

The GABA_A Receptor: Subunit-Dependent Functions and Absence Seizures

C. Guin-Ting Wong, M.Sc.^{1,4}
O. Carter Snead III, M.D., F.R.C.P.(C)^{1,2,3,4}

¹Department of Pharmacology and ²Department of Pediatrics, University of Toronto, and Faculty of Medicine, ³Division of Neurology, ⁴Brain and Behavior Research Program, The Hospital for Sick Children, Toronto, Ontario, Canada

GABA_A receptors on thalamic relay and reticular (nRT) neurons play a critical role in thalamocortical mechanisms underlying absence seizures. Studies with absence seizure-prone rats and transgenic mice have taken advantage of differences in the subunit compositions of GABA_A receptors in the two thalamic cell populations to clarify thalamocortical rhythm generating mechanisms and explain the antiabsence activity of benzodiazepines. The relevance of this work is highlighted by the recent finding of a mutation in the GABA_A receptor $\gamma 2$ subunit in a family with childhood absence seizures.

Introduction

γ -Aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the central nervous system (CNS) where fast inhibitory neurotransmission is mediated by the GABA_A receptor (GABA_AR), a ligand-gated Cl⁻ channel. The GABA_AR is a heteromeric pentamer, the function of which is dependent on subunit composition (1,2). Currently, 20 GABA_AR subunits have been identified in mammalian tissue, including six α , four β , three γ , one δ , one ϵ , one π , one θ , and three ρ subunits (3,4). Based on the presence or absence of a short polypeptide sequence in the second intracellular loop, long and short splice variants, designated L and S, respectively, have been reported for $\gamma 2$, $\beta 2$, and $\beta 4$ subunits. Al-

ternative splicing also results in two different extreme amino-terminal ends for the $\beta 3$ subunit.

The potential combinations of at least 23 subunit forms create a bewildering array of potential subunit combinations; however, in the endoplasmic reticulum and golgi apparatus, a restricted number of subunit combinations assemble together and are properly packaged, processed, and trafficked to the cell surface (5). In mammalian CNS, the GABA_AR is believed to be composed of α , β , or β and θ , plus one or more of the γ , δ , ϵ subunits (3,4). The $\alpha 1\beta 2\beta 2$ combination accounts for approximately 43% of GABA_AR (6), although individual subunits display distinct regional neuronal and subcellular localization characteristics (7–9).

GABA_A R Subtypes in Thalamocortical Circuitry

The GABA_AR is of prime importance in the pathogenesis of absence epilepsy because of its apparent role in the synchronization and desynchronization of thalamocortical circuitry. Perturbation of this process leads to the generation of absence seizures. The oscillatory burst firing of thalamocortical circuitry is attributed to the ability of neurons located in the nucleus reticularis thalami (nRT) to impose their own oscillatory behavior on thalamocortical relay neurons located in the ventral basalis (VB) of the thalamus. The ability of the nRT to switch between oscillatory and burst firing dictates electroencephalographic (EEG) synchronization and desynchronization. The nRT consists of GABAergic neurons that project to the VB, providing inhibitory input. Also, GABAergic nRT neurons project onto one another providing intra-nRT inhibition, which decreases input to the VB. The nRT GABAergic neurons receive glutamatergic inputs from thalamocortical VB fibers and also from corticothalamic fibers projecting back from layer VI of the cerebral cortex (10–17). Within this circuitry, GABA_AR are located on glutamatergic thalamocortical and corticothalamic neurons as well as GABAergic nRT neurons.

The localization of GABA_AR subunit proteins have revealed a complementary distribution within thalamocortical circuitry. The nRT is rich in $\alpha 1$, $\alpha 3$, $\beta 1$, $\beta 3$, and $\gamma 2$ subunits and is virtually devoid of $\beta 2$ and δ subunits. In contrast, the thalamic relay neurons have high levels of $\alpha 1$, $\alpha 4$, $\beta 2$, δ subunits but show no detectable levels of the $\beta 3$ subunit (7). Electrophysiological studies have indicated that inhibitory postsynaptic currents differ in the nRT and thalamic relay neurons (18) a functional difference likely due to the distinct population of GABA_AR subunits in each region.

Address correspondence to Dr. Snead, Division of Neurology, Hospital for Sick Children, 555 University Avenue, Toronto, ON, Canada M5G 1X8; E-mail: csnead@sickkids.ca

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The action of benzodiazepines within thalamocortical circuitry illustrates further the functional differences between GABA_AR localized to the nRT versus those found on thalamic relay neurons. Systematic stimulation of GABA_AR within thalamocortical circuitry via administration of GABA_AR agonists, GABA transaminase inhibitors, or GABA reuptake inhibitors causes an exacerbation of the duration of spike-and-wave discharge (SWD) in pharmacological and genetic animal models of absence seizures (19–23). Because benzodiazepines augment GABA_AR-mediated neurotransmission, these compounds would be predicted to exacerbate absence seizures in a similar fashion; however, this class of drugs has been shown to have both experimental and clinical therapeutic efficacy in the treatment of absence seizures. The reason for this dichotomy is that benzodiazepines enhance GABA_AR-mediated inhibition within the nRT, resulting in a decreased inhibition by the nRT of thalamic relay neurons (24). This specificity of action of benzodiazepines on nRT GABA_AR may be explained by the tissue-specific subunit composition of GABA_AR within the nRT versus those within thalamic relay nuclei. A large number of thalamic relay nuclei contain GABA_AR comprised of the δ subunit; however, GABA_ARs containing the δ subunit are known to be benzodiazepine insensitive. Furthermore, many GABA_AR in thalamic nuclei contain the benzodiazepine-insensitive $\alpha 4$ subunit. Conversely, in the nRT, a large number of GABA_AR contain a benzodiazepine-sensitive $\gamma 2$ subunit in place of the δ subunit, as well as the benzodiazepine-sensitive $\alpha 3$ subunit(7).

Thalamocortical GABA_AR and Absence Seizures

Microinjection studies in the genetic absence epilepsy rats from Strasbourg (GAERS) have helped to define the role of GABA_AR in the thalamocortical circuitry in the pathogenesis of absence seizures in this genetic model. Application of γ -vinyl GABA (GVG), an irreversible GABA transaminase inhibitor, into the nRT increases intra-nRT inhibition and results in less inhibitory input onto thalamic relay neurons. The net result is an inhibition of SWD and attenuation of absence seizures. Conversely, the administration of GVG into thalamic relay nuclei activates GABA_AR on the thalamic relay neurons, resulting in increased inhibitory input and exacerbation of the absence seizures (25).

While GABA_AR agonists exacerbate absence seizures, GABA_AR antagonists, such as pentylenetetrazole, bicuculline, and picrotoxin, fail to block experimental absence seizures (21). Administration of GABA_AR antagonists induce a dose-dependent continuum of seizure types. Low doses induce absence-like seizures; intermediate doses produce clonic seizures responsive to antiabsence drugs, and high doses induce tonic seizures unresponsive to antiabsence drugs (26–29). Administration of

low dose GABA_AR antagonists directly into the nRT results in the enhanced oscillatory synchrony observed in absence seizures, whereas application of GABA_AR antagonists to the relay neurons had no effect (30). However, recently Staak and Pape reported that microinjection of bicuculline into the thalamic relay neurons exacerbated SWD in the WAG/Rij rat model of absence epilepsy (31).

The data suggest that low dose GABA_AR antagonist-induced absence seizures result from the involvement of GABA_AR within the nRT which mediate a decrease in intra-nRT inhibition. Mutagenesis studies have revealed that two key residues in the second transmembrane domain of the $\beta 3$ subunit are essential for high-affinity antagonist binding of tert-butylbicyclophosphorothionate and picrotoxin (32,33). The fact that this high-affinity antagonist binding $\beta 3$ subunit is present in the nRT and not on the thalamic relay neurons may explain the nRT-specific effect of low-dose GABA_AR antagonists in their ability to precipitate absence seizures.

Recently, a mutation in the $\gamma 2$ subunit has been linked to childhood absence of epilepsy and febrile seizures (34). *In vitro* replication of this mutation in recombinant receptors revealed an abolished sensitivity to diazepam. The $\gamma 2$ mutation would be predicted to render GABA_AR in the nRT benzodiazepine-insensitive and thus would be unable to augment intra-nRT inhibition. This study strengthens the concept that physiologically relevant endogenous benzodiazepines exist and play an important role in the prevention of absence seizures in humans.

GABA_AR Mutant Mice

Subunit-Specific Knockouts

Valuable insights regarding GABA_AR-mediated inhibition, subunit assembly, and receptor trafficking have been gained from studies in which the gene for a single subunit has been disrupted, and effectively deleted (35). Currently, knockout mice have been reported for $\alpha 1$, $\alpha 6$, $\beta 2$, $\beta 3$, δ , and $\gamma 2$ subunits. Decreased surface expression of the δ subunit in $\alpha 6$ knockouts concurs with *in vitro* data indicating preferential assembly of these two subunits (36). In addition, the decreased surface expression of all six α subunits in the $\beta 2$ knockout mice suggests that the $\beta 2$ subunit assembles with all six subunits (37).

The major limitations inherent in the gene disruption technique that constrain our ability to interpret phenotypes resulting from knockout techniques are those developmental mechanisms that emerge to compensate for the absence of the gene of interest. For example, although the δ knockout mice displays attenuated sensitivity to the modulatory effects of neurosteroid, there is an associated upregulation of those GABA_AR containing the $\gamma 2$ subunit (38). Similarly, $\alpha 6$

knockout mice have increased sensitivity to diazepam-induced motor impairment, but there is an associated increase in $\beta 3$ -containing GABA_AR, as well as an upregulation of a K⁺ channel (TASK-1) in granule cells.

Near-lethal gene disruptions also present interpretive problems. The $\beta 3$ and $\gamma 2$ knockout mice exhibit extremely high mortality rates, with only a minor percentage of pups surviving. Several of the knockout mice contain a neomycin-resistance cassette used in the gene disruption procedure. This cassette has been shown to impart inhibitory influences on neighboring genes in several nonneuronal systems (39–41). This scenario is likely to explain the widespread decrease of $\alpha 1$ and $\beta 2$ expression observed in the $\alpha 6^{-/-}$ mice because the three subunit genes are located next to each other, in a gene cluster (42). This problem can be avoided in future mutants by strategic removal of the neomycin resistance gene following the creation of the mutant mice, as has been done in the $\alpha 1^{-/-}$ and $\beta 2^{-/-}$ mice (12).

Subtype Specific Benzodiazepine Binding and Knock-In Point Mutation Mice

Benzodiazepines bind to the modulatory site on the GABA_AR in a high-affinity (nM) and low-affinity (uM) manner (43). The GABA_AR with the classic high-affinity benzodiazepine binding site contains an α , β , γ subunit combination. Recombinant receptor studies have indicated that the binding site is located between the interface of the α and γ subunits and that the α subunit is restricted to $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 5$ (44). No benzodiazepine binding is detectable in $\gamma 2^{-/-}$ mice (45).

Recently, single amino acid substitutions in GABA_AR subunits have been incorporated into germlines of mice. This method alters one pharmacological aspect of a subunit while closely preserving the native GABA_AR composition, hence preventing developmental and compensatory *in vivo* responses to a missing subunit. The benzodiazepine-sensitive $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 5$ subunits have a conserved histidine amino acid residue in the binding domain, whereas benzodiazepine-insensitive $\alpha 4$ and $\alpha 6$ subunits have an arginine at the equivalent residue. Point mutations converting the histidine to arginine rendered benzodiazepine-sensitive α subunits to be insensitive *in vitro*. When this point mutation was made in the $\alpha 1$ subunit of a mutant mouse line, benzodiazepines failed to induce sedation and anterograde amnesia and had a reduced anticonvulsant potency (46,47). However, neither diazepam-induced inhibition of REM sleep nor the effect of diazepam on EEG during sleep and wakefulness were altered in the $\alpha 1$ mutants (48). Similar mutations in the $\alpha 2$ and $\alpha 3$ subunits revealed that $\alpha 2$ - and not $\alpha 3$ -containing receptors mediated the anxiolytic response to benzodiazepines (49).

Relevance of GABA_A Mutant Mice to Absence Seizures

Studies of $\beta 3^{-/-}$ mice have provided valuable insight into the role of this subunit in thalamocortical circuitry. GABA_AR-mediated inhibition was essentially abolished within the nRT, but was unaffected in the thalamic relay neurons. This observation is in agreement with the restricted expression of $\beta 3$ containing GABA_AR, in thalamic relay nuclei where the neurons contain primarily the $\beta 2$ subunit. In addition, oscillatory synchrony was greatly intensified in the $\beta 3^{-/-}$ mice. These observations strongly suggest that recurrent inhibitory connections within the nRT act as desynchronizers in thalamocortical circuitry and that $\beta 3$ containing GABA_AR are primarily responsible for GABA_AR-mediated intra-nRT inhibition *in vivo* (30).

The $\beta 2^{-/-}$ mice are an intriguing mutant because loss of the major β subunit in the CNS results in an $\sim 60\%$ loss of GABA_AR throughout the brain; however, neither an overt phenotype nor spontaneous seizures are apparent (37). This lack of a demonstrable phenotype may be explained partially by the existence and potential functional overlap of $\beta 2$ with other β subunits in many neurons. In thalamocortical circuitry, the relay neurons of the $\beta 2^{-/-}$ mice appear to contain only low levels of the $\beta 1$ subunit.

Similarly, $\delta^{-/-}$ mice are of particular interest because of the restricted expression of δ containing GABA_AR in the thalamic relay neurons of the VB versus nRT neurons. These mutant mice are reported to have attenuated responses to neuroactive steroids (38). There are few published data available regarding the effects of chemically induced absence seizures in $\delta^{-/-}$ mice. Neuroactive steroids have been shown to potentiate low-dose pentylenetetrazole-induced absence in $\delta^{+/+}$ but not $\delta^{-/-}$ mice; however, absence seizure activity was measured in these studies by observation of hypoactivity rather than by the more precise method of EEG quantitation.

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