Antiseizure medication in early nervous system development. Ion channels and synaptic proteins as principal targets

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The main strategy for the treatment of epilepsy is the use of pharmacological agents known as antiseizure medication (ASM). These drugs control the seizure onset and improves the life expectancy and quality of life of patients. Several ASMs are contraindicated during pregnancy, due to a potential teratogen risk. For this reason, the pharmacological treatments of the pregnant Women with Epilepsy (WWE) need comprehensive analyses to reduce fetal risk during the first trimester of pregnancy. The mechanisms by which ASM are teratogens are still under study and scientists in the field, propose different hypotheses. One of them, which will be addressed in this review, corresponds to the potential alteration of ASM on ion channels and proteins involved in relevant signaling and cellular responses (i.e., migration, differentiation) during embryonic development. The actual information related to the action of ASM and its possible targets it is poorly understood. In this review, we will focus on describing the eventual presence of some ion channels and synaptic proteins of the neurotransmitter signaling pathways present during early neural development, which could potentially interacting as targets of ASM. This information leads to elucidate whether these drugs would have the ability to affect critical signaling during periods of neural development that in turn could explain the fetal malformations observed by the use of ASM during pregnancy.

KEYWORDS
pregnancy, teratogenicity, neural development, epilepsy, antiseizure medication (ASM)
1 Introduction

Epilepsy is a chronic pathology that affects near 50 million people globally. Its causes include genetic, structural and metabolic aspects, while in a half of reported cases have an undetermined etiology (Pan American Health Organization, 2016). According to the International League Against Epilepsy (ILAE), this disease is defined as a brain disorder characterized by at least one of three conditions. 1) epileptic syndrome diagnostic, 2) exhibit at least two non-induced seizures in a 24-h range, and 3) present at least a 60% probability of generating a new non-induced seizures during the 10 years after the first two seizures (Fisher et al., 2014).

Treatment for epilepsy tries to contain seizures through pharmacologic management, using a set of drugs called antiseizure medication (ASM). Only 70% of affected people respond effectively to ASM, mostly using monotherapy, but it has been documented that between 20%–30% of all those patients do not respond to pharmacological treatments (Pan American Health Organization, 2018; Fattorusso et al., 2021).

Food and Drug Administration (FDA) and European Medicines Agency (EMA) provide a list containing the drugs approved for use, while the choice and concentration of these ASMs vary for each patient based on factors such as type of epilepsy (syndrome), lifestyle, age, seizure frequency and others. Some of the most frequently ASM used are Phenobarbital (PB), Phenytoin (PHT), Carbamazepine (CBZ) and Valproic acid (VPA), from the first generation drugs. Lamotrigine (LTG), Topiramate (TPM), Levetiracetam (LEV) and Gabapentin (GBP) corresponding to second generation and Lacosamide (LCM), Rufinamide (RUF), Cannabinol (CBD) between others from third generation (Hakami, 2021).

The malformations rates decreases with the use of third generation ASM associating a more safe profile to the newer drugs (Tomson et al., 2019). Regarding MCM rate, ASM can be classified as low: ≤3% (OXG, GBP, LTG, LEV); intermediate: 3.1%–6% (TPM, CBZ, PHT); high 6.1%–9% (PB); very high > 9% (VPA) (Abou-Khalil, 2022). Despite this, the use of first generation ASM is still broadly used, not only against epilepsy, but is also use for migraine, mood stabilizer and even pain. In addition, the use of ASM can lead to psycho-behavioral side effects and physical dysfunction, such as irritability, sedation, nausea and others (Johnson, 2019).

Fetal malformations include heart defects, cleft palate and failures related to development of the nervous system, such as neural tube defects (NTDs), all of them classified as major congenital Malformations (MCMs) (Källén et al., 1989; Werler et al., 2011; Wallingford et al., 2013). The relationship between MCM and the use of ASM comes mainly from the three registries: NAAAPR, UK and Ireland and EURAP. Since these antecedents, the teratogenicity in children of pregnant WWE has been associated especially at the use of ASM in high doses (Pennell, 2016). VPA, have cut-offs for higher risks ranging from 500–650 mg/day. A dose-dependent effect was also identified for LTG, CBZ and PB, while the lowest risk was associated with LTG at ≤325 mg/day (Tomson et al., 2019b).

In general, the data shows that elevated MCM rates are associated with the use of high concentrations of VPA and CBZ in comparison with other ASM like LEV (Tomson et al., 2019a). For more detailed information associated with dosage, change in serum levels and bioavailability during pregnancy related with MCM refer to Hakami (2021) and Nucera et al. (2022). In relation with polytherapy, it has been usually considered that multidrug treatments correlate with greater MCMs (Veroniki et al., 2017), nevertheless, more recent studies identify that the specific ASM used is more significant than the number. Once again, the inclusion of VPA was associated with higher prevalence of MCMs (Holmes et al., 2011).

Analyses of teratogenicity in the Central Nervous System (CNS) has been evaluated using frog embryos (Xenopus laevis), showing that exposure in early stages of development, such as neurulation, interferes processes related to cell migration and proliferation generating alterations in glutamate signaling (Sequerra et al., 2018). In addition, autism spectrum disorders (ASD) and intellectual disabilities has been associated with the prenatal exposure of ASM (Bjørk et al., 2022).

Although the mechanisms of ASM to control epilepsy, through ion channels or receptors have been extensively studied, the pathways underlying the teratogenicity during intrauterine development are far for complete. Therefore, it is necessary to investigate the possible association between ASM, ion channels, synaptic proteins and teratogenicity during embryonic development.

2 Main focus

There is large evidence describing teratogenicity in pregnant WWE with ASM treatment during her first trimester of pregnancy (Kilic et al., 2014; Vossler, 2019). Focused on nervous system development, normally its begins with neurulation process in the first month of pregnancy, followed with a series of complex cellular and tissue modifications such as segmentation, migration, differentiation, axonal guidance, synaptogenesis, among others (Knuesel et al., 2014). Based on this information, we could hypothesize that the generation of some neural teratogen alterations would occur due to the disturbance of the ASM with active signaling pathways required for aforementioned biological processes.

3 Early nervous system development

Neurulation is one of the first step for the development of the nervous system in chordates (Colas & Schoenwolf, 2001; Knuesel et al., 2014). This event is preceded by neural induction
(described by Spemann & Mangold on 1924), where a layer of ectodermal cells differentiates and forms the neural plate. This flat layer stretches cephalo-caudally and divides symmetrically while the lateral edges elevate to converge medially and merging to create an internal cavity known as the neural tube. Neurulation takes place in humans during the third week of gestation but its temporary window is specific for each chordates. It is a previous event to synaptogenesis, which occurs around the 20th week of gestation in humans (Knuesel et al., 2014). There is evidence that electrical activity and neurotransmitter signaling is present during neurulation (Root et al., 2008), participating in the regulation of neural plate cell proliferation and migration necessary for the formation of the neural tube (Sequerra et al., 2018; Benavides-Rivas et al., 2020).

Failures in neurulation process leads to NTDs, being anencephaly (erroneous closure of the cranial region) and spina bifida (failure of closure in the caudal zone) (Hughes et al., 2018) the most common malformations. The etiology of NTDs is diverse, involving genetic and environmental factors (Padmanabhan, 2006). Associate to genetic causes, folate deficiency deregulates critical cell remodeling, necessary for this period, like apical constriction (Balashova et al., 2017), while and important environmental factor is the use of ASM during pregnancy (Pippenger, 2003).

Some hypotheses suggests that there is an increase in apoptosis of neural cells results from the interaction of ASM with neurotrophins, NGF and BDNF, interfering with their neuroprotective action (Huang & Reichardt, 2001; Roulett...
et al., 2010). It is also postulated that the deleterious action of excessive free radicals present in ASM-treated women during pregnancy could be the cause for birth defects (Pippenger, 2003). Other studies argue that the teratogenicity of ASM like valproic acid is related to its known inhibitory action on histone deacetylase (HDAC), which leads to indirect changes in DNA methylation and gene expression (Eyal et al., 2004; Smith et al., 2012). Here, we will discuss the possible teratogenic mechanism of ASM through their principal targets.

### 4 General characteristics of ASM

ASMs are drugs used to control epilepsy by reducing the frequency or intensity of seizures. It should be noted that these drugs do not modify disease properties, and instead they intended to stabilize its manifestations controlling epileptic seizures. In fact, currently the term use is ASM in replacement of antiepileptic like before. The principal mechanism of action of ASMs is based on controlling the over-excitability of nervous system by modulating ion channels associated with this function, like voltage-gate channels, selectively permeable to Na⁺, K⁺, and Ca²⁺ and excitatory (glutamate) and inhibitory (GABA) receptors and signaling (Figure 1). In this review, we will focus on describing those proteins that are the principal targets of ASMs to suggest possible interactions between these drugs and embryonic proteins and signaling.

### 5 Channels in early nervous system development

Ion channels allow the passage of Na⁺, K⁺, Ca²⁺ or Cl⁻-ions, modulating the action potential. These channels can be classified into three broad types depending on the stimulus they need to open or close: 1) mechanosensitive channels, 2) ligand-activated channels, and 3) voltage-dependent channels. The voltage-dependent channels are the target of several ASMs listed below.

#### 5.1 Sodium channels

Voltage-gated sodium channels (VGSCs) are composed by one α subunit, with genes encoding the proteins Nav1.1 through Nav1.9. They may also have one or two β subunits encoding the Navβ1 to Navβ4 proteins. In the adult mammalian central nervous system, four of these α subunits are present: Nav1.1, Nav1.2, Nav1.3, and Nav1.6 (Goldin, 2001; Whitaker et al., 2001).

During rat development, Nav1.1 transcripts are first detected before birth on embryonic day 18 (E18) and their levels increase towards adulthood. Nav1.2 begins to be expressed a little earlier at stage E15 with greater levels detected in the spinal cord peaking at postnatal day 7 (P7) and increasing further in other regions. Nav1.3 is robustly expressed at E12 and decreases thereafter reaching a plateau during P7-P15 (Beckh et al., 1989). Relative expression of Nav1.6 transcripts is quite low in embryonic periods in rats, but it increases early after birth (P1) with development (Schaller & Caldwell, 2000).

Studies show that mutations in the Nav1.1, Nav1.2, Nav1.3, Nav 1.6, and Navβ1 subunits correlate with epilepsy (Guo et al., 2008; Larsen et al., 2015; Wolff et al., 2017) and ASMs are aimed to restore normal ion channel activity altered by these mutant subunits.

A depolarization of plasma membrane generates an Action Potential that in turn is transmitted by axonal VGSCs to further spread the seizure activities. Because of this VGSC are the main targets of several ASM like PHT, CBZ, VPA, LMT, OXC, TPM, ESL, RUF, and LCM. Rufinamide (RF) for example, has reported to have a higher affinity for the Nav1.1 and Nav1.6 subunit proteins (Güchrist et al., 2014), and Lacasamide (LCM) exerts inhibitory effects on Nav1.3 and Nav1.7 (Sheets et al., 2008). Here, a valid question is if the VGSC signaling is active and participate in early development. Preliminary, the expression levels of VGSC in early stages of neural development (neurulation) would be weak, then, why some ASM with a sodium channel blocker (SCB) action mechanism displays a teratogen risk. Analyses shows that one possible explanation could be its unspecific action. VPA for example, induce ROS formation and apoptosis and inhibit histone deacetylase (HDAC) (Tomson et al., 2016). CBZ, is a potent enzyme inducer acting through 1A2, 2B6, 2C9, 2C19, and 3A4/5 CYP targets acting directly on endogenous metabolic pathways and also has been documented that enhances adipogenesis inhibiting Wnt/β-Catenin expression (Brodie et al., 2013; Lawthom, 2020). PHT, inhibit non-NMDA glutamate receptors with greater affinity to the Ca²⁺-impermeable AMPA receptors (Dron et al., 2021) and additionally inhibit the cardiac calcium release channels ryanodine receptor 2 (RyR2; Ashna et al., 2020). TPM is an antagonist of AMPA and Kainate receptors, increases GABA(R) responses, inhibits carbonic anhydrase isoenzymes, affects voltage-activated Ca²⁺ channels and interact with protein kinase phosphorylation sites (Bai et al., 2022). LMT inhibits postsynaptic AMPA receptors, N- and P/Q-type calcium channels on presynaptic nerve terminals and glutamate release (Dron et al., 2021). Altogether, shows that probably secondary activities of the SCB could contribute significantly to the teratogen risk.

#### 5.2 Calcium channels

Voltage-gated calcium channels (VGCCs) are composed of an α1 subunit that detects the potential change, forms the pore, and other auxiliary subunits such as α2δ (encoded by four genes: CACNA2D1-4), β (encoded by four genes: CACNB1-4), and γ (encoded by eight known genes
CACNGG1-8) (Catterall, 2000). VGCCs are classified according to the activation of its α1 subunit, channels of high conductance (type L, P/Q, N and R) and low conductance (type T). The L-type include the Cav1.1 to Cav1.4 proteins, the P/Q, N, and R types have only one member each, Cav2.1, Cav2.2, and Cav2.3, respectively, while the T-Type contains the Cav3.1 to Cav3.3 subunits.

Associated to nervous system development, Cav2.1 and Cav2.2 are already functional in St.5-6 In Xenopus laevis embryos (Motin et al., 2007; Cohen-Kutner et al., 2010), and Cav1.2, Cav2.1, Cav2.2 and Cav3.2 channels are present at St.14 in neural cell cultures. It is important to mention that expression of Cav1.2 disappear at St.18 while Cav1.3 show up only from St.22 (Lewis et al., 2014).

In patients with epilepsy, have been detected alterations in several genes encoded by Cav2.1 (Chiozoa et al., 2001; Bomben et al., 2016), Cav2.3 (Weiergraber et al., 2006), Cav3.1 (B. Singh et al., 2007), Cav3.2 (Chen et al., 2003; Ecke et al., 2014), and α2δ subunits encoded by the CACNA2D1 and CACNA2D2 genes (Edvardson et al., 2013; Hino-Fukuyo et al., 2015; Vergult et al., 2015).

Levetiracetam (LEV) and Lamotrigine (LTG) have a higher affinity for Cav2.2 (N-) channels (Wang et al., 1996; Lukyanetz et al., 2002). Topiramate (TPM) exerts part of its function on Cav2.1, Cav2.3 channels and L-type channels (Zhang et al., 2000; Kuzmiski et al., 2005), while Zonisamide (ZNS) inhibits T-type channels (Suzuki et al., 1992). ASM have also been reported as therapeutic targets of VGCC complementary subunits, for example, Pregabalin and Gabapentin bind to α2δ helper subunits encoded by the CACNA2D1 and CACNA2D2 genes (Gee et al., 1996; Hendrich et al., 2008).

One study show that use of 200 μM nifedipine a broadly VGCC blocker generates NTDs inhibiting apical constriction of neural plate cells (Suzuki et al., 2017). Other investigation report that neural tube closure signaling pathway require T-type calcium channels (TTCCs) that controls EphrinA expression and loss of TTCCs produces a failure to seal the anterior neural folds, generating NTDs (Abdul-Wajid et al., 2015). These investigations shows that Ca²⁺ is active and relevant during neurulation through VGCC and that alterations on this signaling lead NTDs, like spine bifida.

### 6 Regulation by neurotransmitters in the early development of the nervous system

#### 6.1 Excitatory glutamatergic transmission

Glutamate is the main excitatory neurotransmitter of the central nervous system. An aberrant enhancement of glutamatergic neurotransmission can result in epileptic activity. In the nervous system, glutamate receptors are divided into metabotropic (mGluR) white eight receptors (mGluR1-R8), and ionotropic (iGluR), which are subdivided into three groups: NMDA (containing the GluN1, GluN2A-2D and GluN3A-3B subunits), AMPA (GluA1-GluA4 subunits), and KAINATE (GluK1 to GluK5 subunits). Several receptors have been associated with epilepsy such us: GluA1, GluA2, GluN1, GluN2A, GluN2B, GluK2 and GluK5 (Smolders et al., 2002; Li et al., 2010; Peret et al., 2014; Egbenya et al., 2018; Zubareva et al., 2018).

Several ASMs target glutamatergic-signaling components. Perampanel (PER) is an AMPA receptor antagonist that decreases the affinity of GluA1/2 and GluA2/3 subunit combinations for glutamate (Augustin et al., 2018; Lange et al., 2019). Lamotrigine also inhibits AMPA channels in a dose-dependent manner (Lee et al., 2008) and topiramate inhibits AMPA and KAINATE receptors (Angehagen et al., 2004).

A study showed that in the neural plate stage of Xenopus laevis (St.13) there is glutamate signaling that regulates Ca²⁺ transients through the GluN1 subunit of NMDARs, which it will be a target of the VPA (Sequerra et al., 2018). In addition, the presence of GluA1 receptor transcripts was described in the same development stages, as GluA2 transcripts begin to be expressed in rats at E18 (Qi et al., 2012) and GluK1 and GluK2 transcripts are present in E17 rats (Joseph et al., 2011). As mentioned early, Ca²⁺ signaling is relevant during neurilallation even before and several glutamate-mediated Ca²⁺ receptors like NMDAR and AMPAR will be active at these stages of nervous system development.

#### 6.2 Inhibitory GABAergic regulation

The main inhibitory neurotransmitter in the brain is γ-aminobutyric acid (GABA), synthesized from glutamate by the enzymes GAD65/67. GABA receptors can be divided into 1 metabotropic [GABA(B)R] coupled to Gai protein, which are composed of the B1 and B2 subunits; and ionotropic [GABA(A) R], which allow the selective passage of Cl⁻, composed by varied heteropentameric subunits (α1-6, β1-4, γ1-3, δ, and ρ) (Bettler & Falder, 2017). A third type of GABA receptor called GABA(A)p [also known as GABA(C)R], is a sub-class of the ionotropic GABA(A)R receptor that presents the ρ subunit, and is expressed principally in the retina (Polenzani et al., 1991). In epilepsy, animal models suggest that alterations in GABA(A)R which contain α1, α5 (Friedman et al., 1994; Hernandez et al., 2019), δ (Dibbens et al., 2004; H.-J. Feng et al., 2006), γ2 (Baulac et al., 2001; Eugène et al., 2007), β1 and β3 (Homanics et al., 1997; Brooks-Kayal et al., 1998; Janve et al., 2016) subunits correlate with seizure states.

Before year 2000, studies showed that the α4, β1, γ1 subunits and the GAD65 and GAD67 enzymes were already present in mice at embryonic E17 (Ma & Barker, 1998). Kaeser and
TABLE 1 Teratogenic and pharmacological mechanism of action of anti-seizure medication.

| Anti-seizure medication, ASM | Major malformation ratea | Molecular targetb | Others molecular targetsc | Use in WWEd |
|-----------------------------|--------------------------|------------------|--------------------------|-------------|
| Phenobarbital, PB           | High                     | (+) GABA(A)R     | SCB, (-) NMDAR           | Avoid       |
| Phenytoin, PHT              | Intermediate             | SCB              | (-) AMPAR, (-) RyR2      | Avoid       |
| Valproate, VPA              | Very high                | SCB              | (+) GABA transmission, (-) HDAC, (-) TCA enzymes, (-) NMDAR | With caution |
| Carbamazepine, CBZ          | Intermediate             | SCB              | (+) GABA(A)R conductance, (-) Wnt/β-Catenin expression, adipsogeny, modulation purinergic and serotoninergic transmission | With caution |
| Oxcarbazepine, OXC          | Low                      | SCB              | (-) Voltage-activated calcium currents | With caution |
| Lamotrigine, LTG            | Low                      | SCB              | (-) N- and P/Q-type Ca2+ channels, (-) AMPAR | Recommend |
| Topiramate, TPM             | Intermediate             | SCB              | (+) GABA(AR), (-) AMPAR/KaiRs, (-) Carbonic Anhydrase, VGCC, PK phosphorylation | With caution |
| Levetiracetam, LEV          | Low                      | SV2              | (-) KaiRs                | Recommend |
| Lacosamide, LCM             | Low                      | SCB              | Carbonic anhydrase (probably) | Insufficient data |

aData are extracted from North American and European registries, Abou-Khalil BW, 2019.

bExtracted from Sills and Rogawski, 2020; Hakami, 2021; Lawthom, 2020; Tomson et al., 2016; Dron et al., 2021 and Stefani et al. (1995).

cExtracted from Nucera et al., 2022.

Abbreviations: WWE, women with epilepsy; (+), activator; (-), inhibitor; SCB, sodium channel blocker; NMDAR, N-methyl-D-aspartate receptor; AMPAR, α-aminoadipic-5-trans-aminomethyl-4-isoxazolepropionic acid receptor; RyR2, ryanodine receptor 2; HDAC, Histone deacetylases; TCA, tricarboxylic acid cycle; GABA(A)R, gamma-aminobutyric acid receptor type A; KaiR, Kainate receptor; VGCC, voltage gated calcium channel; PK, protein kinase.

7 Other mechanisms of regulation of ASMs: Synaptic vesicles

Synaptic Vesicle Protein 2 (SV2) family are proteins with vesicular localization that participate in neurotransmitter release. In vertebrates, there are three isotypes (SV2A, SV2B, and SV2C) (Bajjalieh et al., 1994; Abdellah et al., 2004; Gregory et al., 2006; Zody et al., 2006). SV2A is the most ubiquitously expressed in the brain, while SV2B has a more restricted expression pattern and SV2C is poorly expressed in the brain, because is highly present in the basal ganglia (Bajjalieh et al., 1994; Janz & Sudhof, 1999; Dardou et al., 2011; Crèvecoeur et al., 2013; Edvinsson et al., 2015; Steinberg et al., 2016). It has been seen that all these isotypes are closely related to the protein Synaptotagmin, a Ca2+ sensor belonging to the SNARE complex, in a binding site inhibited by Ca2+, in addition, the SV2A and SV2C isoforms present an additional site of interaction (Schivell et al., 2005).

SV2A knockout mice exhibit a high number of seizures and die by third week of their life, while SV2B knockout animals are viable and do not present severe phenotypic characteristics (Crowder et al., 1999; Janz et al., 1999; Venkatesan et al., 2012). In addition, SV2B levels are decreased in epileptic models and SV2A can be decreased or increased in some epileptic patients (Contreras-García et al., 2018, Contreras-García et al., 2021, Crèvecoeur et al., 2014; Feng et al., 2009; Hanaya et al., 2012; Ohno et al., 2009; Shi et al., 2015) which challenges the understanding of the role of SV2 in epilepsy.

The mechanisms by which ASMs might alter the levels or function of these proteins are still under study. The drug Levetiracetam (LEV) exerts its mechanism of action specifically on SV2A proteins (Lynch et al., 2004; Nowack et al., 2011), and a recently developed drug Brivaracetam.
malformations of the nervous system. An additional hypothesis is related with the action of ASM and secondary targets. Almost all ASM, interact with additional proteins different to the principal targets (Table 1) and the possibility to interfere with signals different to Na⁺ channels, Ca²⁺ channels, glutamate and GABA receptors and synaptic vesicles proteins (SV2) increases significantly. For example, VPA present at least four targets besides Na⁺ channel, including HDAC, ROS generation, TCA enzymes and GABAergic system. Similarly, TPM affects Na⁺ channels, GABA augmentation, AMPAR and KainRs. Then, exist a good association between drugs development and safety profile, whose older drugs (first generation) are more unsafe that new (third generation). In correlation, first ASM have more targets possible versus new drugs, restricting the alterations of multiple signaling.

Despite the development of new ASM, investigations of third generation ASM can generate nervous system malformations in vitro. LCM and its metabolites may have teratogenic effects on the developing mice embryos, reflected in embryonic lethality and malformations, as well as behavioral and histological alterations (López-escobar et al., 2020). Then, LCM generates growth retardation and major malformations increased in a dose-dependent manner and observed mostly in the supratherapeutic group (Mete et al., 2016). These preclinical data will need to be corroborated with new investigations and clinical studies, which should confirm the potential risk of using LCM and third generation ASMs.

In summary, the expression of diverse channels and receptors in early stages of development should be associate with a functional role during embryogenesis. The comprehensive knowledge of the function of these components as possible targets of ASMs will help to evaluate possible interactions during intrauterine gestation in pregnant WWE. More studies are needed to determine if these interactions occurs in vivo, in order to contribute to the understanding the teratogenic effect of old and new ASM during pregnancy. Finally, in relation with the pathology of epilepsy and seizure onset, it has been shown that the expression of several receptors and ion channels changes with epileptic seizures (Bender et al., 2003) and could be a relevant strategy and target for future analyses of ASM.

Author contributions

IP-B and PC wrote the manuscript. IP-B, JF, GM-C, GY, and PC read, contributed and comment to the manuscript and approved the final version of the manuscript.
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References

Abdullah, Z., Almadi, A., Ahmed, S., Aumable, M., Ainscough, R., Almeida, J., et al. (2004). Finishing the euchromatic sequence of the human genome. Nature 431 (7011), 931–945. doi:10.1038/nature00010

Abdal-Wajid, S., Morales-Diaz, H., Khairallah, S. M., and Smith, W. C. (2015). T-Type calcium channel regulation of neural tube closure and action against AMPA receptors and seizures. Cell Rep. 13 (4), 829–839. doi:10.1016/j.celrep.2015.09.035

Abou-Khalil, B. W. (2022). Update on antiseizure medications 2022. Contin. (Minneapolis) 28 (2), 500–555. doi:10.1221/CON.0000000000010104

Åhenghede, M., Ben-Menachem, E., Shank, R., Rönnbäck, L., and Hansson, E. (2004). Topiramate modulation of kainate-induced calcium currents is inversely related to channel phosphorylation level. J. Neurochem. 88 (2), 320–325. doi:10.1046/j.1471-4159.2004.02186.x

Ashna, A., van Helden, D. F., Dos Remedios, C., Molenaar, P., and Laver, D. R. (2020). Phenyltrim宿 dereases activity of cardiac ryanodine receptor 2; a potential mechanism for its cardioprotective action. Mol. Pharmacol. 97 (4), 250–258. doi:10.1124/mol.119.117721

Augustin, K., Williams, S., Cunningham, M., Devlin, A. M., Friedrich, M., Jayasekera, A., et al. (2018). Perampanel and decanoic acid show synergistic action against AMPA receptors and seizures. Epilepsia 59 (11), e172–e178. doi:10.1111/epi.14578

Bai, Y. F., Zeng, C., Sia, M., and Xiao, B. (2022). Molecular mechanisms of topiramate and its clinical value in epilepsy. Seizure 89, 51–66. doi:10.1016/j.seizure.2022.03.024

Bajjalieh, S., Frantz, G., Weimann, J., McConnell, S., and Scheller, R. (1994). Differential expression of synaptic vesicle protein 2 (SV2) isoforms. J. Neurosci. 14 (9), 5223–5235. doi:10.1523/JNEUROSCI.14-09-05223.1994

Balashova, O. A., Visina, O., and Borodinsky, L. N. (2017). Folate receptor 1 is differentially expressed in neuronal cell line C6 glioma cells of human and experimental epileptic hippocampus. J. Neurosci. 23 (17), 6826–6836. doi:10.1523/JNEUROSCI.23-17-06826.2003

Betterl, B., and Fakler, B. (2017). Ionotropic AMPA-type glutamate and metabotropic GABAB receptors: Determining cellular physiology by proteomes. Curr. Opin. Neurobiol. 45, 16–23. doi:10.1016/j.conb.2017.02.011

Björk, M. H., Zega, H., Leinonen, M. K., Cohen, J. M., Dreier, J. W., Furu, K., et al. (2022). Association of prenatal exposure to antiepileptic medication with risk of autism and intellectual disability. JAMA Neurol. 79, 672–681. doi:10.1001/jamanetw.2022.1269

Bomben, V. C., Aiba, I., Qian, J., Mark, M. D., Herlitz, S., and Noebels, J. L. (2016). Isolated P/Q calcium channel deletion in layer VI corticothalamic neurons generates absence epilepsy. J. Neurosci. 36 (2), 405–418. doi:10.1523/JNEUROSCI.2555-15.2016

Briner, W. (2001). The effect of GABA receptor ligands in experimental spina bifida occulta. BMC Pharmacol. 1, 2. doi:10.1186/1471-2210-1-2

Brodie, M. J., Mintzer, S., Pack, A. M., Gidal, B. E., Vecht, C. J., and Schmidt, D. (2013). Enzyme induction with antiepileptic drugs: Cause for concern? Epilepsia 54 (1), 11–27. doi:10.1111/j.1528-1167.2012.03671.x

Brooks-Kayal, A. R., Shumate, M. D., Jin, H., Richter, T., and Coulter, D. A. (1998). Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. Nat. Med. 4 (10), 1166–1172. doi:10.1038/2661

Caruncho, I., Pieri, M., Ciotti, M. T., Albo, F., and Zona, C. (2007). Modulation of AMPA receptors in cultured cortical neurons induced by the antiepileptic drug levetiracetam. Epilepsia 48, 654–662. doi:10.1111/j.1528-1167.2006.00973.x

Catterall, W. (2000). Structure and regulation of voltage-gated Ca2+ channels. Annu. Rev. Cell Dev. Biol. 16, 521–555. doi:10.1146/annurev.cellbio.16.1.521

Chen, Y., Yu, J., Pan, H., Zhang, Y., Wu, H., Xu, K., et al. (2003). Association between genetic variation of CACNA1H and childhood absence epilepsy. Ann. Neurol. 54 (2), 239–243. doi:10.1002/ana.10607

Chioza, B., Wåker, H., Nashef, L., Blower, J., McCormick, D., Sham, P., et al. (2001). Association between the alpha(1A) calcium channel gene CACNA1A and idiopathic generalized epilepsy. Neurology 56 (9), 1245–1246. doi:10.1212/WNL.56.9.1245

Cohen-Kutner, M., Nachmanni, D., and Atlas, D. (2010). CaV2.1 (P/Q channel) interaction with synaptic proteins is essential for depolarization-evoked release. Channels 4 (4), 266–277. doi:10.4161/chann.4.4.12130

Colas, J.-F., and Schoenwolf, G. C. (2001). Towards a cellular and molecular understanding of neurulation. Dev. Dyn. 221 (2), 117–145. doi:10.1002/dvdy.1144

Contreras-García, I. J., Gómez-Lira, G., Phillips-Farlin, B. V., Pichardo-Macias, I. A., García-Cruz, M. E., Chávez-Pacheco, I. J., et al. (2021). Synaptic vesicle protein 2A expression in glutamatergic terminals is associated with the response to levetiracetam treatment. Brain Sci. 11 (5), 531. doi:10.3390/brainsci11050531

Contreras-García, I. J., Pichardo-Macias, I. A., Santana-Gómez, C. E., Sánchez-Huerta, K., Ramirez-Hernández, R., Gómez-González, B., et al. (2018). Differential expression of synaptic vesicle protein 2A after status epilepticus and during epilepsy in a lithium-pilocarpine model. Epilepsy Behav. 88, 283–294. doi:10.1016/j.yebeh.2018.08.023

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glychrist, J., Dutton, S., Diaz-Bustamente, M., McPherson, A., Olivares, N., Kalia, J., et al. (2014). Na v 1.1 modulation by a novel triazole compound attenuates epileptic seizures in rodents. ACS Chem. Biol. 9 (5), 1204–1212. doi:10.1021/acschembio.140088p

Goldin, A. L. (2001). Resurgence of sodium channel research. Annu. Rev. Physiol. 63 (1), 871–894. doi:10.1146/annurev.physiol.63.1.871

Gregory, S. G., Barlow, K. F., McEly, K. E., Kaul, R., Swarbrick, D., Dunham, A., et al. (2006). The DNA sequence and biological annotation of human chromosome 1. Nature 441 (7091), 315–321. doi:10.1038/nature04772

Gao, F., Yu, N., Cai, J.-Q., Quinn, T., Zong, Z.-H., Zeng, Y.-J., et al. (2008). Voltage-gated sodium channel Nav1.1, Nav1.3 and beta1 subunit were up-regulated in the hippocampus of spontaneously epileptic rat. Brain Res. Bull. 75 (1), 179–187. doi:10.1016/j.brainresbull.2007.10.005

Hakami, T. (2021). Neuropharmacology of antiseizure drugs. Neuropharmacol. Rep. 41, 336–351. doi:10.1016/j.neurep.2019

Hanaya, R., Hosoyama, H., Sugata, S., Tokioudome, M., Hirano, H., Tokimura, H., et al. (2012). Low distribution of synaptic vesicle protein 2A and synaptotagmin-1 in the cerebral cortex and hippocampus of spontaneously epileptic rats exhibiting both tonic convulsion and absence seizure. Neuroscience 221, 12–20. doi:10.1016/j.neuroscience.2012.06.058

Hendrich, J., Van Minh, A. T., Heblich, F., Nieto-Rostro, M., Watschinger, K., Striesnig, J., et al. (2008). Pharmacological disruption of calcium channel trafficking by the alpha7delta ligand gabapentin. Proc. Natl. Acad. Sci. U. S. A. 105 (9), 3628–3633. doi:10.1073/pnas.0709831105

Hernandez, C. C., Xiangwei, W., Hu, N., Shen, D., Shen, W., Lagrange, A. H., et al. (2019). Altered inhibitory synapses in de novo GABRA5 and GABRA1 mutations associated with early onset epileptic encephalopathies. Brain 142 (7), 1938–1954. doi:10.1093/brain/awz212

Hino-Fukuyo, N., Kiikai, A., Ari-i-Chinou, N., Niishiro, T., Sato, R., Suzuki, T., et al. (2015). Genomic analysis identifies candidate pathogenic variants in 9 of 18 patients with unexplained West syndrome. Hum. Genet. 134 (6), 649–658. doi:10.1007/s00439-015-1555-5

Holmes, L. B., Mittenhof, R., Shen, A., Smith, C. R., and Hernandez-Diaz, S. (2011). Fetal effects of anticonvulsant polytherapies: Different risks from different drug combinations. Arch. Neurol. 68 (10), 1275–1281. doi:10.1001/archneur.2011.133

Homanics, G. E., Delovery, T. M., Firestone, L. L., Quinlan, J. J., Handforth, A., Harrison, N. L., et al. (1997). Mice devoid of gamma-aminobutyric type A receptor beta2 subunit exhibit a reduction in GABA-coupled channel currents and impairments in platelet, and hypersensitive behavior. Proc. Natl. Acad. Sci. U. S. A. 94 (8), 4143–4148. doi:10.1073/pnas.94.8.4143

Huang, E. J., and Reichardt, L. F. (2001). Neurotrophins: Roles in neuronal development and function. Annu. Rev. Neurosci. 24 (1), 677–736. doi:10.1146/annurev.neuro.24.1.677

Hughes, A., Greene, N. D. E., Capp, A. J., and Galea, G. L. (2018). Valproic acid disrupts the biomechanics of late spinal neural tube closure in mouse embryos. Mech. Dev. 149, 20–26. doi:10.1016/j.mdev.2017.12.001

Jané, V. S., Hernandez, C. C., Verderi, K. M., Hu, N., and Macdonald, R. L. (2016). Epileptic encephalopathy de novo GABRB mutations impair gamma-aminobutyric acid type A receptor function. Ann. Neurol. 79 (3), 806–825. doi:10.1002/ana.24631

Janz, R., Goda, Y., Geppert, M., Missler, M., and Südhof, T. C. (1999). SV2A and SV2C are synaptic vesicle proteins with an inactivating and a non-inactivating function. J. Neurosci. 19 (1), 40–50. doi:10.1016/S0270-7159(98)01004-X

Johnson, E. L. (2019). Seizures and epilepsy. Med. Clin. North Am. 103 (2), 309–324. doi:10.1016/j.mcna.2018.10.002

Joseph, D. J., Williams, D. J., and MacDermott, A. B. (2011). Modulation of neurite outgrowth by activation of calcium-permeable kainate receptors expressed by rat nociceptive-like dorsal root ganglion neurons. Dev. Neurobiol. 71 (10), 818–835. doi:10.1002/dneu.20906

Kaiser, G. E., Babo, B. A., and Salia, M. S. (2011). Cloning and characterization of GABAA alpha subunits and GABAB subunits in Xenopus laevis during development. Dev. Dyn. 240 (4), 862–873. doi:10.1002/dvdy.22580

Kallén, B., Robert, E., Mastroiacovo, P., Martinez-Frias, M. L., Castilla, E. E., and Cocchi, G. (1999). Anticonvulsant drugs and malformations is there a drug specificity? Eur. J. Epidemiol. 15 (5), 31–36. doi:10.1007/s004640050401

Kilic, D., Petersen, H., Kiersgaard, M. I. S., Parner, E. T., Vestergaard, M., Sørensen, M. J., et al. (2014). Birth outcomes after prenatal exposure to antiepileptic
contribute to neural tube closure. Development 144 (7), 1307–1316. doi:10.1242/dev.141952

Suzuki, S., Kawakami, K., Nishimura, S., Watanabe, Y., Yagi, K., Scino, M., et al. (1992). Zonisamide blocks T-type calcium channel in cultured neurons of rat cerebral cortex. Epilepsy Res. 12 (1), 21–27. doi:10.1016/0920-1211(92)90087-A

Tomson, T., Battino, D., Bonizzi, E., Craig, J., Lindblout, D., Perucca, E., et al. EURAP Study Group (2019a). Declining malformation rates with changed antiepileptic drug prescribing: An observational study. Neurology 93 (9), e831–e840. doi:10.1212/WNL.0000000000008001

Tomson, T., Battino, D., Bromley, R., Kochen, S., Meador, K., Pennell, P., et al. (2019b). Management of epilepsy in pregnancy: A report from the international League against epilepsy task force on women and pregnancy. Epileptic Disord. 21 (6), 497–517. doi:10.1684/epid.2019.1105

Tomson, T., Battino, D., and Perucca, E. (2016). Valproic acid after five decades of use in epilepsy: Time to reconsider the indications of a time-honoured drug. Lancet. Neurol. 15 (2), 210–218. doi:10.1016/S1474-4422(15)00334-2

Venkatesan, K., Alix, P., Marquet, A., Douagnie, M., Niespodziany, I., Rogister, B., et al. (2012). Altered balance between excitatory and inhibitory inputs onto CA1 pyramidal neurons from SV2A-deficient but not SV2B-deficient mice. J. Neurosci. Res. 90 (12), 2317–2327. doi:10.1002/jnr.23111

Vergili, S., Dheedene, A., Meurs, A., Faes, F., Janssens, S., et al. (2015). Genomic aberrations of the CACNA2D1 gene in three patients with epilepsy and intellectual disability. Eur. J. Hum. Genet. 23 (5), 628–632. doi:10.1038/ejhg.2014.141

Veroniki, A. A., Cogo, E., Rios, P., Strauss, S. E., Finkelstein, Y., Kreley, R., et al. (2017). Comparative safety of antiepileptic drugs during pregnancy: A systematic review and network meta-analysis of congenital malformations and prenatal outcomes. BMC Med. 15 (1), 95. doi:10.1186/s12196-017-0845-1

Vossler, D. G. (2019). Comparative risk of major congenital malformations with 8 different antiepileptic drugs: A prospective cohort study of the eurap registry. Epilepsy Curr. 19 (2), 83–85. doi:10.1177/1535759719835353

Wallingford, J. B., Niswander, L. A., Shaw, G. M., and Finnell, R. H. (2013). The continuing challenge of understanding, preventing, and treating neural tube defects. Science 339 (6123), 1222002. doi:10.1126/science.1222002

Wang, S.-J., Huang, C.-C., Hsu, K.-S., Tsai, J.-J., and Gean, P.-W. (1996). Inhibition of N-type calcium currents by lamotrigine in rat amygdalar neurons. NeuroReport 7 (18), 3037–3040. doi:10.1097/00001756-199611250-00048

Weiringer, M., Henry, M., Krieger, A., Kamp, M., Radhakrishnan, K., Hescheler, J., et al. (2006). Altered seizure susceptibility in mice lacking the Cav2.3 E-type Ca2+ channel. Epilepsia 47 (5), 839–850. doi:10.1111/j.1528-1167.2006.00541.x

Werler, M. M., Ahrens, K. A., Bosco, J. L. F., Mitchell, A. A., Anderka, M. T., Gilboa, S. M., et al. (2011). Use of antiepileptic medications in pregnancy in relation to risks of birth defects. Ann. Epidemiol. 21 (11), 842–850. doi:10.1016/j.annepidem.2011.08.002

Whitaker, W. R. J., Faull, R. L. M., Waldvogel, H. J., Plumpton, C. J., Emson, P. C., and Clare, J. J. (2001). Comparative distribution of voltage-gated sodium channel proteins in human brain. Brain Res. Mol. Brain Res. 88 (1–2), 37–53. doi:10.1016/S0169-328X(00)00289-8

Wolf, M., Johanesen, K. M., Hedrich, U. B. S., Masnada, S., Rubboli, G., Gardella, E., et al. (2017). Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. Brain 140 (5), 1316–1336. doi:10.1093/brain/awx054

Wood, M. D., and Gillard, M. (2017). Evidence for a differential interaction of brivaracetam and levetiracetam with the synaptic vesicle 2A protein. Epilepsia 58 (2), 255–262. doi:10.1111/epi.13638

Yang, X., Bognar, J., He, T., Mohamed, M., Niespodziany, I., Wolf, C., et al. (2015). Brivaracetam augments short-term depression and slows vesicle recycling. Epilepsia 56 (12), 1899–1909. doi:10.1111/epi.13223

Zeller, A., Arras, M., Jurd, R., and Rudolph, U. (2007). Identification of a molecular target mediating the general anesthetic actions of pentobarbital. Mol. Pharmacol. 71 (3), 852–859. doi:10.1124/mol.106.030049

Zhang, X., Velumian, A. A., Jones, O. T., and Carlen, P. L. (2000). Modulation of high-voltage-activated calcium channels in dentate granule cells by topiramate. Epilepsia 41 (1), 52–60. doi:10.1111/j.1528-1157.2000.tb01273.x

Zody, M. C., Garber, M., Sharpe, T., Young, S. K., Rowen, L., O’Neill, K., et al. (2006). Analysis of the DNA sequence and duplication history of human chromosome 15. Nature 440 (7084), 671–675. doi:10.1038/nature04601

Zubareva, O. E., Kovalenko, A. A., Kalemenev, S. V., Schwarz, A. P., Karyakin, V. B., and Zeitsiev, A. V. (2018). Alterations in mRNA expression of glutamate receptor subunits and excitatory amino acid transporters following pilocarpine-induced seizures in rats. Neurosci. Lett. 686, 94–100. doi:10.1016/j.neulet.2018.08.047