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RNA editing of the $GABA_A$ receptor α3 subunit alters the functional properties of recombinant receptors

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ABSTRACT

RNA editing provides a post-transcriptional mechanism to increase structural heterogeneity of gene products. Recently, the α3 subunit of the $GABA_A$ receptors has been shown to undergo RNA editing. As a result, a highly conserved isoleucine residue in the third transmembrane domain is replaced with a methionine. To determine the effect of this structural change on receptor function, we compared the GABA sensitivity, pharmacological properties and macroscopic kinetics of recombinant receptors containing either the edited or unedited forms of the α3 subunit along with β3 and γ2L. Editing substantially altered the GABA sensitivity and deactivation rate of the receptors, with the unedited form showing a lower GABA EC_{50} and slower decay. Comparable effects were observed with a mutation at the homologous location in the α1 subunit, suggesting a common role for this site in regulation of channel gating. Except for the response to GABA, the pharmacological properties of the receptor were unaffected by editing, with similar enhancement by a variety of modulators. Since RNA editing of the α3 subunit increases through development, our findings suggest that GABAergic neurotransmission may be more effective early in development, with greater GABA sensitivity and slower decay rates conferred by the unedited α3 subunit.

The process of RNA editing is very common in the mammalian nervous system and provides a post-transcriptional mechanism for altering protein structure. Many proteins important for development and function in the nervous system are regulated through the action of RNA-specific adenosine deaminases (ADARs) (Mattick and Mehler, 2008). Several of these gene products are ion channels, including the ligand-gated glutamate receptors. The editing of the AMPA and kainate subtypes of glutamate receptors causes a genomically encoded glutamine residue to be replaced by an arginine within the second transmembrane domain (Sommer et al., 1991). This structural change results in reduced channel permeability to divalent cations (Burnashev et al., 1992). Editing of the glutamate receptor subunits is vital for normal brain development and function, as mice engineered to produce only the unedited GluR2 subunit do not survive into adulthood (Brusa et al., 1995). Recently it was reported that the α3 subunit of the $GABA_A$ receptor (GABAR) also undergoes RNA editing by ADARs (Ohlson et al., 2007; Rula et al., 2008). The result is a change in amino acid sequence within the third transmembrane domain (TM3) that replaces a highly conserved isoleucine residue with a methionine that is found only in the α3 subunit (Fig. 1). The extent of this editing is developmentally regulated, with about 50% of the sites edited in the newborn rat brain increasing to nearly 100% edited in the adult. RNA editing has been shown to occur at four sites within the homologous RDL subunit of *Drosophila* (Hoopengardner et al., 2003; Es-Salah et al., 2008), but had not previously been shown in the vertebrate GABARs.

The GABARs are ligand-gated, chloride-permeable ion channels responsible for fast inhibitory neurotransmission. The GABARs exhibit substantial structural heterogeneity through the expression of at least 16 different subunits in the mammalian brain (Whiting et al., 1999). There are six different subtypes within the α subunit family, each of which has a distinct, developmentally regulated, pattern of expression (Laurie et al., 1992a,b; Wisden et al., 1992). The α3 subunit is one of the predominant α subunits in the embryonic brain, where it is widely and highly expressed. As development progresses, the α3 subunit is largely replaced by the α1 subunit, and its expression is restricted primarily to cortical neurons in the adult (Laurie et al., 1992a,b; Wisden et al., 1992). Production of the α3 subunit can be influenced by pathological changes in the brain. An increase in α3 mRNA is observed during epileptogenesis (Brooks-Kayal et al., 1998) while a reduction is often observed following seizure onset (Poulter et al., 1999; Loup et al., 2006). Animals lacking the α3

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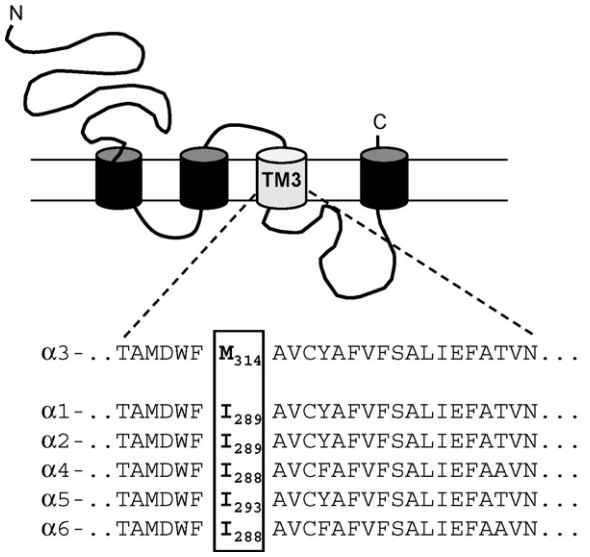

Fig. 1. Mutation site. The amino acid sequence of the 3rd transmembrane domain (TM3) for each of the α subunits is shown beneath the schematic of the subunit structure. The isoleucine/methionine residue affected by RNA editing (number 314 of the mature α3 rat peptide) is boxed and indicated in bold. Sequence alignment from Tyndale et al. (1995).

subunit exhibit abnormalities in sensorimotor processing similar to those observed in schizophrenic patients (Yee et al., 2005). Drugs selectively targeting the α3-containing receptors are under investigation for treatment of anxiety and chronic pain (Dias et al., 2005; Knabl et al., 2008).

A recent report by Rula et al. (2008) showed that editing of the α3 subunit alters some of its kinetic properties. To further examine how the change in amino acid sequence created by RNA editing alters the function of α3-containing GABARs, we compared the pharmacological and electrophysiological properties of receptors containing either the unedited (Iso) or the edited (Met) forms of the subunit. In addition, we made the same residue change within the α1 subunit to determine if this site plays a general role in controlling GABAR function. The subunits were transiently transfected into HEK-293T cells and the chloride currents in response to GABA measured through whole-cell and excised patch recordings.

1. Methods

1.1. Transfection of mammalian cells

Full-length cDNAs in pCMVNeo (Dr. Robert Macdonald, Vanderbilt University) expression vectors were transfected into the human embryonic kidney cell line HEK-293T (GenHunter, Nashville, TN). For selection of transfected cells, the plasmid pHook™-1 (Invitrogen) containing cDNA encoding the surface antibody sFv was also transfected into the cells (Chesnut et al., 1996). Cells were maintained in Dulbecco's modified Eagle medium (DMEM) plus 10% fetal bovine serum, 100 IU/ml penicillin and 100 μg/ml streptomycin. Cells were passaged by a 5 min incubation with 0.05% trypsin/0.02% EDTA solution in phosphate buffered saline (10 mM Na_2HPO_4, 150 mM NaCl, pH 7.3).

The cells were transfected using calcium phosphate precipitation. Plasmids encoding GABAR subunit cDNAs were added to the cells in 1:1:1 ratios of 2 μg each. 1 μg of a plasmid encoding a surface antibody (pHook) was also transfected as a marker for transfection. Following a 4–6 h incubation at 3% CO_2, the cells were treated with a 15% glycerol solution in BBS buffer (50 mM BES(N,N-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid), 280 mM NaCl, 1.5 mM Na_2HPO_4) for 30 s. The selection procedure for pHook expression was performed 44–52 h later. The cells were passaged and mixed with 3–5 μl of magnetic beads coated with antigen for the pHook antibody (approximately 6×10^5 beads) (Chesnut et al., 1996). Following a 30–60 min incubation to allow the beads to bind to positively transfected cells, the beads and bead-coated cells were isolated using a magnetic stand. The selected cells were resuspended into DMEM, plated onto glass coverslips treated with poly L-lysine and coated with collagen and used for recordings 18–28 h later.

1.2. Electrophysiological recording solutions and techniques

For all recordings the external solution consisted of (in mM): 142 NaCl, 8.1 KCl, 6 $MgCl_2$, 1 $CaCl_2$, and 10 HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) with pH 7.4 and osmolarity adjusted to 295–305 mOsm. Recording electrodes were filled with an internal solution of (in mM); 153 KCl, 1 $MgCl_2$, 5 K-EGTA (ethylene glycol-bis (β-aminoethyl ether N,N,N′N′-tetra-acetate), 2 MgATP and 10 HEPES with pH 7.4 and osmolarity adjusted to 295–305 mOsm. GABA was diluted into external solution from freshly made or frozen stocks in water. Drugs were diluted from freshly made stocks in DMSO and were obtained from commercial sources. Patch pipettes were pulled from borosilicate glass with an internal filament (World Precision Instruments, Sarasota, FL) on a two-stage puller (Narishige, Japan) to a resistance of 5–10 MΩ. For whole-cell recordings GABA was applied to cells using a stepper solution exchanger with a complete exchange time of <50 ms (open tip, SF-77B, Warner Instruments, Hamden, CT). For macropatch recordings the 3-barrel square glass was pulled to a final size near 200 μm. 10–90% rise times of the junction potential at the open tip were consistently faster than 400 μs and were tested using a diluted external solution. There was a continuous flow of external solution through the chamber. Currents were recorded with an Axon 200B (Foster City, CA) patch clamp amplifier.

1.3. Construction of mutated subunit cDNAs

Point mutations were generated using the QuikChange procedure and products (Stratagene, La Jolla, CA). Oligonucleotide primers were synthesized and DNA sequencing was performed by the University of South Carolina DNA core facility (Columbia, SC).

1.4. Analysis of whole-cell and macropatch currents

Whole-cell currents were analyzed using the programs Clampfit (pClamp8 suite, Axon Instruments, Foster City, CA) and Prism (Graphpad, San Diego, CA). Concentration-response data was fit with a four-parameter logistic equation. All fits were made to normalized data with current expressed as a percentage of the maximum peak response to GABA for each cell.

Macropatch currents were digitized at 10 kHz and analyzed with the pClamp8.0 suite of programs (Axon Instruments). The deactivation or desensitization rate was determined by fitting the decay current with the Levenberg–Marquardt least squares method with increasing numbers of exponential functions until additional components did not significantly improvement of the fit (*F* test of the sum of squared residuals). In all cases the decay was best fit with the sum of two components with a correlation coefficient for the fit greater than 0.90. Student's paired or unpaired *t*-tests were performed using the Instat program (Graphpad) with a significance level of $p < 0.05$. The logs of the GABA EC_{50} measurements were used for statistical comparison.