Pathophysiological role of extrasynaptic $GABA_A$ receptors in typical absence epilepsy

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Abstract

GABA is the principal inhibitory neurotransmitter in the mammalian CNS. It acts via two classes of receptors, the $GABA_A$, a ligand gated ion channel (ionotropic receptor) and the metabotropic G-protein coupled $GABA_B$ receptor. While synaptic $GABA_A$ receptors underlie classical 'phasic' $GABA_A$ receptor-mediated inhibition, extrasynaptic $GABA_A$ receptors ($eGABA_AR$) mediate a new form of inhibition, termed 'tonic' $GABA_A$ inhibition. The subunit composition of $eGABA_ARs$ differs from those present at the synapse, resulting in pharmacologically and functionally distinct properties. In this mini-review the findings presented at the 2nd Neuroscience Day meeting held last July in Malta will be summarised. Particular emphasis will be given to the important pathophysiological role of $eGABA_AR$ within thalamocortical circuits as a major player in nonconvulsive absence epilepsy. The new findings presented at the conference suggest that enhanced tonic inhibition is a common cause of seizures in several animal models of absence epilepsy and may provide new targets for therapeutic intervention.

Keywords

$GABA_A$ receptors, patch-clamp recording, spike and wave discharges, absence epilepsy.

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Introduction

The action of γ-aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the brain, is mediated by ligand-gated ion channels and metabotropic receptors known as $GABA_A$ and $GABA_B$ receptors, respectively.[1] $GABA_A$ receptors are pentameric assemblies of subunits that form a central ion channel which opens upon GABA binding, increasing membrane permeability to both bicarbonate and chloride ions.[1] This typically occurs during a transient rise in GABA concentration within the synaptic cleft and the activation of postsynaptic receptors, caused by the release of GABA from presynaptic terminals. This resulting brief change in membrane conductance is the underlying factor in "phasic" $GABA_A$ergic inhibition and generation of a "classical" inhibitory postsynaptic potential (IPSP). In this case, both the increase in conductance (that causes shunting of excitatory inputs) and the hyperpolarisation (that reduces depolarisations) contribute to the 'inhibitory' effect of GABA, thereby reducing the probability that an action potential will be initiated. A hyperpolarizing GABA response might not be inhibitory if it triggers hyperpolarization-activated excitatory conductances to produce rebound spikes.[2] Moreover, the response to GABA itself can be depolarizing. This is true for most immature neurons that lack K^+-Cl^- cotransporter (KCC2) and instead accumulate Cl^- via the Na^+-K^+-coupled co-transporter (NKCC1),[3] and also occurs in some mature neurons.[4]

There is a great potential for heterogeneity in GABA receptor assembly since nineteen $GABA_A$ receptor subunits have been cloned from the mammalian CNS (α(1–6), β(1–3), γ(1–3), δ, ε, θ, π, ρ(1–3)). Significantly, the postsynaptic densities of $GABA_A$-ergic synapses are highly enriched with receptors, including α(1–3), α6, β(2-3), and γ2 subunits[5] suggesting that these subunits form the $GABA_A$ receptors responsible for classical "phasic" inhibition. A different subtype of $GABA_A$ receptor has also been described, previously known as the $GABA_C$

receptor, recently renamed $GABA_{A-\rho}$ since exclusively composed of 3 ρ subunits. Stimulation of $GABA_{A-\rho}$ receptors produces a slow to initiate, but sustained in duration, chloride current and has 10 times more affinity for GABA[6].

In addition to phasic inhibition, it has recently been shown that $GABA_A$ receptor activation can occur in a much more spatially and temporally diffuse manner.[1] This been identified in different brain areas such as the cerebellum,[7] hippocampus[8] and thalamus,[9-11] where the very low concentrations of GABA (in the range of nM) found in the extracellular space, can activate persistently a population of nonsynaptic $GABA_A$ receptors, resulting in a "tonic" increase in membrane conductance. These peri or extrasynaptic $GABA_A$ receptors ($eGABA_ARs$) are excellent sensors for extracellular GABA and have indeed a significantly higher affinity for GABA compared to those receptors located at the synapses and in addition a slower rate of desensitization.[1,12-14] However, as recently shown, significant desensitization of $eGABA_ARs$ can also occur at ambient GABA concentrations in the visual thalamus.[15] The differences in the properties of synaptic $GABA_ARs$ and $eGABA_ARs$ is a result of receptor subunit composition, particularly the inclusion of the δ subunit in the dentate gyrus granule cells, cerebellar granule cells, thalamocortical (TC) and some cortical neurons[8-11,16,17] and α5 subunits in CA1 and CA3 hippocampal pyramidal cells.[18-20] The δ subunit containing $eGABA_ARs$ coassemble with 2 α (α4 or α6) and 2 β subunits. The α5 subunit containing $eGABA_AR$ usually coassemble with α, β and γ2 subunit that is typically located at the synaptic space. α1 and α2 subunits as well as β3 subunits are also found at extrasynaptic locations on the soma of hippocampus CA1 pyramidal neurons suggesting that these subunits may also contribute to $eGABA_AR$ signalling and perhaps confer specific pharmacological properties.[21]

In vitro studies have demonstrated that thalamic TC neurons of the dorsal lateral geniculate[9] and the ventrobasal (VB) nucleus[9-11] have robust $GABA_A$-ergic tonic currents in rodents, which are largely mediated by α4β2δ subunit-containing receptors. Electrophysiological evidence corroborated by high expression of the δ-subunit containing $GABA_AR$ in all thalamic nuclei.[17,22-24] Mounting evidence in recent years has made it clear that $eGABA_ARs$-mediated tonic inhibition plays a paramount role in modulating neuronal excitability. It is likely that this uninterrupted GABA conductance controls the overall gain of the neuronal input-output.[25]

It is noteworthy that $eGABA_ARs$ tonic inhibition may not remain constant in its magnitude over time but fluctuates in relation to extracellular GABA concentration.[26] Moreover, this tonic outward background current can be modulated by noradrenalin, dopamine and serotonin (unpublished observations), neurosteroids and alcohol. As far as the thalamus is concerned, tonic currents resulting from activation of $eGABA_ARs$ are responsible for 80-90% of total $GABA_A$ receptor-mediated inhibition.[9,11] Further, it has been suggested that tonic conductance in TC neurons may be larger than in other regions expressing $eGABA_ARs$ including the cerebellum and dentate gyrus.[11] $eGABA_ARs$ might play a role in switching the behavioural state-dependent TC neuron firing modes[9] and modulating the temporal precision of rebound low-threshold Ca^{2+} spikes (LTS).[23] Given the integral role of TC neurons in generating low-frequency (<4 Hz) oscillations in corticothalamic circuits,[9,11] it seems likely that $eGABA_ARs$ are significant in the modulation of slow wave sleep (SWS) activity. Furthermore, the potential importance of these receptors in typical absence epilepsy has recently been described.[27]

Typical absence epilepsy, characterised by the regular occurrence of nonconvulsive seizures that result in periods of sudden and brief loss of consciousness, appears in the electroencephalogram (EEG) as generalized synchronous, and bilateral "spike (or polyspike) and slow wave discharges (SWDs)". The SWDs occur at frequencies between 2.5-4 Hz.[28,29] Childhood epilepsy accounts for 2-10% of childhood epilepsies, with annual incidence of 2-8 per 100,000. Age of onset is typically 4-8 years (peak at 6 years) and seizure frequency may be as high as several hundred events per day.[29] These absence seizures are neither triggered by visual or other sensory stimuli nor usually associated with neurometabolic or neurophysiological deficits, a factor which is thought relevant to ~70% spontaneous remission rates in adolescence.[29,30] Absence seizures are generated in reciprocal cortico-thalamo-cortical networks.[29] The thalamus is required for the full expression of seizures in terms of both electrographic and behavioural aspects,[31] although it is not directly involved in seizure initiation.

Recent imaging investigations have challenged the notion that typical absence seizure is 'generalized'. According to the "theory of the initiation site", seizure genesis seems to occur as a result of aberrant electrical activity in discrete cortical regions, i.e. the perioral region of the somatosensory cortex and the frontal and parietal cortices in animal models and humans, respectively.[32-37]

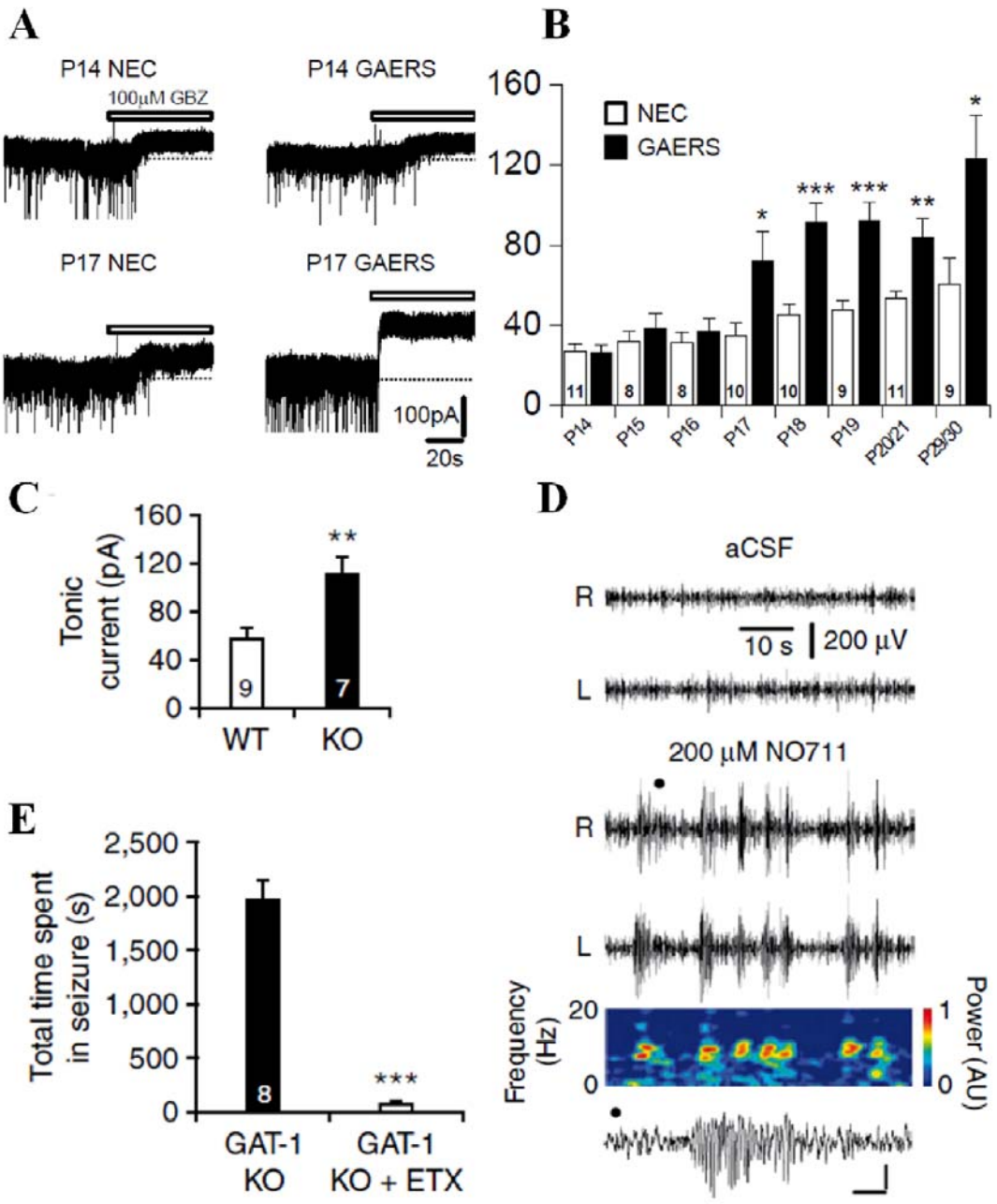

Figure 1: *Increased tonic $GABA_A$ inhibition in genetic and pharmacological models of absence seizures.*

A. Representative current traces from thalamocortical neurons of P14 (top) and P17 (bottom) NEC rats and GAERS, indicating the presence of tonic $GABA_A$ currents after the focal application of 100 μM gabazine (GBZ, white bars). Dotted lines indicate the continuation of the initial baseline current for each neuron.

B. Comparison of the tonic current amplitude in NEC rats and GAERS at the indicated ages.

C. Comparison of tonic current amplitude in wild type (WT) and GAT–1–knockout (KO) mice. Numbers of recorded neurons are as indicated.

D. Simultaneous, bilateral EEG traces from a normal Wistar rat after intrathalamic administration of artificial cerebrospinal fluid (aCSF, top traces) and then 200 μM NO711 (bottom traces). Second from bottom is a spectrogram for the lowest trace L. At the bottom is an enlargement of the single SWD, indicated BY (•). Calibration bars for the enlarged SWD; vertical 200 μV, horizontal 1 s.

E. Comparison of the effect of ethosuximide (ETX) (200 mg/kg/ip) on the total time (1 h) spent in seizure. Number of recorded mice is as indicated. Modified from[27].

Tonic eGABA$_A$R-Mediated Inhibition in Absence Epilepsy: Experimental evidence

We have recently showed that the tonic GABA$_A$ current in TC neurons of the VB thalamus results were enhanced in several different genetic (Figure 1) (i.e. the polygenic GAERS model and the monogenic models stargazer and lethargic mice) as well as pharmacological (i.e. gamma-hydroxybutyrate, GHB, and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-o, THIP) models of absence seizures.[27] In addition, eGABA$_A$ conductance is increased in TC neurons of GABA transporter-1 (GAT-1) knock-out (KO) mice, which we showed to have SWDs and behavioural arrest typical of absence epilepsy.[27] In GAERS, the tonic GABA$_A$ inhibition in VB TC neurons develops gradually after birth, reaching the maximal values at the postnatal day 29/30 doubling the tonic inhibition recorded in NEC, the nonepileptic control strain. The pathological enhancement of tonic GABA inhibition during development in GAERS may be proepileptogenic rather than simply due to the stressful effects of the seizures, since it is already present at postnatal day 17 before the seizure onset that happens around postnatal day 30 (Figure 1A,B). In addition, the monogenic lethargic and stargazer mice showed a similar significant enhancement of tonic current in TC neurons after seizure onset.[27]

The aberrant increase of tonic GABA$_A$ currents in VB TC neurons seems to be independent to changes in vesicular GABA release, expression of δ-subunit containing eGABA$_A$Rs or synaptic GABA$_A$R. Strikingly, a dysfunction of GAT-1 expressed on astrocytes in the thalamus has been revealed in all the genetic animal models of absence epilepsy tested. Therefore, a deficiency in astrocytic GABA uptake through the GAT-1 seems to be a common pathological mechanism shared by different animal models.

These results raise the possible scenario in which absence seizures are due to an impairment of glial GAT-1 in the VB of the thalamus that leads to an increase in extrasynaptic GABA concentration and, in turn, an aberrant enhancement of tonic eGABA$_A$ current. Enhanced tonic GABA$_A$ inhibition in TC neurons would generate typical absence seizures.

This hypothesis is confirmed by our recent experimental findings[27]. GAT-1 KO mice display enhanced tonic GABA$_A$ currents in TC neurons and express ethosuximide-sensitive typical absence seizures. Furthermore, infusion by reverse microdialysis of the GAT-1 blocker NO-711 in the VB of nonepileptic Wistar rats induced ethosuximide-sensitive typical absence seizures (Figure 1D).[27] In addition, systemic administration of the proepiloptegenic GHB failed to induce absence seizures in $\delta^{-/-}$ mice, which exhibit a nearly ablated tonic GABA$_A$ inhibition in TC neurons. Intrathalamic injection of a δ subunit-specific antisense oligodeoxynucleotide strongly decreased both tonic GABA$_A$ current and spontaneous seizures in GAERS.[27] Finally, local administration of THIP, a selective agonist for the δ-subunit containing eGABA$_A$R, in the VB of normal Wistar rats induced ethosuximide-sensitive absence seizures.[27]

In spite of our recent findings, there are quite a number of important unresolved issues surrounding the tonic GABA$_A$ conductance in absence epilepsy that have yet to be addressed experimentally. For example, we do not know exactly how the increase of the GABA$_A$ tonic conductance in VB cell might produce the genesis of SWDs. We can only speculate since final evidence is missing. What we know is that SWDs of typical absence epilepsy appear to be initiated in deep layers of the cortex, where rhythmic paroxysmal depolarisations occur in phase with the EEG spike.[31,39] Strong rhythmic input to thalamic nuclei is provided by the action potentials associated with these synchronous depolarisations. This strong converging corticothalamic input produces bursts of excitatory postsynaptic potentials that trigger T-type Ca^{2+}-channel-mediated LTS and bursts of action potentials in NRT neurons. Conversely, both monosynaptic excitation directly from corticothalamic inputs and disynaptic inhibition via the NRT are received by TC neurons. During ictal activity, TC neurons are generally silent[31,37,39] as result a result of a much stronger corticothalamic excitatory inputs into NRT neurons compared to TC neurons.[40] It is therefore probable that during SWDs the strong NRT GABAergic input into TC neurons activating eGABA$_A$Rs produces a corresponding increase in tonic current. Hypothetically, this could in turn contribute to the observed inhibition of TC neuron output during ictal activity.

Concluding remarks

The evidence presented at the Second Neuroscience Day at Malta University suggests that in both pharmacological and genetic models of typical absence seizures the augmented tonic GABA$_A$ inhibition in TC neurons may lead to the expression of SWDs. Another important discovery is the prominent role played by glial cells in absence epilepsy. Ambient GABA levels around TC neurons is abnormally increased due to reduced GABA uptake by astrocytic GAT-1, enhancing eGABA$_A$R function. Strikingly, this type of nonconvulsive epilepsy seems to have, therefore, a glial aetiology rather than a neuronal one. We still do not know the exact cause of GAT-1 hypofunction. Nevertheless, we could not reveal any decreased thalamic or cortical expression of GAT-1 mRNA or protein levels or the presence of any genetic variants in GAT-1 cDNA from different animal models. We can hypothesize that the GAT-1 impairment could be related to its cytoplasmatic mobilization or phosphorylation processes.

In conclusion, development of antagonist, or even better, inverse agonists selective for eGABA$_A$Rs may have potential therapeutic value in absence epilepsy.

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