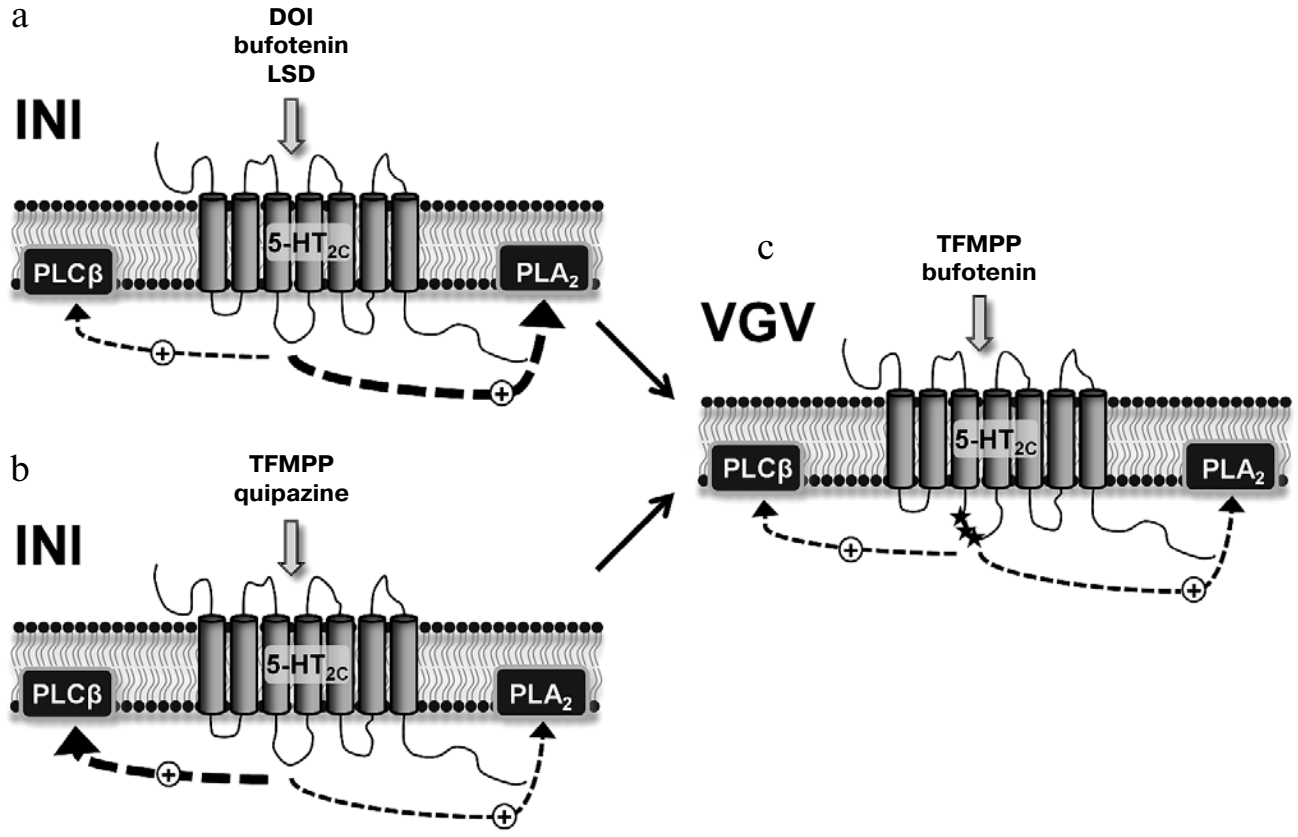


**Fig. 4.** Agonist-directed trafficking of receptor stimulus (ADTRS) is abolished in the fully edited 5-$HT_{2C}$ receptor isoform. a) The agonists DOI, bufotenin, and LSD preferentially activate the $PLA_2$-AA pathway in the unedited receptor. b) In the unedited receptor 5-$HT_{2C-INI}$ the agonists TFMPP and quipazine preferentially activate the PLC-IP pathway. c) In the fully edited receptor isoform 5-$HT_{2C-VGV}$ the pathway preferences of TFMPP and bufotenin are abolished. The PLC-IP and the $PLA_2$-AA pathway are activated at a similar level.

cascades in a cell: the phospholipase C (PLC) pathway and the phospholipase $A_2$ ($PLA_2$) pathway, leading to the release of $IP_3$ or AA, respectively (Fig. 3). The physiological agonist 5-HT shows a similar potency to activate the PLC-IP pathway and the $PLA_2$-AA pathway when tested in a heterologous expression system [42]. In contrast, other ligands of the 5-$HT_{2C}$ receptor preferentially activate one or the other pathway. For example, binding of the hallucinogenic drugs DOI (R(−)-2,5-dimethoxy-4-iodoamphetamine), bufotenin, and LSD to the 5-$HT_{2C}$ receptor leads to a stronger activation of the $PLA_2$-AA than PLC-IP pathway (Fig. 4a). Other drugs (e.g. TFMPP and quipazine), however, preferentially activate the PLC-IP pathway (Fig. 4b) [42]. This effect is called "agonist-directed trafficking of receptor stimulus" (ADTRS) [43] meaning that the receptor response is, at least in part, dependent on the type of ligand. Berg et al. made the interesting observation that RNA editing of the 5-$HT_{2C}$ receptor alters the relation, in which ligands favor activation of the PLC-IP or the $PLA_2$-AA pathway [44]. For example, bufotenin and TFMPP applied to the fully edited receptor isoform 5-$HT_{2C\text{-}VGV}$ activate the two different pathways to the same extent (Fig. 4c), suggesting that the ADTRS is abolished by the full editing of the receptor (see asterisks, Fig. 4c) [44].

On the other hand, RNA editing of a single amino acid is sufficient to introduce ADTRS. RNA editing at amino acid position 156, for example, changes the 5-$HT_{2C}$ receptor response to two strong agonists. While WAY 161503 and Ro 60-0175 are equally effective to activate both pathways (similar to 5-HT) (Fig. 5a), the stimulation of the 5-$HT_{2C\text{-}VNI}$ isoform with these drugs leads to a preferential activation of the PLC-IP pathway (Fig. 5b) [45]. Berg et al. assume that a reduced relative efficacy of the receptor-coupling to the $PLA_2$-AA pathway is the underlying mechanism [45].

These observations point out that differences in the editing status can control the ADTRS phenomenon. In contrast to the aforementioned changes in affinity, this influences not only the extent of drug action but also the quality of the response, as different intracellular pathways can be activated. Consequently, the effects of drugs that target the 5-$HT_{2C}$ strongly depend on editing status of the 5-$HT_{2C}$ receptors expressed in the respective cell.

**Disease-associated changes in the 5-$HT_{2C}$ editing status.** A small increase of the RNA editing ratio at the A site was described in psychiatric suicide victims diagnosed with schizophrenia or major depression, predicting an increase in the occurrence of the 5-$HT_{2C\text{-}VNI}$ isoform [36]. According to the phenomenon described above, this decreases the preference of the receptor to couple to the $PLA_2$-AA. Gurevich et al. analyzed suicide victims diagnosed with major depression and found elevated levels of E site editing and a trend towards elevated C site editing in suicide victims, while D site editing was decreased [46]. In contrast to that, schizophrenic patients show a tendency for decreased editing ratios of the 5-$HT_{2C}$ receptors, with significantly decreased B site editing [47]. These and further studies with depressive, schizophrenic, and bipolar affective patients are very well reviewed by Gardiner et al. and Iwamoto et al. [48, 49]. Summarizing, it seems that there is an overall trend to increased editing of the 5-$HT_{2C}$ receptor in suicide victims (irrespective of their diagnosis) augmenting the number of receptor subtypes with less effective responses [48, 49]. The direct association between major mental disorders (bipolar disorder, major depression, and schizophrenia) and altered RNA editing status of the 5-$HT_{2C}$ receptor is still equivocal and requires further studies.

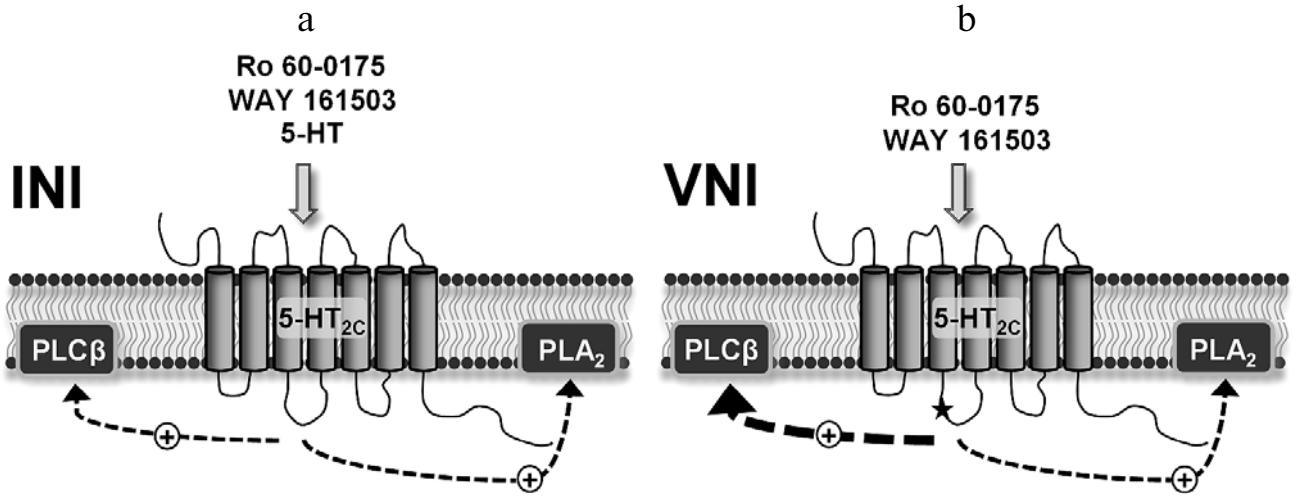


**Fig. 5.** Agonist-directed trafficking of receptor stimulus (ADTRS) can be induced by RNA editing of a single amino acid. a) In the non-edited receptor isoform 5-$HT_{2C\text{-}INI}$ the agonists 5-HT, WAY 161503, and Ro 60-0175 activate the two different signal transduction pathways with equal relative potencies, indicated by arrows of the same thickness. b) Applied to the edited isoform 5-$HT_{2C\text{-}VNI}$ the agonists WAY 161503 and Ro 60-0175 more potently activate the PLC-IP pathway.

The fact that RNA editing of the 5-$HT_{2C}$ receptor changes the basal receptor activity, the action of atypical antipsychotics, and hallucinogenic drugs like LSD, together with the observed changes of the editing status in suicide victims, highlights the relevance of 5-$HT_{2C}$ editing for the etiology and the pharmacological treatment of psychiatric disorders.

**Drugs alter the RNA editing ratio of 5-$HT_{2C}$ receptors.** Drug therapy with selective serotonin reuptake inhibitors (SSRIs) like fluoxetine alters the RNA editing pattern of 5-$HT_{2C}$ receptors: a decrease in E and C site editing and an increase in D site editing were observed after 28 days of fluoxetine treatment [46]. These changes in RNA editing ratios are in the opposite direction to the alterations that were observed by the same group in suicide victims (see above) [46]. The authors suggest that this reversion in RNA editing levels might be part of the therapeutic outcome of fluoxetine treatment in patients with major depression. In addition, the authors also warn that studies about 5-$HT_{2C}$ RNA editing in humans might be influenced by the precedent drug treatment of the patients [46]. As not all drugs have been studied for their effects on 5-$HT_{2C}$ editing, the relevance of the pharmacological treatment for the outcome of disease related studies of RNA editing levels is not always predictable.

It is clear however that the interaction between 5-$HT_{2C}$ receptor editing and drugs works in both directions. Meaning, the intake of pharmacological substances can significantly alter the editing pattern of the receptor, and conversely the editing pattern regulates the ligand affinity and the type of receptor response.

## A SINGLE EDITING SITE IN THE Kv1.1 CHANNEL MODULATES PORE BLOCK BY DRUGS AND LIPIDS

**Structure and function of Kv channels.** Kv channels are essential for the regulation of the resting membrane potential and the shape and duration of action potentials. The large number of voltage gated potassium channels are categorized into 12 families (Kv1-Kv12) [50]. The functional channels are composed of four α-subunits, and only α-subunits of the same Kv channel family assemble to form functional channels [50]. The different combinations of α-subunits lead to a large diversity of possible Kv channel tetramers. In addition, Kvβ-subunits can associate with Kv1 channels influencing their current kinetics and trafficking characteristics [51].

**RNA editing of Kv1.1 channels regulates the current kinetics.** The *Drosophila* channel *Shaker* is the ortholog of the human Kv1 channel family members. *Shaker* undergoes RNA editing at five different sites [52]. In contrast, only one of these RNA editing sites was found in human Kv1 channels: the I400V site of the Kv1.1 subunit (Fig. 6a) [52, 53]. The same editing site was also found in Kv1.1 channels of other species like mouse and rat, indicating a phylogenetic conservation [54]. The edited amino acid I400 is located in the channels pore-forming S6 segment (Fig. 6a), a region critical for the interaction with inactivation particles and open channel blockers [55].

Several functional consequences of this RNA editing have been described so far. As mentioned above,

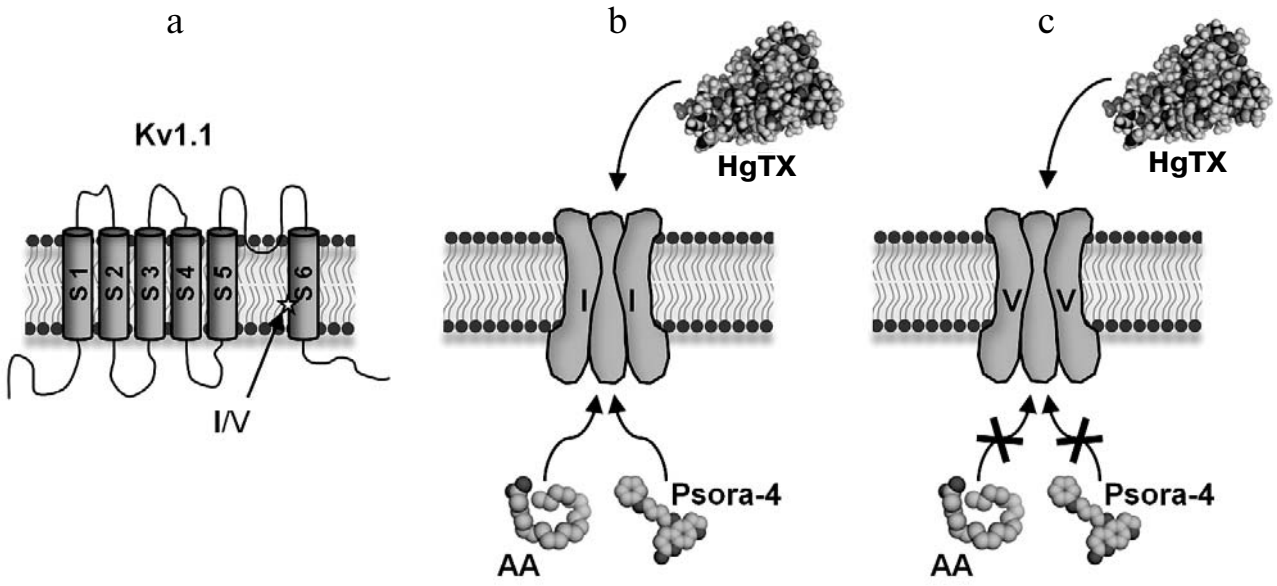


**Fig. 6.** The I400V RNA editing of human Kv1.1 channels alters their sensitivity to different blockers. a) RNA editing of the human Kv1.1 channel results in an isoleucine (I) to valine (V) exchange (I400V) in the pore-forming S6 segment. b) Unedited Kv1.1 channels are sensitive to block by arachidonic acid (AA), Psora-4 and Hongotoxin (HgTX). c) I400V edited Kv1.1 channels are insensitive to block by arachidonic acid (AA) and Psora-4 but sensitive to block by Hongotoxin (HgTX) which blocks from the extracellular side.

Kvβ1 subunits converse the slowly inactivating human Kv1.1 channel into a rapidly inactivating channel [51]. This effect is caused by the N-terminal inactivation particle of Kvβ1 subunits, which occludes the open channel pore of Kv1 channels. Kv1.1 channels with an I400V editing have a massively accelerated recovery from Kvβ1-induced inactivation or block. This faster off-rate (unbinding) of the inactivation domain of Kvβ1 was explained by a reduced affinity of Kvβ1 to the edited pore cavity [54].

**I400V editing of Kv1.1 reduces the potency of Kv1 channel blockers.** The pore lining residues of the S6 segment, including the four isoleucines of the I400V editing site, are important for the binding of Kv channel blockers [56-58]. In a recent publication, we have shown that the I400V editing of Kv1.1 reduces the affinity of Kv channel blockers [59]. We found that the $IC_{50}$ values of the Kv channel blockers Psora-4 [60] and 4-AP [61] were increased 70- and 68-fold, respectively, when Kv1.1 was I400V edited [59]. We found that I400V-edited Kv1.1 subunits form heteromers with other Kv1.x subunit members. If I400V-edited Kv1.1 subunits are incorporated into heteromeric Kv1.x channels, the pharmacology will be changed in a dominant-negative manner [59]. I400V subunits seem to act as a poisonous pill introducing the reduced drug-affinity into tetrameric Kv1.x channels. Thus, the pharmacology of blockers entering the inner channel pore is drastically changed by the I400V editing of Kv1.1 channels (Fig. 6, b and c). However, the block of toxins that bind to the extracellular side of the channel (outer vestibule) is unaffected by the I400V editing in the central cavity (Fig. 6, b and c) [59].

**I400V editing reduces the block of Kv1 channels by endogenous lipids.** Highly unsaturated fatty acids (HUFAs) modulate Kv1 channels [59]. HUFAs are incorporated in lipid membranes and can be released through different signal transduction pathways [62, 63]. Arachidonic acid, for example, induces a fast inactivation in delayed rectifier Kv channels [64]. We have recently shown that arachidonic acid acts as an open channel blocker of Kv1 channels and that a plugging of the pore underlies the previously observed induction of inactivation (Fig. 6b) [59]. The single amino acid exchange (I400V) in the Kv1.1 channel pore, caused by the RNA editing, leads to a massive reduction in the potency of arachidonic acid to block the channels (Fig. 6c) [59]. The reduction in lipid affinity of I400V edited Kv1.1 channels was also observed for the HUFAs docosahexaenoic acid (DHA) and eicosatetraynoic acid (ETYA) and for anandamide (AEA) [59].

**I400V RNA editing of Kv1.1 has to be considered for *in vitro* and *in vivo* studies.** The effects of I400V editing on the pharmaco-sensitivity of Kv1.1 channels have to be considered for studies of the brain, as the changed pharmacology can prompt misleading interpretations of the results. For example, 4-AP is commonly used to identify Kv1 channels in native tissue, as they are more potently blocked by 4-AP than channels of the other Kv families. High I400V editing ratios can generate 4-AP insensitive Kv1.x channels, which are subsequently interpreted as members of the other Kv families. The fact that the Kv1.1 editing status determines the proportion of Kv1 channels sensitive to block was recently proven by experiments with acute isolated neurons from different brain regions: Here, the Kv1.1 editing ratio of the neurons correlated with the resistance of their outward potassium currents to block by Psora-4 but not by Hongotoxin [59].

**Physiological and pathophysiological relevance of I400V editing of Kv1.1 channels.** Future investigation will be necessary to prove the physiological relevance of the lipid regulation of Kv1.1 channels and how this effect is modulated by RNA editing. The Kv1.1 subunit is widely expressed in the brain and its subcellular localization (soma, axon, dendrite, or synaptic terminal) depends on the brain region and type of neuron [65]. The extent of I400V editing also strongly depends on the brain region [52, 59]. The complex interplay of subcellular localization, editing status, and the sensitivity to block by lipids could be an instrument to fine-tune the electrophysiological behavior of neurons.

The first study to show a disease-related relevance of altered Kv1.1 editing was recently published by our group [66]. In chronic epileptic rats (kainic acid model) 4-AP does not trigger seizure-like events, as it does in control animals. Analyzing this epilepsy model, we found an increased I400V editing ratio for Kv1.1 channels of the entorhinal cortex. Our *in vitro* experiments point out that these increased editing ratios can evoke Kv1 channels with a reduced 4-AP sensitivity. If the altered Kv1.1 editing ratio contributes to the etiology of the epilepsy, or if it is a consequence of chronic epilepsy, remains to be elucidated.

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