Published in final edited form as:

Neuropharmacology. 2013 March ; 66: 330–338. doi:10.1016/j.neuropharm.2012.05.044.

Potential of mGlu5 receptors with the novel enhancer, VU0360172, reduces spontaneous absence seizures in WAG/Rij rats

V. D'Amore^{a,1}, I. Santolini^{a,1}, C.M. van Rijn^b, F. Biagioni^a, G. Molinaro^a, A. Prete^{a,c}, P.J. Conn^{d,e}, C.W. Lindsley^{d,e}, Y. Zhou^{d,e}, P.N. Vinson^{d,e}, A.L. Rodriguez^{d,e}, C.K. Jones^{d,e}, S.R. Stauffer^{d,e}, F. Nicoletti^{a,f}, G. van Luijtelar^b, and R.T. Ngomba^{a,*}

^aI.R.C.C.S., NEUROMED, Neuropharmacology Unit, Parco Tecnologico, Località Camerelle 86077, Pozzilli, Isernia, Italy ^bDonders Centre for Cognition, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, The Netherlands ^cChild Neurology and Sleep Pediatric Centre, Ospedale S. Andrea, Rome, Italy ^dDepartment of Pharmacology, Vanderbilt University, Nashville, TN 37232, USA ^eVanderbilt Program in Drug Discovery, Nashville, TN 37232, USA ^fDepartment of Physiology and Pharmacology, University "Sapienza", Rome, Italy

Abstract

Absence epilepsy is generated by the cortico-thalamo-cortical network, which undergoes a finely tuned regulation by metabotropic glutamate (mGlu) receptors. We have shown previously that potentiation of mGlu1 receptors reduces spontaneous occurring spike and wave discharges (SWDs) in the WAG/Rij rat model of absence epilepsy, whereas activation of mGlu2/3 and mGlu4 receptors produces the opposite effect. Here, we have extended the study to mGlu5 receptors, which are known to be highly expressed within the cortico-thalamo-cortical network. We used presymptomatic and symptomatic WAG/Rij rats and aged-matched ACI rats. WAG/Rij rats showed a reduction in the mGlu5 receptor protein levels and in the mGlu5-receptor mediated stimulation of polyphosphoinositide hydrolysis in the ventrobasal thalamus, whereas the expression of mGlu5 receptors was increased in the somatosensory cortex. Interestingly, these changes preceded the onset of the epileptic phenotype, being already visible in pre-symptomatic WAG/Rij rats. SWDs in symptomatic WAG/Rij rats were not influenced by pharmacological blockade of mGlu5 receptors with MTEP (10 or 30 mg/kg, i.p.), but were significantly decreased by mGlu5 receptor potentiation with the novel enhancer, VU0360172 (3 or 10 mg/kg, s.c.), without affecting motor behaviour. The effect of VU0360172 was prevented by co-treatment with MTEP. These findings suggest that changes in mGlu5 receptors might lie at the core of the absence-seizure prone phenotype of WAG/Rij rats, and that mGlu5 receptor enhancers are potential candidates to the treatment of absence epilepsy.

Keywords

Absence epilepsy; WAG/Rij rats; mGlu5 receptor; VU0360172; MTEP

1. Introduction

Typical absence epilepsy is a generalized non convulsive form of epilepsy characterized by bilaterally synchronous spike and wave discharges (SWDs) that spreads all-over the cortex as seen on the electroencephalogram (reviewed by Blumenfeld, 2005). The origin of the generation of SWDs has led to several debates on which anatomical area might be held as an initiation site and pioneer studies have tentatively put together the importance of the thalamus (Jasper and Kershman, 1941) and the cerebral cortex (Avoli and Gloor, 1982) as key players (Meeren et al., 2005). Nowadays it seems well established in rodent genetic models that SWDs are generated in the deep cortical layers of the somatosensory cortex (Meeren et al., 2002; Polack et al., 2009) that is part of cortico-thalamo-cortical loop that comprises besides the cortical pyramidal neurons and GABAergic interneurons, also thalamic relay neurons, and inhibitory neurons of the reticular thalamic nucleus (nRT). These nRT neurons are also connected to each other via inhibitory GABAergic synapses as well as by gap junctions (Blumenfeld, 2005). The cortex (layer V and VI) send excitatory glutamatergic projections to the nRT and thalamic relay neurons; the latter neurons send excitatory glutamatergic projections to cortical pyramidal neurons and nRT. The nRT neurons send inhibitory GABAergic connections to thalamic relay neurons but not to the cortex (Zhang and Jones, 2004; Blumenfeld, 2005; van Luijtelaar and Sitnikova, 2006).

Metabotropic glutamate (mGlu) receptors within the cortico-thalamo-cortical loops are particularly positioned to modulate rather than mediate synaptic transmission and are therefore potential targets for the controlling of SWDs (reviewed by Ngomba et al., 2011a). Group-I mGlu receptors (mGlu1 and mGlu5 receptors) are coupled to Gq/G₁₁ proteins and are predominantly localized in the peripheral portion of postsynaptic densities. Their activation stimulates polyphosphoinositide hydrolysis with formation of inositol-1,4,5-trisphosphate and diacylglycerol, and also regulate the activity of different types of Ca²⁺ and K⁺ potassium channels (reviewed by Nicoletti et al., 2011). mGlu1 and mGlu5 receptors show a different pattern of distribution in the cortico-thalamo-cortical network, which suggests distinct rather than complementary functions of these two receptor subtypes. High densities of mGlu1 receptors are found on dendrites of thalamic relay neurons that receive afferent input from cortical layer VI neurons (Ferraguti et al., 2008; reviewed by Ngomba et al., 2011a). In the cortex, mGlu1 receptors appear to be exclusively expressed postsynaptically on GABAergic interneurons (Stinehelfer et al., 2000). Moreover, it has been shown that activation of mGlu5 receptors has an excitatory effect similar to that mediated by mGlu1 receptors and that these receptors can participate in sensory responses of thalamic relay cells (Salt and Binns, 2000). Thalamic relay neurons also expressed mGlu5 receptors at dendrites postsynaptic to cortical inputs (Romano et al., 1995; Liu et al., 1998). Neurons of the nRT also show moderate-to-low expression of mGlu5 receptors (Romano et al., 1995; Lourenço Neto et al., 2000). In the cerebral cortex, mGlu5 receptors 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). We have recently examined the role of mGlu1 receptors in absence seizures using Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats as a genetic model of absence epilepsy. WAG/Rij rats develop spontaneous SWDs at >2 months of age, and at 5–6 months of age they all have hundreds of SWDs per day (Coenen and van Luijtelaar, 1987; Schridde and van Luijtelaar, 2004). In these rats, generalized bilateral SWDs are characterized by a frequency of 7–9 Hz and duration of 1–30 s (mean 5–6 s), and are associated with behavioural manifestations mimicking those of absences in humans (Coenen and van Luijtelaar, 2003; van Luijtelaar and Sitnikova, 2006). SWDs in WAG/Rij rats are reduced by classical anti-absence drugs, such as ethosuximide and valproate, and are increased by vigabatrin, phenytoin and carbamazepine, which also worsen absence seizures in humans (Bouwman et al., 2007; Coenen and van Luijtelaar, 2003; Thomas et al., 2006). WAG/Rij rats represent a model of

absence epilepsy endowed with construct, face, and pharmacological validity. We have reported that mGlu1 receptors are down-regulated in the ventral basal thalamus of symptomatic WAG/Rij rats, and that pharmacological potentiation of mGlu1 receptors reduces SWDs in these rats (Ngomba et al., 2011b). We have now extended the study to mGlu5 receptors, finding that receptor expression and function change in the thalamus and somatosensory cortex of both pre-symptomatic and symptomatic WAG/Rij rats, and that a selective mGlu5 receptor enhancer significantly reduce absence seizures without affecting motor behaviour.

2. Materials and methods

2.1. Drugs

VU0360172 (N-cyclobutyl-6-[2-(3-fluorophenyl)ethynyl]pyridine-3-carbox amide) was obtained from Vanderbilt University Medical Center, JNJ16259685 ((3,4-dihydro-2H-pyranol[2,3-b]quinolin-7yl)(cis-4-ethoxycyclohexyl)methanone), DHPG(3,5-dihydroxyphenylglycine), MTEP 3-((2-Methyl-1,3-thiazol-4-yl)ethynyl) pyridine hydrochloride, and diazepam were purchased from Tocris Cookson Ltd. (Bristol, UK); VU0360172 was dissolved in 10% Tween 80, and injected s.c.. MTEP was dissolved in saline, and injected i.p..

2.2. Animals

Male WAG/Rij and ACI (Agouti Copenhagen Irish) rats were kept under environmentally controlled conditions (ambient temperature = 22 °C, humidity = 40%) in a room with reversed light–dark cycle (light on from 8:00 p.m. to 8:00 a.m.), with food and water *ad libitum*. The EEG study was carried out during the dark phase of the cycle in which WAG/Rij rats have the largest amount of SWDs (van Luijtelaar and Coenen, 1988). All animals were handled prior to EEG registrations. For biochemical studies, age-matched ACI rats were used as controls. ACI rats have no or only very few SWDs and the lowest number of SWDs of all inbred strains investigated as was assessed in a 48 h EEG evaluation study (Inoue et al., 1990) and in all cases they have much less SWDs than WAG/Rij rats of the same age (Schridde and van Luijtelaar, 2004). Therefore, ACI rats are commonly used as controls in experiments with WAG/Rij rats (Laso et al., 1992; Ngomba et al., 2008; van de Bovenkamp-Janssen et al., 2006; van Rijn et al., 2010). We used rats of both strains at 2 and 8 months of age. WAG/Rij rats of 2 months of age do not show SWDs as yet, and, therefore, are considered “pre-symptomatic”. All 8 months old WAG/Rij rats have about 16–20 SWDs per hour, which is a few hundred SWDs per day, and are thus defined as “symptomatic”. The study was performed in accordance with the guidelines of the European Community for the use of experimental animals and approved by the local ethics committee for animal studies (RU-DEC).

2.3. Immunohistochemistry

Brains from WAG/Rij and ACI rats were fixed in Carnoy's solution (60% ethanol, 10% acetic acid, and 30% chloroform), embedded in paraffin and cut into sections of 10 µm. Deparaffinised sections were pre-incubated for 1 h in normal goat serum for 1 h (vector laboratories, Burlingame, CA), then incubated overnight with polyclonal rabbit anti-mGluR5 (1:100; Millipore, Billerica, MA) antibody dissolved in 10% of PBS and then for 1 h with secondary biotin-coupled anti-rabbit antibody (1:200; Vector Laboratories, Burlingame, CA). 3,3-Diaminobenzidine tetrachloride (Sigma Aldrich, Milan, Italy) was used for detection. Control staining was carried out without the primary antibody. The intensity of mGlu5 immunoreactivity in cortical and thalamic regions (see Fig. 1) was quantified by measuring the relative optical densities. Brain coronal sections of 350 µm were sliced from bregma –1.3 mm to –4.8 mm.

Images were acquired at low magnification (2.5×) and the densitometric analysis was performed by assessing the intensity of the background values (i.e., the optical density measured in unlabelled areas present in the section, such as corpus callosum) by using Zeiss Axio Imager M1 microscope equipped with a motorized stage and focus control system (Zeta axis), and with a digital video camera.

2.4. Western blot analysis of mGlu5 receptors

Eight male WAG/Rij and eight male age-matched control ACI rats, 2 or 8-month old, were anesthetized with ether, decapitated and brains were rapidly removed and frozen. Brains were coded and the codes were released after Western blot analysis. Whole brains were placed on a cryostat and tissue that comprises the principal area of the cortico-thalamo-cortical network were dissected out using the coronal diagrams (between coordinates from bregma −1.88 mm and −3.80 mm) from Paxinos and Watson atlas (2005) as a guide. For cortical areas, we dissected a part of the cortex that included the primary somatosensory cortex (S1) and another portion of the cortex that included the primary motor cortex (M1). Cortical areas 2 mm anterior and posterior from the above mentioned cortical areas were dissected and used as control regions. A thalamic portion containing ventrobasal thalamic nuclei (VB) was also dissected. The expression of mGlu5 receptor proteins was estimated by Western blot analysis, using a specific rabbit polyclonal antibody (1:1.000, Upstate Biotechnology, Lake Placid, NY) and a mouse monoclonal antibody to label α -actin (1:100.000, Sigma, St. Louis, MO). Brain tissues were homogenized at 4 °C in 50 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA, 1% Triton X-100, 1 mM PMSF, 1 μ g/ml aprotinin, 1 μ g/ml pepstatin, and 1 μ g/ml leupeptin. Proteins were resuspended in SDS-bromophenol blue reducing buffer with 40 mM DTT. Western blot analyses were carried out using 8% SDS polyacrylamide gels which were electro-blotted on immunoblot PVDF membranes (BioRad, Milano, Italy); filters were blocked overnight in TBS-T buffer containing 5% non fatty milk. Blots were incubated for 1 h with rabbit polyclonal anti-mGlu5 antibodies and mouse monoclonal antibody to label α -actin. Filters were washed with TBS-T buffer and then incubated for 1 h with secondary antibodies (peroxidase-coupled anti-rabbit or anti-mouse, Amersham, Piscataway, NJ) diluted 1:7000 with TBS-T buffer. Immunostaining was revealed by enhanced ECL. Immunoreactive protein bands were quantified using the densitometry method (Scion image software, <http://rsb.info.nih.gov/niimage/>). Values were obtained by calculating the ratio between the area under the curve (AUC) of the optical density of mGlu5 signal and the AUC of the house keeping protein α -actin for each lane.

2.5. Measurement of DHPG-stimulated polyphosphoinositide (PI) hydrolysis in living rats

ACI and WAG/Rij rats ($n = 5$ animals per group) were anesthetized with ketamine (100 mg/kg) plus xylazine (10 mg/kg) and injected with [3 H] inositol (2 μ Ci/5 μ l/2 min, i.c.v.). Twenty-four hours later, rats were treated with lithium ions (administered as LiCl, 10 mmol/kg, s.c.) to inhibit the conversion of inositol monophosphate (InsP) into free inositol. The mGlu1/5 receptor agonist, DHPG (500 nmol/5 μ l saline containing 50% dimethyl sulfoxide) was injected i.c.v., 1 h after LiCl injection. Control rats were injected with the vehicle alone. The selective mGlu1 receptor antagonist JNJ16259685 (10 mg/kg) and diazepam (10 mg/kg) used as an anti-convulsant drug, were injected i.p. 30 min before DHPG. Rats were killed 1 h after treatment with DHPG or vehicle. The sensorymotor cortex and the thalamus were quickly removed and stored at −80 °C. On the day of the assay, tissue was sonicated in 1.25 ml of water containing 10 mM LiCl. After centrifugation at 10,000 g for 20 min, the [3 H]InsP present in the supernatant was separated by anion exchange chromatography in 10 ml columns containing 1.5 ml of Dowex 1-X-8 resin (formate form, 100–200 mesh; Bio-Rad). Columns were washed twice with water, once with a solution of 5 mM sodium tetraborate and 40 mM sodium formate to elute cyclic InsP and glycerophosphoinositols,

and then with 6.5 ml of 0.2 M ammonium formate and 0.1 M formic acid for the elution of InsP (Nicoletti et al., 1986). Total radioactivity in the brain regions was determined by counting a 100 μ l aliquot of the whole homogenate.

2.6. EEG recordings

Male WAG/Rij rats (8-month old, group numbers are presented in the legend of Fig. 4) with a mean body weight of 390 ± 20 g, were used. Animals were individually housed in Macrolon cages in a room illuminated with white lights from 8:00 p.m. to 8:00 a.m. A permanent cortical tripolar electrode set was implanted under complete isoflurane anaesthesia, one electrode into the frontal region (coordinates with the skull surface flat and from bregma zero-zero, (AP +2.0; L -2.5), and the other in the parietal region (A -6.0; L -4.0) (Paxinos and Watson, 2005). The ground electrode was placed over the cerebellum. After the surgery, animals were allowed to recover for two weeks. Rats were put into transparent recording cages, connected to an EEG cable which allowed free movement, and habituated to the experimental conditions for 12 h. The EEG was filtered (only frequencies between 0.1 and 100 Hz), were allowed to pass, digitalized with a sample frequency of 256 Hz, and stored for off-line analysis using Windaq system (DATAQ Instruments, Akron, OH, USA). Baseline EEG was recorded during the dark period (9:00–11:00 a.m.) for 2 h prior to injection and thereafter, following the systemic injection of the selective mGlu5 receptor positive allosteric modulator, VU0360172 (3 or 10 mg/kg, s.c., dissolved in tween) and the mGlu5 receptor antagonist, MTEP (10 or 30 mg/kg, i.p., dissolved in saline), or vehicle in a volume of 1 ml either s.c. or i.p.. The post injection EEG was registered during the next 5 h. SWDs were marked at visual inspection based on commonly used criteria: trains of sharp spikes and slow waves lasting minimally 1 s, an amplitude of the spikes at least twice the background, frequency of the SWDs between 7 and 10 Hz and an asymmetric appearance of the SWDs (van Luijckelaar and Coenen, 1986). Ten randomly selected SWDs of eight rats in the third hour of injection of the highest dose of VU0360172 and ten SWDs in the control group (tween) were analysed with respect to the frequency spectrum of SWDs (Brain Vision Analyser, Brain Products GmbH, Gilching, Germany).

2.7. Animal behaviour: recording of spontaneous motor activity

Spontaneous motor activity in symptomatic WAG/Rij rats was recorded with an analogic passive infrared detector (PIR) (Luna PR, Rokonet Electronics LTD, Rishon Le Tzion, Israel) (van Rijn et al., 2010) during the EEG recording session. The analogue signal was digitalized simultaneously with the EEG signal. In order to check whether the drugs induced behavioural effects and whether the putative drug effects on SWDs could be ascribed to effects on behaviour considering the intimate relationship between the behavioural state of the animal and the occurrence of SWDs (Drinkenburg et al., 1991; Smyk et al., 2011), the amount of bodily movements in the baseline and after drug administration were quantified by calculating the mean of the PIR signal per hour.

2.8. Statistical analysis

Data are expressed as means $\pm$ SEM. Statistical analysis for Western blot and immunohistochemistry was carried out by a two-way ANOVA, with the rat strain and age as independent factors, while Student's *t*-test was used for the analysis of DHPG-stimulated PI hydrolysis. Statistical analysis of EEG and behavioural data was carried out by ANOVA for repeated measures followed by the Bonferroni's test to isolate the differences.

3. Results

3.1. Expression of mGlu5 receptors in the cortico-thalamo-cortical network of ACI and WAG/Rij rats at 2 and 8 months of age

Immunohistochemical analysis of mGlu5 receptors in the sensorymotor cortex and thalamus of ACI and WAG/Rij rats at 2 and 8 months of age is shown in Fig. 1A and D, respectively. In the motor and somatosensory cortices of non-epileptic ACI rats, expression of mGlu5 receptors decreased with age, being lower at 8 than at 2 months of age. In contrast, expression of mGlu5 receptors in the motor and somatosensory cortices of WAG/Rij rats did not change with age. Eight-month old symptomatic WAG/Rij rats showed a significant increase in mGlu5 receptor immunoreactivity in the motor and somatosensory cortices as compared to age-matched ACI rats (Fig. 1B and C). A trend to an increased immunoreactivity was also seen in 2-month old presymptomatic WAG/Rij rats with respect to age-matched ACI rats. However, this difference did not reach statistical significance (Fig. 1B and C). In contrast, 8-month old WAG/Rij rats showed a reduction in mGlu5 receptor immunoreactivity in both the nRT and ventrobasal thalamic nuclei (VB). In the nRT, mGlu5 receptor immunoreactivity was significantly lower in 8-month old WAG/Rij rats than in 2-month old WAG/Rij rats (Fig. 1E). In the VB, a significant reduction was found between 8-month old WAG/Rij rats and age-matched ACI rats (Fig. 1F). A nonsignificant trend to a reduction in mGlu5 receptor immunoreactivity was also seen in the VB of 2-month old WAG/Rij rats, as compared to ACI rats of corresponding age (Fig. 1F).

We also carried out immunoblot analysis of mGlu5 receptors on protein extracts from the motor cortex, somatosensory cortex, and VB of ACI and WAG/Rij rats. Immunoblots showed a major band at about 130 kDa corresponding to the mGlu5 receptor monomers. Data of Western blot analysis were in line with those obtained by immunohistochemistry showing an increased mGlu5 receptor expression in the motor and somatosensory cortices, and a reduced expression in the VB of 8-month old symptomatic WAG/Rij rats as compared to age-matched ACI rats (Fig. 2A–F). The only difference was that these changes reached statistical significance also in 2-month old WAG/Rij rats (vs. age-matched ACI rats) (Fig. 2A–F). Thus, at least immunoblot data suggest that changes of mGlu5 receptors in the cortico-thalamic-cortical network of WAG/Rij rats precede the onset of SWDs.

3.2. Changes in mGlu5 receptor-mediated PI hydrolysis in the cortex and thalamus of WAG/Rij rats

We used an *in vivo* method to assess the mGlu5-receptor mediated PI hydrolysis in the sensorymotor cortex and thalamus of ACI and WAG/Rij rats (see Fig. 3A for experimental details). Rats were infused i.c.v. with the mixed mGlu1/5 receptor agonist, DHPG (500 nmol/5 μ l), and the component of PI hydrolysis mediated by mGlu5 receptors was isolated by co-injecting the rats with the mGlu1 receptor antagonist, JNJ16259685 (10 mg/kg, i.p.).

There was no significant change in DHPG-stimulated PI hydrolysis in the sensorymotor cortex between WAG/Rij and ACI rats, although a trend to an increase was seen in WAG/Rij rats (Fig. 3B). In contrast, a large reduction in DHPG-stimulated PI hydrolysis was seen in the thalamus of WAG/Rij rats (Fig. 3C), in close agreement with receptor expression data.

3.3. Pharmacological potentiation of mGlu5 receptors reduced spike and wave discharges (SWDs) in WAG/Rij rats

Acute treatment with MTEP (10 or 30 mg/kg, i.p.), a selective negative allosteric modulator (NAM) of mGlu5 receptors (Anderson et al., 2002; Cosford et al., 2003), did not change the incidence and mean duration of SWDs in symptomatic WAG/Rij rats (Fig. 4A and B). We next examined whether pharmacological potentiation of mGlu5 receptors could affect SWDs

by injecting rats with the mGlu5 receptor enhancer, VU0360172. This drug was used at doses (3 or 10 mg/kg, s.c.) that are known to be centrally active (Rodriguez et al., 2010). Pharmacological enhancement of mGlu5 receptors significantly reduced the incidence of SWDs in a dose-dependent manner in the first 2 h after administration, and the effect of the highest dose lasted up to 4 h after drug injection (Fig. 4C). Treatment with VU0360172 also reduced the mean duration of SWDs to a similar extent as the incidence of SWDs (Fig. 4D). To exclude unknown off-target effects of VU0360172, the compound was also injected at the dose of 3 mg/kg, s.c., in WAG/Rij rats pretreated with MTEP (10 mg/kg, i.p.) 15 min earlier. As shown in Fig. 4E and F, the effect of VU0360172 was antagonized by MTEP, demonstrating that the drug reduced absence seizures by amplifying the endogenous activation of mGlu5 receptors.

Spectral analysis of the EEG during SWDs after the highest dose of VU0360172 showed that the peak frequency of the SWDs was 7.9 Hz, and did not differ from the peak frequency detected in WAG/Rij rats treated with vehicle (data not shown).

3.4. Pharmacological potentiation of mGlu5 receptors did not affect motor behaviour

To exclude that changes in SWD induced by VU0360172 were secondary to changes in motor behaviour, we measured motor activity in 8-month old WAG/rj rats for 7 h following the injection of vehicle or VU0360172 (3 or 10 mg/kg, s.c.). ANOVA for repeated measures showed a significant effect of time ($F = 5.77$, $df\ 5,14$, $p < 0.001$), with no drug effect and no drug $\times$ time interaction (Fig. 5).

4. Discussion

A major finding here was that pharmacological enhancement of mGlu5 receptor activity caused a robust and dose-dependent reduction in the incidence and mean duration of SWDs in WAG/Rij rats, which have pharmacological validity as a model for absence epilepsy. In contrast, pharmacological blockade of mGlu5 receptors did not affect absence seizures. A different scenario occurs in models of convulsive seizures, where pharmacological blockade of mGlu5 receptors is usually protective (Chapman et al., 2000; Lojková and Mares, 2005; Jesse et al., 2008). WAG/Rij rats also showed a reduced expression and function of mGlu5 receptors in the thalamus and an increased receptor expression in the sensorymotor cortex. Immunoblot data show that in WAG/Rij rats changes in mGlu5 receptor expression are antecedent to the onset of absence seizures, and, therefore, are not secondary to seizure activity. This is peculiar because changes in the expression of mGlu1 receptors in the thalamus only occur in symptomatic WAG/Rij rats (Ngomba et al., 2011b), whereas expression of mGlu4 receptors show opposite modifications in pre-symptomatic and symptomatic WAG/Rij rats (Ngomba et al., 2008).

We wish to highlight that the reduced function of mGlu5 receptors in the thalamus was seen using an *in vivo* method that allows the assessment of agonist-stimulated PI hydrolysis without the biases inherent to the conventional assay of PI hydrolysis in brain slices. We suggest that a reduced expression and function of mGlu5 receptors in the thalamus is a molecular determinant of the pathological phenotype of WAG/Rij rats, and that pharmacological amplification of thalamic receptors that are still present and functional is sufficient to significantly reduce absence seizures.

If so, it is not surprising that MTEP had no effect on SWDs because thalamic mGlu5 receptors were already hypofunctional in WAG/Rij rats. It is worth mentioning that another mGlu5 receptor NAM, 2-methyl-6-(phenylethynyl)pyridine (MPEP) inhibits SWDs in lethargic mice (Chapman et al., 2000), which represent an atypical model of absence epilepsy and bear a mutation in the $\alpha 4$ subunit of voltage-sensitive calcium channels

(Burgess et al., 1999). However, MPEP is also a weak mGlu4 receptor PAM (Mathiesen et al., 2003) and mGlu4 receptors are involved in the generation of absence seizures (Snead et al., 2000; Ngomba et al., 2008). MPEP had no effect on acutely induced SWDs by low doses of the GABA_A receptor antagonist, pentylenetetrazole, in immature rats (Lojková and Mares, 2005). The logical conclusion is that the role of mGlu5 receptors in different seizure and epilepsy models is not uniform, and the effect of mGlu5 receptor NAMs critically depends on the genetic background of the animals, the age and/or the type of absence seizures (e.g., spontaneous vs. drug-induced).

The mGlu5 receptor is physically linked to the NR2 subunit of N-methyl-D-aspartate (NMDA) receptors through a chain of scaffolding proteins that include PSD95, Shunk, and Homer (Tu et al., 1999). Furthermore, activation of mGlu5 receptors facilitates the activation of NMDA receptors, and *vice versa* (Doherty et al., 1997; Ugolini et al., 1999; Awad et al., 2000; Attucci et al., 2001; Mannaioni et al., 2001; Pisani et al., 2001; Alagarsamy et al., 2005). This interaction cannot explain the protective effect of VU0360172 that we have observed here because it is the NMDA receptor blockade that reduces absence seizures in WAG/Rij rats (Peeters et al., 1990; Coenen and van Luijtelaar, 2003). Either neuronal mGlu5 receptors act independently of the NMDA receptors in regulating synaptic activity in the cortico-thalamo-cortical network, or glial mGlu5 receptors (Liu et al., 1998) have a role in regulating the expression of high affinity glutamate transporters (Aronica et al., 2003) or signalling molecules that may affect the function of thalamic or cortical neurons.

An attractive hypothesis is that activation of mGlu5 receptors in VB restrains GABA release and therefore protects against SWDs through a mechanism mediated by an enhanced formation of endocannabinoids with ensuing activation of presynaptic CB1 cannabinoid receptors (Varma et al., 2001; Jung et al., 2005; Uchigashima et al., 2007; Won et al., 2009; Izumi and Zorumski, 2012). Interestingly, pharmacological activation of CB1 receptors reduces absence seizures in WAG/Rij rats (van Rijn et al., 2010). It is worth noting that systemic injection of drugs that enhance synaptic GABA levels, such as tiagabine and vigabatrin, enhance SWDs in GAERS and WAG/Rij rats (Coenen et al., 1995; Depaulis and van Luijtelaar, 2006; Bouwman et al., 2007), and similar effects are seen following bilateral injections of γ -vinyl GABA or the GABA_A receptor agonist, muscimol, into the medial part of the ventral lateral thalamus containing thalamic relay nuclei (Liu et al., 1991). In contrast, injections of γ -vinyl GABA or muscimol into the nRT suppress SWDs (Liu et al., 1991). Local injections with VU0360172 in the VB or nRT in combination with CB1 receptor antagonists and/or GABAergic drugs may help to clarify the precise mechanism whereby pharmacological enhancement of mGlu5 receptors reduces absence seizures in WAG/Rij rats.

We have also found an increased expression of mGlu5 receptors in the sensorymotor cortex of WAG/Rij rats, although receptor function was not significantly enhanced. The significance of this finding is uncertain because pharmacological blockade of mGlu5 receptors did not affect absence seizures. We cannot exclude that an enhanced expression of mGlu5 receptors in the cerebral cortex represents a compensatory protective mechanism against SWDs that was further amplified by VU0360172. Again, experiments in which drugs are microinfused in the main stations of the cortico-thalamic-cortical network are necessary to disclose the precise role of mGlu5 receptors in the regulation of absence seizures.

The evidence that a selective mGlu5 receptor enhancer significantly reduced absence seizures in WAG/Rij rats without affecting motor behaviour is of great interest from a therapeutic standpoint. If activation of a single receptor type is warranted, receptor

enhancers are advantageous over orthosteric agonists because they recruit exclusively those receptors that are endogenously activated (i.e., they act in an activity-dependent manner). This places mGlu5 receptor enhancers in the right position as novel anti-absence drugs, which might correct the abnormalities in mGlu5 receptor function without recruiting “silent” mGlu5 receptors that could generate dose-related adverse effects. It should be highlighted that more than 20% of patients with absence epilepsy and those with atypical absence seizure are refractory to conventional medication (Mikati and Holmes, 1997; Panayiotopoulos, 1999; Ollivier et al., 2009). Speculatively, mGlu5 receptor PAMs could be developed for the treatment of patients with absence epilepsy refractory to conventional medications, since subtype selective ligands of mGlu receptors are now under Phase I and II clinical studies for the treatment of neurological and psychiatric disorders (Niswender and Conn, 2010).

Acknowledgments

We also wish to thank Elly Willemsvan Bree, Hans Krijnen and Saskia Hermeling for biotechnical assistance.

References

- Alagarsamy S, Saugstad J, Warren L, Mansuy IM, Gereau RW 4th, Conn PJ. NMDA-induced potentiation of mGluR5 is mediated by activation of protein phosphatase 2B/calceinurin. *Neuropharmacology*. 2005; 49:135–145. [PubMed: 16005030]
- Anderson JJ, Rao SP, Rowe B, Giracello DR, Holtz G, Chapman DF, Tehrani L, Bradbury MJ, Cosford ND, Varney MA. [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*. 2002; 303:1044–1051. [PubMed: 12438526]
- Aronica E, Gorter JA, Ijlst-Keizers H, Rozemuller AJ, Yankaya B, Leenstra S, Troost D. Expression and functional role of mGluR3 and mGluR5 in human astrocytes and glioma cells: opposite regulation of glutamate transporter proteins. *Eur J Neurosci*. 2003; 17:2106–2118. [PubMed: 12786977]
- Attucci S, Carlà V, Mannaioni G, Moroni F. Activation of type 5 metabotropic glutamate receptors enhances NMDA responses in mice cortical wedges. *Br J Pharmacol*. 2001; 132:799–806. [PubMed: 11181420]
- Avoli M, Gloor P. Interaction of cortex and thalamus in spike and wave discharges of feline generalized penicillin epilepsy. *Exp Neurol*. 1982; 76:196–217. [PubMed: 7084360]
- Awad H, Hubert GW, Smith Y, Levey AI, Conn PJ. Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. *J Neurosci*. 2000; 20:7871–7879. [PubMed: 11050106]
- Blumenfeld H. Cellular and network mechanisms of spike-wave seizures. *Epilepsia*. 2005; 46:21–33. [PubMed: 16302873]
- Bouwman BM, Suffczynski P, Midzyanovskaya IS, Maris E, van den Broek PL, van Rijn CM. The effects of vigabatrin on spike and wave discharges in WAG/Rij rats. *Epilepsy Res*. 2007; 76:34–40. [PubMed: 17643266]
- Burgess DL, Biddlecome GH, McDonough SI, Diaz ME, Zilinski CA, Bean BP, Campbell KP, Noebels JL. Beta subunit reshuffling modifies N- and P/Q-type Ca²⁺ channel subunit compositions in lethargic mouse brain. *Mol Cell Neurosci*. 1999; 13:293–311. [PubMed: 10328888]
- Chapman AG, Nanan K, Williams M, Meldrum BS. 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). *Neuropharmacology*. 2000; 39:1567–1574. [PubMed: 10854901]
- Coenen AM, van Luijckelaar EL. The WAG/Rij rat model for absence epilepsy: age and sex factors. *Epilepsy Res*. 1987; 1:297–301. [PubMed: 3143552]

- Coenen AM, Blezer EH, van Luijtelaar EL. Effects of the GABA-uptake inhibitor tiagabine on electroencephalogram, spike-wave discharges and behaviour of rats. *Epilepsy Res.* 1995; 21:89–94. [PubMed: 7588592]
- Coenen AM, van Luijtelaar EL. Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. *Behav Genet.* 2003; 33:635–655. [PubMed: 14574120]
- Cosford ND, Roppe J, Tehrani L, Schweiger EJ, Seiders TJ, Chaudary A, Rao S, Varney MA. [3H]-methoxymethyl-MTEP and [3H]-methoxy-PEPy: potent and selective radioligands for the metabotropic glutamate subtype 5 (mGlu5) receptor. *Bioorg Med Chem Lett.* 2003; 13:351–354. [PubMed: 12565928]
- Depaulis, A.; van Luijtelaar, G. Genetic models of absence epilepsy in the rat. In: Pitkänen, A.; Schwartkroin, PA.; Moshé, SL., editors. *Models of Seizures and Epilepsy.* Elsevier; Amsterdam: 2006. p. 233–248.
- Doherty AJ, Palmer MJ, Henley JM, Collingridge GL, Jane DE. (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) activates mGlu5, but no mGlu1, receptors expressed in CHO cells and potentiates NMDA responses in the hippocampus. *Neuropharmacology.* 1997; 36:265–267. [PubMed: 9144665]
- Drinkenburg WH, Coenen AM, Vossen JM, van Luijtelaar EL. Spike-wave discharges and sleep-wake states in rats with absence epilepsy. *Epilepsy Res.* 1991; 9:218–224. [PubMed: 1743184]
- Ferraguti F, Crepaldi L, Nicoletti F. Metabotropic glutamate 1 receptor: current concepts and perspectives. *Pharmacol Rev.* 2008; 60:536–581. [PubMed: 19112153]
- Inoue M, Peeters BW, van Luijtelaar EL, Vossen JM, Coenen AM. Spontaneous occurrence of spike-wave discharges in five inbred strains of rats. *Physiol Behav.* 1990; 48:199–201. [PubMed: 2122483]
- Izumi Y, Zorumski CF. NMDA receptors, mGluR5, and endocannabinoids are involved in a cascade leading to hippocampal long-term depression. *Neuropsychopharmacology.* 2012; 37:609–617. [PubMed: 21993209]
- Jasper HH, Kershman J. Electroencephalographic classification of the epilepsies. *Arch Neurol Psychiatry Chicago.* 1941; 45:903–943.
- Jesse CR, Savegnago L, Rocha JB, Nogueira CW. Neuroprotective effect caused by MPEP, an antagonist of metabotropic glutamate receptor mGluR5, on seizures induced by pilocarpine in 21-day-old rats. *Brain Res.* 2008; 10:197–203. [PubMed: 18242586]
- Jung KM, Mangieri R, Stapleton C, Kim J, Fegley D, Wallace M, Mackie K, Piomelli D. Stimulation of endocannabinoid formation in brain slice cultures through activation of group I metabotropic glutamate receptors. *Mol Pharmacol.* 2005; 68:1196–1202. [PubMed: 16051747]
- Laso W, Przewłocka B, Van Luijtelaar EL, Coenen AM, Przewłocki R. Endogenous opioid peptides in brain and pituitary of rats with absence epilepsy. *Neuropeptides.* 1992; 21:147–152. [PubMed: 1630600]
- Liu Z, Vergnes M, Depaulis A, Marescaux C. Evidence for a critical role of GABAergic transmission within the thalamus in the genesis and control of absence seizures in the rat. *Brain Res.* 1991; 545:1–7. [PubMed: 1650272]
- Liu XB, Muñoz A, Jones EG. Changes in subcellular localization of metabotropic glutamate receptor subtypes during postnatal development of mouse thalamus. *J Comp Neurol.* 1998; 395:450–465. [PubMed: 9619499]
- Lojková D, Mares P. Anticonvulsant action of an antagonist of metabotropic glutamate receptors mGluR5 MPEP in immature rats. *Neuropharmacology.* 2005; 49:219–229. [PubMed: 15950993]
- Lourenço Neto F, Schadrack J, Berthele A, Zieglgänsberger W, Tölle TR, Castro-Lopes JM. Differential distribution of metabotropic glutamate receptor subtype mRNAs in the thalamus of the rat. *Brain Res.* 2000; 854:93–105. [PubMed: 10784111]
- Mannaioni G, Marino MJ, Valenti O, Traynelis SF, Conn PJ. Metabotropic glutamate receptors 1 and 5 differentially regulate CA1 pyramidal cell function. *J Neurosci.* 2001; 21:5925–5934. [PubMed: 11487615]
- Mathiesen JM, Svendsen N, Bräuner-Osborne H, Thomsen C, Ramirez MT. Positive allosteric modulation of the human metabotropic glutamate receptor 4 (hmGluR4) by SIB-1893 and MPEP. *Br J Pharmacol.* 2003; 138:1026–1030. [PubMed: 12684257]

- Meeren HK, Pijn JP, van Luijtelaar EL, Coenen AM, Lopes da Silva FH. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. *J Neurosci*. 2002; 22:1480–1495. [PubMed: 11850474]
- Meeren H, van Luijtelaar G, Lopes da Silva F, Coenen A. Evolving concepts on the pathophysiology of absence seizures: the cortical focus theory. *Arch Neurol*. 2005; 62:371–376. [PubMed: 15767501]
- Mikati MA, Holmes GL. Lamotrigine in absence and primary generalized epilepsies. *J Child Neurol*. 1997; 1:29–37.
- Ngomba RT, Ferraguti F, Badura A, Citraro R, Santolini I, Battaglia G, Bruno V, De Sarro G, Simonyi A, van Luijtelaar G, Nicoletti F. Positive allosteric modulation of metabotropic glutamate 4 (mGlu4) receptors enhances spontaneous and evoked absence seizures. *Neuropharmacology*. 2008; 54:344–354. [PubMed: 18022649]
- Ngomba RT, Santolini I, Salt TE, Ferraguti F, Battaglia G, Nicoletti F, van Luijtelaar G. Metabotropic glutamate receptors in the thalamocortical network: strategic targets for the treatment of absence epilepsy. *Epilepsia*. 2011a; 52:1211–1222. [PubMed: 21569017]
- 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. Protective role for type-1 metabotropic glutamate receptors against spike and wave discharges in the WAG/Rij rat model of absence epilepsy. *Neuropharmacology*. 2011b; 60:1281–1291. [PubMed: 21277877]
- Nicoletti F, Iadarola MJ, Wroblewski JT, Costa E. Excitatory amino acid recognition sites coupled with inositol phospholipid metabolism: developmental changes and interaction with alpha 1-adrenoceptors. *Proc Natl Acad Sci U S A*. 1986; 83:1931–1935. [PubMed: 2869493]
- Nicoletti F, Bockaert J, Collingridge GL, Conn PJ, Ferraguti F, Schoepp DD, Wroblewski JT, Pin JP. Metabotropic glutamate receptors: from the workbench to the bedside. *Neuropharmacology*. 2011; 60:1017–1041. [PubMed: 21036182]
- Niswender CM, Conn PJ. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu Rev Pharmacol Toxicol*. 2010; 50:295–322. [PubMed: 20055706]
- Ollivier ML, Dubois MF, Krajcinovic M, Cossette P, Carmant L. Risk factors for valproic acid resistance in childhood absence epilepsy. *Seizure*. 2009; 18:690–694. [PubMed: 19836978]
- Panayiotopoulos CP. Typical absence seizures and their treatment. *Arch Dis Child*. 1999; 81:351–355. [PubMed: 10490445]
- Paxinos, G.; Watson, C. *The Rat Brain in the Stereotaxic Coordinates*. Academic Press Ltd; London: 2005.
- Peeters BW, van Rijn CM, Vossen JM, Coenen AM. Involvement of NMDA receptors in non-convulsive epilepsy in WAG/Rij rats. *Life Sci*. 1990; 47:523–529. [PubMed: 2169562]
- Pisani A, Gubellini P, Bonsi P, Conquet F, Picconi B, Centonze D, Bernardi G, Calabresi P. Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. *Neuroscience*. 2001; 106:579–587. [PubMed: 11591458]
- Polack PO, Mahon S, Chavez M, Charpier S. Inactivation of the somatosensory cortex prevents paroxysmal oscillations in cortical and related thalamic neurons in a genetic model of absence epilepsy. *Cereb Cortex*. 2009; 19:2078–2091. [PubMed: 19276326]
- 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. 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 anti-psychotic activity. *Mol Pharmacol*. 2010; 78:1105–1123. [PubMed: 20923853]
- Romano C, Sesma MA, McDonald CT, O'Malley K, Van den Pol AN, Olney JW. Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. *J Comp Neurol*. 1995; 355:455–469. [PubMed: 7636025]
- Salt TE, Binns KE. Contributions of mGlu1 and mGlu5 receptors to interactions with N-methyl-D-aspartate receptor-mediated responses and nociceptive sensory responses of rat thalamic neurons. *Neuroscience*. 2000; 100:375–380. [PubMed: 11008175]

- Schridde U, van Luijtelaar G. The influence of strain and housing on two types of spike-wave discharges in rats. *Genes Brain Behav.* 2004; 3:1–7. [PubMed: 14960010]
- Smyk MK, Coenen AM, Lewandowski MH, van Luijtelaar G. Endogenous rhythm of absence epilepsy: relationship with general motor activity and sleep-wake states. *Epilepsy Res.* 2011; 93:120–127. [PubMed: 21146957]
- Snead OC 3rd, Banerjee PK, Burnham M, Hampson D. Modulation of absence seizures by the GABA(A) receptor: a critical role for metabotropic glutamate receptor 4 (mGluR4). *J Neurosci.* 2000; 20:6218–6224. [PubMed: 10934271]
- Stinehelfer S, Vruwink M, Burette A. Immunolocalization of mGluR1alpha in specific populations of local circuit neurons in the cerebral cortex. *Brain Res.* 2000; 861:37–44. [PubMed: 10751563]
- Sun QQ, Zhang Z, Jiao Y, Zhang C, Szabó G, Erdelyi F. Differential metabotropic glutamate receptor expression and modulation in two neocortical inhibitory networks. *J Neurophysiol.* 2009; 101:2679–2692. [PubMed: 19244358]
- Thomas P, Valton L, Genton P. Absence and myoclonic status epilepticus precipitated by antiepileptic drugs in idiopathic generalized epilepsy. *Brain.* 2006; 129:1281–1292. [PubMed: 16513683]
- Tu JC, Xiao B, Naibitt S, Yuan JP, Petralia RS, Brakeman P, Doan A, Aakalu VK, Lanahan AA, Sheng M, Worley PF. Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. *Neuron.* 1999; 23:583–592. [PubMed: 10433269]
- Uchigashima M, Narushima M, Fukaya M, Katona I, Kano M, Watanabe M. Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. *J Neurosci.* 2007; 27:3663–3676. [PubMed: 17409230]
- Ugolini A, Corsi M, Bordi F. Potentiation of NMDA and AMPA responses by the specific mGluR5 agonist CHPG in spinal cord motoneurons. *Neuropharmacology.* 1999; 38:1569–1576. [PubMed: 10530818]
- van de Bovenkamp-Janssen MC, van der Kloet JC, van Luijtelaar G, Roubos EW. NMDA-NR1 and AMPA-GluR4 receptor subunit immunoreactivities in the absence epileptic WAG/Rij rat. *Epilepsy Res.* 2006; 69:119–128. [PubMed: 16487682]
- van Luijtelaar EL, Coenen AM. Two types of electrocortical paroxysms in an inbred strain of rats. *Neurosci Lett.* 1986; 70:393–397. [PubMed: 3095713]
- van Luijtelaar EL, Coenen AM. Circadian rhythmicity in absence epilepsy in rats. *Epilepsy Res.* 1988; 2:331–336. [PubMed: 3143564]
- van Luijtelaar G, Sitnikova E. Global and focal aspects of absence epilepsy: the contribution of genetic models. *Neurosci Biobehav Rev.* 2006; 30:983–1003. [PubMed: 16725200]
- van Rijn CM, Gaetani S, Santolini I, Badura A, Gabova A, Fu J, Watanabe M, Cuomo V, van Luijtelaar G, Nicoletti F, Ngomba RT. WAG/Rij rats show a reduced expression of CB1 receptors in thalamic nuclei and respond to the CB1 receptor agonist, R(+)WIN55,212-2, with a reduced incidence of spike-wave discharges. *Epilepsia.* 2010; 51:1511–1521. [PubMed: 20132294]
- Varma N, Carlson GC, Ledent C, Alger BE. Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. *J Neurosci.* 2001; 21:RC188. [PubMed: 11734603]
- Wijetunge LS, Till SM, Gillingwater TH, Ingham CA, Kind PC. mGluR5 regulates glutamate-dependent development of the mouse somatosensory cortex. *J Neurosci.* 2008; 28:13028–13037. [PubMed: 19052194]
- Won YJ, Puhl HL 3rd, Ikeda SR. Molecular reconstruction of mGluR5a-mediated endocannabinoid signaling cascade in single rat sympathetic neurons. *J Neurosci.* 2009; 29:13603–13612. [PubMed: 19864572]
- Zhang L, Jones EG. Corticothalamic inhibition in the thalamic reticular nucleus. *J Neurophysiol.* 2004; 91:759–766. [PubMed: 14586030]

Fig. 1. Immunohistochemical analysis of mGlu5 receptors in the cortico-thalamic-cortical network of ACI and WAG/Rij rats. Representative mGlu5 receptor immunostaining in the cortex (M1 and S1) and thalamus (nRT and VB) of ACI and WAG/Rij rats at 2 and 8 months of age are shown in (A) and (D), respectively. The densitometric analysis was performed in M1, S1, nRT and VB (the areas are delineated with black discontinuous illustrative boxes in A, D). Data of densitometric analysis are shown in (B, C, E, F). Values are means $\pm$ S.E.M. and were calculated from the average of 5 coronal sections for each animal ($n = 6$ animals per group). * $p < 0.05$ vs. the corresponding values obtained in the same strain at 2 months, # $p < 0.05$ vs. the corresponding values obtained in ACI rats at 8 months.

Fig. 2.

Immunoblot analysis of mGlu5 receptors in the ventrobasal thalamus and sensorymotor cortex of ACI and WAG/Rij rats. Representative immunoblot of mGlu5 receptors in the ventrobasal thalamic nuclei (A), somatosensory cortex (C) and motor cortex (E) of 2- and 8-month old ACI or WAG/Rij rats are shown. Densitometric analysis is shown in (B), (D), and (F), respectively. Statistical analysis was carried out by two-way ANOVA, with the rat strain and age as variable factors. Values are means $\pm$ S.E.M. ($n = 8$ animals per group). Post hoc analysis: $*p < 0.05$ vs. age-matched control ACI rats. In each individual blot, protein extracts from rats of each experimental group were loaded in order to obtain relative comparisons among the groups.

Fig. 3.

Changes in mGlu5 receptor-mediated PI hydrolysis in the sensorymotor cortex and thalamus of WAG/Rij rats. *In vivo* measurements of PI hydrolysis in the sensorymotor cortex and thalamus of 8-month old WAG/Rij rats and age-matched controls (ACI rats) was carried out as outlined in (A). PtdIns-4,5, P_2 = phosphatidylinositol-4,5-bisphosphate; Ins-1,4,5- P_3 = inositol-1,4,5-trisphosphate; InsP = inositolmonophosphate. DHPG-stimulated PI hydrolysis in the sensorymotor cortex (B) and in thalamus (C) of WAG/Rij rats ($n = 5$ animals per group). * $p < 0.05$ (Student's t test) vs. values obtained in controls rats. Basal values of $[^3\text{H}]\text{InsP}$ formation normalized by the amount of total radioactivity in each sample were 1.45 ± 0.17 and 1.40 ± 0.15 (means $\pm$ S.E.M.) in the sensorymotor cortex of ACI and WAG/Rij rats, respectively. Basal values of $[^3\text{H}]\text{InsP}$ formation normalized by the amount of total radioactivity in each sample were 1.68 ± 0.08 and 1.72 ± 0.12 (means $\pm$ S.E.M.) in the thalamus of ACI and WAG/Rij rats, respectively.

Fig. 4.

Pharmacological potentiation of mGlu5 receptors reduces spike and wave discharges (SWDs) in WAG/Rij rats. Pharmacological potentiation of mGlu5 receptors reduces both number and mean duration of SWDs in symptomatic WAG/Rij rats (C, D). No effect of MTEP alone (A, B) or combined with VU0360172 (E, F) on number and mean duration of SWDs. Values are means $\pm$ S.E.M. of 4/8 animals (MTEP 10 mg/kg = 4 rats, MTEP 30 mg/kg = 6 rats, VU0360172 3 or 10 mg/kg = 6 rats, MTEP + VU0360172 = 8 rats). $p < 0.05$ (ANOVA for repeated measures followed by Bonferroni's test) vs. the corresponding values at baseline (T_0) (*) or vs. the corresponding values obtained in WAG/Rij rats treated with vehicle (#).

Fig. 5. Assessment of motor behaviour in 8-month WAG/Rij rats treated with vehicle or VU0360172. Values are means + S.E.M. ($n = 6$ rats per group). ANOVA for repeated measures showed a significant effect of time with no drug effect and no drug $\times$ time interaction.