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The $\alpha_2\delta$ Subunit and Absence Epilepsy: Beyond Calcium Channels?

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Abstract: Background: Spike-wave discharges, underlying absence seizures, are generated within a cortico-thalamo-cortical network that involves the somatosensory cortex, the reticular thalamic nucleus, and the ventrobasal thalamic nuclei. Activation of T-type voltage-sensitive calcium channels (VSCCs) contributes to the pathological oscillatory activity of this network, and some of the first-line drugs used in the treatment of absence epilepsy inhibit T-type calcium channels. The $\alpha_2\delta$ subunit is a component of high voltage-activated VSCCs (i.e., L-, N-, P/Q-, and R channels) and studies carried out in heterologous expression systems suggest that it may also associate with T channels. The $\alpha_2\delta$ subunit is also targeted by thrombospondins, which regulate synaptogenesis in the central nervous system.

Objective: To discuss the potential role for the thrombospondin/$\alpha_2\delta$ axis in the pathophysiology of absence epilepsy.

Methods: We searched PubMed articles for the terms “absence epilepsy”, “T-type voltage-sensitive calcium channels”, “$\alpha_2\delta$ subunit”, “ducky mice”, “pregabalin”, “gabapentin”, “thrombospondins”, and included papers focusing this Review’s scope.

Results: We moved from the evidence that mice lacking the $\alpha_2\delta$-2 subunit show absence seizures and $\alpha_2\delta$ ligands (gabapentin and pregabalin) are detrimental in the treatment of absence epilepsy. This suggests that $\alpha_2\delta$ may be protective against absence epilepsy via a mechanism that does not involve T channels. We discuss the interaction between thrombospondins and $\alpha_2\delta$ and its potential relevance in the regulation of excitatory synaptic formation in the cortico-thalamo-cortical network.

Conclusion: We speculate on the possibility that the thrombospondin/$\alpha_2\delta$ axis is critical for the correct functioning of the cortico-thalamo-cortical network, and that abnormalities in this axis may play a role in the pathophysiology of absence epilepsy.

Keywords: $\alpha_2\delta$ subunit, T-type voltage-sensitive Ca$^{2+}$ channels, non-T-type voltage-sensitive Ca$^{2+}$ channels, ducky mice, pregabalin, gabapentin, absence epilepsy, thrombospondins.

1. T-TYPE VOLTAGE-SENSITIVE CALCIUM CHANNELS: THE KEY MOLECULAR PLAYERS IN ABSENCE EPILEPSY

Absence epilepsy is the most common form of pediatric epilepsy, with an estimated annual incidence of 1-8 per 100,000 children aged 4-15 years, and accounting for 10-17% of all cases of childhood-onset epilepsy [1]. Childhood and juvenile absence epilepsy belongs to a group of epileptic syndromes of genetic origin classified as “idiopathic generalized epilepsies” (IGEs) by the International League Against Epilepsy [2]. Absence seizures, the hallmark of absence epilepsy, are characterized by transient lapses of consciousness associated with bilateral, symmetrical and generalized spike-wave discharges (SWDs) at the electroencephalogram (EEG), generated by pathological oscillations in a cortico-thalamo-cortical network with a cortical origin. This SWDs generating network includes interconnected pyramidal cells and GABAergic interneurons in the somatosensory cortex (SSCtxt), thalamocortical relay...
cells in the ventrobasal (VB) thalamic nuclei, the reticular thalamic nucleus (nRT), and their connecting pathways [3-6] (Fig. 1). Highly excitable cells in deep layers of the SSCtx send excitatory fibers to both the VB thalamic nuclei and nRT. VB neurons, in turn, send excitatory fibers to the cortical pyramidal neurons and to GABAergic nRT neurons, which are interconnected by gap junctions. nRT GABAergic neurons send their axons to VB thalamic nuclei, but not to the SSCtx. The activity of nRT neurons is regulated by GABA_A receptors targeted by the antiabsence drug, clonazepam [3, 7-10] (Fig. 1). Genetic models of absence epilepsy include tottering, leaner, roller and rocker mice carrying mutations in the gene encoding for the α1 subunit of P/Q-type voltage-sensitive calcium channels (VSCCs), stargazer mice lacking the protein stargazin, lethargic and ducky mice carrying mutations in the genes encoding the β1 and α2δ subunits of VSCCs, respectively [9], and Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats, which develop spontaneous absence seizures with age [6].

Activation of T-type VSCCs contributes to the generation of pathological oscillations underlying SWDs. These channels are activated at negative membrane potentials and show a fast voltage-dependent inactivation with respect to other VSCCs. Owing to these peculiar functional properties, T channels are involved in the generation of repetitive firing in the cortico-thalamo-cortical network [11, 12]. In the pathophysiology of SWDs, an increased excitatory drive from the SSCtx to the nRT generates pathological bursts of nRT GABAergic neurons leading to GABA_A receptor-mediated inhibitory postsynaptic potentials (IPSPs) in VB thalamic neurons. The resulting hyperpolarization enhances the activity of T-type VSCCs in VB thalamic neurons producing the pathological 3-4 Hz oscillations that are typical of absence seizures in humans and 7-11 Hz in the genetic rat models [3]. Based on pioneering in vitro studies, it is thought that the therapeutic activity of ethosuximide in absence epilepsy occurs via the inhibition of low-threshold Ca²⁺ current (I_T) in the thalamus and SSCtx [13-15].

There are three subtypes of T-type calcium channels: Ca₃.1, Ca₃.2 and Ca₃.3, containing the pore-forming α₁G, α₁H and α₁L subunits, respectively [16]. Ca₃.2 and Ca₃.3 channels are expressed in nRT neurons and at least Ca₃.3 has been implicated in the generation of nRT bursts. Ca₃.1 channels are highly expressed in thalamocortical neurons, and all types of T channels are found in cortical neurons [17-19]. Studies carried out in WAG/Rij rats support the role of T channels in the pathophysiology of absence epilepsy. Changes in the transcript and protein levels of Ca₃.1 have been found in thalamic nuclei of presymptomatic WAG/Rij rats, and I_T current density was increased in thalamic neurons of WAG/Rij rats as compared to age-matched non epileptic controls [20, 21].

2. ROLE FOR THE α₂δ SUBUNIT IN THE MODULATION OF VSCCS

VSCCs are formed by a multimolecular complex that includes the α, β, γ, and α₂δ subunits [22]. The α₁ subunit forms the ion channel and accounts for the basic properties of the channel. Based on the specific type of the α₁ subunit, VSCCs are classified into T-type (containing the α₁G, α₁H and α₁L subunits), L-type (containing the α₁C, α₁D, α₁S, and α₁F subunits), P/Q-type (containing the α₁A subunit), N-type (containing the α₁B subunit), and R-type (containing the α₁E subunit) [16]. There are four isoforms of the intracellular β subunit, and eight isoforms of the transmembrane γ subunits, which do not confer a particular specificity to the VSCCs, although the γ subunit is preferentially found in L-type channels of the skeletal muscle [23, 24]. The γ₂ subunit belongs to the group of transmembrane AMPA-receptor regulating proteins (TARPs) and is also named stargazin [25, 26]. Interestingly, stargazer mice lacking the γ₂ subunit show a severe neurological phenotype characterized by ataxia and absence epilepsy [27-30]. Stargazin levels are altered in the SSCtx of GAERS, but not in the cortex of WAG/Rij rats [31].

The transmembrane α₂δ subunit is formed by the α₂ and δ subunits covalently linked by a disulfide bridge. Interestingly, the two subunits are generated by a single gene product, which is post-translationally cleaved. The α₂ subunit is glycosylated and interacts extracellularly with the α₁ subunit. The δ subunit has a single transmembrane domain and interacts with anchoring intracellular proteins via the C-terminal region [32]. There are four isoforms of the α₂δ subunit, produced by different genes. α₂δ-1, originally described in the skeletal muscle, is ubiquitous; α₂δ-2 and α₂δ-3 are specifically found in neurons, whereas α₂δ-4 is non-neuronal.

**Fig. (1).** Cortico-thalamo-cortical network underlying absence seizures. VB = VVentrobasal thalamic nuclei; nRT = reticular thalamic nucleus. GABAergic neurons projecting from the nRT to the VB.
The αδ-1 protein is mainly present in axon terminals of excitatory neurons [33], whereas the αδ-2 protein is expressed by cerebellar Purkinje cells [34]. The αδ-3 protein is ubiquitously expressed in the forebrain [33].

The αδ subunit regulates the activity of VSCCs by increasing current amplitude and causing a shift of voltage-dependent activation towards more hyperpolarized membrane potentials [35-39]. In addition, the αδ subunit regulates cellular trafficking and membrane expression of VSCCs. Accordingly, transient expression of the αδ-1, -2, and -3 subunits in cultured hippocampal neurons targets P/Q channels in presynaptic terminals, thereby enhancing depolarization-evoked neurotransmitter release [40]. This mechanism may be particularly relevant under pathological conditions, as suggested by the evidence that the αδ-1 subunit translocates from neuronal cell bodies of dorsal root ganglia to axon terminals in the superficial layers of the dorsal horns of the spinal cord in a mouse model of neuropathic pain [41].

3. THE AMBIGUOUS ROLE FOR THE αδ SUBUNIT IN ABSENCE EPILEPSY

3.1. Studies on the Association Between the αδ Subunit with T Channels in Heterologous Expression Systems

It is unclear to date whether native T channels associate with the αδ subunit. All available studies were generated in heterologous expression systems and existing data are controversial. Overexpression of αδ in NG108 cells influences the voltage-dependence of activation of T-type calcium channels although the presence of endogenous high voltage-activated (HVA) channels in these cells is a confounding factor [42]. Using transfected monkey COS-7 cells and Xenopus oocytes co-expressing αδ and α1G subunits the same authors found that the αδ subunit enhances membrane insertion or stabilization of Ca3.1 T channels without changing the voltage-dependence or kinetics of Ca2+ currents [43], as opposed to data obtained in NG108 cells [42]. A potential role for the αδ subunit in the regulation of membrane insertion of T channels was also suggested by the use of GFP-tagged Ca3.2 channels in various expression systems [44], and by co-immunoprecipitation in osteocyte membranes [45]. Contrasting findings were obtained in transfected HEK293 cells, where the same authors reported either no or small effects of the αδ subunit on voltage-dependence or inactivation kinetics of T channel [46-48]. These findings raise the possibility that αδ may associate with, and facilitate the activation of, T channels in neurons. However, this remains to be determined taking into account that HVA channels expressed in neurons may compete with T channels for the interaction with the αδ subunit.

3.2. The Ducky Mouse: An Intriguing Example of Absence Epilepsy Associated with the Lack of αδ Subunit

The association between the Cacna2d2 gene and childhood absence epilepsy suggests a role for the αδ subunit in the pathophysiology of absence seizures [49]. Because the αδ subunit facilitates the activity of T channels (see above) it is reasonable to predict that the lack of the αδ subunit confers protection against absence seizures. In contrast, ducky mice, characterized by a spontaneous loss-of-function mutation of the Cacna2d2 gene, show generalized bilateral SWDs with a frequency of 5-7 Hz, and are considered as a genetic model for absence epilepsy [34]. Ducky mice also show severe ataxia, dyskinesias, dysgenesis of the cerebellum and other central nervous system (CNS) regions, axonal dystrophy and demyelination [50]. Another spontaneous mutation of the Cacna2d2 gene (entla) encodes a mutant form of the αδ-2 protein with an intact C-terminus. This mutation also causes an atypical form of generalized absence epilepsy in mice [51], as does a targeted knockout of Cacna2d2 [52]. The epileptic phenotype of mice lacking αδ-2 is counterintuitive if one hypothesizes that the αδ subunit positively modulates T-type calcium channels in neurons. However, we cannot exclude that αδ-2 and T channels are not expressed in the same neuronal compartments and that the lack of αδ-2 in ducky mice causes absence seizures through mechanisms that do not involve T channels, e.g., via functional abnormalities of HVA VSCCs.

3.3. Lack of Therapeutic Activity of the two αδ Ligands, Gabapentin and Pregabalin, in Absence Epilepsy

Gabapentin and pregabalin have been developed for the treatment of focal epilepsy and are currently used in the treatment of neuropathic pain, migraine, anxiety disorders, and bipolar disorders [53, 54]. Gabapentin and pregabalin bind selectively to αδ-1 and αδ-2 with nanomolar affinity [55-58]. An arginine residue in position 217 of αδ-1 is part of the binding pocket, and binding of both [3H] gabapentin and [3H] pregabalin is largely reduced in the brain regions of transgenic mice with the αδ-1 R217A mutation [38, 59-62]. Interaction of gabapentin and pregabalin with αδ has two major effect on VSCCs: (i) a reduced membrane localization of αδ and α1 subunits; and (ii) a reduced calcium channel current. It is generally believed that both effects contribute to the therapeutic action of gabapentin and pregabalin in focal epilepsy, neuropathic pain, and generalized anxiety disorder [61, 63].

By interacting with the αδ subunit, gabapentin and pregabalin are expected to have therapeutic potential in absence epilepsy as a result of T channel inhibition. In contrast, clinical studies have consistently shown that the two drugs have no activity in absence epilepsy [64, 65], and gabapentin can precipitate absence and myoclonic status epilepticus [66-68]. This paradoxical aggravation, that usually results in suble or overt increased seizure activity and worsening of EEG abnormalities, is increasingly recognized as a serious and common problem [67]. Moreover, clinical and EEG pitfalls in the diagnosis of epilepsy may often result in erroneous classification and treatment choices. In animal studies, pregabalin did not affect the incidence of SWDs in GAERS [69] and gabapentin had no effect on absence-like seizures in the lethargic mouse model of human absence epilepsy [70].

4. POTENTIAL EXPLANATIONS FOR THE AMBIGUOUS ROLE OF THE αδ SUBUNIT IN ABSENCE EPILEPSY

4.1. Modulation of Non-T-type VSCCs

One possible explanation for the epileptic phenotype of ducky and entla mice is that the loss of αδ subunit causes
absence seizures by restraining the activity of HVA Ca\(^{2+}\) channels (P/Q, N, L and R channels). Although T channels are one of the major players in the generation of pathological oscillations in the cortico-thalamo-cortical network, a large body of evidence suggests that HVA channels are also involved in the pathophysiology of absence seizures. Pharmacological activation of L-type HVA Ca\(^{2+}\) channels alters the firing rate of thalamocortical neurons [71], and systemic administration of L-type Ca\(^{2+}\) channel blocker, nimodipine, increases the frequency of SWDs. In contrast, a decreased incidence of SWDs was found after intracerebroventricular injection of the L-type Ca\(^{2+}\) channel opener, BAY K8644, which could also antagonise the effects of nimodipine on SWDs [72, 73]. R-type channels are also involved in the generation of oscillatory burst discharges in nRT neurons [74], and administration of the selective R-type Ca\(^{2+}\) blocker, conotoxin GVIA, reduces SWDs [73]. The N-type Ca\(^{2+}\) channel blocker, α-conotoxin MVIIA, can also reduce the number of SWDs [75]. Mutations of the \(\text{Cacn}a1a\) gene encoding for the \(\alpha_1\) subunit of P/Q-type Ca\(^{2+}\) channels are associated with absence seizures in rodents and humans [76-78]. Genetic deletion of the \(\text{Cacn}a1a\) gene in cortical layer VI neurons enhances T-type Ca\(^{2+}\) currents in thalamic relay and nRT neurons, thereby generating synchronous SWDs that are inhibited by ethosuximide [79]. It is possible that in ducky mice a reduced expression/activity of HVA VSCCs generates absence seizures either directly or as a result of a compensatory increase in the activity of T-type calcium channels.

4.2. Beyond VSCCs: The \(\alpha_2\delta\) Subunit Mediates the Action of Thrombospondins in the CNS

4.2.1. Physiology of Thrombospondins in the CNS

The \(\alpha_2\delta\) subunit is the receptor for a class of proteins, called thrombospondins (TSPs), which were first isolated from platelets stimulated with thrombin and show a widespread distribution in various organs, including the CNS. TSPs are a large oligomeric extracellular matrix (ECM) proteins, found in vertebrates and lower metazoa [80-82]. The human TSP protein family consists of five members (TSP-1, TSP-2, TSP-3, TSP-4, and TSP-5, or COMP, cartilage oligomeric matrix protein) which are subdivided into two groups [80, 83, 84] on the basis of their oligomerization state and domain structure. Subgroup A includes TSP-1 and TSP-2, which form homotrimers; subgroup B includes TSP-3, TSP-4, and TSP-5, which form homopentamers [85]. The various isoforms of TSPs are the products of different genes, and display differential non-overlapping functions [86].

TSPs primarily regulate cell-cell and cell-extracellular matrix interactions [87] acting through a number of ECM proteins and cell surface receptors and controlling cytoskeletal dynamics, cell migration, and cell attachment [88]. All these activities regulate many aspects of cell phenotype and contribute to angiogenesis and wound healing, vessel wall biology and synaptogenesis [84].

Astrocyte-secreted TSP-1, TSP-2 promote synaptogenesis in cultured hippocampal neurons [89] and retinal ganglion cells [90], and TSP-1 can also promote neuronal migration [91] and axonal growth [92]. TSP-1 and TSP-2 are highly expressed during development [89, 90, 93] and acts in specific time windows to promote synaptogenesis in the developing brain. TSP-4 is exclusively expressed by mature astrocytes, and represents the mature TSP isofrom in the CNS [88].

Eroglu et al. [94] have shown for the first time that the synaptogenic epidermal growth factor (EGF)-like repeat domains of TSPs interact with the VWF-A (von Willebrand factor) domain of \(\alpha_2\delta\)-1. This interaction mediates the synaptogenic activity of TSPs. Accordingly, overexpression of \(\alpha_2\delta\)-1 enhanced synaptogenesis in mice, and the \(\alpha_2\delta\) ligand, gabapentin, restrained the synaptogenic activity of TSPs [94].

4.2.2. Potential Role for Thrombospondins in the Pathophysiology of Absence Epilepsy

A potential link between alterations in synaptogenesis and absence epilepsy is suggested by the evidence that stargazin mutation in mice causes an impairment in synaptic formation and maturation in the cerebellum [95]. In addition, double mutant \(zzi/zzi,\ tm/tm\) rats, which spontaneously develop absence seizures after 6 weeks of age, show a low expression of the synaptic vesicle proteins, SV2A and synaptotagmin-1 [96]. We have found a selective reduction of the transcript encoding for TSP-1 in the ventrobasal thalamus of presymptomatic and symptomatic WAG/Rij rats [97], which model human absence epilepsy [98, 99].

An attractive hypothesis is that the TSP/\(\alpha_2\delta\) axis plays a key role in synaptic formation within the cortico-thalamo-cortical network that lies at the core of the pathophysiology of absence epilepsy [3] and that defects in either TSPs or \(\alpha_2\delta\) disrupt synaptic organization in the circuit resulting into absence seizures. TSPs may have a broader role in epileptogenesis that involves other types of epilepsy. Mendus et al. [100] have recently found that mice with genetic deletion of TSP-1 show an increased sensitivity to chemical kindling induced by repeated administrations of a subconvulsive dose of pentylentetrazole (PTZ). TSP-1 knockout mice developed generalized tonic-clonic seizures in response to the second administration of PTZ, whereas at least eight administrations were required to produce the same phenotype in wild-type mice [100]. Interestingly, TSP-1 knockout mice also showed a reduced expression of both \(\alpha_2\delta\)-1 and \(\alpha_2\delta\)-2 subunits in the frontal cortex [100].

In conclusion, the \(\alpha_2\delta\) subunit subserves pleiotropic functions that may be relevant for the pathophysiology of different type of epilepsy including absence epilepsy and may be targeted by therapeutic intervention. The role of \(\alpha_2\delta\) subunit in absence epilepsy cannot be reconducted to the modulation of T-type channels, and may involve other types of VSCCs. In addition, increasing evidence suggests that the TSP/\(\alpha_2\delta\) axis can be dysfunctional in absence epilepsy and other forms of epilepsy (Fig. 2). Abnormalities in either TSPs and/or \(\alpha_2\delta\) subunit may contribute to the process of epileptogenesis, which is ultimately responsible for the recurrence of epileptic seizures. If proven to be correct, this may lay the groundwork for the design of novel disease modifying drugs that are directed towards mechanisms that lie at the core of epileptogenesis.
Fig. (2). Potential mechanisms explaining why genetic deletion or pharmacological inhibition of the $\alpha_2$δ subunit facilitates the generation of absence seizures.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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