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Influence of isradipine, niguldipine and dantrolene on the anticonvulsive action of conventional antiepileptics in mice

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Abstract

We report the effects of two new dihydropyridine derivatives, isradipine (4-(4′-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid methylisopropylester) and niguldipine (1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinecarboxylic acid 3-(4,4-diphenyl-1-piperidinyl)-propyl methyl ester hydrochloride), and of dantrolene (1-{(5-{*p*-nitrophenyl}furfurylidene)-amino}hydantoin sodium, an inhibitor of Ca^{2+} release from intracellular stores) on the protective efficacy of antiepileptic drugs against maximal electroshock-induced seizures. It was shown that dantrolene (5–20 mg/kg), isradipine (5–10 mg/kg) and niguldipine (up to 2.5 mg/kg) did not influence the electroconvulsive threshold in mice, although a higher dose of niguldipine (5 mg/kg) significantly elevated it. Dantrolene (10–20 mg/kg) and isradipine (1 mg/kg) did not affect the anticonvulsive activity of conventional antiepileptic drugs. In contrast, niguldipine (2.5–5 mg/kg) impaired the protective action of carbamazepine and phenobarbital. No effect of niguldipine (2.5–5 mg/kg) was observed upon the anticonvulsive efficacy of diphenylhydantoin and valproate. BAY k-8644 (methyl-1,4-dihydro-2,6-dimethyl-5-nitro-4-{(2-trifluoromethyl)-phenyl}-pyridine-5-carboxylate, an L-type Ca^{2+} channel agonist) did not reverse the action of niguldipine alone or the niguldipine-induced impairment of the anticonvulsive action of carbamazepine and phenobarbital. Niguldipine did not influence the free plasma levels of carbamazepine and phenobarbital, so a pharmacokinetic interaction is not probable. The results suggest that in contrast to the anticonvulsive activity of niguldipine against electroconvulsions, this Ca^{2+} channel inhibitor significantly weakened the protective action of both carbamazepine and phenobarbital. These effects do not seem to result from the blockade of voltage-dependent Ca^{2+} channels. Isradipine and dantrolene did not have a modulatory action on the threshold for electroconvulsions or on the anticonvulsive activity of antiepileptic drugs. It may be concluded that the use of niguldipine, isradipine, and dantrolene in epileptic patients seems questionable. © 1997 Elsevier Science B.V. All rights reserved.

Keywords: Dihydropyridine; Dantrolene; Anti-epileptic; Electroshock, maximal; Seizure

1. Introduction

Antiepileptic drugs may exert their anticonvulsant activity at the ionic level, either by indirect or direct actions on neuronal ion conductances (Porter, 1989). Among voltage-gated cationic ion channels, those for Na^+, K^+ and Ca^{2+} are of special interest (Faingold, 1992). Voltage-gated Ca^{2+} channels have been divided into four main types (T, N, L and P), all of which have been determined in neurons (Faingold, 1992). Several clinically established antiepileptics are thought to interact with voltage-gated Ca^{2+} channels. Thus, diphenylhydantoin, carbamazepine, barbiturates and benzodiazepines have been observed to reduce Ca^{2+} influx into synaptic terminals, although in some cases the concentrations required were in excess of therapeutic levels (Faingold, 1992). The effect on N channels may explain the ability of these drugs to reduce transmitter release, since an important function of N channels has been proposed to involve mediation of the presynaptic release of neurotransmitters. Diphenylhydantoin is reported to suppress N-type and T-type Ca^{2+} currents (Faingold, 1992). Finally, several antiepileptic drugs, i.e. diphenylhydantoin, carbamazepine and benzodiazepines, have been reported to interact with the intracellular effects of Ca^{2+}, e.g., by inhibiting calmodulin activation of calmodulin kinase II. This effect of antiepileptics could be involved in their actions on cell functions and synaptic excitability (De Lorenzo, 1988).

Various Ca^{2+} channel inhibitors have been examined for anticonvulsant activity. For instance, in audiogenic seizures the phenylalkylamines (e.g., verapamil) are completely ineffective, while dihydropyridines (nifedipine,

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nicardipine, nimodipine and nitrendipine) and the diphenylalkylamine (flunarizine) and, less potently so, the benzothiazepine derivative, diltiazem, exert anticonvulsant effects against clonic and/or tonic seizures (De Sarro et al., 1988). Contradictory data exist in relation to convulsions resulting from the stimulation of NMDA receptors. On one hand, Dolin et al. (1988) reported on the proconvulsive activity of dihydropyridines in seizures induced by either systemic or intracerebral *N*-methyl-D,L-aspartic acid, and on the other hand, Palmer et al. (1993) showed anticonvulsant effects of dihydropyridines and other Ca^{2+} channel inhibitors against this systemic convulsant. In other studies, the dihydropyridine derivatives were shown to inhibit pentylenetetrazol-induced seizures and electroconvulsions, but to be ineffective against strychnine- and aminophylline-induced seizures (Czuczwar et al., 1990a; Dolin et al., 1988; Jagiełło-Wójtowicz et al., 1991; Meyer et al., 1990). Finally, Ca^{2+} channel inhibitors have been found to potentiate the potency of common antiepileptic drugs, both against maximal electroshock- and pentylenetetrazol-induced convulsions (Czuczwar et al., 1990a,b, 1992; Gasior et al., 1996). It is noteworthy that the Ca^{2+} channel inhibitors evaluated for anticonvulsant effects primarily block the L-type channel. Dihydropyridines, at high doses, may also act on T-type channels (Faingold, 1992).

The present study assessed the efficacy of a combined treatment with antiepileptic drugs and two new dihydropyridines, isradipine (4-(4′-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid methylisopropylester) and niguldipine (1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinecarboxylic acid 3-(4,4-diphenyl-1-piperidinyl)-propyl methyl ester hydrochloride) or dantrolene (1-{(5-{*p*-nitrophenyl}furfurylidene)-amino}hydantoin sodium, an inhibitor of Ca^{2+} release from intracellular stores) (Mody and MacDonald, 1995), in terms of anticonvulsive activity and adverse effects.

2. Materials and methods

2.1. Animals and experimental conditions

The experiments were carried out on female Albino Swiss mice weighing 20–25 g. The animals were housed in colony cages with free access to food (chow pellets) and tap water. The experimental temperature was 21 ± 1°C and mice were on a natural light-dark cycle. The experimental groups consisting of 8–12 animals were chosen by means of a randomized schedule.

2.2. Drugs

Diphenylhydantoin sodium, carbamazepine (both drugs purchased from Sigma, St. Louis, MO, USA), phenobarbital sodium (Polfa, Kraków, Poland), valproate magnesium (Polfa, Rzeszów, Poland), two dihydropyridines, isradipine and niguldipine hydrochloride, dantrolene sodium and BAY k-8644 (methyl-1,4-dihydro-2,6-dimethyl-5-nitro-4-{(2-trifluoromethyl)-phenyl}-pyridine-5-carboxylate, an L-type Ca^{2+} channel agonist; all four substances from Research Biochemicals International, Natick, MA, USA) were used in this study. Diphenylhydantoin, carbamazepine, isradipine, niguldipine and dantrolene were suspended in a 1% solution of Tween 81 (Loba Chemie, Vienna, Austria). Valproate and phenobarbital were brought into solution with sterile saline. All drugs, except for BAY k-8644 which was given subcutaneously, were administered intraperitoneally in a volume of 10 ml/kg, diphenylhydantoin 120 min, phenobarbital 60 min, valproate, carbamazepine, isradipine, niguldipine, dantrolene 30 min and BAY k-8644 15 min before electroconvulsions and behavioral tests. Doses of drugs refer to their free forms.

2.3. Acute toxicity

Toxicity was assessed by the method of Litchfield and Wilcoxon (1949) and is presented as respective LD_{50} values calculated from the mortality of mice after 24 h. A drug at its LD_{50} is expected to cause 50% lethality in tested animals. To estimate LD_{50} values (in mg/kg), at least four groups consisting of eight mice were pretreated with different doses of a drug and observed 1 and 24 h after drug administration. Afterwards, an intensity-response curve was calculated on the basis of the percentage of dead mice after 24 h.

2.4. Electroconvulsions

Electroconvulsions were produced according to Swinyard et al. (1952), using ear-clip electrodes and alternating current delivered by a Hugo Sachs (Type 221, Freiburg, Germany) generator, the stimulus duration being 0.2 s. Tonic hindlimb extension was taken as the endpoint. The convulsive threshold was evaluated as CS_{50}, which is the current strength (in mA) necessary to produce tonic hindlimb extension in 50% of the animals tested. To estimate the convulsive threshold, at least four groups of mice (8–10 animals per group) were challenged with electroshocks of various intensities. Subsequently, an intensity-response curve was calculated on the basis of the percentage of mice showing convulsions. In order to evaluate the respective ED_{50} values (in mg/kg), mice pretreated with different doses of an antiepileptic drug were challenged with maximal electroshock (25 mA). Again, at least four groups of mice, consisting of 8–10 animals, were used to estimate each ED_{50} value. A dose-effect curve was constructed, based on the percentage of mice protected.

2.5. Chimney test

The effects of antiepileptic drugs on motor impairment were quantified with the chimney test of Boissier et al. (1960). In this test, animals had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm length). Motor

impairment was indicated by the inability of the mice to climb backwards up the tube within 60 s. The results were shown as a percentage of animals which failed to perform the test.

2.6. Estimation of the plasma levels of carbamazepine and phenobarbital

Mice were injected with either vehicle + carbamazepine (or phenobarbital) or niguldipine + one of these two antiepileptic drugs. The animals were killed by decapitation at times scheduled for the convulsive test and blood samples of approximately 1 ml were collected into Eppendorf tubes. Samples of blood were centrifuged at 10 000 r.p.m. (Abbott centrifuge; Irving, TX, USA) for 3 min and plasma samples were pipetted into a micropartition system MPS-1 (Amicon, Danvers, MA, USA) for separation of free from protein-bound microsolutes. The MPS-1 tubes were then centrifuged at 3000 r.p.m. (MPW-360 centrifuge; Mechanika Precyzyjna, Warsaw, Poland) for 10 min and the filtrate samples of 50 μl were applied to Abbott system cartridges. The free plasma levels of carbamazepine and phenobarbital were measured by immunofluorescence, with the use of an Abbott TDx analyzer (Abbott, Irving, TX, USA). Plasma levels of both antiepileptic drugs were expressed in μg/ml of plasma as means ± S.D. of at least seven determinations.

2.7. Statistics

LD_{50}, CS_{50} or ED_{50} values and statistical analysis of the results, obtained in the electroconvulsive tests, were calculated by using a computer program based on the method of Litchfield and Wilcoxon (1949). The results obtained in the chimney test were compared statistically by using Fisher's exact probability test. Plasma levels of antiepileptic drugs were evaluated with unpaired Student's *t*-test.

3. Results

3.1. Acute toxicity

LD_{50} values of isradipine, niguldipine and dantrolene were 16.3, 86.9 and > 500 mg/kg, respectively.

3.2. Effects of isradipine, niguldipine and dantrolene upon the electroconvulsive threshold

Niguldipine (5 mg/kg), applied 30 min, 60 min and 120 min before the test, raised the electroconvulsive threshold from 5.5 to 7.0, 6.9 and 6.7 mA, respectively. However, this Ca^{2+} channel inhibitor did not affect the threshold when injected 180 min before the test. Niguldipine at 2.5 mg/kg did not influence the electroconvulsive threshold (Table 1).

Isradipine (5–10 mg/kg) and dantrolene (5–20 mg/kg), administered 30 min, 60 min, 120 min and 180 min prior to the test, did not influence the electroconvulsive threshold (results not shown in Table 1). In subsequent experiments, isradipine was administered at the dose of 1 mg/kg, 30 min before the test, because of its low LD_{50} value.

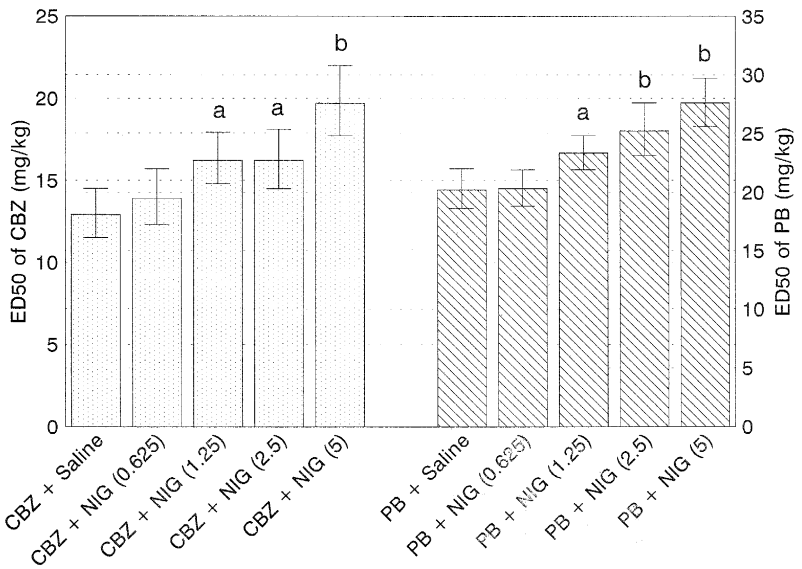

Fig. 1. Effect of niguldipine on the anticonvulsive activity of carbamazepine or phenobarbital against maximal electroshock-induced seizures in mice. Bars represent ED_{50} values in mg/kg. Error bars show 95% confidence limits for the ED_{50} values. All compounds were injected intraperitoneally in a single dose, phenobarbital 60 min, carbamazepine and niguldipine 30 min before the test. The doses of niguldipine are shown below each bar in mg/kg. The calculation of the ED_{50} values and statistical analysis were performed according to Litchfield and Wilcoxon (1949). [a] $P < 0.05$, [b] $P < 0.001$ vs. respective control group. Abbreviations: CBZ, carbamazepine; PB, phenobarbital; NIG, niguldipine.

Table 1
Influence of niguldipine on the convulsive threshold at various times after injection

Treatment (mg/kg)	Time (min)			
	30	60	120	180
Vehicle	5.5 (4.9–6.2)	5.5 (4.9–6.2)	5.4 (4.8–6.1)	5.3 (4.7–6.0)
Niguldipine (2.5)	5.6 (5.0–6.4)	5.7 (5.0–6.4)	N.D.	N.D.
Niguldipine (5)	7.0 [b] (6.2–7.9)	6.9 [b] (6.2–7.8)	6.7 [a] (6.0–7.4)	5.6 (5.0–6.4)

Niguldipine was administered intraperitoneally. Table data are CS_{50} values (in mA) with 95% confidence limits in parentheses. CS_{50} values and statistical analysis were calculated according to Litchfield and Wilcoxon (1949). [a] $P < 0.05$, [b] $P < 0.01$ vs. respective control groups; N.D., not determined.

Table 2
Influence of BAY k-8644 on the convulsive threshold and the anticonvulsant action of niguldipine

BAY k-8644 (mg/kg)	Niguldipine (mg/kg)	
	0	5
0	4.2 (3.4–5.2)	6.0 [a] (5.2–6.9)
5	3.7 (3.1–4.5)	6.7 [b] (6.1–7.4)

BAY k-8644 was given subcutaneously and niguldipine intraperitoneally, 15 and 30 min before the test, respectively. Table data are CS_{50} values (in mA) with 95% confidence limits in parentheses. [a] $P < 0.01$, [b] $P < 0.001$ vs. control group. See also Table 1.

Table 3
Influence of BAY k-8644 on the niguldipine-induced reduction of the protective activity of carbamazepine or phenobarbital

Treatment (mg/kg)	ED_{50} (mg/kg)	
	Carbamazepine	Phenobarbital
Vehicle	14.2 (12.8–15.9)	20.5 (19.0–22.1)
Niguldipine (2.5)	17.3 [a] (15.9–18.8)	24.9 [b] (23.1–26.7)
BAY k-8644 (0.5)	15.3 (14.0–16.8)	19.9 (18.3–21.7)
BAY k-8644 (0.5) + niguldipine (2.5)	17.7 [a] (16.1–19.4)	25.2 [b] (23.1–27.6)

Presented values are ED_{50} values with 95% confidence limits in parentheses (Litchfield and Wilcoxon, 1949). [a] $P < 0.01$, [b] $P < 0.001$ vs. control group. For treatment times refer to Fig. 1 and Table 2.

3.3. Influence of isradipine, niguldipine and dantrolene upon the protective activity of antiepileptic drugs against maximal electroshock-induced seizures in mice

Niguldipine (1.25, 2.5 and 5 mg/kg) significantly elevated the ED_{50} of carbamazepine from 12.9 to 16.2, 16.2 and 19.7 mg/kg, respectively. Niguldipine (1.25, 2.5 and 5 mg/kg) also impaired the protective activity of phenobarbital, as reflected by an increase in its ED_{50} value from 20.2 to 23.3, 25.2 and 27.6 mg/kg, respectively (Fig. 1). However, niguldipine (5 mg/kg) was without an effect on the antielectroshock efficacy of either diphenylhydantoin or valproate (Fig. 2). Neither isradipine (1 mg/kg) nor dantrolene (10–20 mg/kg) affected the protective activity

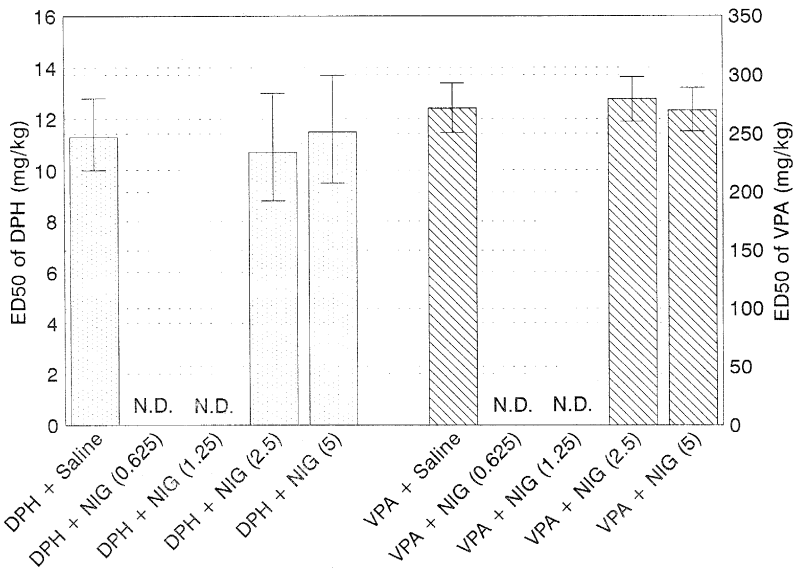

Fig. 2. Influence of niguldipine on the protective action of diphenylhydantoin or valproate against maximal electroshock-induced seizures in mice. Bars represent ED_{50} values in mg/kg. Error bars show 95% confidence limits for the ED_{50} values. All compounds were injected intraperitoneally in a single dose, diphenylhydantoin 120 min, valproate and niguldipine 30 min before the test. The doses of niguldipine are shown below each bar in mg/kg. The calculation of the ED_{50} values and statistical analysis were performed according to Litchfield and Wilcoxon (1949). Abbreviations: DPH, diphenylhydantoin; VPA, valproate; NIG, niguldipine; N.D., not determined.

of the antiepileptic drugs studied (results not shown in the figures).

3.4. Influence of BAY k-8644 on the convulsive threshold and the anticonvulsive action of carbamazepine and phenobarbital against maximal electroshock

Bay k-8644 (5 mg/kg) did not affect the convulsive threshold and the anticonvulsive action of niguldipine against electroconvulsions (Table 2). Moreover, BAY k-8644 (0.5 mg/kg) did not influence the protection offered by carbamazepine or phenobarbital against maximal electroshock. Finally, this Ca^{2+} channel activator did not reverse the niguldipine-induced impairment of the anticonvulsant efficacy of either carbamazepine or phenobarbital (Table 3).

3.5. Chimney test

When applied at doses equal to their ED_{50} values against maximal electroshock-induced convulsions, carbamazepine (12.9 mg/kg) and phenobarbital (20.2 mg/kg) did not influence the performance of mice in the chimney test. Also niguldipine (2.5 mg/kg) did not cause any motor disturbances. The combined treatment with niguldipine (2.5 mg/kg) and carbamazepine (16.2 mg/kg), at doses providing 50% protection against maximal electroshock, resulted in a slight motor impairment, when compared to carbamazepine alone given at its ED_{50} of 12.9 mg/kg. However, carbamazepine alone (16.2 mg/kg) also produced motor disturbances (Table 4). Dantrolene (20 mg/kg) caused strong motor impairment in 75% of mice. The combined treatment of dantrolene (20 mg/kg) with phenobarbital (18 mg/kg) or carbamazepine (13.2 mg/kg), at doses providing 50% protection against maximal electroshock, also resulted in significant motor disturbances (87.5 and 75% of mice impaired, respectively – results not shown in Table 4).

Table 4
Motor impairment after administration of antiepileptic drugs, niguldipine or a combination of niguldipine and an antiepileptic

Treatment (mg/kg)	*n*	Mice impaired (%)
Vehicle	12	0
CBZ (12.9)	12	25
CBZ (16.2)	12	33.3 [a]
CBZ (16.2) + niguldipine (2.5)	12	41.6 [a]
PB (20.2)	12	16.6
PB (24.9)	12	25
PB (24.9) + niguldipine (2.5)	12	25
Niguldipine (2.5)	12	0

The results of the chimney test are expressed as the percentage of animals that failed to perform this test (see Section 2). [a] $P < 0.05$ vs. saline-treated group (Fisher's exact probability test), *n*, number of animals. Antiepileptics at lower doses and the combined treatment provided 50% protection against maximal electroshock. Treatment times are listed in Fig. 1.

Table 5
Influence of niguldipine on the free plasma levels of carbamazepine and phenobarbital in mice

Treatment (mg/kg)	Niguldipine (mg/kg)	
	0	2.5
Carbamazepine (16.2)	2.25 ± 0.24	2.29 ± 0.21
Phenobarbital (24.9)	19.36 ± 1.52	18.39 ± 1.35

Presented values are the means (in μg/ml) of eight determinations ± S.D. Unpaired Student's *t*-test was used for statistical analysis of the data. For treatment times see Fig. 1.

3.6. Influence of niguldipine on the free plasma levels of carbamazepine and phenobarbital

Niguldipine (2.5 mg/kg) did not alter the free plasma levels of carbamazepine (16.2 mg/kg) and phenobarbital (24.9 mg/kg; Table 5).

4. Discussion

In the present study, only niguldipine raised the electroconvulsive threshold; isradipine and dantrolene were without effect. The niguldipine-induced increase in the electroconvulsive threshold was not reversed by BAY k-8644 (5 mg/kg), so the anticonvulsive activity of niguldipine is probably independent of Ca^{2+} channel blockade. It is noteworthy that BAY k-8644 (5 mg/kg) significantly reduced the anticonvulsive action of flunarizine against electroconvulsions (Gasior et al., 1995). It was previously documented that nicardipine potentiated the efficacy of carbamazepine against maximal electroshock, whilst nimodipine possessed a broader spectrum of activity, additionally enhancing the protection offered by diphenylhydantoin. However, nicardipine distinctly increased the level of carbamazepine, while nimodipine did not affect the levels of either antiepileptic in plasma. The combined treatment with these Ca^{2+} channel inhibitors and antiepileptic drugs, at doses providing 50% protection against maximal electroshock, did not significantly affect the motor performance of mice in the chimney test, when compared to antiepileptics given alone at their ED_{50} values (Czuczwar et al., 1992). Niguldipine did not influence the protective activity of valproate or diphenylhydantoin, but reduced the antielectroshock potency of carbamazepine and phenobarbital against maximal electroshock. Moreover, this Ca^{2+} channel inhibitor did not alter the free plasma levels of either carbamazepine or phenobarbital, so a pharmacokinetic interaction, at the time intervals scheduled for the convulsive test at least, is not probable. BAY k-8644 (1–5 mg/kg) impaired per se the protective efficacy of phenobarbital and carbamazepine (Gasior et al., 1995). Consequently, to evaluate an involvement of Ca^{2+}

channel blockade in the niguldipine-induced reduction of the protective effects of carbamazepine and phenobarbital, BAY k-8644 was used in the dose of 0.5 mg/kg. At this dose BAY k-8644 did not affect the niguldipine-induced decrease in the anticonvulsive activity of carbamazepine or phenobarbital so the involvement of Ca^{2+} channel blockade in this effect seems doubtful.

Carbamazepine and phenobarbital are thought to exert some of their protective activity via Ca^{2+} channels (Gasior et al., 1995). Furthermore, most whole cell currents during normal neuronal function are considered to be carried by dihydropyridine-insensitive N-type channels (Nowycky et al., 1985). But during strong and sustained depolarization, such as that observed during initiation and propagation of ictal activity, much of the voltage-sensitive Ca^{2+} influx is believed to be mediated by L-type channels (Fox et al., 1987). In this context, the reduction by niguldipine of carbamazepine's or phenobarbital's anticonvulsive potential against maximal electroshock was completely unexpected.

Isradipine and dantrolene (ineffective on the threshold alone) did not alter the protective efficacy of antiepileptic drugs against maximal electroshock-induced seizures. Dantrolene was administered in doses up to 20 mg/kg, because of the strong motor impairment induced per se. In combination studies, isradipine was applied at the dose of 1 mg/kg, because of its low LD_{50}. Among other Ca^{2+} channel inhibitors, only verapamil did not influence the anticonvulsant activity of conventional antiepileptics against pentylenetetrazol- and maximal electroshock-induced convulsions (Czuczwar et al., 1990a,b). According to Hamann et al. (1983), this effect might be due to the limited penetration of this Ca^{2+} antagonist into the brain. Whether isradipine shares a similar pharmacokinetic profile remains an open question.

Recent findings indicate that a large proportion of the neurotoxic Ca^{2+} that enters nerve cells, following depolarization, originates from an intracellular Ca^{2+} pool. The release of Ca^{2+} from this pool is sensitive to the skeletal muscle relaxant dantrolene. It is possible that the initial Ca^{2+} entry might be augmented by the release of Ca^{2+} from intracellular stores, which may lead to neurotoxicity (Mody and MacDonald, 1995). In our study, dantrolene affected neither the electroconvulsive threshold nor the protective activity of conventional antiepileptic drugs against maximal electroshock. This may suggest that Ca^{2+} release from intracellular stores may have nothing to do with the efficacy of antiepileptic drugs. However, it is interesting to note that the combination of dantrolene and antiepileptic drugs resulted in a considerable impairment of motor performance.

In conclusion, our study is the first one reporting that a dihydropyridine Ca^{2+} channel inhibitor, niguldipine, is able to reduce the anticonvulsant action of some conventional antiepileptic drugs. This may lead to the assumption that Ca^{2+} channel inhibitors do not generally enhance the anticonvulsive action of antiepileptic drugs, which was found to be the case for different combinations with antiepileptic drugs (Czuczwar et al., 1990a,b, 1992; Gasior et al., 1996). Consequently, the selection of Ca^{2+} channel inhibitors to be used in clinical trials, in our opinion, should be based upon experimental data. To a certain degree, a correlation exists between the results of experimental studies and clinical data. For instance, flunarizine potentiates the anticonvulsive activity of all major antiepileptics against maximal electroshock-induced seizures in mice (Czuczwar et al., 1992). When used as an adjuvant, flunarizine diminishes the incidence of seizures in therapy-resistant patients with partial complex seizures (Overweg et al., 1984). These results were completed by Binnie et al. (1985), who showed that the adverse effects of such combinations (drowsiness and gain of weight) were not serious and moreover, flunarizine remained without effect upon the plasma levels of antiepileptic drugs. However, there is also a report pointing to a low effectiveness of flunarizine co-administered with antiepileptic drugs in patients with intractable epilepsy (Nakane et al., 1989). Nimodipine is much less potent than flunarizine when combined with antiepileptic drugs against electroconvulsions in mice, and the clinical use of nimodipine is not recommended (Czuczwar et al., 1992). Clinical data indicate that this Ca^{2+} channel inhibitor, as an add-on therapy in refractory epilepsy, is not a useful adjuvant anticonvulsant agent (Larkin et al., 1991). The results of the present study clearly indicate that the use of niguldipine, isradipine, and dantrolene in epileptic patients appears questionable.

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