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European Journal of Pharmacology 430 (2001) 251–259

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AMPA and $GABA_B$ receptor antagonists and their interaction in rats with a genetic form of absence epilepsy

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Received 25 July 2001; received in revised form 5 September 2001; accepted 7 September 2001

Abstract

The effects of combined and single administration of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist, 7,8-methylenedioxy-1-(4-aminophenyl)-4-methyl-3-acetyl-4,5-dihydro-2,3-benzodiazepine (LY 300164), and of the $GABA_B$ receptor antagonist γ-aminopropyl-*n*-butyl-phosphinic acid (CGP 36742), on spontaneously occurring spike-wave discharges were investigated in WAG/Rij rats. LY 300164 had minor effects; only the highest dose (16 mg/kg) reduced the number of spike-wave discharges in a short time window. CGP 36742 was more effective as it significantly reduced the number of spike-wave discharges and shortened their duration at the doses of 25 and 100 mg/kg. The ED_{50} values for the inhibition of spike-wave discharges by LY 300164 and CGP 36742 in a time window 30–60 min after injection were 15.5 and 16.6 mg/kg, respectively. The ED_{50} of CGP 36742 was reduced to 8.0 mg/kg when this antagonist was administered in combination with LY 300164 (6 mg/kg). The interaction between the two antagonists appeared to be additive according to isobolographic analysis. Importantly, CGP 36742 and LY 300164 administered either alone or in combination had no apparent effects on behavior. These results may provide information for a rational approach to polytherapy for the treatment of generalized absence epilepsy.

Keywords: CGP 36742; LY 300164; AMPA/kainate receptor; $GABA_B$ receptor; Absence epilepsy; WAG/Rij, rat; Isobologram

1. Introduction

It is well recognized that synchronized burst-firing within a thalamocortical circuit generates spike-wave discharges, which underlie generalized absence epilepsy. The two main neurotransmitter pathways involved in these pathological thalamocortical projections are glutamate and γ-aminobutyric acid (GABA) (for reviews see Coenen et al., 1992; Caddick and Hosford, 1996; Danober et al., 1998; Renier and Coenen, 2000).

The thalamic relay nuclei and the reticular thalamic nuclei play a pivotal role in the generation of oscillations that form the basis for the spike-wave discharges. α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors appear to mediate fast transmission in the thalamus (Salt and Eaton, 1996) while the reticular thalamic nuclei contain GABA-ergic neurons. Also, according to data derived from the computational models that reproduce spike-wave oscillations, both AMPA and $GABA_B$ receptors interact during the generation and propagation of the oscillations (Destexhe, 1999; Destexhe et al., 1996). Thalamocortical relay cells can elicit AMPA-mediated excitatory postsynaptic potentials in the reticular thalamic nuclei, while the latter neurons elicit $GABA_A$- and $GABA_B$-related inhibitory postsynaptic potentials. Un-

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01393-0

der these circumstances, the interaction between thalamocortical relay cells and reticular thalamic nuclei is capable of generating continuous oscillations, an action observed from cortical electroencephalogram (EEG). Moreover, intracortical excitatory connections, mediated by AMPA receptors, lead to the increases in cortical excitability that leads to the spike-wave discharges (Destexhe, 1999). $GABA_B$ conductances are tonically activated due to their slow kinetics, which generate a prolonged hyperpolarization in thalamocortical neurons. The hyperpolarization supports the maintenance of oscillations, whereas decreases in $GABA_B$ conductance strongly reduce the occurrence of the spike-wave discharges (Destexhe, 1999).

$GABA_B$ receptor antagonists have been shown to be effective in animal models of absence epilepsy (Marescaux et al., 1992; Snead, 1992; Puigcerver et al., 1996). For example, γ-aminopropyl-(diethoxymethyl)-phosphinic acid (CGP 35348), a selective $GABA_B$ receptor antagonist, is active against the spike-wave discharges in Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and old Wistar rats. Recently, a novel, orally active $GABA_B$ receptor antagonist, γ-aminopropyl-*n*-butyl-phosphinic acid (CGP 36742) (Mondadori et al., 1993), was shown to decrease significantly the spike-wave discharges in GAERS (Vergnes et al., 1997).

Experimental data about the role of AMPA receptor antagonists in animal models of absence epilepsy seem to be lacking. 6-Cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX), non-NMDA receptor antagonist causes a dose-dependent decrease in the spike-wave discharges, with no behavioral changes (Ramakers et al., 1991). AMPA produces a dose-dependent increase in the spike-wave discharges, whereas its non-selective antagonist, glutamic acid diethyl ester, decreases the spike-wave discharges in WAG/Rij rats. Kainate has no effect on the occurrence of the spike-wave discharges (Peeters et al., 1994b). On the other hand, there is abundant data concerning the AMPA receptor antagonists as highly effective anticonvulsant agents (Tarnawa and Vizi, 1998). Moreover, these types of compounds such as 7,8-methylenedioxy-1-(4-aminophenyl)-4-methyl-3-acetyl-4,5-dihydro-2,3-benzodiazepine (LY 300164) proved to posses a wide anticonvulsant spectrum (Bialer et al., 2001).

From the above-mentioned studies, it is likely that two different neurotransmitter systems are involved in controlling the spike-wave discharges activity and that a combination of drugs which act on these systems is a good basis for rational polytherapy in absence epilepsy. More specifically, we hypothesize that combining the effects of two novel AMPA and $GABA_B$ receptor antagonists may lead to a beneficial therapy of absence epilepsy. We tested this hypothesis using an isobolographic method in the WAG/Rij inbred strain of rats, which is recognized as an animal model of human absence epilepsy (Van Luijtelaar and Coenen, 1986; Coenen et al., 1992; Renier and Coenen, 2000).

2. Materials and methods

2.1. Animals and experimental conditions

Experiments were performed in 32, 6-month-old male WAG/Rij rats weighing between 290 and 350 g. They were maintained on a reversed 12-h light–dark cycle with light on at 19:00 h. Each rat received up to three drug treatments with at least a 5-day interval between each treatment. All animal experiments were done according to the Helsinki declaration and conducted in accordance with the guidelines of the European Community Council directive 86/609/EEC. A local ethical committee approved the experimental protocol.

2.2. Surgery and EEG recording

We used aseptic procedures and halothane anesthesia to implant EEG electrodes (MS333/2-A; Plastics One, VA, USA) on the cortical surface: one on the frontal region (coordinates with skull surface flat and bregma zero–zero: A2.0 L3.5) and a second one on the parietal region (A-6.0 L4.0). We placed a reference electrode in the cerebellum (Van Luijtelaar and Coenen, 1986). The rats were allowed to recover from surgery for 10 days before experimentation began. On the experimental day, the rats were placed in recording cages ($25 \times 35 \times 30$ cm) and connected with leads through swivels to an amplifier and a computer-based data acquisition system. The CODAS system (Dataq Instruments, OH, USA) was used to monitor the EEG. The EEG signals were sampled with a frequency of 200 Hz and recorded for 1 h before (baseline) and 4 h after drug or vehicle administration. We analyzed the EEG visually using criteria for the spike-wave discharges according to that described by Van Luijtelaar and Coenen, 1986. Although the statistical analysis was based on 1-h baseline and 4-h post-injection recordings, the visual presentation of the spike-wave discharges data was limited to the baseline and the first 2-h post-injection.

2.3. Behavioral observation

We observed the rat's behavior continuously for a 30-min period, beginning 30 min after injection when the plasma levels of both drugs are high (Eckstein and Swanson, 1995; Steulet et al., 1996). The recorded behavior was divided into two major categories: active behavior (walking, rearing, sniffing, grooming, eating and drinking) and passive behavior (sitting, lying and standing still).

2.4. Drugs

CGP 36742 (Novartis, Basle, Switzerland), the $GABA_B$ receptor antagonist, was dissolved in saline. LY 300164 (Eli Lilly, IN, USA), the AMPA receptor antagonist, was suspended in a 1% solution of Tween 80 (Sigma, MO,

USA). Both drugs were administered intraperitoneally (i.p.) in a volume of 2 ml/kg. Control animals were treated with either saline or 1% Tween 80. To obtain dose–response relationships, the animals were treated with three doses of CGP 36742 (6.125, 25 and 100 mg/kg) or LY 300164 (1, 4 and 16 mg/kg). In the polytherapy experiments, the rats were given three doses of CGP 36742 (3, 9 and 27 mg/kg) and one fixed dose of LY 300164 (6 mg/kg).

2.5. Statistics

The number and duration of the spike-wave discharges obtained from EEG recordings as well as the behavioral performance of animals were analyzed using one-way analysis of variance (either dose or time) followed by Bonferroni's multiple comparison when appropriate. The numbers of the spike-wave discharges were fitted into the sigmoid E_{max} model and the ED_{50} with 95% confidence intervals (CIs) were calculated as described by Roks et al. (1999). The ED_{50} was regarded as the dose necessary to obtain 50% inhibition of the spike-wave discharges. The type of interaction between drugs as to their anti-absence activity was analyzed by plotting the ED_{50} values on the isobologram (Tallarida et al., 1989).

3. Results

3.1. Effects of LY 300164 on the spike-wave discharges

LY 300164 had no significant effects upon the number of the spike-wave discharges during the post-injection period with pooled data compared to baseline. However, at the 16 mg/kg dose, LY 300164 showed a significant time effect [$F(3,36) = 3.9,\ P < 0.001$], Bonferroni test revealed a decrease ($P < 0.001$) in the number of the spike-wave discharges at 30–60-min post-injection compared to vehicle-treated rats (Fig. 1). LY 300164 did not influence the mean duration of the spike-wave discharges (Fig. 2).

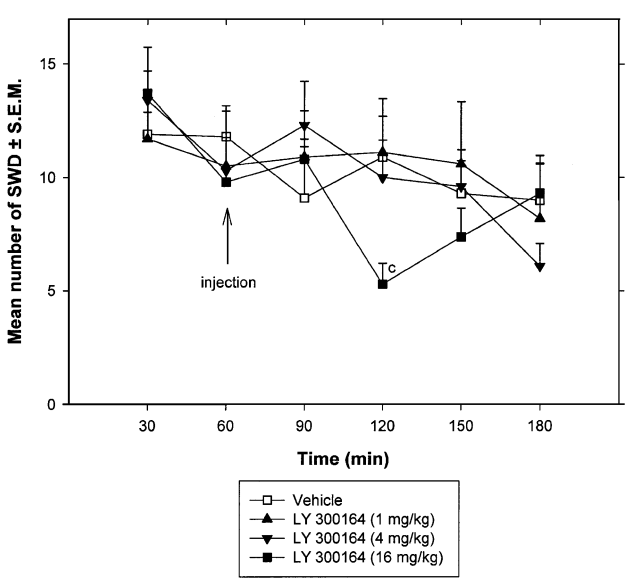

Fig. 1. Effects of LY 300164 on the number of the spike-wave discharges (SWD) in the electroencephalogram (EEG) during a 1-h baseline period and 2 h after the injection. Each data point represents the mean number of SWD ± S.E.M for 10 rats in 30-min intervals. $^{c}P < 0.001$ vs. vehicle-treated rats (Bonferroni's multiple comparison test).

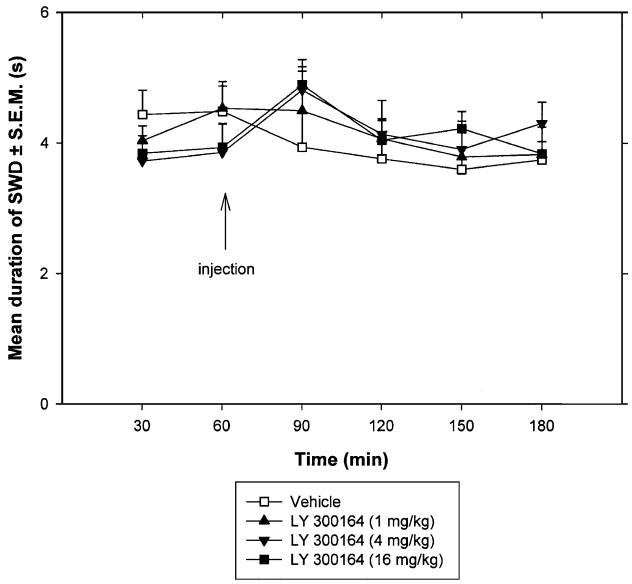

Fig. 2. Effects of LY 300164 on the mean duration of the spike-wave discharges (SWD) in the electroencephalogram (EEG) during a 1-h baseline period and 2 h after the injection. Each data point represents the mean duration of SWD ± S.E.M for 10 rats in 30-min intervals.

3.2. Effects of CGP 36742 on the spike-wave discharges

The effects of CGP 36742 on the number of the spike-wave discharges in the cortical EEG are shown in Fig. 3. Analysis of variance based on the post drug recording period showed a significant drug effect [$F(3,36) = 17.04$, $P < 0.001$]. Bonferroni's multiple comparison test revealed a dose-dependent reduction in the number of the spike-wave discharges after CGP 36742 treatment. More specifically, the groups receiving CGP 36742 in the doses of 25 and 100 mg/kg had significantly less spike-wave discharges than did the control animals ($P < 0.001$). The lowest dose of CGP 36742 (6.125 mg/kg) remained without a significant effect in this regard (Fig. 3).

There were significant changes of CGP 36742 activity over time as was revealed by a within group analysis. Compared to the baseline (Fig. 3), CGP 36742 at the dose of 25 mg/kg was active against the spike-wave discharges starting from 30 up to 150 min after injection. At the dose of 100 mg/kg, it became active immediately after injection and this activity was observed up to 240 min.

Fig. 4 shows that the mean duration of the spike-wave discharges was significantly shortened after the administration of CGP 36742 [$F(3,36) = 22.7,\ P < 0.001$]. However,

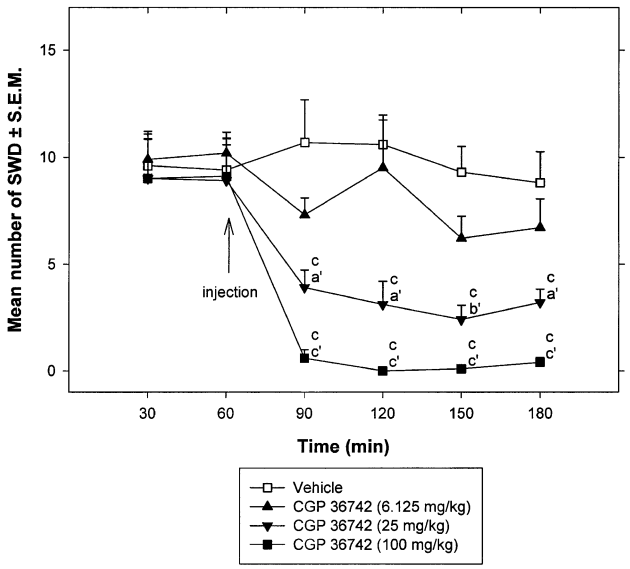

Fig. 3. Effects of CGP 36742 on the number of the spike-wave discharges (SWD) in the electroencephalogram (EEG) during a 1-h baseline period and 2 h after the injection. Each data point represents the mean number of SWD ± S.E.M of 10 rats in 30-min intervals. $^{c}P < 0.001$ vs. vehicle-treated rats; $^{a\prime}P < 0.05$, $^{b\prime}P < 0.01$, $^{c\prime}P < 0.001$ vs. baseline EEG recording (Bonferroni's multiple comparison test).

Bonferroni's multiple comparison test showed that only at the dose of 100 mg/kg did it significantly decrease the mean duration when compared to control animals ($P < 0.001$) and baseline values ($P < 0.001$). The reduction occurred immediately after the injection and lasted 180 min. Lower doses of CGP 36742 did not affect the mean duration of the spike-wave discharges (Fig. 4).

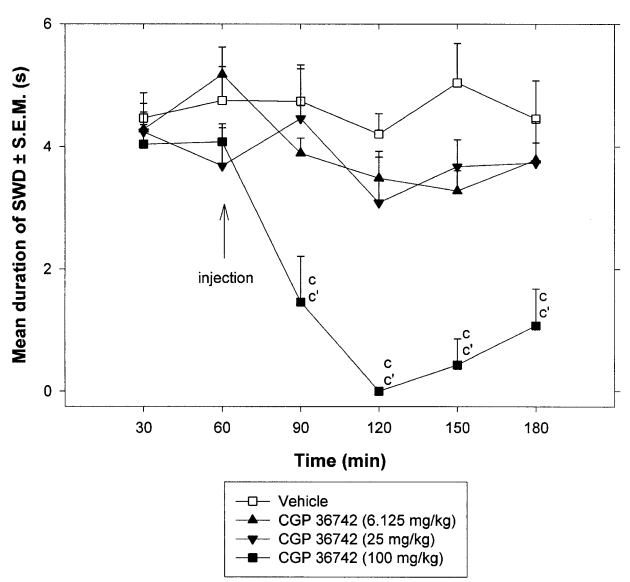

Fig. 4. Effects of CGP 36742 on the mean duration of the spike-wave discharges (SWD) in the electroencephalogram (EEG) during a 1-h baseline period and 2 h after the injection. Each data point represents the mean duration of SWD ± S.E.M for 10 rats in 30-min intervals. $^{c}P < 0.001$ vs. vehicle-treated rats; $^{c\prime}P < 0.001$ vs. baseline EEG recording (Bonferroni's multiple comparison test).

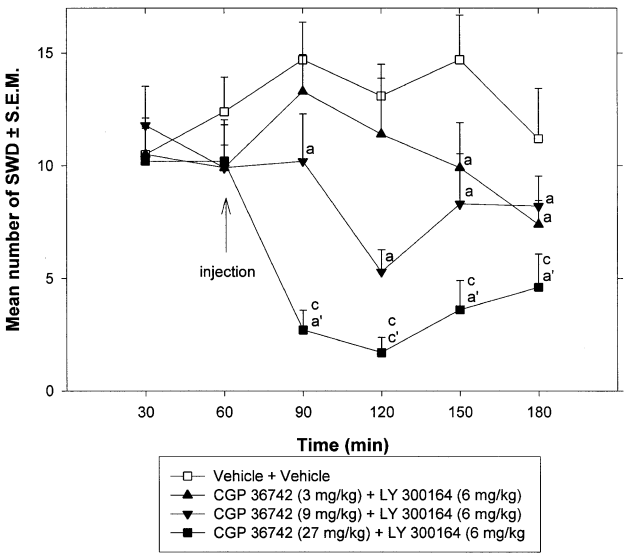

Fig. 5. Effects of combined treatment with CGP 36742 and LY 300164 (6 mg/kg) on the number of the spike-wave discharges (SWD) in the electroencephalogram (EEG) during a 1-h baseline period and 2 h after the injection. Each data point represents the mean number of SWD ± S.E.M of 10 rats in 30-min intervals. $^{a}P < 0.05$, $^{c}P < 0.001$ vs. vehicle-treated rats; $^{a\prime}P < 0.05$, $^{c\prime}P < 0.001$ vs. baseline EEG recording (Bonferroni's multiple comparison test).

3.3. Effects of combined treatment with LY 300164 and CGP 36742 on the spike-wave discharges

When combined with a dose of LY 300164 (6 mg/kg), CGP 36742 showed a dose-dependent inhibition of the

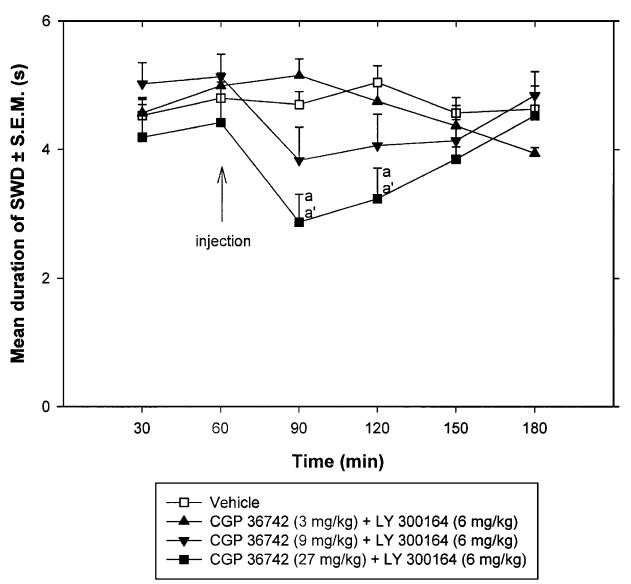

Fig. 6. Effects of combined treatment with CGP 36742 and LY 300164 (6 mg/kg) on the mean duration of the spike-wave discharges (SWD) in the electroencephalogram (EEG) during a 1-h baseline period and 2 h after the injection. Each data point represents the mean duration of SWD ± S.E.M for 10 rats in 30-min intervals. $^{a}P < 0.05$ vs. vehicle-treated rats; $^{a\prime}P < 0.05$ vs. baseline EEG recording (Bonferroni's multiple comparison test).

spike-wave discharges [$F(3,36) = 14.8$, $P < 0.001$] (Fig. 5). Bonferroni's post-hoc test revealed that groups receiving LY 300164 (6 mg/kg) with CGP 36742 at 3, 9 or 27 mg/kg had significantly less spike-wave discharges than did vehicle-treated groups and also when compared with baseline recording (Fig. 5).

The mean duration of the spike-wave discharges was shortened after the combined LY 300164/CGP 36742 treatment [$F(3,36) = 3.99$; $P < 0.05$]. Bonferroni's post-hoc test revealed that the spike-wave discharges duration was shortened after treatment with the highest doses of CGP 36742 and LY 300164 (Fig. 6).

3.4. Isobolographic analysis

Fig. 7 shows that the ED_{50} of CGP 36742 for inhibiting the number of the spike-wave discharges 30 to 60 min following administration was 16.6 (14.8–18.9) mg/kg, whereas the ED_{50} for LY 300164 was 15.5 (13.1–18.3) mg/kg. When combined with LY 300164 (6 mg/kg), the ED_{50} of CGP 36742 was 8.0 (5.0–12.0) mg/kg. Theoretical additive ED_{50} and 95% confidence intervals for this combination were estimated to be 10.0 (8.0–12.7) mg/kg. The experimental ED_{50} lies below the isobolographic line, which may suggest supra-additivity. However, the experimental and theoretical 95% confidence intervals overlap to a great extent (Fig. 7). Therefore, the observed interaction seems to be additive.

3.5. Behavioral observation

The analysis of variance did not reveal any significant differences in active or passive behavior after LY 300164 (up to 16 mg/kg) treatment (data not shown). Similarly,

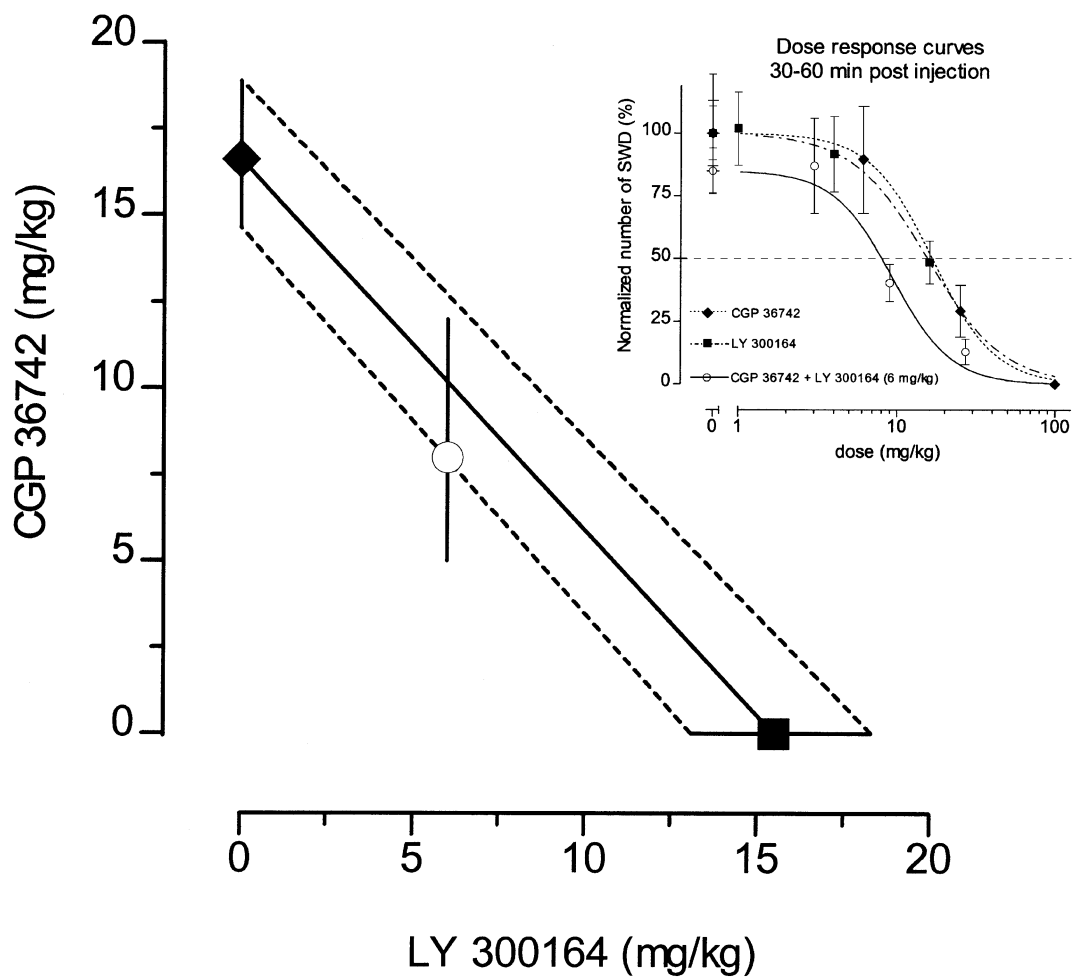

Fig. 7. Isobologram for the anti-absence interaction between CGP 36742 and LY 300164. The ED_{50} value of CGP 36742 is plotted on the ordinate (closed diamond) and that of LY 300164 on the abscissa (closed square). The ED_{50} values (for the inhibition of SWD) were obtained by fitting data to the sigmoid E_{max} model, for the data from the time period 30–60-min post-injection (see graph insert and Materials and methods). The straight line connecting the two plotted ED_{50} values is the isobolographic line, while dotted lines represent 95% confidence intervals (CIs). The experimental ED_{50} of the combination of CGP 36742 and LY 300164 (6 mg/kg) is plotted as the open circle and 95% CIs as the solid vertical line. If the experimentally determined ED_{50} lies on the isobolographic line, then the drug effects are additive. If the ED_{50} lies below this line, supra-additivity is assumed and when the ED_{50} lies above the isobolographic line, there is infra-additivity. However, when 95% CIs of the experimentally obtained combination overlap with the respective intervals of the isobolographic line, then the interaction is regarded as statistically non-significant. In such case, the observed interaction seems to be additive.

CGP 36742 up to 100 mg/kg remained without any marked effect (data not shown). The combined treatments with LY 300164 (6 mg/kg) and CGP 36742 (up to 27 mg/kg) also had no significant influence on behavior (data not shown).

4. Discussion

The current study confirmed the high anti-absence activity of $GABA_B$ receptor antagonists. Surprisingly, the non-competitive AMPA receptor antagonist showed only weak effects against the spike-wave discharges in WAG/Rij rats. These drugs did not affect the morphology of the spike-wave discharges in the EEG recording; only their number and duration were changed. We suggest that the anti-absence potencies of $GABA_B$ and AMPA receptor antagonists are additive. Additivity implies that the effect of the combination of these two compounds is equal to that predicted from individual dose–response curves. No synergy or antagonism was found in the present study. It is possible that a pharmacokinetic interaction is responsible for this additive effect. However, such an interaction seems unlikely since LY 300164 did not influence the free plasma levels of various antiepileptic drugs while it enhanced their anticonvulsant potential at the same time (Czuczwar et al., 1998; Borowicz et al., 1999, 2000, 2001).

CGP 36742, the $GABA_B$ receptor antagonist, was previously reported to be effective against the spike-wave discharges in GAERS (Vergnes et al., 1997). The present results confirm a strong efficacy of CGP 36742 to suppress the spike-wave discharges and shorten its duration. CGP 36742 was active at lower doses in WAG/Rij rats than for GAERS. The highest anti-absence activity of CGP 36742 was observed 60 min after administration and this was similar to that reported for GAERS. The peak activity in WAG/Rij rats correlated well with the peak plasma level of this compound, which occurs between 0.5 and 1 h after i.p. administration (Steulet et al., 1996). CGP 36742 was less potent than other phosphinic antagonists of $GABA_B$ receptor to depress a slow inhibitory postsynaptic potential in vitro (Pozza et al., 1999). However, it is orally active (Mondadori et al., 1993), penetrates the blood–brain barrier and is relatively long-acting (Steulet et al., 1996). Indeed, its anti-absence activity in the WAG/Rij rat was evident up to 4 h following the injection.

LY 300164 was used as a non-competitive AMPA receptor antagonist that belongs to the 2,3-benzodiazepine derivatives. It is more potent than its well-recognized parent substance, (1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI 52466) (Donevan et al., 1994; Rammes et al., 1996). 2,3-Benzodiazepine derivatives antagonize AMPA receptor-mediated responses by a novel allosteric blocking mechanism. These compounds are effective in many animal seizure models (Tarnawa and Vizi, 1998). In fact, LY 300164 was effective against maximal electroshock- and pentylenetetrazol-induced seizures with the ED_{50} doses of 4.6 and 116.8 mg/kg, respectively. Moreover, it attenuated both chemically and electrically kindled seizures within a 12.5–20-mg/kg dose range (Bialer et al., 2001).

In vitro studies have shown that NMDA, AMPA and metabotropic receptors are involved in excitatory synaptic responses recorded from thalamocortical neurons (McCormick, 1992; McCormick and von Krosigk, 1992). Moreover, enhanced AMPA receptor-mediated transmission was recorded in cortical pyramidal neurons of GAERS (Avanzini et al., 1996). This observation supports the hypothesis that AMPA receptor-mediated transmission may contribute to the enhancement of cortical excitability and, as a consequence, to the spike-wave discharges generation (Danober et al., 1998). In the current study, LY 300164 had weak activity against the spike-wave discharges in WAG/Rij rats. Considering the ubiquity of AMPA receptors in sensory thalamo-cortical cells, the involvement of sensory relay nuclei in this type of epilepsy (Inoue et al., 1993), and the assumed substantial role of thalamo-cortical and cortical neuroexcitability that leads to the spike-wave discharges (Avanzini et al., 1996; Destexhe, 1999), stronger effects of the new AMPA receptor antagonist may have occurred. The weak effects may raise doubts about a suggested role of AMPA receptors in generating the spike-wave discharges. Nevertheless, significant effects were obtained in the present study. The effect of LY 300164 was observed in a short time window: the first hour after the injection. LY 300164, like other 2,3-benzodiazepines, is relatively short-acting in rodents (De Sarro et al., 1995; Lodge et al., 1996; Czuczwar et al., 1998), whereas their activity is considerably longer in non-rodent species (Smith et al., 1991; Engberg et al., 1993). The peak plasma level of LY 300164 in Fisher rats occurs about 0.5 h following oral administration, whereas the pharmacologically inactive *N*-acetyl metabolite showed its highest plasma concentration 1.5 h later (Eckstein and Swanson, 1995). The half-life after oral administration of LY 300164 in rats is approximately 1.3 to 2.5 h (Leander, personal communication).

LY 300164 significantly reduced the number of the spike-wave discharges only at the highest dose, 16 mg/kg. This contrasts with previously reported data about another AMPA receptor antagonist, CNQX, which appeared to be more potent than LY 300164 (Ramakers et al., 1991). The difference in anti-absence activity may involve a different mechanism of AMPA receptor blockade, since CNQX acts in a competitive manner. The effects of CNQX in WAG/Rij rats were attenuated by injection of NMDA, AMPA and kainate. Therefore, it is possible that CNQX could affect both NMDA- and AMPA/kainate receptor-mediated neurotransmission (Ramakers et al., 1991). Interactions between NMDA and non-NMDA receptors were further examined in WAG/Rij rats (Peeters et al., 1994a). 2-Amino-7-phosphonoheptanoate (APH) and kynurenic

acid completely blocks the stimulating action of NMDA on the spike-wave discharges (Peeters et al., 1994a). On the other hand, the AMPA-induced increase in the spike-wave discharges is abolished by glutamic acid diethyl ester and kynurenic acid (Peeters et al., 1994a). These data may suggest a pathophysiological link between NMDA- and non-NMDA-mediated transmission relating to absence epilepsy. Another study of Peeters et al. (1994b) revealed that glutamic acid diethyl ester dose dependently decreases the number of the spike-wave discharges in WAG/Rij rats and that these effects were blocked by AMPA. In general, these data may suggest a contribution of all three types of ionotropic glutamate receptors in absence epilepsy. However, low selectivity of CNQX and glutamic acid diethyl ester does not fully justify a simple conclusion about the role of the AMPA receptor in the generation of the spike-wave discharges.

It was suggested that simultaneous inhibition of several different neurotransmitter pathways involved in epileptogenesis might allow better seizure control than selective antagonism of a particular receptor (Löscher, 1998). This assumption seems especially important in absence epilepsy. Others have suggested that $GABA_B$ and excitatory amino acid receptors in reciprocally coupled populations of excitatory thalamocortical and inhibitory reticular thalamic neurons play a main role in the generation of the spike-wave discharges (Golomb et al., 1996; Destexhe, 1999; Destexhe et al., 1996). Therefore, blockade of both types of receptors may lead, not only to a more pronounced anti-absence effect when compared to that of blockade of an individual receptor, but also to the formations of a theoretical basis for rational polytherapy. Indeed, our results indicate that there is additivity between $GABA_B$ and AMPA receptor antagonists in the WAG/Rij model of absence epilepsy. Consequently, given together, these antagonists provide the same inhibition of the spike-wave discharges at doses lower than those used alone. Similar effects, that involved NMDA and AMPA receptor antagonists, were observed in amygdala-kindled and electrically induced seizures (Löscher et al., 1993; Czuczwar et al., 1995). Interestingly, a drug (LU 73068; 4,5-dihydro-1-methyl-4-oxo-7-trifluoromethyl-imidazo[1,2α]quinoxaline-carbonic acid) that combines an antagonistic action at both glycine/NMDA and AMPA receptors, was a potent anticonvulsant agent in kindled rats, whereas L-701,324 (glycine/NMDA receptor antagonist; 7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-quinoline-(2(1*H*)one) and NBQX (AMPA receptor antagonist; 6-nitro-7-sulfamoylbenzo(*f*)quinoxaline-2,3-dione), when administered alone, had no anticonvulsant activity (Potschka et al., 1998).

It is interesting that CGP 36742 and LY 300164, when injected alone or in combination, did not significantly affect behavioral parameters in WAG/Rij rats. Perhaps more specific behavioral tests would have reveal side-effects characteristic of the compounds used in the current study. Generally, $GABA_B$ receptor antagonists do not affect locomotor activity (Mead and Little, 1995) and appear to enhance cognition and memory (Mondadori et al., 1993, 1996). Non-competitive AMPA receptor antagonists (including LY 300164) attenuate amphetamine-and MK-801 (dizocilpine)-induced hyperactivity and decrease spontaneous locomotion in mice (Vanover, 1998). However, GYKI 52466 has no effects on spontaneous locomotor activity in rats (Danysz et al., 1994). The most serious adverse effect of 2,3 benzodiazepines is ataxia, which occurs at doses just above those that produce anticonvulsive activity (Tarnawa and Vizi, 1998). Indeed, LY 300164 was reported to induce ataxia and hypomotility in rats at the doses of 25 and 60 mg/kg and in monkeys at dosage higher than 5 mg/kg. The side-effects were transient and the animals recovered fully within 4 h (Bialer et al., 2001). Even at the highest dose of LY 300164 (16 mg/kg) used in this study, there were no signs of ataxia in WAG/Rij rats. This contrasts with marked behavioral changes induced by MK-801, the non-competitive NMDA receptor antagonist, in the WAG/Rij rats (Peeters et al., 1989).

In conclusion, the current results advocate a role for the involvement of $GABA_B$ and AMPA receptors in generalized absence epilepsy. Further, according to isobolographic analysis, the antagonists of the two receptors show additive effects against the spike-wave discharges in WAG/Rij rats. We suggest that $GABA_B$ and AMPA receptor-mediated neurotransmission regulates the occurrence of the spike-wave discharges in an additive manner.

Acknowledgements

The generous gifts of LY 300164 by Ms. Margaret H. Niedenthal and Dr. David Leander (Eli Lilly, Indianapolis, IN, USA) and CGP 36742 by Dr. Barbara Willi (Novartis, Basel, Switzerland) are greatly appreciated. The authors are grateful to Ms. Elly Willems, Mr. Jean Paul Dibbets and Mr. Hans Krijnen for their skilful technical assistance. The visit of R.K. to Nijmegen was supported by the CISP.

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