

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/175798>

Please be advised that this information was generated on 2021-06-19 and may be subject to change.

Article 25fa pilot End User Agreement

This publication is distributed under the terms of Article 25fa of the Dutch Copyright Act (Auteurswet) with explicit consent by the author. Dutch law entitles the maker of a short scientific work funded either wholly or partially by Dutch public funds to make that work publicly available for no consideration following a reasonable period of time after the work was first published, provided that clear reference is made to the source of the first publication of the work.

This publication is distributed under The Association of Universities in the Netherlands (VSNU) 'Article 25fa implementation' pilot project. In this pilot research outputs of researchers employed by Dutch Universities that comply with the legal requirements of Article 25fa of the Dutch Copyright Act are distributed online and free of cost or other barriers in institutional repositories. Research outputs are distributed six months after their first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and/or copyrights owner(s) of this work. Any use of the publication other than authorised under this licence or copyright law is prohibited.

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please contact the Library through email: copyright@ubn.ru.nl, or send a letter to:

University Library
Radboud University
Copyright Information Point
PO Box 9100
6500 HA Nijmegen

You will be contacted as soon as possible.

Chapter 11

Is There a Future for mGlu5-Positive Allosteric Modulators in Absence Epilepsy? A Comparison with Ethosuximide

Gilles van Luijtelaar, Valerio D'Amore, Ines Santolini,
and Richard T. Ngomba

Abstract Ethosuximide is the drug of choice in the treatment of various types of absence seizures. However, there is plenty of room for other anti-absence drugs, considering that not all subjects (57–74%) become seizure-free and about 47% of ethosuximide therapy fails. New anti-absence drugs may target or modulate glutamatergic and/or GABAergic neurotransmission, the key players in the circuitry involved in the cortico-thalamo-cortical oscillations responsible for the highly stereotyped spike-wave discharges (SWDs). Cortical highly excitable cells in the focal region form the trigger for the occurrence of SWDs. In contrast, enhanced tonic inhibition is dominant in the thalamus. Biochemical studies have shown that symptomatic WAG/Rij rats differ from age-matched controls in metabotropic glutamate (mGlu) receptor expression and function: mGlu5 receptor expression and function are increased in the somatosensory cortex, and mGlu1 receptor expression is decreased in the thalamus. The two group I mGlu receptor-positive allosteric

G. van Luijtelaar, Ph.D. (✉)
Donders Centre of Cognition, Radboud University, Nijmegen, The Netherlands
e-mail: g.vanLuijtelaar@donders.ru.nl

V. D'Amore, Ph.D., Pharm.D.
Donders Centre of Cognition, Radboud University, Nijmegen, The Netherlands

I.R.C.C.S. NEUROMED, Neuropharmacology Unit, Parco Tecnologico,
Località Camerelle, 86077 Pozzilli, IS, Italy
e-mail: damorevalerio@gmail.com

I. Santolini, M.S., Pharm.D.
I.R.C.C.S. NEUROMED, Neuropharmacology Unit, Parco Tecnologico,
Località Camerelle, 86077 Pozzilli, IS, Italy
e-mail: ines.santolini@gmail.com

R.T. Ngomba, Pharm.D., M.R.Pharms., Ph.D.
I.R.C.C.S. NEUROMED, Neuropharmacology Unit, Parco Tecnologico,
Località Camerelle, 86077 Pozzilli, IS, Italy

School of Pharmacy in College of Science, University of Lincoln, Lincoln LN6 7TS, UK
e-mail: richardngomba@hotmail.com

modulators (PAMs) VU0360172 and RO0711401 have an interesting profile in acute and (sub)chronic pharmacological studies and produce a dose-dependent decrease of SWDs. Moreover, both compounds are effective in reducing SWDs in the cortex and thalamus. Interestingly, the GABA reuptake blocker tiagabine reduces SWDs in the cortex and not in the thalamus, while the efficacy of ethosuximide is higher in the cortex than in the thalamus. It is thought that VU0360172 stimulates cortex GABA interneurons, which inhibit highly excitable cortical neurons in the focal area. In the thalamus, VU0360172 most likely reduces tonic inhibition. Thus, group I mGlu receptor PAMs might be further developed as anti-absence drugs, with putative disease-modifying effects on epileptogenesis. The preclinical profile of group I mGlu receptor PAMs deserves to be further explored in models of generalized epilepsy and focal types of epilepsy.

Keywords VU0360172 • Positive allosteric modulator • Group I mGlu receptors • Absence epilepsy • Absence seizures • WAG/Rij • Genetic absence model • Antiepileptic drug development

11.1 Introduction

Childhood absence epilepsy (CAE) is the most common form of pediatric epilepsy, accounting for 10–17% of all cases. It typically begins between 4 and 8 years of age and is characterized by brief staring spells, often with the eyes fluttering and the child being unresponsive to external stimuli. Children may experience dozens to hundreds of these episodes per day. Ethosuximide (Etx), dating back to the 1950s, and valproate acid (VPA) have been for many decades and still are the drugs of first choice for absence seizures (Wallace 1986), while the relatively new antiepileptic drug lamotrigine was proposed and approved only in the mid-1990s (Frank et al. 1999; Posner et al. 2005). It was only recently that the efficacy, safety, and tolerability of the three compounds were compared in a large multicentered study. In 2010 a double-blind, randomized, comparative controlled study, including 446 patients, examined the efficacy and tolerability of Etx, VPA, and lamotrigine. At the week 16–20 visit, subjects on Etx (53%) and VPA (58%) had significantly higher freedom from failure rates than subjects on lamotrigine. Subjects on Etx also had significantly less attentional dysfunction compared to subjects on VPA (Glauser et al. 2010; Tenney and Glauser 2013). This combination of findings implied Etx as the optimal initial monotherapy for CAE. Despite these rather favorable outcomes and Etx being the “winner” at week 16–20, Etx therapy failed in 47% of subjects: 14% due to seizures and 24% due to intolerable side effects, while 13% withdrew from the study. Epidemiologic cohort studies showed only seizure freedom in the range from 21 to 74%. In five prospective cohort studies, the proportion of seizure-free subjects was 57–74%. Although labeled as a “benign” syndrome, the clinical course of CAE is variable, and remission rates are far lower than in other classic benign

idiopathic epilepsies such as benign rolandic epilepsy (Teoh and Chan 1975). Although a complete discussion of the adverse effects is beyond the scope of this review – the reader is referred to a recent review by Gören and Onat (2007) – the usage of Etx has been associated with commonly observed dose-dependent side effects related to the gastrointestinal tract, central nervous system, and hematopoiesis.

Most of the recent gained insights in the neurobiology of absence epilepsy were obtained in the genetic rodent models, in which GAERS and WAG/Rij rats were most commonly used. Both models mimic each other, and both are well described and have construct, face, and predictive validity (van Luijtelaar and Coenen 1986; Coenen and van Luijtelaar 2003; van Luijtelaar and Sitnikova 2006; Depaulis and van Luijtelaar 2006). In the last 10 years, a new dimension to the pharmacological treatment of absence epilepsy has been added by the proposal that Etx has, in WAG/Rij and in GAERS besides anti-absence, also antiepileptogenic effects when treatment started early and lasted 4 months (Blumenfeld et al. 2008; Sarkisova 2010; Russo et al. 2010; van Luijtelaar et al. 2013; Dezsi et al. 2013) and that the comorbidity of absence epilepsy with a mild form of depression-like behavior, typically for the WAG/Rij strain (Sarkisova and van Luijtelaar 2011), was prevented as a consequence of antiepileptogenesis by Etx (Sarkisova et al. 2010). Most relevant from a theoretical but also from a translational point of view is that remission has been found to be more than likely in high-compliance absence patients that were treated with Etx (Berg et al. 2014). It can be concluded that Etx is the most precious tool in the treatment of absence epilepsy and that it is certainly not without problems, including the relative high failure rate, and its putative adverse effects suggest that there must be room for new and better anti-absence drugs. Since Etx is the drug of choice, new and better anti-absence drugs must be compared with Etx.

11.2 The Assumed Working Mechanisms of ETX

It has long been thought that the action of Etx is to block I_h currents in thalamic-cortical (TC) relay cells and, consequently, the burst firing mode of these cells. The burst firing of TC cells was assumed to be the neurophysiological substrate of SWD activity in cortico-thalamo-cortical pathways. These ideas were developed in the beginning of 1990s based on *in vitro* neurophysiologic studies using voltage-clamp techniques in acutely isolated neurons of the ventrobasal complex of the thalamus from rats and guinea pigs. Therapeutically relevant concentrations of Etx induced a reduction of the low-threshold Ca^{2+} current, which was most pronounced at more hyperpolarized potentials first in the thalamic relay nuclei with no change in its kinetics or steady-state properties (Coulter et al. 1989; Macdonald and Kelly 1993; White 1999); the later was also found for neurons in the reticular thalamic nucleus (RTN) (Huguenard and Prince 1994). It was generally felt that virtually all thalamic neurons are endowed with a prominent low-threshold Ca^{2+} conductance that is of sufficient magnitude to generate a low-threshold Ca^{2+} spike (LTS) with its

accompanying burst firing mode of thalamic cells (Jahnsen and Llinás 1984a, b; Deschênes et al. 1984). Later, it was established that this conductance is crucial for the generation of normal thalamo-cortical oscillations such as sleep spindles (Steriade and Llinás 1988) and for the occurrence of delta waves, typical of deep slow sleep (Domich et al. 1986; Crunelli et al. 2014). The possibility was raised that this conductance may also play a key role in the development of pathological SWDs, and this idea was thought to be a sufficient explanation for the working mechanism of Etx in reducing SWDs in absence epilepsy patients.

However, Etx also decreases persistent Na^+ - and Ca^{2+} -activated K^+ currents in thalamic and layer V cortical pyramidal neurons (Leresche et al. 1998; Crunelli and Leresche 2002a), which may also explain the decrease in burst and increase in tonic firing. This newly discovered mechanism of Etx casts doubts on the hypothesis that only a reduction of I_T in thalamic neurons underlies the therapeutic action of this anti-absence medicine (Crunelli and Leresche 2002b). In addition, there is evidence in a genetic absence epilepsy rat model that Etx reduces cortical γ -aminobutyric acid (GABA) levels (Greenhill et al. 2012). Also, elevated glutamate levels in the primary motor cortex of rats with absence epilepsy (but not in normal animals) are reduced by Etx. In addition, whole-cell patch-clamp studies revealed an increase in spontaneous GABA release by Etx concurrent with no change in glutamate release at synapses in the rat's entorhinal cortex in vitro (Greenhill et al. 2012). This was reflected in a substantial rise in the ratio of network inhibition to excitation and a concurrent decrease in excitability of neurons embedded in this network. These authors concluded that Etx directly elevates synaptic inhibition in the cortex next to its well-established effects on ion channels and that both factors contribute to the well-known anti-absence effects. Interestingly, an increase in synaptic inhibition of cortical cells was also found after tiagabine administration by the same authors.

The notion that Etx facilitates cortical inhibition is relevant considering that there is now good evidence that generalized absence seizures in the genetic models, but also in a model in which systemic administration of low-dose PTZ induces absence-like seizures, are initiated at a cortical focus in the deep layers of the perioral region of the somatosensory cortex (Meeren et al. 2002; Polack et al. 2007). Subsequent studies revealed that Etx exerted an immediate and strong anti-absence action when injected in the somatosensory cortex but not the cortex in general (Manning et al. 2004; Polack and Charpier 2009). Moreover, in vivo studies showed that the majority (60%) of cat TC neurons are completely silent during SWDs (Steriade and Contreras 1995), and in GAERS, no LTSs were recorded in TC neurons during the majority (90%) of SWDs (Pinault et al. 1998; Slaght et al. 2002). However, hyperpolarized mediated LTSs and the burst firing mode were seen in RTN during SWDs in GAERS (Slaght et al. 2002), and indeed microinfusion of Etx into the ventral basal complex of the thalamus produced only a weak and delayed anti-absence effect, while the effects in the RTN were of a larger magnitude. In all, the outcomes of these studies question the notion that Etx exerts its therapeutic effect to a large extent on the relay nuclei of the thalamus (Manning et al. 2004) and only by blocking I_T channels.

Hypotheses on the working mechanisms of Etx can also be inferred from drug interaction studies. Moreover, drug interaction studies are clinically relevant considering that monotherapy often fails, and seizure control can be only achieved with polytherapy. In order to evaluate the combination of Etx with other anti-absence drugs, the single and combined effects of VPA acid and Etx have been studied. The incidence of SWDs was the readout variable. The median effective doses (ED_{50} values) in this model were 121 and 21.5 mg/kg for VPA and Etx, respectively (van Rijn et al. 2004). When the agents were administered together, the interaction was shown to be infra-additive: the combination was less effective in diminishing the incidence of SWDs in WAG/Rij rats than could be anticipated and based on the net effect of the drugs administered alone. This is considered as indirect evidence that the two anti-absence drugs share some common cellular or molecular mechanism, perhaps blocking Na^+ channels or increasing GABA levels in the cortex.

11.3 New Anti-absence Drugs

The search for new anti-absence drugs has been concentrated on drugs that target the circuitry in which the absence epilepsy characteristic SWDs are elicited, maintained, and aborted. For typical absence epilepsy, it is undoubtedly the reciprocally interconnected cortico-thalamo-cortical network including the RTN (van Luijtelaaar and Sitnikova 2006; Blumenfeld 2005; Lüttjohann and van Luijtelaaar 2015; Stefan and Lopes da Silva 2013); for atypical absence epilepsy, it is more likely a hippocampal, thalamo-cortical circuit (Onat et al. 2013). GABA and glutamate are the major neurotransmitter systems within the interconnected cortex and thalamus. Therefore, it seems logical to investigate the role of these neurotransmitter systems on the incidence of SWDs. In the mid-1990s, drugs affecting ionotropic glutamate receptors were in the focus of interest, and as was expected, all subclasses of antagonists reduced SWDs, while agonists enhanced SWDs in the WAG/Rij absence model (Peeters et al. 1990; Ramakers et al. 1991; Jakus et al. 2004; van Luijtelaaar and Zobeiri 2014). However, many of the ionotropic receptor antagonist compounds had toxic effects, and despite their anti-absence and anticonvulsant action, most of the ionotropic glutamate antagonists were not further developed as antiepileptic drugs.

Since targeting mGluRs might be less toxic than targeting ionotropic glutamate receptors, we decided to focus on the role of mGluRs, also considering that drugs targeting mGluR have been shown to exert pro- or antiepileptic effects in various seizure and epilepsy models (Alexander and Godwin 2006). More specifically, orthosteric antagonists of group I mGluRs reduced motor seizures induced by sound stimulation in DBA mice as well as nonconvulsive (absence) seizures in the lethargic mice (Chapman et al. 1999, 2000) – the latter is a genetic absence model with ataxia and hypoactivity – while the nonselective orthosteric agonist DHPG targeting both type 1 and 5 receptors enhanced convulsions elicited by audiogenic stimulation in DBA mice (Moldrich et al. 2003). The role of orthosteric agonists on nonconvulsive

epilepsy has not been investigated. Interestingly, besides orthosteric agonists and antagonists, also selective positive and negative allosteric modulators (PAMs and NAMs) are currently developed and available. These PAMS and NAMS might be even more subtle in inducing changes. A second reason was that mGluRs are widely distributed in the cortico-thalamo-cortical networks (Ngomba et al. 2011a), the brain circuitry in which SWDs originate, are maintained, and end (Cheong et al. 2009; Lüttjohann and van Luijtelaar 2015). These networks consist of a large number of subtypes of mGluRs, and they are selectively present in different parts of the circuitry, offering possibilities for selective targeting of disease-modified (sub)systems. Here we focus specifically on the role of group I mGluRs, with their two subtypes (1 and 5), in the pathogenesis of absence epilepsy and whether drugs affecting this system might be used for anti-absence therapy. It is known that group I mGluRs are involved in absence epilepsy because knockdown as well as whole-animal knockout of PLC β 4, a protein involved in the group I signaling pathway, induced spontaneous SWDs in mice with simultaneous behavioral arrest. In addition, the susceptibility to drug-induced SWDs was increased in these knockout mice, indicating that the deletion of thalamic PLC β 4 leads to the genesis of absence seizures (Cheong et al. 2009). Interestingly, PLC β 4 colocalizes with mGlu1 receptors and mediates mGlu1 receptor signaling in thalamic nuclei (Miyata et al. 2003; Watanabe et al. 1998). Also, the pharmacological studies of Chapman et al. (1999, 2000; Moldrich et al. 2003) on the lethargic mice absence model suggest a role of group I receptors in nonconvulsive seizures mediated in thalamo-cortical networks.

11.4 Neurochemical Studies

As mentioned above, mGluRs are widely distributed in the cortico-thalamo-cortical networks (Ngomba et al. 2011a). In the thalamus, group I mGluRs are located on relay neurons in the ventrobasal thalamus postsynaptic to the cortical inputs (Ferraguti et al. 2008; Romano et al. 1995; Liu et al. 1998). Moderate-to-low mGlu5 receptor mRNA and protein levels are expressed in RTN neurons (Romano et al. 1995; Lourenço Neto et al. 2000), whereas these neurons do not express mGlu1R mRNA (Shigemoto et al. 1992). mGlu1Rs in the cortex are located postsynaptically on GABAergic interneurons (Stinehelfer et al. 2000), and mGlu5Rs are expressed by pyramidal neurons postsynaptic to thalamo-cortical projections (Wijetunge et al. 2008), as well as by interneurons (Romano et al. 1995; Sun et al. 2009). Group I mGluRs are coupled to G $_q$ /G $_{11}$ proteins, and their activation stimulates polyphosphoinositide hydrolysis with formation of inositol-1,4,5-trisphosphate and diacylglycerol and also regulates the activity of different types of Ca $^{2+}$ and K $^{+}$ channels (Nicoletti et al. 2011).

Several studies on expression and function were performed in the WAG/Rij model in order to assess the role of mGluRs in the pathophysiology of nonconvulsive epilepsy. In the WAG/Rij rat absence model, the SWDs start to become present

in the cortical EEG from 2 to 3 months of age and onward. The analysis of expression of mGluRs was carried out by using symptomatic WAG/Rij rats (minimally 6 months of age) and presymptomatic WAG/Rij rats (not more than 2 months of age) which were compared with age-matched control non-epileptic ACI rats. Various parts of the cortico-thalamo-cortical network, such as RTN, ventrobasal thalamic nuclei, somatosensory cortex, and motor cortex, were inspected (Ngomba et al. 2011b; D'Amore et al. 2013). The functional activity of group I mGluRs was examined by using an *in vivo* method that allowed measurements of agonist-stimulated PI hydrolysis after incorporation of [3H]inositol into the phospholipids of living rats (Molinaro et al. 2009). mGlu1 or mGlu5 receptor function was evaluated in the thalamus and in the somatosensory cortex and compared to age-matched controls.

Both the expression and function of mGlu1 and mGlu5 receptors were reduced in the thalamus of symptomatic WAG/Rij rats compared to age-matched non-epileptic ACI rats. Lower levels of group I subtype protein receptors in the thalamus of the symptomatic WAG/Rij rats were confirmed by immunohistochemical analysis (Ngomba et al. 2011b). Moreover, these data showed that the reduced expression of mGlu1 α receptors found in symptomatic WAG/Rij rats was observed in the dorsal and medial nuclei, but not in the ventroposterolateral thalamus. Furthermore, no mGlu1 α receptor mRNA was observed in the RTN, and no change in mGlu1 α receptor signaling was detected in the somatosensory cortex of symptomatic WAG/Rij rats compared to age-matched control ACI rats. The level of mGlu1 α receptors in the thalamus and the developmental changes in expression in different thalamic nuclei were also investigated by Karimzadeh et al. (2016) in presymptomatic and symptomatic WAG/Rij rats and compared with age-matched non-epileptic wistar controls. These authors found that the protein level of mGlu1 α receptors in the thalamus of the symptomatic WAG/Rij rats was lower than in non-epileptic animals. In addition, the number of mGlu1 α receptors in different thalamic nuclei was lower in the 6-month-old WAG/Rij compared to age-matched wistar control rats. The gene expression of mGlu1 α receptor was also significantly lower in 6-month-old WAG/Rij rats in the lateral dorsal part of the thalamus compared to all other groups. In contrast, the expression of mGlu5Rs was increased either in the somatosensory or motor cortex of symptomatic WAG/Rij rats, as assessed by immunohistochemical and Western blot analysis (D'Amore et al. 2013). In all, these neurochemical data demonstrate that absence epilepsy in this genetic model is accompanied by significant changes in expression and function of group I mGluR in relevant parts of the SWD-generating circuit.

11.5 Acute and Subchronic Pharmacological Studies

Considering our aim to evaluate putative new treatments for absence epilepsy, the effects of selected group I and subgroup 1 PAMs on the occurrence of spontaneous absence seizures were first investigated (Ngomba et al. 2011b). Adult symptomatic rats of the WAG/Rij strain were treated systemically with RO0711401 (3, 10, or 30

mg/kg, s.c.), a selective PAM at mGlu1Rs (Vieira et al. 2009). The outcomes indicated that 3 mg/kg of RO0711401 could only abolish the early stress-related increase in the incidence of SWDs; 10 mg/kg was required for a substantial reduction in the incidence of SWDs; and 30 mg/kg could reduce the incidence as well as the mean duration of trains of SWDs. To further demonstrate a protective role for mGlu1Rs against SWDs, the same rats were systemically injected with JNJ16259685 (2.5 or 5 mg/kg, i.p.), a selective NAM of mGlu1 receptors. Treatment with JNJ16259685 increased the incidence of SWDs in a dose-dependent manner (Ngomba et al. 2011b). In fentanyl-anesthetized WAG/Rij rats, a microinjection of the selective mGlu1 α receptor agonist DHPG in the lateral dorsal thalamus reduced the mean duration of SWDs, and the selective antagonist LY367385 increased the amplitude of the spikes of the SWDs and the mean duration of SWDs in symptomatic WAG/Rij rats. These results were in line with the results obtained in the free-moving WAG/Rij rats (Karimzadeh et al. 2016).

Group I (mGluR1 and mGluR5) receptors show a different pattern of distribution in the C-T-C network, which suggests distinct rather than complementary functions of these two receptor subtypes. Therefore, our pharmacological investigation was extended to mGlu5 receptors. The pharmacological enhancement of mGluR5 activity with the PAM VU0360172 at doses of 3 and 10 mg/kg, s.c., known to be centrally active (Rodriguez et al. 2010), caused a robust and dose-dependent reduction in the incidence and mean duration of SWDs. The acute treatment with MTEP (10 or 30 mg/kg, i.p.), a selective NAM of mGlu5 receptors (Anderson et al. 2002; Cosford et al. 2003), did not change the incidence and mean duration of the SWDs (D'Amore et al. 2013).

From a therapeutic standpoint, it is important to establish whether tolerance is developed to the action of these putative anti-absence drugs. Effective doses of VU0360172 (3 mg/kg, s.c.) and RO0711401 (10 mg/kg, s.c.) were administered to rats twice daily for 10 days, and incidence of SWDs was determined across this period (D'Amore et al. 2014, 2013). As expected (Ngomba et al. 2011b), both mGlu1 and mGlu5 receptor PAMs suppressed the incidence of SWDs in the first 2 days of treatment. The anti-absence effect of the mGlu5 receptor PAM (VU0360172) persisted largely over the 10-day administration period with only a small sign of tolerance. In contrast, rats quickly developed complete tolerance to RO0711401 from the third day of treatment. Next, it was wondered whether chronic treatment with RO0711401 or VU0360172 would cause desensitization of mGlu1 and mGlu5Rs in symptomatic WAG/Rij rats and in non-epileptic age-matched wistar control rats (D'Amore et al. 2014). Chronic administration of VU0360172 increased the expression of mGlu5 receptors in the thalamus and in the cortex of WAG/Rij rats without changing the expression of mGlu1 α receptors. Treatment with RO0711401 enhanced the expression of both mGlu1 α and mGlu5 receptors in the thalamus and cortex of WAG/Rij rats. Opposite data were obtained in non-epileptic wistar rats, in which repeated injections of the two PAMs downregulated the expression of mGlu1 α and mGlu5Rs. RO0711401 changed the expression of both the mGlu1 α and the mGlu5Rs in WAG/Rij and wistar rats, whereas VU0360172 selectively changed the expression of mGlu5Rs only (D'Amore et al. 2014). These pharmacody-

dynamic and accompanying pharmacokinetic studies could not explain the large differences between VU0360172 and RO0711401 in terms of tolerance to the SWD-suppressing effects.

All these outcomes contribute to highlight the key role of group I mGluRs in the pathophysiology of absence seizures, at least in the spontaneous absence seizure model rats of the WAG/Rij strain. Moreover, they suggest that chronic administration of group I PAMS may change the expression of mGlu1a and mGlu5Rs in the cortex and thalamus, that these changes might be different for the two different PAMS, and that the genetic context (WAG/Rij vs lethargic mice) may also be crucial for the changes in receptor function after repeated administration.

11.6 Local Injection Studies

The next step was a C-T-C circuit demarcation strategy adopted by independent intracortex or intra-thalamus (ventral basal complex) microinfusions to investigate site-specific effects on the regulation of SWDs. WAG/Rij rats received bilateral microinfusion of RO0711401 or VU030172. The two drugs were equally effective in reducing the incidence of SWDs when injected into the cortex. Both drugs were also effective in reducing SWD incidence when they were infused in the thalamus, although the mGlu5 PAM VU036012 displayed somewhat greater thalamic efficacy than the mGlu1 PAM RO0711401 (D'Amore et al. 2015).

Dysfunction of glutamatergic or GABAergic neurotransmission is supposed to be a cause of the initiation and spread of seizures. Moreover, glutamate and GABA interact at many locations within the C-T-C network. Therefore, the effects of tiagabine – a GABA reuptake inhibitor at the neuronal or glial GAT-1 transporter – locally administered to the cortex and thalamus was investigated. Cortical administration of tiagabine resulted in a dose-dependent decrease in the incidence of SWDs, an effect similar to the effects of the group I PAMs. In contrast, intra-thalamic injections of tiagabine showed a dose-dependent increase in the incidence of SWDs, an effect opposite to that obtained with the PAMs.

Next, the interaction between GABA and glutamate was investigated. More precisely, it was established whether and in which direction an increased availability of (extra)synaptic GABA influences responses to VU0360172 in the thalamus and cortex. Combined microinjections of VU0360172 and tiagabine were carried out. Co-administration of VU0360172 and tiagabine reduced the incidence of SWDs when injected in the cortex, an effect similar to the effects of both compounds alone. Moreover, an indication was obtained for subadditive effects of the two drugs. In contrast, completely opposite effects were observed after co-administration to the thalamus: a decrease followed by an increase in the incidence of SWDs was seen, suggesting that the effects of VU0360172 were completely blocked in the presence of increased GABA (D'Amore et al. 2015).

In all the above mentioned pharmacological experiments with PAMs and NAMs, the behavior of the rats was quantified with the aid of a calibrated infrared movement

detector; it allowed the quantification of the amount of activity during the EEG recording session. In none of the experiments described above did VU0360172 affect the behavior of the rats.

It can be concluded that these pharmacological studies demonstrate dose-dependent SWD decreasing effects of both PAMs, minimal tolerance in a twice daily 10-day administration protocol of VU0360172, and the quick development of tolerance to RO0711401. Local injections of both PAMs demonstrated that the compounds are effective in reducing SWDs in the cortex and thalamus. Effects on behavior were not noticed. In all, VU0360172 has a good preclinical profile as an anti-absence drug in the WAG/Rij absence model.

11.7 Comparison Between Ethosuximide and VU0360172

The preclinical profile of VU0360172 as an anti-absence drug, as described here, seems promising, but this specific PAM has not been tested yet in other assays and models. Etx has a 60-year-long tradition, and much is known regarding its clinical efficacy and adverse effects. Etx has the best clinical profile among available anti-absence drugs, and new anti-absence drugs have to be compared with it. Recent reviews also point toward the limitations of Etx. These limitations clearly demonstrate the need for other treatment options. Many earlier *in vivo* and *in vitro* neurophysiologic studies on healthy animals, such as rats, cats, and ferrets, with pharmacologically induced seizures (e.g. Coulter et al. 1989, Huguenard and Prince 1994; Deschênes et al. 1984; von Krosigk et al 1993; Porcello et al. 2003) have found that Etx acts in the thalamus and by blocking Ca^{2+} channels, both in the relay nuclei and in the RTN. The comparative cortex-thalamus local injection studies of Manning et al. (2004) in the GAERS model and the outcomes of the neurophysiologic studies of the Pinault and Crunelli groups (Leresche et al. 1998; Crunelli and Leresche 2002b; Pinault et al. 1998) have yielded a somewhat different focus, regarding the location where Etx exerts its anti-absence effect, the assumed role of thalamic bursting neurons during SWDs, and the mechanisms of action. Relevant in this context is that these authors did most of their experiments *in vivo* in the well-characterized GAERS model and not on slices from healthy animals. Etx exerts its action predominantly in the cortical focal area in the somatosensory cortex, not in other cortical regions in the GAERS model. The action of Etx was also demonstrated in the thalamic ventral basal complex and RTN (Manning et al. 2004; Richards et al. 2003), although its efficacy in the thalamus was clearly less (Fig. 11.1).

The comparison of the local (cortical and thalamic) effects of both drugs is presented in Fig. 11.1. Both Etx and VU0360172 show a good SWD-suppressing effect in the cortex; however, the SWD-suppressing effects of VU0360172 in the thalamus seem to be more pronounced than those of Etx, although it is risky to compare results of different studies.

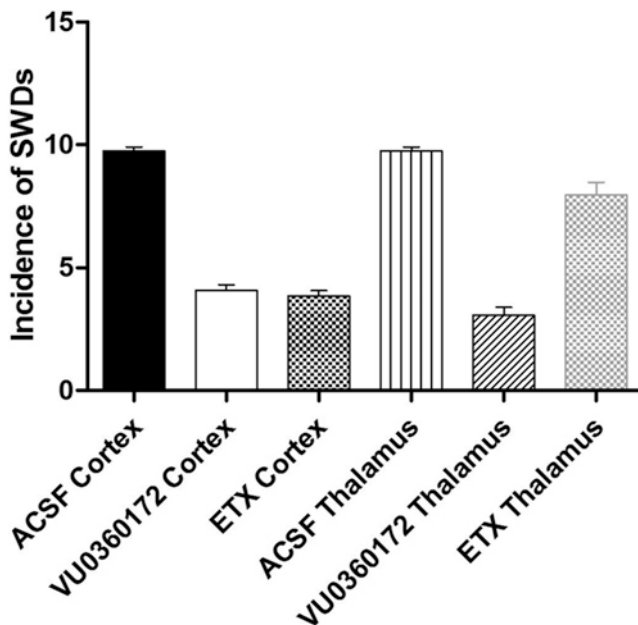


Fig. 11.1 Representative figure: Incidence of SWDs 30 min after bilateral microinfusion of artificial cerebrospinal fluid (ACSF), Ethosuximide (ETX), or VU3601726 in the thalamus and cortex (After Manning et al. 2004; D'Amore et al. 2015)

Our results show that VU360172 acts well in two target areas, while this seems less to be the case for EtX. VU360172 and RO0711401 were equally effective in the somatosensory cortex and ventral basal part of the thalamus. This strongly suggests that both PAMs may target both locations equally and effectively and that this may contribute to successful seizure suppression of both group I PAMs.

11.8 Discussion

There is plenty of evidence that group I mGluR PAMs aggravate glutamatergic neurotransmission: binding of these PAMs increases the affinity of glutamate-site agonists at its extracellular N-terminal binding site, group I PAMs potentiate synaptically evoked mGlu1 receptor responses in rat brain slices (Knoflach et al. 2001), and mGlu1R stimulation enhances excitability in layer V cortical neurons without any effect on GABAergic neurotransmission (Bandrowski et al. 2003). Therefore, at first glance, the SWD-reducing effects of the PAMs VU360172 and RO0711401 seem puzzling considering that the glutamatergic ionotropic receptor agonists aggravate SWDs in the genetic rodent models (Peeters et al. 1990; Ramakers et al.

1991; Jakus et al. 2004; van Luijtelaar and Zobeiri 2014). However, the outcomes of our local injection study might give two clues why this PAM reduces SWDs. The first regard the outcomes of cortical injections of the PAM alone, tiagabine alone, and the combination of both drugs. The PAM VU0360172 had, when injected in the focal area, the same action as tiagabine, a drug known to block GABAergic reuptake. The combined administration showed subadditive effects, suggesting that both drugs targeted the same mechanism. We proposed that VU0360172 and RO0711401, its receptors being present postsynaptically on GABAergic interneurons, stimulate the activity of GABAergic interneurons, facilitating the release of GABA and diminishing SWDs. This would fit nicely with the view that SWDs in the rodent models are due to enhanced local cortical excitation and reduced local inhibition (van Luijtelaar and Sitnikova 2006; Lüttjohann et al. 2011). Whether this mechanism of VU0360172 is only valid for the cortex of genetic epileptic rats, with their epilepsy-related changes in cortical mGlu5 receptor expression and function, as mentioned in the section on biochemistry, needs to be established. The second clue comes from the thalamic injection studies. Group I PAMs infused in the thalamus suppress SWDs. Here it is thought that they reduce the assumed increased tonic inhibition (Cope et al. 2009), a second reason for SWDs to occur in the genetic rat models. Also, our data on local infusion of tiagabine, showing a dose-dependent increase in SWDs, are in full agreement with the tonic inhibition hypothesis of Cope and collaborators.

From a drug development perspective, it is important to mention that none of the local injection studies were effects on the motor system of the rats observed, suggesting that the motor system does not seem to be largely affected by group I PAMs. This is in clear contrast to the large effects of some of the ionotropic glutamatergic agonists, such as MK801. The lack of behavioral effects in our EEG study suggests that the anti-absence effects of the PAMs are not secondary to changes in behavior or sleep–wake behavior, as far as this could be inferred from our quantitative behavioral measurements. Interestingly, VU0360172 did not affect spontaneous locomotor activity in an open field, but it did dose-dependently reduce hyperlocomotion induced by amphetamine (Rodriguez et al. 2010). Tolerance toward the anti-absence action of a twice daily 10-day drug administration regimen was minimal with VU0360172 in contrast to RO0711401. With the latter drug, tolerance was absent in the first 2 days, but on the third day (after five injections), it was complete. Neurotoxic studies with higher doses of VU0360172, such as the measurement of a motor coordination in a rotor-rod task, measures on sedation, and analgesia of higher doses of the drug, are indicated. To establish whether the compound affects cognition is challenging. Fortunately, group I PAMs have already been proposed for the treatment of psychosis and cognitive disturbances in schizophrenic patients, since they enhance certain forms of neural plasticity via LTD and LTD, learning, and memory processes (Rush et al. 2002). The latter properties were ascribed to the ability of PAMs to indirectly potentiate the function of NMDA receptors; our cortical injection studies suggest that the action of group I PAMs might, in addition to this well-known effect, facilitate cortical GABAergic transmission. The drug deserves and needs to be investigated for its putative antiepileptic

action in other models of seizure and epilepsy. This is also indicated considering the interaction between VU0360172 and tiagabine, as found in the thalamus. The balance between glutamate and GABA is disturbed in many types of epilepsy, including mesial temporal lobe epilepsy. Also in these models, group I PAMs may increase the diminished GABAergic inhibition. In vivo imaging of mGluR5 via microPET/CT revealed regional changes of mGluR5-binding potential of the rat brain in a pilocarpine-induced epilepsy model. The temporal and spatial changes in mGluR5 availability suggest an abnormal glutamatergic network during epileptogenesis (Choi et al. 2014).

Antiepileptic drugs are often given in combination, considering that monotherapy might often be insufficient for full seizure control. However, many antiepileptic drugs are enzyme inducing and lower the efficacy of other medications. Etx showed an infra-additive effect with VPA (van Rijn et al. 2004); drug combination studies with group I PAMs are necessary. A first drug interaction study on VU0360172 with chronically administered Etx showed that it kept its anti-absence action but without time and dose dependency (D'Amore et al. 2016). The data also suggested that the PAM can be used as an adjunct therapy in patients with absence epilepsy, as well as being used in monotherapy. Whether our group I PAM has disease-modifying properties that stop epileptogenesis is also important. Beneficial effects on cell proliferation by a mGluR1 α agonist after episodes of early-life status epilepticus were recently described (Friedman et al. 2016). It might be relevant to investigate the disease-modifying properties of group I PAMs in other models as well.

It needs to be added that the results obtained on the direction of the effects of group I PAMs and NAMs on absence epilepsy as described here (PAMs reduce, NAMs enhance, or have no effect) are opposite to what has been described in another genetic model: the lethargic mice. A discussion on the validity of the GAERS and WAG/Rij models vs the lethargic mouse model is out of the scope of this paper; it is well known that the latter has a complex phenotype (besides absences seizures, it presents with chronic ataxia, hypoactivity, and transient attacks of severe dyskinetic motor behavior) next to a large GAD(67) expression in thalamic cells and disturbances in the GABA_B receptor system accompanied by an involvement of T-type voltage-gated Ca²⁺ channels. It is obvious, though, that differences in the direction of the effects of the mGlu modulators in the two genetic absence models could be due to the genotype and to different causes of the absence seizures. Another possibility is that the PAMs and NAMs act differently in the two species because of changes in group I receptor expression and function in absence epilepsy-relevant parts of the brain. In order to solve the issue regarding the opposite direction of the effects in the two models, the same compounds should be investigated in the two models.

Although the used PAM is considered selective for mGlu5R, VU0360172 does not interact in a fully competitive manner with the prototypic allosteric binding site, in contrast to some other recently synthesized mGlu5 PAMs (Rook et al. 2015). Whether this property contributes to its anti-absence action in vivo remains to be established. If so, this would offer unique chances for discovering new mechanisms of absence seizures.

References

- Alexander GM, Godwin DW (2006) Metabotropic glutamate receptors as a strategic target for the treatment of epilepsy. *Epilepsy Res* 71:1–22
- Anderson JJ, Rao SP, Rowe B, Giracello DR, Holtz G, Chapman DF, Tehrani L, Bradbury MJ, Cosford ND, Varney MA (2002) [³H]Methoxymethyl-3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine binding to metabotropic glutamate receptor subtype 5 in rodent brain: in vitro and in vivo characterization. *J Pharmacol Exp Ther* 303:1044–1051
- Bandrowski AE, Huguenard JR, Prince DA (2003) Baseline glutamate levels affect group I and II mGluRs in layer V pyramidal neurons of rat sensorimotor cortex. *J Neurophysiol* 89:1308–1316
- Berg AT, Levy SR, Testa FM, Blumenfeld H (2014) Long-term seizure remission in childhood absence epilepsy: might initial treatment matter? *Epilepsia* 55:551–593
- Blumenfeld H (2005) Cellular and network mechanisms of spike-wave seizures. *Epilepsia* 46(Suppl 9):21–33
- Blumenfeld H, Klein JP, Schridde U, Vestal M, Rice T, Khera DS, Bashyal C, Giblin K, Paul-Laughinghouse C, Wang F, Phadke A, Mission J, Agarwal RK, Englot DJ, Motelow J, Nersesyian H, Waxman SG, Levin AR (2008) Early treatment suppresses the development of spike-wave epilepsy in a rat model. *Epilepsia* 49:400–409
- Chapman AG, Yip PK, Yap JS, Quinn LP, Tang E, Harris JR, Meldrum BS (1999) Anticonvulsant actions of LY 367385 ((+)-2-methyl-4-carboxyphenylglycine) and AIDA ((RS)-1-aminoindan-1,5-dicarboxylic acid). *Eur J Pharmacol* 368:17–24
- Chapman AG, Nanan K, Williams M, Meldrum BS (2000) Anticonvulsant activity of two metabotropic glutamate group I antagonists selective for the mGlu5 receptor: 2-methyl-6-(phenylethynyl)-pyridine (MPEP), and (E)-6-methyl-2-styryl-pyridine (SIB 1893). *Neuropharmacol* 39:1567–1574
- Cheong E, Zheng Y, Lee K, Lee J, Kim S, Sanati M, Lee S, Kim YS, Shin HS (2009) Deletion of phospholipase C beta4 in thalamocortical relay nucleus leads to absence seizures. *Proc Natl Acad Sci U S A* 106:21912–21917
- Choi H, Kim YK, Oh SW, Im HJ, Hwang DW, Kang H, Lee B, Lee YS, Jeong JM, Kim EE, Chung JK, Lee DS (2014) In vivo imaging of mGluR5 changes during epileptogenesis using [¹¹C] ABP688 PET in pilocarpine-induced epilepsy rat model. *PLoS One* 9(3):e92765
- Coenen AL, van Luijtelaar EL (2003) Genetic animal models of absence epilepsy: a review of the WAG/Rij strain of rats. *Behav Genet* 33:635–655
- Cope DW, Di Giovanni G, Fyson SJ, Orbán G, Errington AC, Lorincz ML, Gould TM, Carter DA, Crunelli V (2009) Enhanced tonic GABAA inhibition in typical absence epilepsy. *Nat Med* 15:1392–1398
- Cosford ND, Roppe J, Tehrani L, Schweiger EJ, Seiders TJ, Chaudary A, Rao S, Varney MA (2003) [³H]-methoxymethyl-MTEP and [³H]-methoxy-PEPy: potent and selective radioligands for the metabotropic glutamate subtype 5 (mGlu5) receptor. *Bioorg. Med. Chem Lett* 13:351–354
- Coulter DA, Huguenard JR, Prince DA (1989) Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons. *Ann Neurol* 25:582–593
- Crunelli V, Leresche N (2002a) Childhood absence epilepsy: genes, channels, neurons and networks. *Nat Rev Neurosci* 3:371–382
- Crunelli V, Leresche N (2002b) Block of thalamic T-Type Ca²⁺ channels by Ethosuximide is not the whole story. *Epilepsy Curr* 2:53–56
- Crunelli V, David F, Leresche N, Lambert RC (2014) Role for T-type Ca²⁺ channels in sleep waves. *Pflugers Arch* 466:735–745
- D'Amore V, Santolini I, van Rijn CM, Biagioni F, Molinaro G, Prete A, Conn PJ, Lindsley CW, Zhou Y, Vinson PN, Rodriguez AL, Jones CK, Stauffer SR, Nicoletti F, van Luijtelaar G, Ngomba RT (2013) Potentiation of mGlu5 receptors with the novel enhancer, VU0360172, reduces spontaneous absence seizures in WAG/Rij rats. *Neuropharmacol* 66:330–338

- D'Amore V, Santolini I, Celli R, Lionetto L, De Fusco A, Simmaco M, van Rijn CM, Vieira E, Stauffer SR, Conn PJ, Bosco P, Nicoletti F, van Luijtelaar G, Ngomba RT (2014) Head-to-head comparison of mGlu1 and mGlu5 receptor activation in chronic treatment of absence epilepsy in WAG/Rij rats. *Neuropharmacol* 85:91–103
- D'Amore V, von Randow C, Nicoletti F, Ngomba RT, van Luijtelaar G (2015) Anti-absence activity of mGlu1 and mGlu5 receptor enhancers and their interaction with a GABA reuptake inhibitor: effect of local infusions in the somatosensory cortex and thalamus. *Epilepsia* 56:1141–1151
- D'Amore V, Raaijmakers RH, Santolini I, van Rijn CM, Ngomba RT, Nicoletti F, van Luijtelaar G (2016) The anti-absence effect of mGlu5 receptor amplification with VU0360172 is maintained during and after antiepileptogenesis. *Pharmacol Biochem Behav* 146-147:50–59
- Depaulis A, van Luijtelaar G (2006) Genetic animal models of absence epilepsy in the rat. In: Pitkanen A, Schwartzkroin PA, Moshe SL (eds) *Models of seizures and epilepsy*. Elsevier, Amsterdam, pp 233–248
- Deschênes M, Paradis M, Roy JP, Steriade M (1984) Electrophysiology of neurons of lateral thalamic nuclei in cat: resting properties and burst discharges. *J Neurophysiol* 51:1196–1219
- Dezsi G, Ozturk E, Stanic D, Powell KL, Blumenfeld H, O'Brien TJ, Jones NC (2013) Ethosuximide reduces epileptogenesis and behavioral comorbidity in the GAERS model of genetic generalized epilepsy. *Epilepsia* 54:635–643
- Domich L, Oakson G, Steriade M (1986) Thalamic burst patterns in the naturally sleeping cat: a comparison between cortically projecting and reticularis neurones. *J Physiol Lond* 379:429–449
- Ferraguti F, Crepaldi L, Nicoletti F (2008) Metabotropic glutamate 1 receptor: current concepts and perspectives. *Pharmacol Rev* 60:536–581
- Frank LM, Enlow T, Holmes GL, Manasco P, Concannon S, Chen C, Womble G, Casale EJ (1999) Lamictal (lamotrigine) monotherapy for typical absence seizures in children. *Epilepsia* 40:973–979
- Friedman LK, Sharma A, Corcia M, Webster T, Qazi L, Simsovits D, Khalil Y, Hu S, Kantrowitz M, Hong H (2016) Selective inhibition of metabotropic glutamate type 1 alpha receptor (mGluR1 α) reduces cell proliferation and migration following status epilepticus in early development. *Int J Dev Neurosci* 54:6–21
- Glauser TA, Cnaan A, Shinnar S, Hirtz DG, Dlugos D, Masur D, Clark PO, Capparelli EV, Adamson PC, Childhood Absence Epilepsy Study Group (2010) Ethosuximide, valproic acid, and lamotrigine in childhood absence epilepsy. *N Engl J Med* 362:790–799
- Gören MZ, Onat F (2007) Ethosuximide: from bench to bedside. *CNS Drug Rev* 13:224–239
- Greenhill SD, Morgan NH, Massey PV, Woodhall GL, Jones RS (2012) Ethosuximide modifies network excitability in the rat entorhinal cortex via an increase in GABA release. *Neuropharmacol* 62:807–814
- Huguenard JR, Prince DA (1994) Intrathalamic rhythmicity studied in vitro: nominal T-current modulation causes robust antioscillatory effects. *J Neurosci* 14:5485–5502
- Jahnsen H, Llinás R (1984a) Electrophysiological properties of guinea-pig thalamic neurones: an in vitro study. *J Physiol* 349:205–226
- Jahnsen H, Llinás R (1984b) Voltage-dependent burst-to-tonic switching of thalamic cell activity: an in vitro study. *Arch Ital Biol* 122:73–82
- Jakus R, Graf M, Ando RD, Balogh B, Gacsalyi I, Levay G, Kantor S, Bagdy G (2004) Effect of two noncompetitive AMPA receptor antagonists GYKI 52466 and GYKI 53405 on vigilance, behavior and spike-wave discharges in a genetic rat model of absence epilepsy. *Brain Res* 1008:236–244
- Karimzadeh F, Modarres Mousavi SM, Ghadiri T, Jafarian M, Soleimani M, Sadeghi SM, Mesgari M, Joghataei MT, Gorji A (2016) The modulatory effect of metabotropic glutamate receptor type-1 α on spike-wave discharges in WAG/Rij Rats. *Mol Neurobiol* 16. [Epub ahead of print]
- Knoflach F, Mutel V, Jolidon S, Kew JN, Malherbe P, Vieira E, Wichmann J, Kemp JA (2001) Positive allosteric modulators of metabotropic glutamate 1 receptor: characterization, mechanism of action, and binding site. *Proc Natl Acad Sci U S A* 98:13402–13407

- Leresche N, Parri HR, Erdemli G, Guyon A, Turner JP, Williams SR, Aprodini E, Crunelli V (1998) On the action of the anti-absence drug ethosuximide in the rat and cat thalamus. *J Neurosci* 18:4842–4853
- Liu XB, Muñoz A, Jones EG (1998) Changes in subcellular localization of metabotropic glutamate receptor subtypes during postnatal development of mouse thalamus. *J Comp Neurol* 395:450–465
- Lourenço Neto F, Schadrack J, Berthele A, Zieglgänsberger W, Tölle TR, Castro-Lopes JM (2000) Differential distribution of metabotropic glutamate receptor subtype mRNAs in the thalamus of the rat. *Brain Res* 854:93–105
- Lüttjohann A, van Luijtelaar G (2015) Dynamics of networks during absence seizure's on- and offset in rodents and man. *Front Physiol* 6:16
- Lüttjohann A, Zhang S, de Peijper R, van Luijtelaar G (2011) Electrical stimulation of the epileptic focus in absence epileptic WAG/Rij rats: assessment of local and network excitability. *Neurosci* 188:125–134
- Macdonald RL, Kelly KM (1993) Antiepileptic mechanisms of action. *Epilepsia* 34(Suppl 5):S1–S8
- Manning JP, Richards DA, Leresche N, Crunelli V, Bowerly NG (2004) Cortical-area specific block of genetically determined absence seizures by ethosuximide. *Neuroscience* 123:5–9
- Meeren HK, Pijn JP, Van Luijtelaar EL, Coenen AM, Lopes da Silva FH (2002) Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. *J Neurosci* 22:1480–1495
- Miyata M, Kashiwadani H, Fukaya M, Hayashi T, Wu D, Suzuki T, Watanabe M, Kawakami Y (2003) Role of thalamic phospholipase C[β]4 mediated by metabotropic glutamate receptor type 1 in inflammatory pain. *J Neurosci* 23:8098–8108
- Moldrich RX, Chapman AG, De Sarro G, Meldrum BS (2003) Glutamate metabotropic receptors as targets for drug therapy in epilepsy. *Eur J Pharmacol* 476(1-2):3–16
- Molinaro G, Traficante A, Riozzi B, Di Menna L, Curto M, Pallottino S, Nicoletti F, Bruno V, Battaglia G (2009) Activation of mGlu2/3 metabotropic glutamate receptors negatively regulates the stimulation of inositol phospholipid hydrolysis mediated by 5-hydroxytryptamine_{2A} serotonin receptors in the frontal cortex of living mice. *Mol Pharmacol* 76:379–387
- Ngomba RT, Santolini I, Salt TE, Ferraguti F, Battaglia G, Nicoletti F, van Luijtelaar G (2011a) Metabotropic glutamate receptors in the thalamocortical network: strategic targets for the treatment of absence epilepsy. *Epilepsia* 52:1211–1222
- Ngomba RT, Santolini I, Biagioni F, Molinaro G, Simonyi A, van Rijn CM, D'Amore V, Mastroiacovo F, Olivieri G, Gradini R, Ferraguti F, Battaglia G, Bruno V, Puliti A, van Luijtelaar G, Nicoletti F (2011b) Protective role for type-1 metabotropic glutamate receptors against spike and wave discharges in the WAG/Rij rat model of absence epilepsy. *Neuropharmacol* 60:1281–1291
- Nicoletti F, Bockaert J, Collingridge GL, Conn PJ, Ferraguti F, Schoepp DD, Wroblewski JT, Pin JP (2011) Metabotropic glutamate receptors: from the workbench to the bedside. *Neuropharmacology* 60:1017–1041
- Onat FY, van Luijtelaar G, Nehlig A, Snead OC 3rd (2013) The involvement of limbic structures in typical and atypical absence epilepsy. *Epilepsy Res* 103:111–123
- Peeters BW, van Rijn CM, Vossen JM, Coenen AM (1990) Involvement of NMDA receptors in non-convulsive epilepsy in WAG/Rij rats. *Life Sci* 47:523–529
- Pinault D, Leresche N, Charpier S, Deniau JM, Marescaux C, Vergnes M, Crunelli V (1998) Intracellular recordings in thalamic neurones during spontaneous spike and wave discharges in rats with absence epilepsy. *J Physiol* 509:449–456
- Polack PO, Charpier S (2009) Ethosuximide converts ictogenic neurons initiating absence seizures into normal neurons in a genetic model. *Epilepsia* 50:1816–1820
- Polack PO, Guillemain I, Hu E, Deransart C, Depaulis A, Charpier S (2007) Deep layer somatosensory cortical neurons initiate spike-and-wave discharges in a genetic model of absence seizures. *J Neurosci* 27:6590–6599

- Porcello DM, Smith SD, Huguenard JR (2003) Actions of U-92032, a T-type Ca²⁺ channel antagonist, support a functional linkage between I(T) and slow intrathalamic rhythms. *J Neurophysiol* 89:177–185
- Posner EB, Mohamed K, Marson AG (2005) Systematic review of treatment of typical absence seizures in children and adolescents with ethosuximide, sodium valproate or lamotrigine. *Seizure* 14:117–122
- Ramakers GM, Peeters BW, Vossen JM, Coenen AM (1991) CNQX, a new non-NMDA receptor antagonist, reduces spike wave discharges in the WAG/Rij rat model of absence epilepsy. *Epilepsy Res* 9:127–131
- Richards DA, Manning JP, Barnes D, Rombola L, Bowery NG, Caccia S, Leresche N, Crunelli V (2003) Targeting thalamic nuclei is not sufficient for the full anti-absence action of ethosuximide in a rat model of absence epilepsy. *Epilepsy Res* 54:97–107
- Rodriguez AL, Grier MD, Jones CK, Herman EJ, Kane AS, Smith RL, Williams R, Zhou Y, Marlo JE, Days EL, Blatt TN, Jadhav S, Menon UN, Vinson PN, Rook JM, Stauffer SR, Niswender CM, Lindsley CW, Weaver CD, Conn PJ (2010) Discovery of novel allosteric modulators of metabotropic glutamate receptor subtype 5 reveals chemical and functional diversity and in vivo activity in rat behavioral models of anxiolytic and antipsychotic activity. *Mol Pharmacol* 78:1105–1123
- Romano C, Sesma MA, McDonald CT, O'Malley K, Van den Pol AN, Olney JW (1995) Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. *J Comp Neurol* 355:455–469
- Rook JM, Tantawy MN, Ansari MS, Felts AS, Stauffer SR, Emmitte KA, Kessler RM, Niswender CM, Daniels JS, Jones CK, Lindsley CW, Conn PJ (2015) Relationship between in vivo receptor occupancy and efficacy of metabotropic glutamate receptor subtype 5 allosteric modulators with different in vitro binding profiles. *Neuropsychopharmacology* 40:755–765
- Rush AM, Wu J, Rowan MJ, Anwyl R (2002) Group I metabotropic glutamate receptor (mGluR)-dependent long-term depression mediated via p38 mitogen-activated protein kinase is inhibited by previous high-frequency stimulation and activation of mGluRs and protein kinase C in the rat dentate gyrus in vitro. *J Neurosci* 22:6121–6128
- Russo E, Citraro R, Scicchitano F, De Fazio S, Di Paola ED, Constanti A, De Sarro G (2010) Comparison of the antiepileptogenic effects of an early long-term treatment with ethosuximide or levetiracetam in a genetic animal model of absence epilepsy. *Epilepsia* 51:1560–1569
- Sarkisova K, van Luijtelaaar G (2011) The WAG/Rij strain: a genetic animal model of absence epilepsy with comorbidity of depression [corrected]. *Prog Neuropsychopharmacol Biol Psychiatry* 35:854–876
- Sarkisova KY, Kuznetsova GD, Kulikov MA, van Luijtelaaar G (2010) Spike-wave discharges are necessary for the expression of behavioral depression-like symptoms. *Epilepsia* 51:146–160
- Shigemoto R, Nakanishi S, Mizuno N (1992) Distribution of the mRNA for a metabotropic glutamate receptor (mGluR1) in the central nervous system: an in-situ hybridization study in adult and developing rat. *J Comp Neurol* 322:121–135
- Slaght SJ, Leresche N, Deniau JM, Crunelli V, Charpier S (2002) Activity of thalamic reticular neurons during spontaneous genetically determined spike and wave discharges. *J Neurosci* 22:2323–2334
- Stefan H, Lopes da Silva FH (2013) Epileptic neuronal networks: methods of identification and clinical relevance. *Front Neurol* 4:8
- Steriade M, Contreras D (1995) Relations between cortical and thalamic cellular events during transition from sleep patterns to paroxysmal activity. *J Neurosci* 15(1 Pt 2):623–624
- Steriade M, Llinás RR (1988) The functional states of the thalamus and the associated neuronal interplay. *Physiol Rev* 68:649–742
- Stinehelfer S, Vruwink M, Burette A (2000) Immunolocalization of mGluR1alpha in specific populations of local circuit neurons in the cerebral cortex. *Brain Res* 861:37–44
- Sun QQ, Zhang Z, Jiao Y, Zhang C, Szab G, Erdelyi F (2009) Differential metabotropic glutamate receptor expression and modulation in two neocortical inhibitory networks. *J Neurophysiol* 101:2679–2692

- Tenney JR, Glauser TA (2013) The current state of absence epilepsy: can we have your attention? *Epilepsy Curr* 13:135–140
- Teoh PC, Chan HL (1975) Lupus-scleroderma syndrome induced by ethosuximide. *Arch Dis Child* 50:58–61
- van Luijtelaar EL, Coenen AM (1986) Two types of electrocortical paroxysms in an inbred strain of rats. *Neurosci Lett* 70:393–397
- van Luijtelaar G, Sitnikova E (2006) Global and focal aspects of absence epilepsy: the contribution of genetic models. *Neurosci Biobehav Rev* 30:983–1003
- van Luijtelaar G, Zobeiri M (2014) Progress and outlooks in a genetic absence epilepsy model (WAG/Rij). *Curr Med Chem* 21:704–721
- van Luijtelaar G, Mishra AM, Edelbroek P, Coman D, Frankenmolen N, Schaapsmeeders P et al (2013) Anti-epileptogenesis: electrophysiology, diffusion tensor imaging and behaviour in a genetic absence model. *Neurobiol Dis* 60:126–138
- van Rijn CM, Sun MS, Deckers CL, Edelbroek PM, Keyser A, Renier W, Meinardi H (2004) Effects of the combination of valproate and ethosuximide on spike wave discharges in WAG/Rij rats. *Epilepsy Res* 59:181–189
- Vieira E, Huwyler J, Jolidon S, Knoflach F, Mutel V, Wichmann J (2009) Fluorinated 9H-xanthene-9-carboxylic acid oxazol-2-yl-amides as potent, orally available mGlu1 receptor enhancers. *Bioorg Med Chem Lett* 19:1666–1669
- von Krosigk M, Bal T, McCormick DA (1993) Cellular mechanisms of a synchronized oscillation in the thalamus. *Science* 261:361–364
- Wallace SJ (1986) Use of ethosuximide and valproate in the treatment of epilepsy. *Neurol Clin* 4:601–616
- Watanabe M, Nakamura M, Sato K, Kano M, Simon MI, Inoue Y (1998) Patterns of expression for the mRNA corresponding to the four isoforms of phospholipase C beta in mouse brain. *Eur J Neurosci* 10:2016–2025
- White HS (1999) Comparative anticonvulsant and mechanistic profile of the established and newer antiepileptic drugs. *Epilepsia* 40(Suppl 5):S2–10
- Wijetunge LS, Till SM, Gillingwater TH, Ingham CA, Kind PC (2008) mGluR5 regulates glutamate-dependent development of the mouse somatosensory cortex. *J Neurosci* 28:13028–13137