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ScienceDirect
Neuropharmacology 51 (2006) 1058–1067
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Effects of non-competitive AMPA receptor antagonists injected into some brain areas of WAG/Rij rats, an animal model of generalized absence epilepsy

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Received 7 December 2005; received in revised form 24 May 2006; accepted 29 June 2006

Abstract

CFM-2 [1-(4-aminophenyl)-3,5-dihydro-7,8-dimethoxy-4H-2,3-benzodiazepin-4-one] and THIQ-10c [*N*-acetyl-1-(4-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline], are two non-competitive 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid (AMPA) receptor antagonists, which demonstrated to antagonize generalized tonic-clonic seizures in different animal models. We have evaluated the effects of such compounds in a genetic animal model of absence epilepsy, the WAG/Rij rat. Animals were focally microinjected into specific brain areas of the cortico-thalamic circuit in order to evaluate the effects of these compounds on the number and duration of epileptic spike-wave discharges (SWDs) and better characterize the role of AMPA neurotransmission in this animal model. The focal microinjection of the two AMPA antagonists into some thalamic nuclei (ventralis posteromedialis (VPM), reticularis (NRT), ventralis posterolateralis (VPL) and the primary somatosensory forelimb region (S1FL)) was, generally, not able to significantly modify the occurrence of SWDs. Whereas, both compounds were able to reduce the number and duration of SWDs dose-dependently when microinjected into the peri-oral region of the primary somatosensory cortex (S1po). These findings suggest that AMPA receptor antagonists might play a role in absence epilepsies and that it might depend on the involvement of specific neuronal areas.

Keywords: Absence seizures; AMPA receptor antagonists; CFM-2; THIQ-10c; WAG/Rij rat; Thalamus; Primary somatosensory cortex

1. Introduction

Generalized absence seizures in humans are characterized by brief periods of behavioural arrest, inability to answer questions and occasional automatism. These seizures are accompanied by a characteristic electroencephalographic (EEG) pattern defined by 3–5 Hz spike and wave discharges (SWDs) (Snead, 1992; Kerr and Ong, 1995).

Excessive glutamatergic neurotransmission is understood to be one of the primary pathological mechanisms behind the aetiology of numerous types of epilepsy (Chapman, 1998; Meldrum et al., 1999). The excitatory system seems to play an identical role in the two main kinds of epilepsy: convulsive or tonic-clonic generalized epilepsy and non-convulsive or absence epilepsy. Generally, glutamate agonists facilitate and antagonists reduce both convulsive and non-convulsive epilepsy (Coenen and van Luijtelaar, 2003).

Rats of the Wistar Albino Glaxo from Rijswijk (WAG/Rij) inbred strain with spontaneous bilaterally synchronous generalized SWDs are recognized as a good genetic model of

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0028-3908/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2006.06.014

human absence epilepsy (Coenen et al., 1992; van Luijtelaar et al., 1989). Electrophysiological studies indicated that abnormal discharges on the EEG are generalized and cortico-thalamic network is primarily involved (Seidenbecher and Pape, 2001; Coenen and van Luijtelaar, 2003; Landisman et al., 2002; Blumenfeld and McCormick, 2000; Steriade, 2001; Snead, 1995). Several neurotransmitters that regulate thalamocortical functions, also including glutamate and GABA, play significant role in the pathophysiology of this type of epilepsy (Snead, 1995; Staak and Pape, 2001; Crunelli and Leresche, 1991; Knight and Bowery, 1992). WAG/Rij rats represent a useful model of gene-linked absence epilepsy (Coenen and van Luijtelaar, 2003).

In previous reports, the effects of (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine maleate (MK-801), a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist were investigated in WAG/Rij rats, and a reduction of the number and mean cumulative duration of SWDs was found (Filakovszky et al., 2001; Peeters et al., 1989). In addition, in a recent study, Kaminski et al. (2001) examined the effect of the AMPA/kainate receptor antagonist, LY 300164 (talampanel or GYKI 53773) in the same model. These authors found weak anti-absence effects of this compound. In particular, only the highest dose tested (16 mg/kg) caused a significant decrease in the number of SWDs during a short time window (30–60 min post-injection). More recently, Jakus et al. (2004) examined the effect of other two AMPA/kainate receptor antagonists, GYKI 52466 and GYKI 53405, the racemate of talampanel, in the same model. These authors reported weak effects of both compounds on SWDs even if GYKI 52466 was able to significantly decrease the number of SWDs induced by 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPTA), a 5-HT_{1A} receptor antagonist.

The purpose of the present study was to ascertain the activity of two non-competitive AMPA receptor antagonists, CFM-2 [1-(4-aminophenyl)-3,5-dihydro-7,8-dimethoxy-4H-2,3-benzodiazepin-4-one] and THIQ-10c [*N*-acetyl-1-(4-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline] against absence seizures in the WAG/Rij rat model and investigate the role of AMPA receptors in specific brain areas primary involved in the generation of absence seizures. In previous studies, both compounds have been demonstrated to act selectively as non-competitive antagonists of AMPA receptors (Chimirri et al., 1997; Gitto et al., 2003) and to possess anticonvulsant properties in various generalized tonic-clonic animal models of seizures (De Sarro et al., 1995, 2003). Therefore, the effects of a focal bilateral administration of various doses of such compounds into some selected thalamic nuclei (nucleus ventralis posteromedialis thalami (VPM), nucleus reticularis thalami (NRT), nucleus ventralis posterolateralis thalami (VPL)) on the typical epileptic SWDs of WAG/Rij rats was investigated. In addition, in order to better evaluate the corticothalamic circuit, focal bilateral microinjection of the same AMPA antagonists was also carried out into the peri-oral (S1po) or the forelimb (S1FL) region of the primary somatosensory cortex. Thalamic sites have been chosen according to previous reports demonstrating that nuclei of the ventrobasal complex, such as nucleus ventralis posteromedialis thalami (VPM) and nucleus ventralis posterolateralis thalami (VPL) and the nucleus reticularis thalami (NRT) are essential for generation and maintenance of absence seizures (Meeren et al., 2005; Danober et al., 1998). The cortical sites have been chosen on a recent demonstration that, in the WAG/Rij rat, an initial site of absence seizures is present in the primary somatosensory cortex (S1po) (Meeren et al., 2002, 2005).

2. Materials and methods

2.1. Animals

Male WAG/Rij rats of 8–9 months of age and a body weight of 280–340 g were purchased from Harlan Italy (Correzzana, Milan, Italy). Rats were housed three or four per cage (350 × 530 mm long × 180 mm high) under stable conditions of humidity (60 ± 5%) and temperature (21 ± 2 °C) and were kept under a reversed light/dark (12/12 h) cycle (light on at 19:00 h). All rats were given free access to food and water until the time of experiments. Procedures involving animals and their care were conducted in conformity with the international and national law and policies (European Communities Council Directive of 24th November 1986, 86/609EEC). Animals were used only for this protocol, and at the end of the experiments, injection sites were verified by histology inspection following focal injection of Evan's blue dye.

2.2. Experimental design

CFM-2, THIQ-10c or vehicle were administered in epileptic animals; the administration was performed focally into specific brain areas in a volume of 0.5 μl/side/rat. Control animals received equal volumes of vehicle (40% solution of 2-hydroxypropyl-γ-cyclodextrin, Research Biochemicals International, Natick, USA) at the respective times before tests.

2.3. Experimental protocol in WAG/Rij rats

All WAG/Rij rats were chronically implanted, under chloral hydrate anaesthesia (400 mg/kg i.p.; Carlo Erba, Milan, Italy), using a Kopf stereotaxic instrument, with five cortical electrodes for EEG recordings and two guide cannulae for focal administration. Stainless steel screw electrodes were implanted on the dura mater over the cortex: two in the frontal region (coordinates with skull surface flat and bregma zero-zero: AP = 2; L = ± 2.5) and two in the parietal region (AP = − 6; L = ± 2.5) according to the atlas coordinates of Paxinos and Watson (1986). The ground electrode was placed over the cerebellum. The electrodes' leads were soldered to a miniature connector, which was head mounted with cranioplastic cement and mounting screws. Cannulae were stereotaxically implanted respectively into the: nucleus ventralis posteromedialis thalami (VPM, AP = > − 3.3; L = ± 2.6; H = 6 mm from bregma), nucleus reticularis thalami (NRT, AP = − 2.8; L = ± 3.4; H = 5.8 mm from bregma), or nucleus ventralis posterolateralis thalami (VPL, AP = − 2,3; L = ± 2.8; H = 6 mm from bregma) and into the peri-oral region of the primary somatosensory cortex (S1po, AP = > − 2.1; L = ± 5.5; H = 4 mm from bregma) or the primary somatosensory forelimb region (S1FL, AP = > − 2.1; L = ± 3; H = 2 mm from bregma) according to the coordinates of the atlas by Paxinos and Watson (1986). All animals were allowed at least 1 week of recovery and usually handled twice a day. In order to habituate the animals to the recording conditions, the rats were connected to recording cables for at least 3 days before the experiments. The animals were attached to a multichannel amplifier (Astro-Med, West Warwich, USA) by a flexible recording cable and an electric swivel, fixed above the cages, permitting free movements. Separate groups of rats (6 rats for each dose if not differently indicated) were used to determine the effects of vehicle, CFM-2 or THIQ-10c.

2.4. Drug administration and EEG recording protocol

At least a week after surgery animals that displayed spontaneous bilateral SWDs have been randomly divided in groups of 8–10 and assigned to each drug; in each group, rats have been treated in a randomized order with the different doses of the drug under study or with vehicle, with an interval of at least 3 days between injections. Following this protocol every animal has been injected at the most 3 times, the randomization aimed to obtain a schedule with the administration of increasing doses to every animal and avoiding the microinjection of the same dose to the same animal. The interval of three days between injections was sufficient for recovery and this was confirmed by recordings with no injections on the second day of interval (>48 h after last injection). The results of our histological examination showed no gross physical damage which is also confirmed by the fact that SWDs still occurred normally in this animal. Every recording session lasted 6 h: 1 h baseline recording without injection, and 5 h recording after the focal administration of vehicle, CFM-2 or THIQ-10c. Injection cannula was lowered 2 mm beyond the edge of the guide cannula to the nuclei. The vehicle or drug was infused in a volume of 0.5 μl/side via a Hamilton syringe connected to a CMA/100 infusion pump. The injection cannula was withdrawn 1 min following infusion.

EEG signals were amplified and conditioned by analog filters (filtering: below 1 Hz and above 30 Hz at 6 dB/octave) and subjected to an analogue-to-digital conversion with a sampling rate of 64 Hz. The quantification of absence seizures was based on the number and the duration of electroencephalogram SWDs, as previously described (Russo et al., 2004; Gareri et al., 2005). Briefly, the number and duration of SWDs for each rat were summarized in 30 min intervals (epochs) for 1 h before and 5 h after drug or vehicle treatment, and scored by visual inspection of the EEG recordings. To assess the pharmacological effect of the compounds over time, each 5 h electroencephalogram recording was divided into 30 min, the cumulated SWDs' duration and number per epoch after drug administration were calculated, and then normalised by dividing with the corresponding duration for the 30 min period after vehicle injection in each animal group and presented as means ± S.E.M. During each recording the behavioural changes after drug treatment in comparison to vehicle were noted. Animals' behaviour was recorded on videotape and analyzed later by two independent persons, in parallel, all of them expert in rat behaviour, according to Coenen and van Luijtelaar (1989) to exclude possible interference between side effects and absence pathology, animals that displayed behavioural side effects have been discarded from data analysis.

2.5. Tissue preparation and Immunohistochemistry

Six WAG/Rij male adult rats (8–9 months old, 280–340 g) were deeply anesthetized with chloral hydrate (400 mg/kg; i.p.) and sacrificed by transcardiac perfusion with cold PBS, pH 7.4 and subsequently with cold 4% paraformaldehyde (PFA) containing 0.2% saturated picric acid in PBS. Brains were removed, post-fixed overnight at 4 °C in the same fixative solution, and then stored in 0.1 M phosphate buffer (PB) containing 20% sucrose overnight at 4 °C. The frozen sections were cut in a coronal plane at a thickness of 50 μm by a microtome. The sections were rinsed in PBS twice and immersed in 0.3% H_2O_2 in PBS for 10 min followed by three rinses in PBS. The sections were blocked with 10% goat serum (GIBCO BRL) in PBS for 1 h and incubated overnight at 4 °C with mouse anti-GluR2 monoclonal antibody (1:100; ZYMED Laboratories Inc., San Francisco) followed by peroxidase-conjugated goat anti-mouse IgG (1:300; Jackson Immuno Research Laboratory) for 3 h at room temperature. The sections were rinsed in acetate-imidazole buffer (50 mM sodium acetate, 10 mM imidazole, pH 7.2 adjusted with glacial acetate acid) and developed in chromagen solution (0.04% diaminobenzidine; DAB), 2.5% $NiSo_4$ and 0.005% H_2O_2 in acetate-imidazole buffer, pH 7.2) for 10–20 min. Following a series of washes in acetate-imidazole buffer, sections were mounted and coversliped. The immunoreactivity of the sections was analyzed under the microscope.

Immunoreactivity of GluR2 was determined by visual inspection of stained sections using light microscopy at 4–100× magnification. A semi-quantitative analysis of the stained sections has also been performed. Three independent specialist in immunohistochemistry have performed a microscopical inspection of the selected brain areas and have given a value to the different areas according to the following scale: (+) no reactivity; (++) low reactivity around cell bodies; (+++) marked staining around cell bodies.

2.6. Test compounds

The non-competitive AMPA receptor antagonists CFM-2 [1-(4-aminophenyl)-3,5-dihydro-7,8-dimethoxy-4H-2,3-benzodiazepin-4-one; MW = 311.3] and THIQ-10c [*N*-acetyl-1-(4-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline; MW = 345.83] were synthesized by Prof. Alba Chimirri group (Department of Medicinal Chemistry, University of Messina, Italy). The AMPA antagonists were dissolved in a 40% 2-hydroxypropyl-γ-cyclodextrin with sonication and mild heating. Vehicle (40% solution of 2-hydroxypropyl-γ-cyclodextrin) was obtained from Research Biochemicals International (Natick, USA).

2.7. Statistical analysis

EEG's recordings were subdivided in 30 min epochs, duration and number of SWDs were treated separately for every epoch. Such values were cumulated and data obtained were expressed as mean ± S.E.M. for every dose of compound. Such data were statistically compared with the respective vehicle control groups using a one-way analysis of variance (ANOVA), being the dose the only variable, followed by multiple comparison by Bonferroni. The data are expressed as means ± S.E.M. All tests used were two-sided and $P < 0.05$ was considered significant. The behavioural response was recorded but not statistically analysed for each animal.

3. Results

3.1. Effects of non-competitive AMPA antagonists, focally administered in thalamic nuclei, on the incidence of absence seizures

Focal administration of both compounds in thalamic nuclei had generally no marked effects on the number and duration of SWDs. In particular, both AMPA antagonists (CFM-2; 0.5, 1, 2 or 4 μg/side and THIQ-10c; 0.1, 0.5, 1 or 2 μg/side) administered into the VPM or VPL did not induce any significant modification in epileptic SWDs in comparison to control group up to the doses tested (Figs. 1 and 2). Whereas the focal microinjection into the NRT of the two highest doses (CFM-2; 2 or 4 μg/side and THIQ-10c; 1 or 2 μg/side) of both compounds induced a slight but significant dose-dependent decrease in both the number and duration of SWDs. This effect was short lasting with a rapid onset, being both SWDs parameters already reduced in the first 30 min epoch, and SWDs values returned to baseline levels after 90 min (Fig. 3). A higher dose injected in the NRT (CFM-2; 5 μg/side and THIQ-10c; 5 μg/side), instead, caused a rapid, non-significant, increase in the number and duration of SWDs and light slow wave sleep accompanied by notable behavioural effects such as sedation and hypomotility, similarly to the effects observed by Jakus and co-workers with the non-competitive AMPA antagonist, GYKI 52466 administered intraperitoneally in the same animal model (Jakus et al., 2004). No further characterization of this data has been performed being the aim of this study to characterize the relevance of AMPA neurotransmission in this model in specific areas during normal activity and not in the presence of side effects,

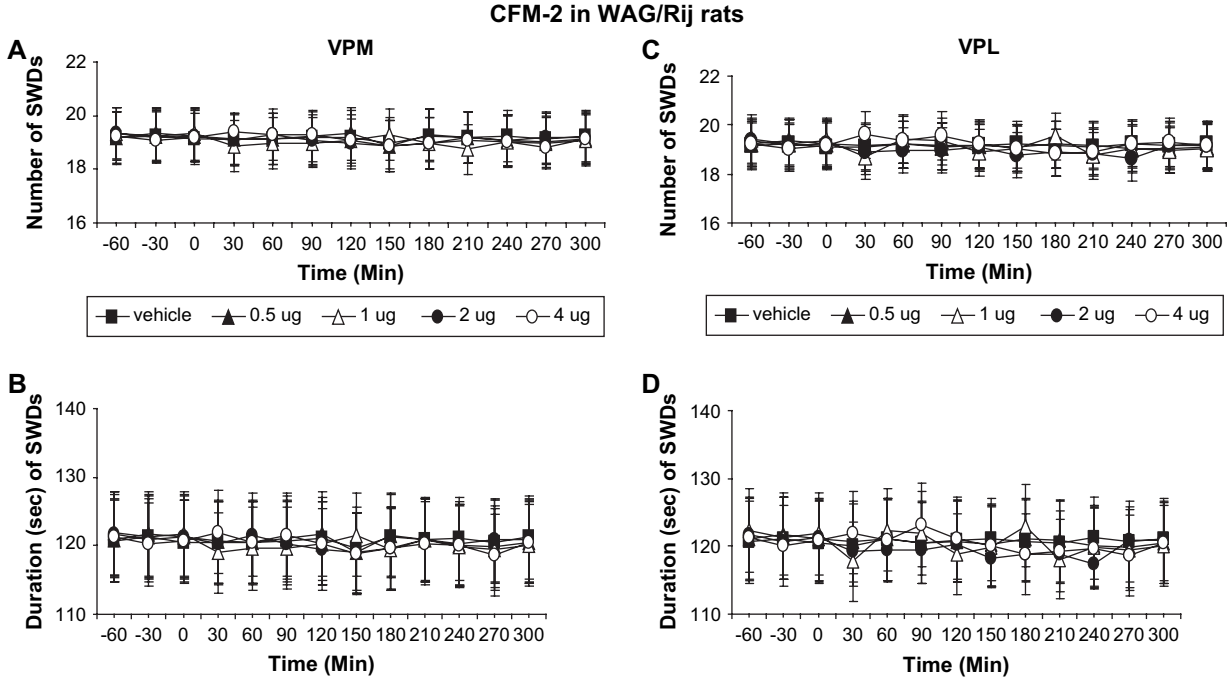

Fig. 1. Effects of the focal administration in the VPM and VPL of various doses of CFM-2 on the number (A, C); and duration (B, D) of SWDs in WAG/Rij rats.

furthermore, with the injection of such a high dose, diffusion to other areas due to the high lipophilicity of these molecules, it is not possible to ascertain if these side effects are related to the specific nucleus or due to diffusion to other areas at active concentration. Infusion of the vehicle into each of the thalamic nuclei produced no effects.

3.2. Effects of non-competitive AMPA antagonists, focally administered into cortical areas, on the incidence of absence seizures

Inversely, the focal administration of both AMPA antagonists, at the same doses injected into thalamic nuclei, into

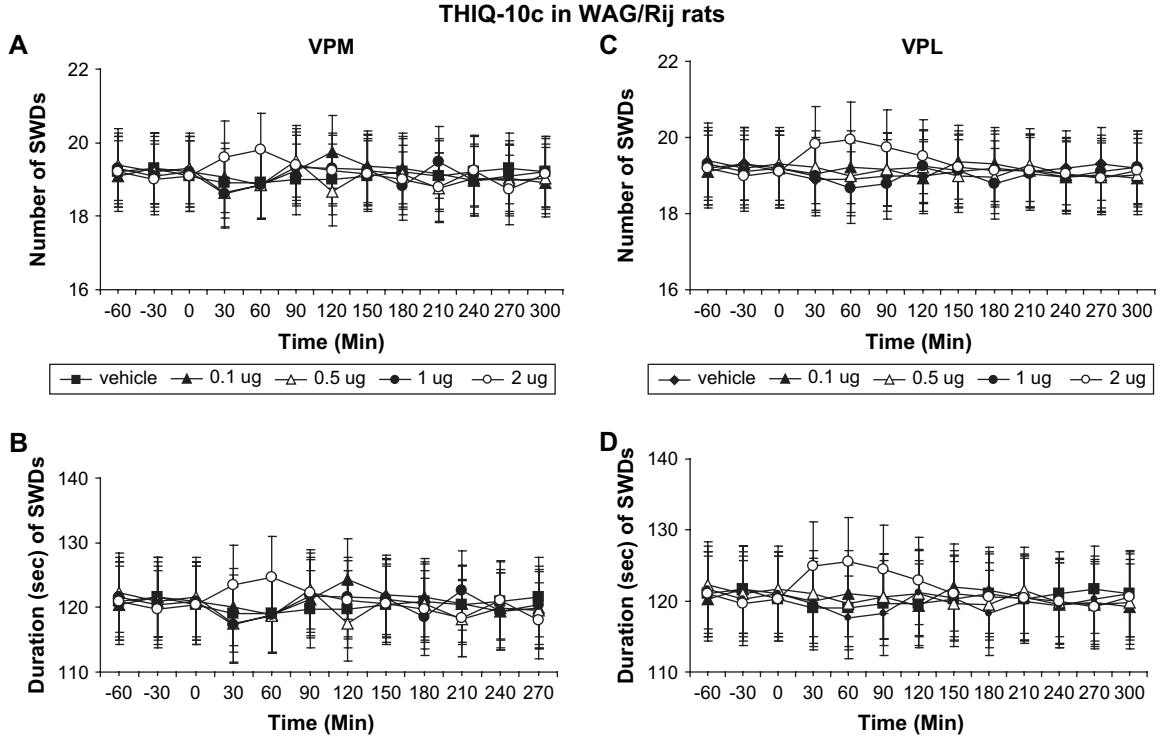

Fig. 2. Effects of the focal administration in the VPM and VPL of various doses of THIQ-10c on the number (A, C); and duration (B, D) of SWDs in WAG/Rij rats.

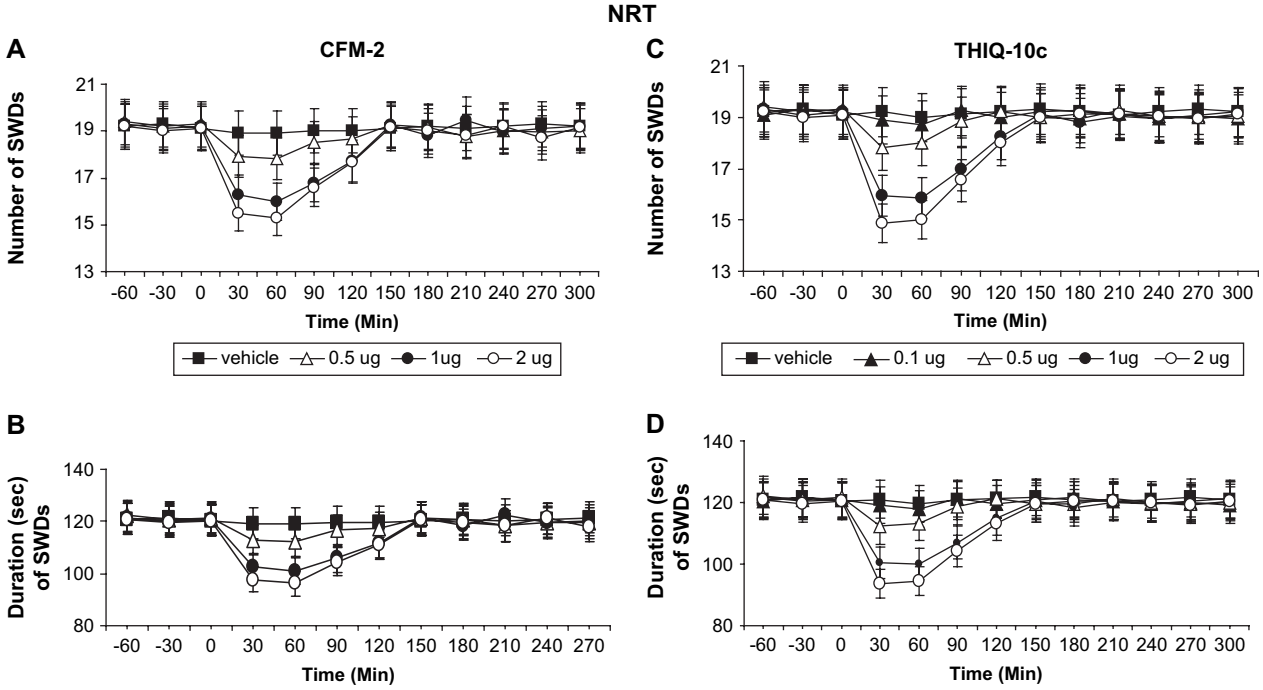

Fig. 3. Effects of the focal administration in the NRT of various doses of CFM-2 and THIQ-10c on the number (A, C); and duration (B, D) of SWDs in WAG/Rij rats.

the S1po induced a dose-dependent decrease in the incidence and duration of epileptic SWDs (Fig. 4). In particular, only the two highest doses induced a significant reduction of both the number and the duration of SWDs, also in this area the microinjection of a higher dose of both compounds induced marked behavioural effects and therefore, was excluded from the pharmacological evaluation, also in this area the effect had a rapid onset, a significant reduction was observed in the first 90 min with the peak of action during the 2nd epoch of the recording. In contrast, targeting the S1FL with both compounds produced no significant effects in SWD activity (Fig. 5).

3.3. Effects of AMPA antagonists on animal behaviour

From the analysis of animal behaviour we have not noticed any variation in comparison to control or vehicle treated animals at all doses studied, unless differently specified for higher doses which, however, have not been considered for further analysis for all the reasons above mentioned.

3.4. Immunohistochemistry

The aim of this set of experiments was to identify a gross difference in the distribution of GluR2 subunit of AMPA receptors within the brain areas in which both non-competitive AMPA antagonists have been microinjected. The microscopical inspection of the selected stained sections indicated that immunostaining for GluR2 is widely distributed in axons and dendrites with no marked apparent differences between the brain areas considered, white substance was completely negative. Differences were noted in the distribution of GluR2 subunits around cell bodies. GluR2 subunits were more concentrated around neurones belonging to cortical areas with no visible differences between S1po and S1FL, however, a more specific evaluation might highlight differences which can be easily overlooked with our protocol. Regarding the thalamic nuclei it appeared that the NRT is the most immunoreactive of all thalamic nuclei considered. GluR2 subunit immunoreactivity seemed to fade starting from the external nuclei going toward the most internal, namely, abundance of GluR2 immunoreactivity followed this order: NRT > VPL > VPM (Figs. 6 and 7, Table 1).

4. Discussion

In our study, we examined the effects of the focal administration into some selected brain areas of two selective non-competitive AMPA receptor antagonists, CFM-2 and THIQ-10c, on the occurrence and duration of spike-wave discharges (SWDs) in WAG/Rij rats to better characterize the role played by AMPA receptors within the thalamo-cortical circuit responsible for absence seizures in this animal model.

WAG/Rij rats show spontaneous SWDs; thus, they serve as a genetic rat model of human absence epilepsy (Coenen and van Luijtelaar, 2003). Absence epilepsy in these animals is characterized by the spontaneous occurrence of bilateral synchronous SWDs, which involve the entire cortical mantle (Snead, 1995). Thalamus, as a pacemaker structure for rhythmic cortical oscillations, is very likely responsible for the primary neuronal dysfunction underlying the generation of SWDs (Avanzini et al., 1993; Steriade, 1997). However, using non-linear association analysis of EEG signals from both

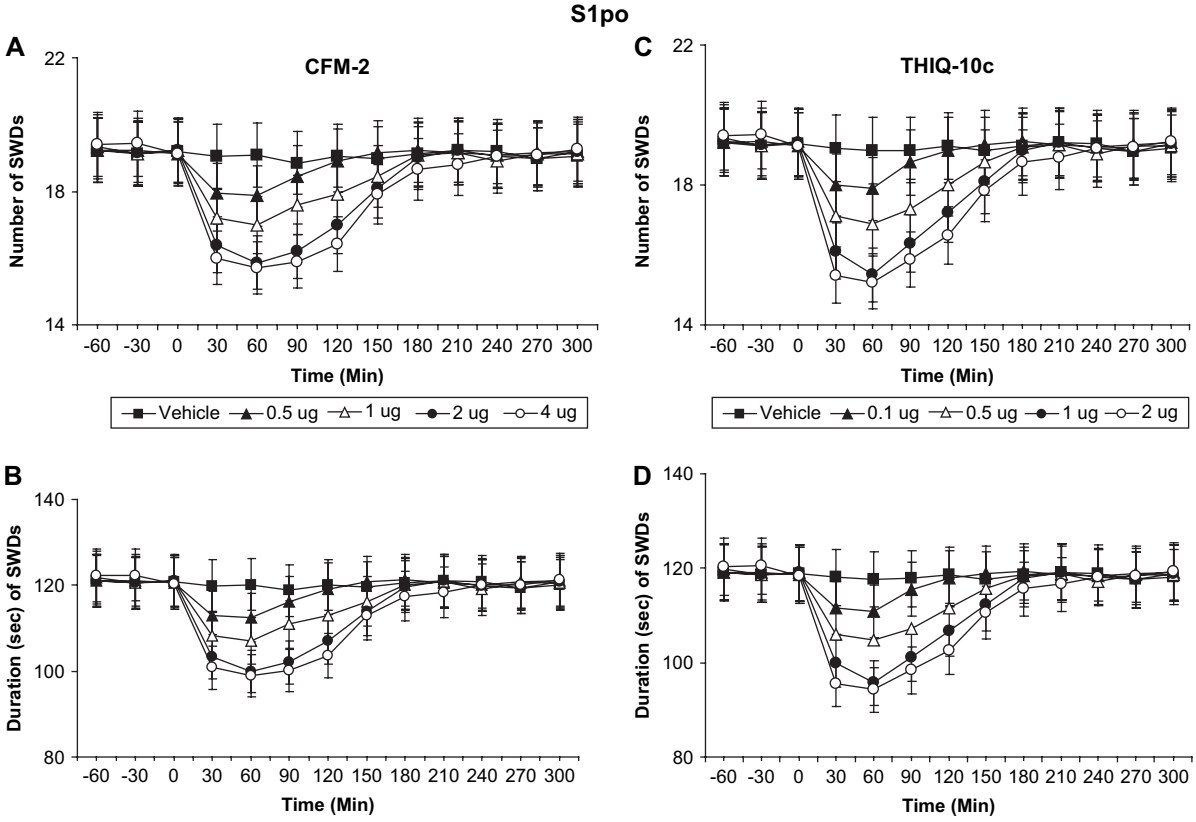

Fig. 4. Effects of the focal administration in the S1po of various doses of CFM-2 and THIQ-10c on the number (A, C); and duration (B, D) of SWDs in WAG/Rij rats.

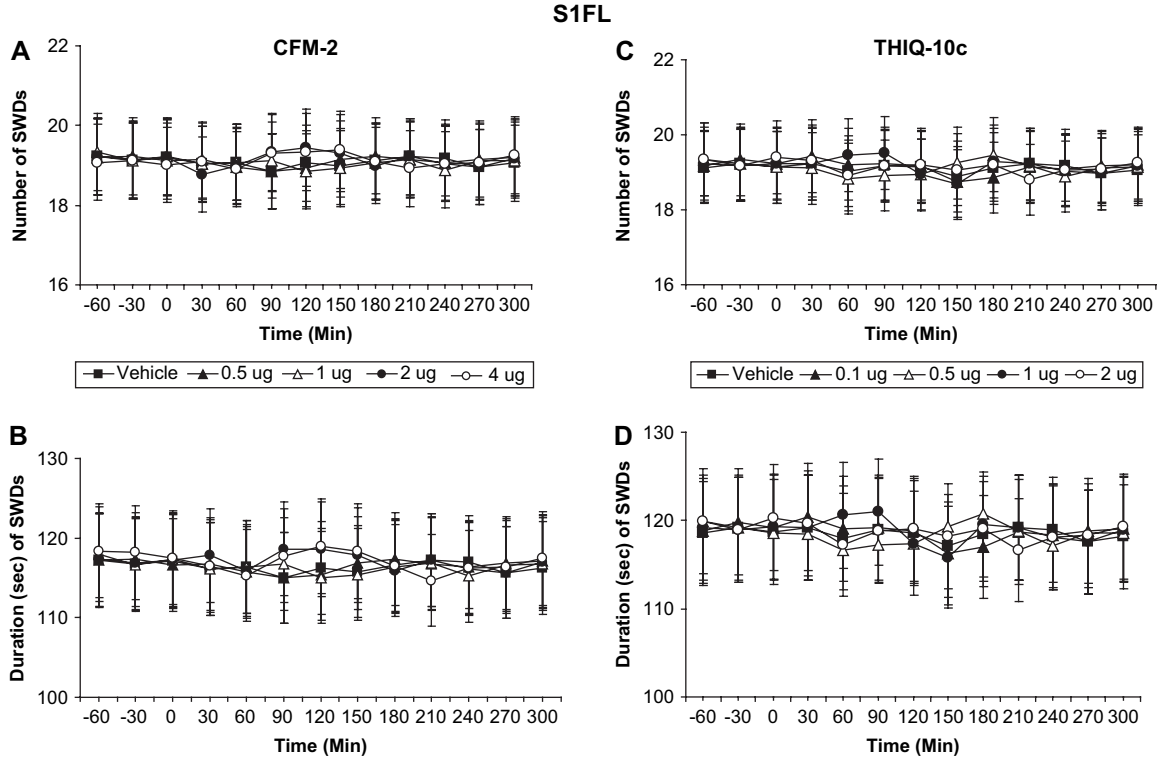

Fig. 5. Effects of the focal administration in the S1FL of various doses of CFM-2 and THIQ-10c on the number (A, C); and duration (B, D) of SWDs in WAG/Rij rats.

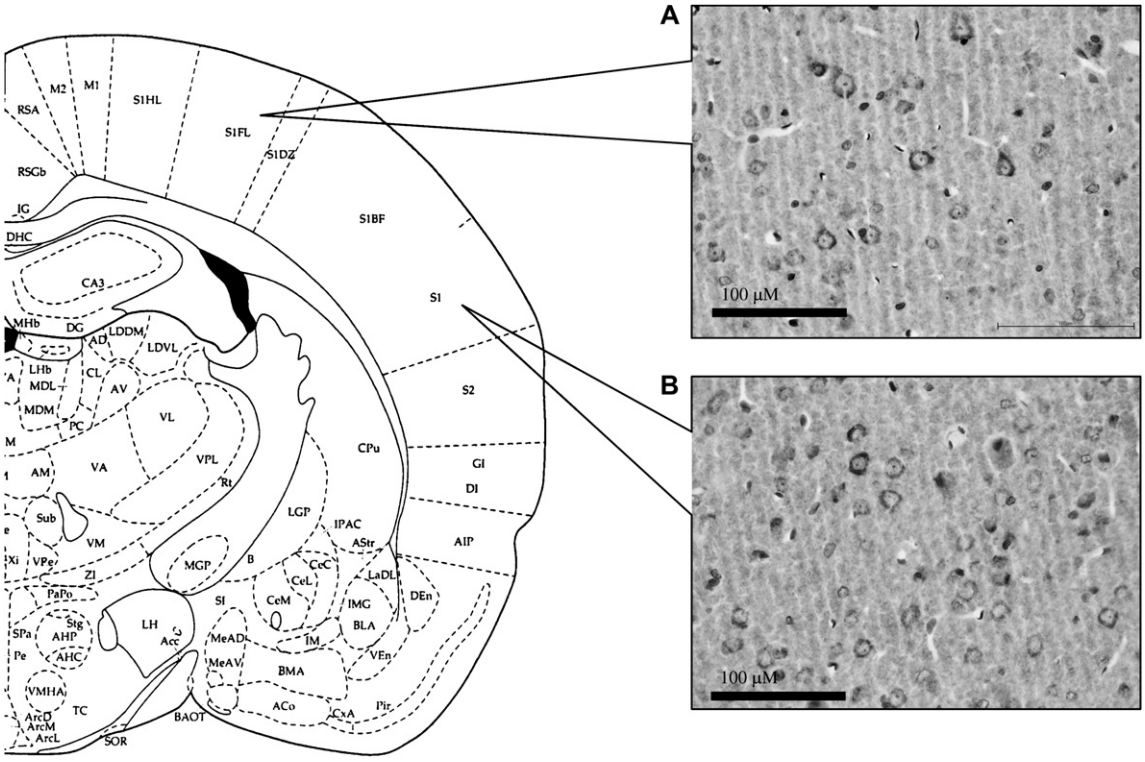

Fig. 6. Expression of GluR2 subunits of AMPA glutamate receptors in: (A) S1FL; and (B) S1po. Images have been obtained at a magnification of 40×, brain image has been adapted from Paxinos and Watson (1986).

thalamic and cortical structures during the first 500 ms of SWDs activity in the WAG/Rij rat, an initial site of absence seizures was recently identified within the primary somatosensory cortex (S1po) (Meeren et al., 2002, 2005). Moreover the microinfusion of ethosuximide into S1po of freely moving GAERS (Genetic Absence Epileptic Rats of Strasbourg) resulted in an immediate and almost complete cessation of seizure activity, comparable to that observed after systemic administration supporting the involvement of S1po as a specific focal origin, in the generation of genetically determined SWDs (Richards et al., 2003; Manning et al., 2004), also phenytoin and lidocaine injected in this area blocked SWDs (van Luijtelaar et al., 2005; Sitnikova and van Luijtelaar, 2005).

Regardless of the primary cause, synaptically released glutamate acting on ionotropic and metabotropic receptors appears to play a major role in the initiation and spread of seizure activity (Chapman, 1998, 2000; Meldrum, 1994, 2000; Meldrum et al., 1999; Moldrich et al., 2003). The role of NMDA receptors has been widely investigated in the epileptogenesis (Bradford, 1995; Meldrum et al., 1999; De Sarro and De Sarro, 1992, 1993); previous studies showed that the NMDA antagonist MK-801 decreases the spontaneous epileptic activity in WAG/Rij rats (Filakovszky et al., 1999, 2001; Peeters et al., 1989, 1990) on the other hand, another study, indicated that i.c.v. or focal intrathalamic NMDA injection in GAERS rapidly and dose-dependently suppressed SWDs at small doses and produced convulsions at high doses (Koerner et al., 1996), similarly to other results obtained in the GHB model of absence epilepsy (Banerjee and Snead, 1992, 1995). Peeters et al. (1994) demonstrated that AMPA administered i.c.v. increases the amount of SWDs in this animal model. Recently, there is abundant data concerning the AMPA receptor antagonists as highly effective anticonvulsant agents (Tarnawa and Vizi, 1998; De Sarro et al., 2003; Gitto et al., 2003, 2004). In a recent study, Kaminski et al. (2001) found weak effects of LY 300164; only the highest dose (16 mg/kg) significantly reduced the number of SWDs in WAG/Rij rats.

In the present study, we found that non-competitive AMPA receptor antagonists, CFM-2 and THIQ-10c, showed different effects depending on the brain site of administration. In particular, when the two AMPA antagonists were administered into the VPM, VPL and S1FL they were not able to modify the characteristic EEG recordings from WAG/Rij rats, at least at the free side-effects doses tested, whereas when administered into the NRT and S1po they induced a significant reduction of both the number and duration of SWDs. This latter effect appeared to be dose-dependent and short lasting.

From these data it appears that these molecules present a selectivity of action for some brain areas, namely S1po and NRT, whereas are not able to affect absence seizures when microinjected in other brain areas still involved in the generation and maintenance of SWD rhythmcity. From previous reports, it appears that AMPA receptor antagonists have no effects on absence seizures when systemically administered in this animal model (Kaminski et al., 2001; Jakus et al., 2004). We obtained identical results administering both CFM-2 or THIQ-10c intraperitoneally (data not published) indicating that AMPA receptors do not appear to play a significant role in absence epilepsy. In contrast, we have observed that

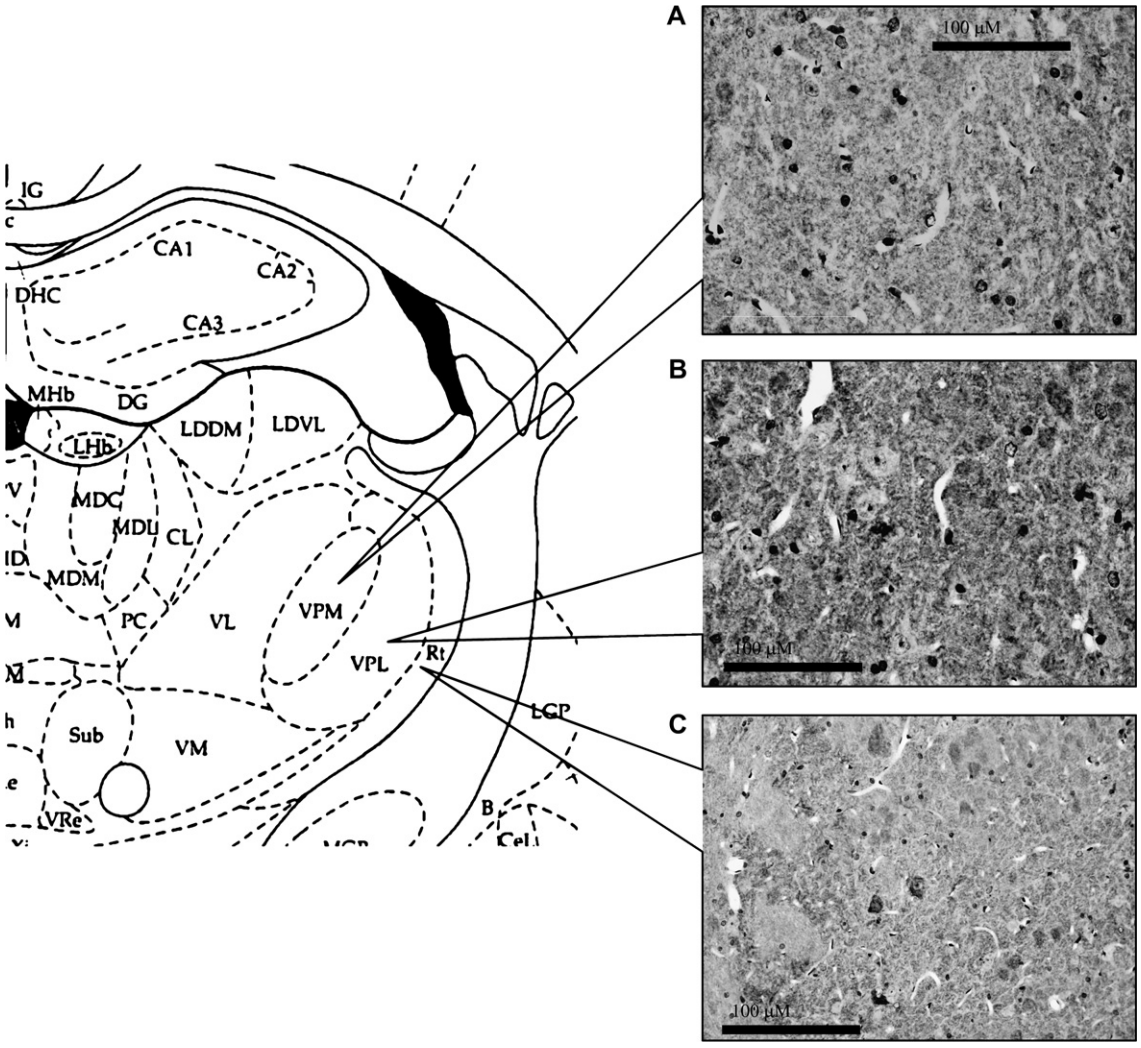

Fig. 7. Expression of GluR2 subunits of AMPA glutamate receptors in: (A) VPM; (B) VPL; and (C) NRT. Images have been obtained at a magnification of 40×, brain image has been adapted from Paxinos and Watson (1986).

targeting specific brain structures gives to this type of molecules a significant activity against absence seizures. This might be explained in terms of selectivity of this molecules and expression of glutamate receptor subunits expressed in the different brain areas, indicating that if it would be possible to target specific areas with selective molecules these might be useful in the treatment of this type of epilepsy, furthermore, these results indicate that, even if AMPA receptors might not play a major role in absence epilepsy, this type of receptors are recruited during absence seizures. This hypothesis is also supported by the findings of Peeters et al. (1994), i.c.v. administration of AMPA increases SWDs, and by other reports indicating that GluR4 subunits are primarily involved in the rhythmicity of the corticothalamic circuit (Golshani et al., 2001; van de Bovenkamp-Janssen et al., 2006).

Table 1
Immunoreactivity to GluR2 subunit in some brain areas of WAG/Rij rats

Brain area	Immunoreactivity to GluR2 subunits
S1po	+++
S1FL	+++
NRT	+++/++
VPL	++/+
VPM	+

Results obtained by a semi-quantitative analysis of stained sections. Values have been obtained by averaging the response of three experts and reported according to the following scale: (+) no reactivity; (++) low reactivity around cell bodies; (+++) marked staining around cell bodies (see also text).

The distribution of AMPA receptor subunits has been widely studied in other strain of rats (for a review see Martin et al., 1993). Immunocytochemistry shows that glutamate receptor subunits are distributed abundantly and differentially within neuronal cell bodies and processes in cerebral cortex, basal ganglia, limbic system, thalamus, cerebellum and brainstem. In neocortex and hippocampus, pyramidal neurones express GluR1 and GluR2/3/4c immunoreactivities; many non-pyramidal, calcium-binding, protein-enriched neurones in cerebral cortex are selectively immunoreactive for GluR1. In striatum, the cellular localizations of GluR1, GluR2/3/4c and GluR4 immunoreactivities are different; in this region,

GluR1 co-localizes with many cholinergic neurones. GluR1 co-localizes with most dopaminergic neurones within the substantia nigra. In several brain regions, astrocytes show GluR4 immunoreactivity (Martin et al., 1993).

Mineff and Weinberg (2000) described a high expression GluR4 AMPA receptor subunit in reticular and ventral posterior thalamic nuclei of the rat, moderate staining for GluR3 and low levels of staining for GluR2, and barely detectable levels of GluR1 immunoreactivity. Furthermore, they found that corticothalamic synapses in the reticular thalamic nucleus contain twice as much GluR2/3, and at least three times more GluR4 protein than do intrathalamic synapses. In the ventral posterior nucleus, corticothalamic synapses contain similar amounts of GluR2/3, but four times more GluR4 than do those from ascending afferents. Corticothalamic synapses in reticular nucleus contain slightly more GluR2/3, and three times more GluR4, than those in ventral posterior nucleus (Mineff and Weinberg, 2000; Spreafico et al., 1994; Golshani et al., 2001).

There is only one very recent report about the distribution of AMPA receptor subunits in the WAG/Rij animal model of absence epilepsy, describing the distribution of GluR4 subunit (van de Bovenkamp-Janssen et al., 2006). They found that 6 month-old WAG/Rij rats express less GluR4 subunit in the S1po and a higher expression in the NRT in comparison to age matched ACI control rats. The two compounds used in this study bind to AMPA receptors independently from the subunit composition, although they have a preferential selectivity for GluR2 receptors (data not yet published). Furthermore, the expression of GluR2 subunits have been demonstrated to be altered in the GHB animal model of absence seizures (Hu et al., 2001). Therefore, it has been decided to perform a set of experiments to characterize the expression of this subunit in the brain of WAG/Rij rats. The results obtained confirm that GluR2 subunits are not expressed around cell bodies in thalamic nuclei with the exception of the NRT, whereas, they are widely expressed in the two cortical sites.

The low selectivity for the GluR2 subunit of our molecules does not permit us to explain their activity in these areas where this subunit is expressed and therefore, further studies, with more selective compounds, are needed from this point of view. It is to be stated that, in contrast to this hypothesis, this two molecules do not have effects on SWDs when systemically injected, however, the inability of AMPA receptor antagonists to significantly modify the SWDs could be due to the fact that after intraperitoneal administration the compounds do not reach high enough concentration in the nuclei and this is also limited by the impossibility of using higher doses for the appearance of side effects. This latter hypothesis is also in agreement with the findings of Kaminski et al. which reported that a high dose of LY 300164 reduces the number of SWDs, furthermore, it is known that in some cases the evaluation of the effects after focal administration can be completely different from the systemic results. Phenytoin has been reported to be ineffective when administered systemically in another model of absence epilepsy, the GAERS (Marescaux et al., 1992), while suppressing SWDs when administered focally in the S1po (van Luijtelaar et al., 2005), similarly, muscimol, i.c.v. administered, increases the number of SWDs in WAG/Rij rats whereas, when administered focally in the lateral part of the thalamus decreases SWD activity (Liu et al., 1991).

In conclusion, being the two non-competitive antagonists effective when microinjected into selective brain areas, it is possible to speculate that in the animal model considered, an over stimulation of AMPA receptors is involved in the generation of absence seizures confirming that glutamatergic neurones are recruited along the cortico-thalamic network involved in the oscillations underlying absence epilepsy (McCormick and Huguenard, 1992; McCormick, 1992; McCormick and von Krosigk, 1993).

Acknowledgments

Financial support from the Italian Ministry of Education, University and Research (MIUR, Cofin 2003, Rome) and the National Research Council (CNR, Rome) is gratefully acknowledged.

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