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Opposite effects of T- and L-type Ca^{2+} channels blockers in generalized absence epilepsy

Gilles van Luijtelaar [a,*], Dorota Wiaderna [b], Christyl Elants [1], Wim Scheenen [c]

[a] *NICI, Department of Comparative and Physiological Psychology, Psychology Laboratory, Nijmegen University, PO Box 9104, 6500 HE Nijmegen, Netherlands*

[b] *Nofer's Institute of Toxicology, £ódź, Poland*

[c] *Department of Cellular Animal Physiology, Nijmegen Institute for Neurosciences, Nijmegen University, Nijmegen, Netherlands*

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Abstract

The role of the T-type Ca^{2+} channel blocker, ethosuximide, the L-type Ca^{2+} channel blocker, nimodipine and L-type Ca^{2+} channel opener, BAY K8644 (1,4 Dihydro-2,6-dimethyl-5-nitro-4-[trifluoromethyl)-phenyl]-3-pyridine carboxylic acid methyl ester), was investigated on spike-wave discharges in WAG/Rij rats. This strain is considered as a genetic model for generalized absence epilepsy. A dose-dependent decrease in the number of spike-wave discharges was found after i.c.v. ethosuximide, an increase after i.p. nimodipine and a decrease after i.c.v. BAY K8644. BAY K8644 was also able to antagonise the effects of nimodipine. Preliminary data were obtained with two conotoxins, MVIIC and GVIA, which block P/Q-type and N-type Ca^{2+} channels, respectively. Only after i.c.v. administration of ω-conotoxin GVIA were the number and duration of spike-wave discharges reduced, but animals showed knock-out lying. The latter suggests behavioural or toxic effects and that the decrease in spike-wave activity cannot unequivocally be attributed to blockade of N-type Ca^{2+} channels.

It can be concluded that T- and L-type Ca^{2+} channel blockers show opposite effects on spike-wave discharges. Furthermore, these effects are difficult to explain in terms of a model for spindle burst activity in thalamic relay cells proposed by McCormick and Bal [Sleep and arousal: thalamocortical mechanisms. Ann. Rev. Neurosci. 20 (1997) 185]. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

There are several indications that Ca^{2+} plays a role in epileptogenesis. It is well known that during a seizure, the intracellular Ca^{2+} concentration increases and the extracellular Ca^{2+} concentration decreases (Heinemann et al., 1977). Next, anticonvulsant drugs, such as phenytoin and carbamazepine, inhibit the influx of Ca^{2+} (Crowder and Bradford, 1987; Yaari et al., 1987). Furthermore, the antiepileptic drug, verapramil, is a rather specific L-type Ca^{2+} channel blocker with clear anticonvulsive properties. Therefore, it seems logical that drugs that block the influx of Ca^{2+} into cells by blockade of Ca^{2+} channels, that is Ca^{2+} channel blockers or antagonists, possess antiepileptic properties (Hoffmeister and Tetterhorn, 1986; De Sarro et al., 1990, 1992a; De Falco et al., 1992; Walden et al., 1992; Czuczwar et al., 1994).

There is direct neurophysiological evidence for a role of a subset of Ca^{2+} channels, the so-called T-type Ca^{2+} channels, in the genesis of thalamocortical oscillations. These oscillations form the basis for the occurrence of spike-wave discharges. The presence of spike-wave discharges in the electroencephalogram is one of the key symptoms of generalized absence epilepsy. It is thought that the low voltage-activated or T-type Ca^{2+} currents are involved in the rhythmic firing properties of thalamic neuronal assemblies. During a transition from the awake state to drowsiness, thalamocortical relay cells are hyperpolarized due to a decrease in excitatory inputs (Steriade et al., 1993; McCormick and Bal, 1997). The hyperpolariza-

* Corresponding author. Tel.: +31-24-361-5621; fax: +31-24-361-6066.

E-mail address: luijtelaar@nici.kun.nl (G. van Luijtelaar).

[1] Christyl Elants is currently at Organon International, Newhouse, Scotland.

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tion acts to de-inactivate the T-type Ca^{2+} current, the membrane is depolarised toward threshold for a burst of Na^{+}/K^{+}-dependent action potentials, the T-type current is inactivated, and repolarisation of the membrane due to inactivation is followed by rebound and hyperpolarization. It has been established that the amplitude of the T-type Ca^{2+} current is increased in the reticular thalamic nucleus in GAERS (genetic absence epileptic rats from Strasbourg) (Avanzini et al. 1993; Tsakiridou et al., 1995), a widely used genetic rat model of generalized absence epilepsy developed by Marescaux and Vergnes (Marescaux et al., 1992; Danober et al., 1998). Finally, the specific anti-absence drug, ethosuximide, blocks in vitro the T-type Ca^{2+} channel in thalamocortical neurons (Coulter et al., 1989; 1990) although another mechanism of action for ethosuximide was proposed recently (Leresche et al., 1998). Finally, it was found that ethosuximide reduces dose dependently the number of spike-wave discharges in WAG/Rij rats (Peeters et al., 1988; Van Luijtelaar et al., 1995).

There has been much less emphasis on the involvement of other than T-type Ca^{2+} channels in processes leading to spike-wave discharges. A role of such channels can be important for theories on the epileptogenesis of absence epilepsy, particularly since there is some evidence that other neuronal voltage-dependent Ca^{2+} channels are involved in the regulation of cellular functions, such as membrane excitability, rhythmic firing and neurotransmitter release. In addition to T-type channels, five other types of mammalian Ca^{2+} channels have been described (Takahashi and Momlyama, 1993; Mori et al., 1996). Recent studies suggest the involvement of other than T-type Ca^{2+} channels in epilepsy, in general, (Lingenhöhl et al., 1997), or in generalized absence epilepsy, in particular, in both patients and rats (Van Luijtelaar et al., 1995; Fletcher and Frankel, 1999; Fletcher et al., 1996; Burgess and Noebels, 1999). A mutation in the alpha1-A P/Q type Ca^{2+} channel gene has recently been associated with inherited neurological diseases, such as ataxia and spike-wave discharges in the tottering mouse mutant (Fletcher et al., 1996; Campbell and Hess, 1999). Another possibility is that loss of a specific subunit could give rise to a channelopathy through a major reduction of a specific Ca^{2+} current and that as a compensatory consequence, more and other Ca^{2+} currents may occur. These findings suggest that Ca^{2+} channels other than T-type Ca^{2+} channels are likely to be involved in the occurrence of spike-wave discharges.

There are surprisingly few studies with specific agonists and antagonists in pure models of absence epilepsy, considering all the molecular work that is currently done (Doyle et al., 1997; Burgess and Noebels, 1999; Campbell and Hess, 1999; Fletcher and Frankel, 1999). BAY K8644 (1,4 Dihydro-2,6-dimethyl-5-nitro-4-[trifluoromethyl)-phenyl]-3-pyridine carboxylic acid methyl ester) is a L-type Ca^{2+} channel agonist. This dihydropyridine derivative increases the opening probability of a particular subclass of Ca^{2+} channels, the L-type Ca^{2+} channel, thereby promoting voltage-dependent Ca^{2+} influx. Nimodipine also belongs to the class of the dihydropyridines. It is also centrally active and acts as a Ca^{2+} channel antagonist by blocking the voltage-dependent L-type Ca^{2+} channel (Suzuki and Rogawski, 1989). BAY K8644 is a specific L-type Ca^{2+} channel agonist and nimodipine is a specific antagonist (Spedding and Paoletti, 1992). Nimodipine is an effective anti-convulsant drug in tonic–clonic seizure models (De Sarro et al., 1990, 1992a; De Falco et al., 1992). In contrast, we have established that the L-type Ca^{2+} channel antagonist, nimodipine, increases the number of spike-wave discharges in acute and chronic experiments (Van Luijtelaar et al., 1994, 1995). In full agreement with this is our finding that the L-type Ca^{2+} channel agonist, BAY K8644, both reduces the number of spike-wave discharges (van Luijtelaar et al., 1995) and induces convulsions (De Sarro et al., 1990, 1992b; Watson and Little, 1994; Van Luijtelaar et al., 1995).

In our previous studies, drugs had been administered systemically and peripheral effects could not be excluded. Also, these studies were done in 2-year-old Wistar rats. This prompted us to investigate the effect of i.c.v. administered L- and T-type Ca^{2+} channel modulators, BAY K8644, nimodipine and ethosuximide on the occurrence of spike-wave discharges in the WAG/Rij strain, a genetic model for generalized absence epilepsy with face, construct and predictive validity (Van Luijtelaar and Coenen, 1986, 1997; Van Luijtelaar et al., 1991a,b; Peeters et al., 1988; Coenen et al., 1992).

Preliminary data were obtained with two conotoxins that inhibit different subtypes of Ca^{2+} channels: ω-conotoxin GVIA and ω-conotoxin MVIIC. The first is an inhibitor of the N-type Ca^{2+} channels and the second is an antagonist of the P/Q-type Ca^{2+} channel. Both types of Ca^{2+} channels can be found in the central nervous system. Recently, it was proposed that in mouse mutants, a deficit in one of the P/Q subunits is connected to the appearance of spike-wave discharges (Fletcher et al., 1996; Fletcher and Frankel, 1999).

2. Materials and methods

The subjects were 40 male WAG/Rij rats born and raised in our laboratory. The rats were housed in pairs until surgery. On the day of surgery they were between 6 and 12 months of age and their body weight ranged from 282 to 410 g. After surgery they were housed singly. Housing was under standard laboratory conditions (food and water were always available ad libitum, light cycle 12 h light/12 h darkness, with bright light on at 2000 h, room temperature was kept at 21°C). All rats were chronically fitted with a tripolar electrode set (Plastic One, MS 333/2A) and a guide cannula (Plastic One, 22GA) under complete Nembutal anesthesia (50 mg/kg, i.p.). The two active electrodes were implanted in the frontal (A 2.0; L 3.5) and

parietal (A 6.0; L 4.0) regions of the cortex and the ground electrode in the cerebellum. The cannula was placed into the left ventricle (A 0.7; L 1.3). All coordinates were taken with bregma zero–zero and skull surface flat. Our protocol complied with the guidelines of the European Community and was approved by our institutional ethics committee.

The electroencephalographic signals were amplified and filtered, only signals between 1 and 100 Hz were allowed to pass, and were digitised (sample rate 200 Hz), monitored, and stored on optical disks for subsequent offline analysis with a CODAS system.

2.1. Procedure

The rats were left undisturbed for at least 1 week after surgery to allow recovery from surgery, next they were moved to a recording cage, adapted to the leads for at least 16 h preceding recording of electroencephalographic activity. Recording always took place between 0900 and 1500 h, in the dark phase of the light–dark cycle. The rats were only used once, except for the experiments with the conotoxins when they had 1 week earlier received nimodipine or BAY K8644 or ethosuximide. They were randomly chosen from among the rats with a reliable electroencephalographic signal.

The i.c.v. injection procedure: the 3.5-mm-length (equal with the cannula) needle was connected to a 10-μl Hamilton syringe through a flexible, polyethylene tube; 5 μl of the drug was administered into the left ventricle at a rate of 1 μl per 30 s. The needle was kept in the guide cannula for another minute.

2.1.1. Drug solution preparation

The dihydropyridine solutions were freshly prepared in a dark room and shielded from light. A mixture of solutol/ethanol/0.9% NaCl (5:5:90) was used. Solutol (polyethylene glycol 660 hydrostearate) was a gift from CNS Research Bayer (Cologne, Germany). Ethosuximide, purchased from RBI, was dissolved in physiological saline. Conotoxin GVIA, purchased from RBI, was dissolved in oxygen-free distilled water. Conotoxin MVIIC, purchased from RBI, was dissolved in physiological saline. All equipment was washed with 0.1% (w/v) polyethyleneimine in order to minimise nonspecific binding of conotoxins to surfaces. The pH of the conotoxin drug solutions was 6.0.

Separate groups of rats ($n = 8$) were used to determine the effect of i.c.v. injection of ethosuximide and BAY K8644. These rats were recorded for 5 h: 1 h baseline without injection, 1 h after the injection of solvent and 3 h after injection of ethosuximide or BAY K8644 in different doses every hour, according to the rules of cumulative dose design. The doses of ethosuximide were 100, 300 and 900 μg/5 μl, the doses of BAY K8644 were 10, 40, 160 μg/5 μl.

2.1.2. Combined effect of the L-type Ca^{2+} channel agonist and antagonist

A separate group of rats ($n = 8$) was recorded for 3 h: 1 baseline h, 1 h after the administration of nimodipine and 1 h after the administration of BAY K8644. Nimodipine was injected i.p. at a dose of 17.6 mg/kg in a volume of 2 ml. BAY K8644 was administered i.c.v. at a dose of 80 μg/5 μl. A cumulative design was not considered appropriate considering possible toxic effects of the two conotoxins. ω-conotoxin GVIA and ω − conotoxin MVIIC were, thus, given at one dose only. The i.c.v. dose for ω − conotoxin GVIA was 0.1 μg/5μl ($n = 8$), the same dose was used for ω − conotoxin MVIIC ($n = 9$). Before administration, a 1-h baseline was recorded, followed by an injection of solvent and 1-h electroencephalographic recording. Next, the animals were injected with a conotoxin and electroencephalographic activity was recorded for the subsequent 3 h.

2.2. Data analyses

In all experiments, the number and duration of spike-wave discharges were visually scored according to criteria described earlier (Van Luijtelaar and Coenen, 1986). The behaviour of the animals was monitored through a window from an adjacent room. Fifteen minutes after every injection, active (locomotor) and passive (immobile sitting or lying) behaviour was scored for 30 min. Behaviour was quantified with the aid of The Observer, a software package for behaviour data (Noldus, 1991). All data (spike-wave discharges and behaviour) were analysed with analyses of variance with time/dose as "within group" factor. If significant overall effects were found, then *t*-tests for dependent groups were subsequently used. A P value smaller than 0.05 was considered significant.

3. Results

3.1. Effects of ethosuximide

The data (Fig. 1) indicate a dose-dependent decrease of the number and total duration of spike-wave discharges. The analyses of variance showed clear effects of ethosuximide administration. First, there was a significant ($F(4,35) = 5.75$; $P < 0.01$) dose (or hour) effect. After 300 μg of the drug, the rats had a significantly smaller number of spike-wave discharges than after baseline, solvent or 100 μg ethosuximide. Additionally, after the highest dose of ethosuximide the number of spike-wave discharges was lower than after baseline, solvent, 100 or 300 μg ethosuximide.

Secondly, there was also a significant effect ($F(4,35) = 5.21$; $P < 0.01$) on the total duration of spike-wave discharges episodes. A dose-related decrease was found: a decrease at all times measured in comparison to the baseline hour was observed. The two highest concentrations of

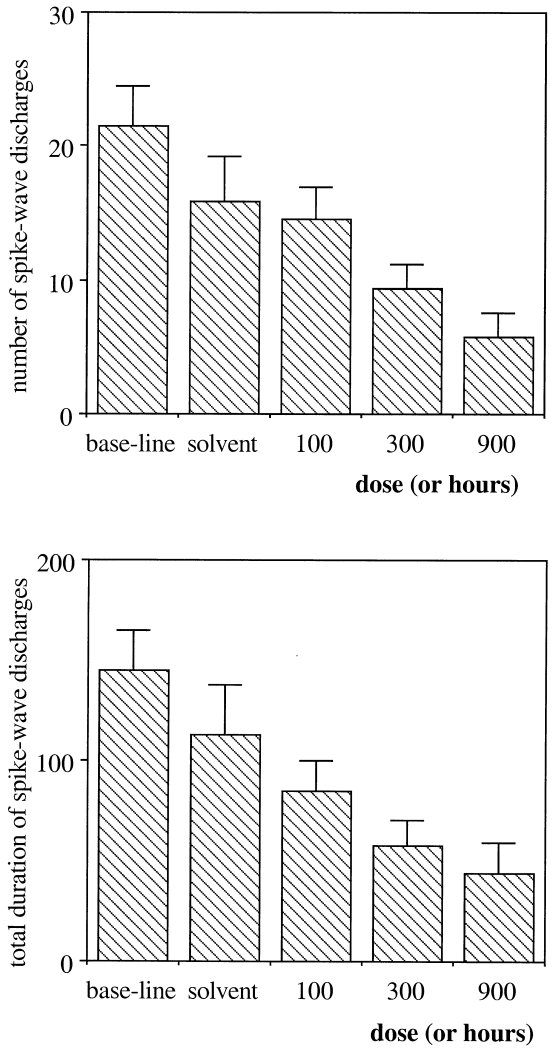

Fig. 1. Dose-related decrease of the number (upper) and total duration (lower plot) of spike-wave discharges in WAG/Rij rats after the i.c.v. administration of the T-type Ca^{2+} channel antagonist, ethosuximide, in a cumulative dose electroencephalographic experiment. Mean and S.E.M. are depicted.

the drug caused the shortening of the total duration of spike-wave discharges and their effects differed significantly from the hour at which the rats had received the solvent. Additionally, there was also a significant decrease in the total duration for the 300 μg group compared to the 100 μg group.

A substantial part of the behavioural variance could be ascribed to the different doses of ethosuximide ($F(4,36) = 3.75$; $P < 0.05$): the duration of passive behaviour was prolonged after 900 μg compared to that after saline and at baseline.

3.2. Effects of BAY K8644

There were significant differences ($F(4,28) = 5.85$; $P < 0.01$) in the number of spike-wave discharges with the different doses of BAY K8644. The two highest doses of the drug were followed by a smaller number of spike-wave discharges than during baseline and after solvent. Additionally, the number of spike-wave discharges was smaller after the injection of 40 μg BAY K8644, compared to that in rats after injection of 10 μg of the drug. There were also differences in the total duration of spike-wave discharges ($F(4,28) = 5.36$; $P < 0.01$). A decrease was found after 40 μg compared to baseline and after 160 μg compared to baseline and saline (Fig. 2). There were no effects of different doses of BAY K8644 on the duration of active and passive behaviour.

3.3. Effects of nimodipine and combined effects of nimodipine and BAY K8644

The number of spike-wave discharges showed significant differences between the hours of the recording

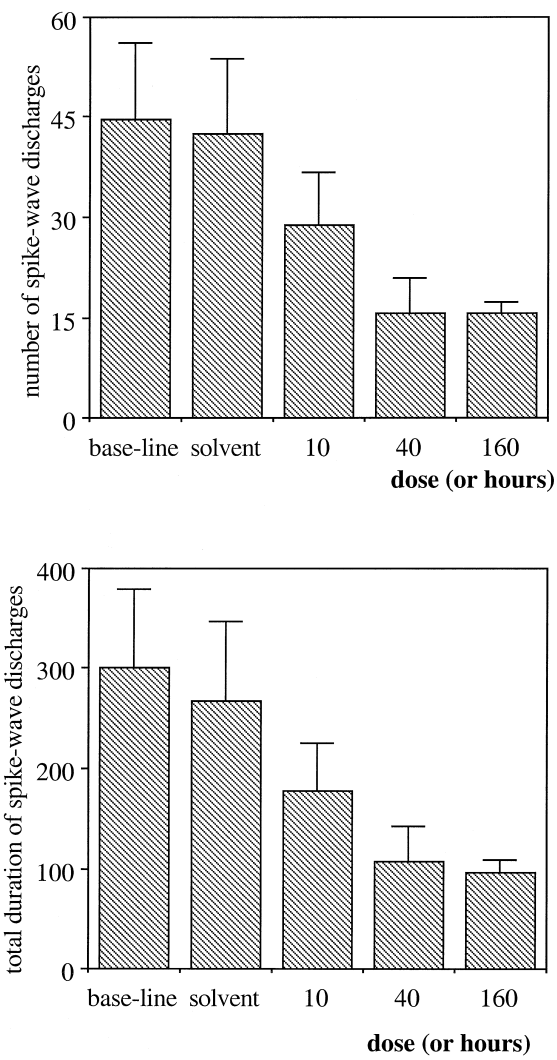

Fig. 2. Dose-related decrease of the number (upper) and total duration (lower plot) of spike-wave discharges in WAG/Rij rats after the i.c.v. administration of the L-type Ca^{2+} channel agonist, BAY K8644, in WAG/Rij rats in a cumulative dose electroencephalographic experiment. Mean and S.E.M. are depicted.

($F(2,21) = 148.38$; $P < 0.01$) (Fig. 3). The number of spike-wave discharges was higher after nimodipine administration than in the predrug control hour. The number of spike-wave discharges was significantly reduced after BAY K8644 administration compared to the previous hour (nimodipine administration) but did not reach the control values. A similar effect ($F(2,21) = 104.6$; $P < 0.01$) was observed for the total duration of spike-wave discharges: there was an increase in total duration of spike-wave discharges after nimodipine in comparison to the previous control hour. A subsequent decrease after BAY K8644 administration was found in comparison with the nimodipine hour (Fig. 3).

Analysis of behaviour also revealed also a significant ($F(2,21) = 10.54$; $P < 0.001$) effect: the mean duration of passive behaviour was longer after nimodipine and BAY K8644 than in the preceding baseline hour.

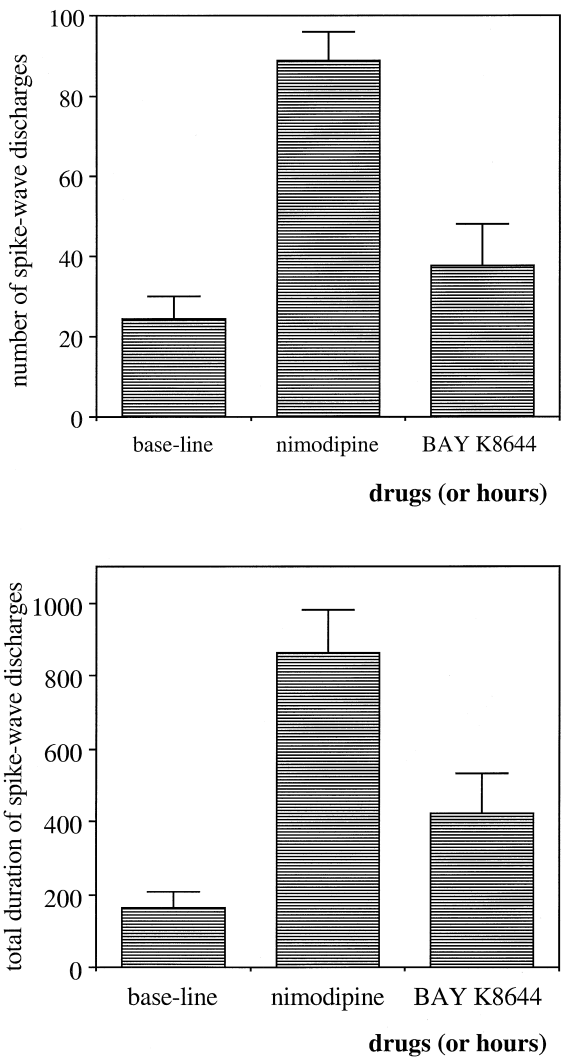

Fig. 3. Effects of L-type Ca^{2+} channel antagonist nimodipine (17.2 mg/kg, i.p.), followed by L-type Ca^{2+} channel agonist, BAY K8644 (80 μg/5 μl, i.c.v.), on the number (upper) and total duration (lower) of spike-wave discharges in WAG/Rij rats. Mean and S.E.M. are depicted.

3.4. Effects of conotoxins

Analysis of electroencephalographic activity after ω-conotoxin, GVIA (N-type Ca^{2+} channel blocker), showed a significant change in spike-wave discharges over time ($F(4,40) = 65.12$; $P < 0.01$). From the post hoc tests, it appeared that the number of spike-wave discharges was decreased after the injection of GVIA compared to both baseline and saline. The decrease persisted in the second and third hour after injection. A similar effect of the drug was found for the total duration of spike-wave discharges ($F(4,40) = 19.89$; $P < 0.0001$). The total duration was decreased for the entire recording period. An effect was also found for the mean duration of spike-wave discharges: the mean duration was found to be reduced in the second hour post-injection compared to that after saline and at baseline.

The rats lay remarkably still after the administration of the compound, but were not asleep (knock-out lying) since there was no large-amplitude slow activity characteristic for non-REM sleep in their electroencephalographic activity. Four rats made additional shaking movements after they tried to change position. During this shaking, the tail rose. The duration of this shaking was always between 10 and 15 s. After this shaking the rats resumed knock-out lying. No changes in the electroencephalographic activity were noticed during these shaking periods. Passive behaviour was increased ($F(2,24) = 22.81$; $P < 0.01$) after ω − conotoxin GVIA compared to that during baseline and after saline.

Electroencephalographic activity after the i.c.v. administration of ω-conotoxin MVIIC (P/Q type Ca^{2+} channel blocker) showed significant effects for number ($F(4,45) = 15.51$; $P < 0.01$), mean ($F(4,45) = 35.56$; $P < 0.01$) and total duration ($F(4,45) = 13.86$; $P < 0.01$). After saline and after the injection with this conotoxin, the number of spike-wave discharges was increased compared to baseline recording. In the second hour, the number was significantly decreased compared to saline and the first conotoxin hour. The mean and total duration of spike-wave discharges were also significantly reduced but only in the second hour after administration. The effects on total duration had disappeared in the third hour. ω-conotoxin MVIIC also had effects on behaviour: the duration of automatic (grooming, digging) behaviour was increased.

3.5. Discussion

It is generally accepted that Ca^{2+} channel blockers act as anticonvulsant drugs (De Sarro et al., 1990; Rogawski and Porter, 1990; Walden et al., 1992; Czuczwar et al., 1994). However, previous experiments performed from this laboratory have shown opposite effects of T- and L-type Ca^{2+} channel blockers in nonconvulsive epilepsy (Van Luijtelaar et al., 1995). Considering these opposite

effects, the fact that Ca^{2+} channel blockers and openers have also peripheral effects (e.g. on skeletal and cardiac muscles and basic systemic parameters, such as blood pressure), and since these compounds might indirectly modulate seizure activity, including spike-wave discharges, through these peripheral effects (Coenen et al., 1992; Gralewicz et al., 1994; Zapater et al., 1998), we attempted to confirm and extend our previous results. Therefore, ethosuximide and BAY K8644 were administered i.c.v. Since it is rather difficult to prepare a clear solution with nimodipine and since a clear solution is essential for i.c.v. administration, it was decided to administer nimodipine only i.p. Another important difference from our previous study was that the subjects were now 1-year-old WAG/Rij rats instead of 2-year-old Wistar rats. WAG/Rij rats of this age are a well-validated model of generalized absence epilepsy. In contrast to some of the mouse mutant models, the WAG/Rij strain of rats is a model of generalized absence epilepsy without other known neurological or cognitive deficits: WAG/Rij rats show normal learning in a two-way active avoidance task and in different spatial memory tasks (Van Luijtelaar and Coenen, 1997). Findings of our earlier study with nimodipine and BAY K8644 were confirmed and extended in WAG/Rij rats (Van Luijtelaar et al., 1995).

The mechanism of action of Ca^{2+} channels depends on their electrophysiological, pharmacological and biophysical properties. Both L, N, P/Q and T-type Ca^{2+} channels are present in the brain. There are more T-type than L-type channels in the thalamus (Carbone and Lux, 1986), a structure that is heavily involved in the generation of spike-wave discharges. Spike-wave discharges are generated by rhythmic interaction between thalamic and cortical areas, a process in which the T-type Ca^{2+} currents are crucial. The suppression of T-type Ca^{2+} currents in thalamic neurons is a mechanism by which ethosuximide controls spike-wave discharges (Coulter et al., 1989, 1990; Yamada et al., 1993; Huguenard and Prince, 1994; Snead, 1995; Varadi et al., 1995), although recently strong doubts were expressed (Leresche et al., 1998; Campbell and Hess, 1999). Leresche and coworkers noticed a lack of action of ethosuximide on low- and high-threshold Ca^{2+} currents. They found instead that ethosuximide decreased the non-inactivating Na^{+} current and acted on the Ca^{2+}-activated K^{+} current, which should explain the decrease in burst firing, the increase in tonic firing and ultimately, the decrease in the number of spike-wave discharges.

Ethosuximide has been the drug of choice for the treatment of absence epilepsy since its introduction in 1960. The majority of the studies and models suggest that it blocks Ca^{2+} entry through the T-type channels. More specifically, the low-threshold Ca^{2+} spike in the thalamic relay cells is suppressed, and burst firing is inhibited. The effect of ethosuximide, a dose-dependent decrease in number and total time of spike-wave discharges, is fully consistent with previous reports, both for humans and rats (in vivo and in vitro) (Peeters et al., 1988; Coulter et al., 1989, 1990; Rogawski and Porter, 1990; Kostyuk et al., 1992; Van Luijtelaar et al., 1995).

The dihydropyridines, including nimodipine, prevent the Ca^{2+} influx through neuronal voltage-dependent L-type Ca^{2+} channels and are effective anticonvulsant drugs in the tonic–clonic phase of convulsive epilepsy. This has been confirmed, mainly in models of complex partial epilepsy and generalized tonic–clonic seizures (De Falco et al., 1992; De Sarro et al., 1992a,b; Czuczwar et al., 1994; Zapater et al., 1998). Interestingly, we had found an opposite effect on nonconvulsive type of epilepsy (Van Luijtelaar et al., 1995) and this is expected also to occur in the nonconvulsive WAG/Rij absence model we now used. The results obtained after the administration of nimodipine in WAG/Rij rats, a dose-dependent increase in the number of spike-wave discharges, confirmed our previous results obtained in 2-year-old Wistar rats (Van Luijtelaar et al., 1995). Therefore, it seems that nimodipine has both anti-convulsant effects in convulsive epilepsy and epileptoformic effects in nonconvulsive types of epilepsy.

The L-type Ca^{2+} channel opener, BAY K8644, is generally considered to be a proconvulsant drug (De Sarro et al., 1992a), although not in all models. It does not induce audiogenic seizures in Genetic Epilepsy Prone Rats and DBA/2 mice and is not effective in the convulsion threshold test in mice (De Sarro et al., 1990; Czuczwar et al., 1994), but convulsions were observed after a high dose (De Sarro et al., 1992b; Czuczwar et al., 1994). In agreement with the latter outcomes, we had observed fatal convulsions in our previous study with old Wistar rats and in the present study, the highest concentration (160 $\mu g/5\mu l$) caused convulsions in WAG/Rij rats. The convulsions did not appear after administration of 80 μg. Even more pronounced than the proconvulsant effects of BAY K8644 was the dose-dependent suppression of spontaneously occurring spike-wave discharges. The present data confirm our previous results: BAY K8644 has, as does nimodipine, opposite effects on convulsive and nonconvulsive epilepsy. It is, on one hand, a proconvulsant drug and, on the other hand, it decreases the number of spike-wave discharges dose dependently and can be, therefore, considered as an anti-absence drug. Thus, BAY K8644 is proconvulsant but reduces spike-wave discharges. The present data, obtained after i.c.v. administration of BAY K8644, showed that the reduction in the number of spike-wave discharges is centrally mediated; there were no changes in duration of passive behaviour and its effects, as reported here, cannot be explained by peripheral actions.

The significant increase in the number and total duration of spike-wave discharges after injection of nimodipine was blocked by the subsequent administration of BAY K8644. It seemed that BAY K8644 reduced the nimodipine-enhanced number of spike-wave discharges. These results were to be expected, considering that L-type Ca^{2+}

channel openers and blockers show opposite effects in models of convulsive and of nonconvulsive epilepsy. The L-type Ca^{2+} channel blocker, flunarizine, has been shown to enhance the threshold for electroconvulsive shocks, whereas BAY K8644 has been shown to lower this threshold (Gasior et al., 1995). In the present experiment, BAY K8644 was given 1 h after nimodipine with the assumption that nimodipine was still effective. This has been clearly demonstrated in 2-year-old Wistar rats: in that study, nimodipine was effective for at least 2 h post-injection (Van Luijtelaar et al., 1995). The fact that BAY K8644 only partially reverses the nimodipine-induced effects can be explained by our experimental protocol and by the properties of the dihydropyridines: dihydropyridines, such as BAY K8644 and nimodipine, can only bind to the L-type Ca^{2+} channel when the channel is in an open state (Hockerman et al., 1997; Striessnig et al., 1998). A previous block of the channels by nimodipine might, therefore, be difficult to reverse with a Ca^{2+} channel agonist. It was shown that in other seizure models, the effects of BAY K8644 could be antagonised by the L-type Ca^{2+} blockers, nimodipine and nitrendipine (De Sarro et al., 1992a). Therefore, it seems that in the case of drugs that modulate L-type Ca^{2+} channels, such as BAY K8644 and nimodipine, there are two possible ways to antagonise epileptic activity. Firstly, BAY K8644 can reduce the epileptoformic effects of nimodipine on nonconvulsive epilepsy. Secondly, nimodipine can inhibit the convulsions evoked by BAY K8644. The present results also showed that the effects of nimodipine i.p. on spike-wave discharges can be antagonised by BAY K8644 administered into the ventricle. This suggests that the effects of both compounds are centrally mediated. The results obtained with nimodipine and BAY K8644 in this and in our previous study suggest that not only the T-type Ca^{2+} currents, but L-type Ca^{2+} currents also, are important in the control of spike-wave discharges. The results also showed that blocking T-type Ca^{2+} channels inhibits, and that blocking L-type channels enhances, spike-wave discharges.

A role for other than the T-type Ca^{2+} channels in generalized absence epilepsy is less well documented, although a role of L-type Ca^{2+} currents in the occurrence of the processes leading to the burst mode of thalamocortical relay cells is acknowledged. Huguenard (1996) proposed that the long duration of the low threshold Ca^{2+} spike could lead to activation or inactivation of other voltage- or-Ca^{2+}-dependent conductance that can interact with the T-type Ca^{2+} current to produce repetitive bursts. McCormick and Huguenard (1992) proposed an interaction between T- and L-type Ca^{2+} currents for burst generation. McCormick and Bal (1997) proposed that depolarisation of the cell membrane of a rhythmic burst firing cell deactivates a portion of the high-threshold spike that was based on activation of the L-type Ca^{2+} current. Their model is based on in vitro studies in the ferret. It emphasises an interaction between relay cells in the lateral geniculate and inhibitory cells in the caudal part of the reticular thalamic nucleus. Their model suggests that L- and T-type Ca^{2+} currents interact to produce the Ca^{2+} spike and that this paves the way for the afterhypolarization, which forms the base for the spindle bursts. However, the McCormick and Bal model does not clearly explain the opposite effects in the electroencephalographic activity we now found after administration of the T-type (a decrease of spike-wave discharges) and L-type (an increase of spike-wave discharges) Ca^{2+} channel blockers. One possibility is that an assumed altered L-type Ca^{2+} channel function in WAG/Rij rats causes these channels to inactivate at a faster rate. Such an enhanced inactivation might enhance Ca^{2+}-sensitive K^+ channels. This, in turn, would lead to a more pronounced hyperpolarization phase. This could allow the activation/inactivation cycle that is observed during spike-wave discharges, consisting of T-type Ca^{2+} channels and K^+ channels, respectively, to activate faster upon inactivation.

A second possibility is that the perigeniculate nucleus in the ferret has anatomical and functional properties different from those of that part of the reticular thalamic nucleus in the rat. Particularly, the presence of dendro-dendritic contacts between neurons in the caudal part of the reticular thalamic nucleus, e.g. the perigeniculate nucleus might be important for the generation of oscillations (Steriade, 1999). These contacts may be crucial for synchronisation of the spindle or spike-wave discharge rhythm in the pacemaking reticular thalamic nucleus with the whole thalamus. Such dendro-dendritic contacts between reticular thalamic neurons have been described in for cats and monkeys, not for rats. Next, preliminary data obtained by us suggest that the caudal part of the reticular thalamic nucleus including the perigeniculate nucleus does not has pacemaker properties (Meeren et al., 1998).

Studies at the molecular level might also give some other clues concerning structure and diversity of Ca^{2+} channels and their role in absence epilepsy (Varadi et al., 1995; Ryan, 1999). Recent application of genetic analysis in different mutant, genetically distinct, autosomal recessive models of absence epilepsy has resulted in the suggestion that these syndromes are caused by mutations in genes encoding three types of Ca^{2+} channel subunits (Ryan, 1999). More specifically, mutations were identified in the genes encoding the α1A and βa4 subunits of voltage-gated P-type Ca^{2+} channels. B subunits normally regulate Ca^{2+} currents via a direct interaction with α1 (pore forming) subunits of these channels (Caddick et al., 1999). The mutated Ca^{2+} channel α1A subunit gene that encodes the pore-forming protein of P/Q-type voltage-dependent Ca^{2+} channels is present in the tottering mice. However, our preliminary data do not suggest a large contribution for this subtype channel since the decrease in the number of spike-wave discharges after the i.c.v. administration of the P/Q Ca^{2+} channel blocker, ω-conotoxin MVIIC, was only found in the second hour after injection. It should be

added, however, that the conotoxin we now used does not block all P/Q Ca^{2+} channels, so the possibility exists that only a subclass of P/Q Ca^{2+} channels is crucial for spike-wave discharge activity. Recently, it appeared that ω-conotoxin MVIIC also blocks N-type Ca^{2+} channels (Herrero et al., 1999). More conclusive in vivo pharmacological experiments will require more selective molecules. Lingenhöhl et al. (1997) found no effects of a P-type Ca^{2+} channel antagonist on electroshock induced convulsions in rats. Some indications were found for a putative role for the N-type Ca^{2+} channel subtype in absence epilepsy, since ω − conotoxin GVIA blocks the N-type Ca^{2+} channels and spike-wave discharges were reduced. N-type Ca^{2+} channels are localised in nerve terminals, whereas L-type Ca^{2+} channels may be localised preferentially in and nearby cell bodies. The number of ω − conotoxin GVIA binding sites in rat brain associated with N-type Ca^{2+} channels is approximately 10 times greater than that of L-type Ca^{2+} channels and ω-conotoxin GVIA binding was not inhibited by various classes of L-type Ca^{2+} channel antagonists (Yamada et al., 1993). Jackson and Scheideler (1996) found that the administration of ω − conotoxin GVIA in DBA 2 mice induced a shaking syndrome. This behaviour consists of persistent whole body shakes, which involved the head, neck and all four limbs. Similar results were found in 4/9 of our rats. All our rats showed immobility, knock-out lying without any sign of sleep as was clear from the electroencephalographic activity. Four of our rats showed body and limb shakes on trying to change their position. After 10 to 15 s, the rats resumed their original knock-out lying position. This particular behaviour of the rats after GVIA makes it difficult to reach conclusions about a putative role for the N-type Ca^{2+} channels on spike-wave discharges, although an anti-absence effects of this type of blocker is still possible.

In summary, the results of our experiment confirm a role of the T-type Ca^{2+} channels and extend our view that T- and L-type Ca^{2+} channel blockers have opposite roles regarding the control of spike-wave discharges, which cannot be due to peripheral effects of the compounds. The way in which L- and T-type Ca^{2+} currents interact in the pathogenesis of the spike-wave discharges characteristic for generalized absence epilepsy does not fit a recently proposed model. A minimal prerequisite for making progress is that outcomes from molecular studies in which deficits for specific receptors are suggested to be crucial for a specific form of epilepsy should be in agreement with data from pharmacological studies. More selective and clean Ca^{2+} channel blockers have to be evaluated in the various genetic absence epilepsy models.

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