

REVIEW

CALCIUM MODULATION IN EPILEPSY

Wojciech Kułak¹, Wojciech Sobaniec^{1, #}, Katarzyna Wojtal², Stanisław J. Czuczwar^{2, 3}

¹Department of Pediatric Neurology and Rehabilitation, Medical University of Białystok, Waszyngtona 17, PL 15-274 Białystok, Poland, ²Department of Pathophysiology, Medical University, Jaczewskiego 8, PL 20-090 Lublin, ³Isotope Laboratory, Institute of Agricultural Medicine, Jaczewskiego 2, PL 20-090 Lublin, Poland

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The ideal antiepileptic drug (AED) should correct the aberrant pathophysiology of epileptogenesis without interfering with normal neurotransmission. A new group of drugs with antiepileptic efficacy, without sedative properties, would be an exciting prospect. Theoretical considerations and results from experimental animal models of epilepsy have put forward the possibility that calcium (Ca^{2+}) antagonists may form such a group. The initiation of epileptogenic activity in the neuron is thought to be connected with the phenomenon known as “intrinsic burst firing”, which is activated by an inward Ca^{2+} current. Ca^{2+} is described as the primary mediator of “excitotoxic” neuronal damage. Both necrotic and apoptotic cell death is associated with Ca^{2+} entry into the cells during status epilepticus. The Ca^{2+} channel blockers depressed epileptic depolarizations of neurons. In this review, we present anticonvulsant effects of cinnarizine, flunarizine, nifedipine, nimodipine, nicardipine, amlodipine, isradipine, niguldipine, diltiazem, verapamil and dantrolene in animal models of seizures. Also, a detailed analysis of interactions between Ca^{2+} blockers and AEDs was performed. Clinical trials in intractable epilepsy support to a certain degree antiepileptic properties of Ca^{2+} antagonists.

Key words: antiepileptic drugs, calcium channel blockers, seizures

[#] correspondence; e-mail: kneur2@wp.pl

Abbreviations: AED(s) – *antiepileptic drug(s)*, AMPA – α -*amino-3-hydroxy-5-methyl-isoxazole propionate*, ATP – *adenosine triphosphate*, ATPase – *adenosine triphosphatase*, BAY k-8644 – *methyl-3-nitro-4-[2-trifluoromethylphenyl]pyridine-5-carboxylate*, BZDs – *benzodiazepines*, CBZ – *carbamazepine*, CGP 40116 *D-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid*, CGP 43487 – *D-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid methyl ester*, CLO – *clonazepam*, CNS – *central nervous system*, EEG – *electroencephalogram*, ETX – *ethosuximide*, FBM – *felbamate*, GABA – γ -*aminobutyric acid*, GYKI 52466 – *1-(4-amino-phenyl)-4-methyl-7,8-methylenedioxy-5-2,3-benzodiazepine*, LP – *lipid peroxides*, LY 235959 – *(-)-3R,4aS,6R,8aR-6-(phosphonomethyl) decahydro-isoquinoline-3-carboxylic acid*, LY 300 164 – *7-acetyl-3-(4-aminophenyl)-8,9-dihydro-8-methyl-7H-1,3-dioxolo[4,5-h][2,3]-benzodiazepine*, MES – *maximal electroshock*, MK-801 – *dizocilpine maleate*, NMDA – *N-methyl-D-aspartate*, PB – *phenobarbital*, PDS(s) – *paroxysmal depolarizing shift(s)*, PHT – *phenytoin*, PTZ – *pentetrazole*, ROCCs – *receptor-operated calcium channels*, VDCCs – *voltage-dependent calcium channels*, VPA – *valproate*

Introduction

An approximately 1% of the world population suffer from epilepsy. At least 20–30% of patients with temporal lobe epilepsy have resistant seizures despite optimal pharmacological therapy [2]. Complex partial seizures are frequently refractory to currently used therapies, with up to 40% of patients demonstrating this seizure type considered difficult to manage [41].

The ideal antiepileptic drug (AED) should correct the aberrant pathophysiology of epileptogenesis without interfering with physiological neurotransmission [19]. A new group of drugs with antiepileptic efficacy, but lacking sedative properties, would be an exciting prospect.

Theoretical considerations and results from experimental animal models of epilepsy have put forward the possibility that calcium (Ca^{2+}) antagonists may form such a group and come closer to the ideal, specifically blocking epileptogenesis [8, 18, 33, 58].

Meyer et al. [43] stated that selective central nervous system Ca^{2+} channel blockers may be a new class of anticonvulsant agents.

In this paper, data on the role of Ca^{2+} and Ca^{2+} antagonists in epilepsy are reviewed.

Calcium and brain damage

Ca^{2+} is a regulator of metabolic pathways and serves important functions as a second messenger, thus the free cytosolic Ca^{2+} concentration must be tightly regulated at around 10^{-7} M. Ca^{2+} influx occurs by voltage dependent calcium channels (VDCCs) and receptor-operated calcium channels (ROCCs), while release from the endoplasmic reticulum is triggered by inositol triphosphate [16, 30]. Efflux of Ca^{2+} occurs by a high affinity-low capacity ATPase (calmodulin-dependent) and by a low affinity-high capacity, electrogenic $\text{Na}^+/\text{Ca}^{2+}$ exchanger. Ca^{2+} -binding proteins buffer any Ca^{2+} entering the cell, or released within the cell. When Ca^{2+} concentrations increase, mitochondria become important in calcium storage. The Ca^{2+} sequestration process is dependent on ATP and during ischemia Ca^{2+} concentration within the cell rises [30, 57].

In the 1980s and 1990s, a great deal of excitement was generated by new insights into new mechanisms of brain damage during hypoxia/ischemia [30, 31]. It was well known that hypoxia/ischemia lasting more than a few minutes could cause irreversible brain damage. Further research indicated that reperfusion might cause more damage than simple hypoxia [30, 53]. The mechanism of reperfusion injury is thought to involve the production of free oxygen radicals. The free radicals induce a chain reaction leading to a breakdown of the neuronal cell membrane (necrotic cell death). Further, free radicals are generated, causing a damage in the original cell that spreads to neighboring cells [30, 53]. The brain uses glucose as its primary energy source. Glutamic acid, or glutamate, is a common metabolite of glucose metabolism. Glutamate is involved in several metabolic processes in the brain. It plays a role as a precursor for the inhibitory neurotransmitter, γ -aminobutyric acid (GABA). Elevated levels of glutamate were associated with increased brain activity. Furthermore, glutamate-induced excitotoxicity is a major mechanism by which neuronal loss may occur [9, 41, 53, 64].

Glutamate receptors can be divided into ionotropic and metabotropic ones. Activation of iono-

tropic glutamate receptors leads to greater permeability of the cell membrane to the Na^+ and Ca^{2+} [9, 54]. There are three types of ionotropic glutamate receptors. These receptors are distinguished by their response to AMPA (α -amino-3-hydroxy-5-methyl-isoxazole propionate), kainic acid (kainate), or NMDA (N-methyl-D-aspartate) [14, 54]. These glutamate receptors are composed of several protein subunits. Four glutamate receptor subunits (GluR1-GluR4) are believed to serve as AMPA receptor subunits, and five receptor subunits are regarded as kainate receptor subunits (GluR5-GluR7 and KA1, KA2). NMDA receptors are assembled from 5 subunits belonging to two families: NMDAR1 and NMDAR2. NMDAR2 family has four members: A, B, C, and D. The NMDA receptor is voltage-dependent and during membrane depolarization, magnesium (Mg^{2+}) is displaced, allowing large amounts of Ca^{2+} enter the cell. Ca^{2+} entry starts a cascade of biochemical reactions within the neuron that leads to its death. Ca^{2+} is described as the primary mediator of "excitotoxic" neuronal damage. Depending on the availability of energy, necrosis, apoptosis or both can occur. Both necrotic and apoptotic cell death is associated with Ca^{2+} entry into the cells, for example, during status epilepticus. This Ca^{2+} overload impairs neuronal viability, leading to oxidative damage, cytoskeletal degeneration, and microtubule dysfunction or protein aggregation. Activation of gelsolin leads to the dismantling of neuronal cytoskeleton. The Ca^{2+} overload may also lead to clustering of procaspases, which promotes the activation of caspases and initiates the execution phase of apoptosis [30, 53, 57].

Calcium and epileptogenicity

In literature there are many observations on Ca^{2+} role in the generation of epileptic activity [15, 55, 59].

A Ca^{2+} flux into the intracellular space represents the first stage of epileptic neuronal events. Experimental studies showed a decrease in the extracellular concentrations of Ca^{2+} [59]. The initiation of epileptogenic activity in the neuron is thought to involve the normal phenomenon known as "intrinsic burst firing", which is activated by an inward Ca^{2+} current [15]. It may lead to synchronous burst firing of multiple neurons. This is associated with the production of a paroxysmal depolarizing shift (PDS), an abnormal long-duration ac-

tion potential, long recognized to accompany seizure initiation and shown to be the cellular abnormality underlying the interictal "spike" in electroencephalogram (EEG) tracings [15, 55]. PDSs may also depend on synchronization of a network of neurons through synaptic mediation. It was demonstrated that Ca^{2+} flux into the presynaptic terminal is an important factor for neurotransmission [55, 59]. De Lorenzo [16] has postulated that Ca^{2+} enters the presynaptic terminal and binds to calmodulin, a major Ca^{2+} receptor protein in the neurons. The Ca^{2+} -calmodulin complex regulates several aspects of synaptic activity, e.g. neurotransmitter release and neuronal function. There are some evidence that carbamazepine (CBZ), phenytoin (PHT) and benzodiazepines (BZDs) inhibit protein phosphorylation stimulated by the Ca^{2+} -calmodulin complex [16]. At a cellular level, antiepileptic drugs were found to decrease neuronal excitability by interacting with VDCCs, by modulating GABA metabolism, transport, and by affecting ionotropic glutamate receptors. There is evidence that anticonvulsant action of PHT and CBZ results from an interaction with Ca^{2+} -VDCCs [59]. Schumacher et al. [55] demonstrated that PHT selectively altered low-voltage-activated Ca^{2+} channels in cultured hippocampal neurons, neuroblastoma cells and rodent thalamic neurons. It was shown that PHT partially antagonized L-type Ca^{2+} current. These authors have found that in human hippocampal granule cells, CBZ inhibited high voltage-activated currents. The concentrations necessary to obtain significant effects are above therapeutic cerebral spinal fluid concentrations and therapeutic whole-brain concentrations.

Epileptic depolarizations of neurons were found to be depressed by Ca^{2+} channel blockers [27, 54].

Calcium antagonism

The Ca^{2+} antagonists exert their effect on " Ca^{2+} channels", the proteins spanning the cell membrane that, when "open", allow passive passage of the Ca^{2+} into the cell [8, 12]. The channels may be opened by the occupation of associated receptors (ROCCs) or by changes in membrane potential including those occurring with depolarization (VDCCs). The L-type Ca^{2+} channel is composed of five different polypeptide subunits, each with different molecular mass. The existence of this channel type has been demonstrated in many regions of the central nervous sys-

tem (CNS) such as the cortex, hippocampus, cerebellum, spinal cord. The Ca^{2+} antagonists affect an $\alpha 1$ subunit of VDCCs (L-channels) that produce a long-lasting inward Ca^{2+} current [27, 60].

The N (high-threshold inactivating) and T (low) type Ca^{2+} currents have also been documented. The T-type current could contribute to rhythmic firing of vertebrate neurons and the N or L type current could be involved in the release of neurotransmitters [8, 59].

In general, it is thought that the blockade of T-type Ca^{2+} channels is associated with efficacy in treating absence seizures, while blockage of high voltage-activated (L-type) Ca^{2+} channels seems to be associated with control of partial seizures with or without secondary generalization. Since Ca^{2+} influx is a necessary step in neurotransmitter release, one consequence of Ca^{2+} channel blockade is reduced release of neurotransmitters, including glutamate. More importantly, elevated levels of intracellular Ca^{2+} are thought to activate numerous Ca^{2+} -dependent processes that lead to cell death. Blockage of Ca^{2+} channels may play a key role in preventing these events [8, 54, 59].

Immunohistochemical studies have identified the P-type channel in the mammalian CNS, which appears to serve both as a generator of intrinsic activity and as a modulator of neuronal integration and transmitter release. Recently, Q- and R-type channels have been discovered [61].

Some Ca^{2+} antagonists may also act by preventing the “ Ca^{2+} overload” not mediated by specific channels, which occurs during epileptic seizures and anoxic conditions [8].

More recently, some human disorders were found to be caused by mutations in VDCCs' genes, and several common elements were noted amongst this otherwise diverse group of diseases. In the CNS, voltage-gated channelopathies may cause seizures, hemiplegic migraine, or episodic ataxia [61].

A common denominator among all epilepsies is an alteration in neuronal excitability, and VDCCs seem to be a logical candidate, a priori, for the underlying molecular defect. For a selected subset of exemplary seizure disorders with autosomal dominant inheritance, genetic loci have been mapped by linkage analysis, and positional cloning strategies have identified mutations in genes coding for VDCCs. For instance a Ca^{2+} channel CACNB4 gene at 2q(22-23/b₄ subunit) was identified [59] to

be associated with familial generalized epilepsy and episodic ataxia.

The genetic analysis in different mutant, genetically distinct, autosomal recessive models of absence epilepsy has suggested that these syndromes are caused by mutations in genes encoding three types of Ca^{2+} channel subunits [61]. More specifically, mutations were identified in the genes encoding the $\alpha 1A$ and $\beta a4$ subunits of P-type VDCCs. The B subunits normally regulate Ca^{2+} currents *via* a direct interaction with $\alpha 1$ (pore forming) subunits of these channels [7].

Cinnarizine and flunarizine

Flunarizine is a difluorinated derivative of cinnarizine.

Experimental studies

The anticonvulsant properties of cinnarizine and flunarizine were first demonstrated by Desmedt et al. [18] using maximal electroshock (MES) or pentetrazole (PTZ) models in rats and MES in mice. Tonic seizures were also inhibited by these Ca^{2+} channel inhibitors in rodent models created with the use of chemical convulsants: D,L-allylglycine, bicuculline, and in the amygdaloid kindling seizure model. In photosensitive baboons, flunarizine completely suppressed myoclonic responses to stroboscopic stimulation [48].

Flunarizine at doses of 15 and 40 mg/kg raised the threshold for electroconvulsions, remaining ineffective at lower doses [11, 21, 62]. The anticonvulsant action of flunarizine against electroconvulsions was significantly reduced by BAY k-8644, the L-type Ca^{2+} channel agonist, indicating a specific mechanism of action of this Ca^{2+} channel inhibitor [23]. It is well documented that flunarizine enhances the anticonvulsive activity of a majority of conventional and potential antiepileptics [LY 300164, LY 235959, GYKI 52466, CGP 43487, CBZ, valproate (VPA), PHT] against MES-induced seizures in mice [11, 20, 21, 62]. However, it does not affect the protective action afforded by felbamate (FBM) and MK – 801 [20, 24]. This Ca^{2+} channel antagonist did not change the free plasma levels of antiepileptic drugs in all above-cited studies. In some cases, the combined treatment of flunarizine with AEDs produced motor impairments (evaluated in the chimney test) and long-term memory deficits (measured in the passive avoid-

ance task), but these effects were comparable to those evoked by AEDs given alone [11, 20, 21].

Sobaniec and Kułak [58] evaluated the anticonvulsant effects of flunarizine against the electrically evoked seizures in rats. Flunarizine, clobazam (1,5-benzodiazepine) and flunarizine + clobazam were administered 1 h before the convulsive test. The duration of seizures was significantly shortened by flunarizine, clobazam and flunarizine + clobazam. The co-administration of clobazam significantly decreased the duration of seizures and their intensity [58].

De Sarro et al. [17], in sound-induced seizures in DBA/2 mice also described the anticonvulsant properties of flunarizine.

Flunarizine (up to 40 mg/kg) *per se* did not suppress PTZ-induced convulsions in mice [22]. It (up to 20 mg/kg) did not modify the anticonvulsive action of ethosuximide (ETX), VPA and clonazepam (CLO) in PTZ-evoked clonic seizures either. Nevertheless, flunarizine alone or combined with ethosuximide (ETX) increased the incidence of tonic seizures and mortality in mice [22].

The anticonvulsant effects of cinnarizine against bicuculline-induced seizures were also demonstrated in a model of generalized tonic-clonic seizures [34].

Clinical studies

In an open study, cinnarizine was administered at a fixed dosage of 2 mg/kg, b.i.d. in 15 epileptic children for three months. The patients had received treatment for at least 6 months (mean 10.5 months) with VPA at a mean dose of 30 mg/kg/day. The complex partial secondarily generalized seizures appeared at a rate of 1-4 incidences per month (mean 1.6). Prolonged administration of cinnarizine insignificantly decreased the VPA levels. Eight patients had a seizure reduction by over 50%. One patient was withdrawn from the study because of increased seizures severity. No other side effects were detected [35].

The half-life of flunarizine is between 3 and 17 h and its distribution volume is around 8 l/kg. The kinetics is linear, with steady-state concentrations proportional to a dose in an individual patient, but there is variation between subjects from 30 to 100 ng/ml on a 10 mg daily dose. In epileptic patients taking other AEDs, steady-state flunarizine concentrations were four to five times lower than in those taking equivalent doses of flunarizine as

monotherapy [48]. This is due to induction of hepatic metabolic enzymes by concomitant treatment with AEDs, such as CBZ or phenytoin (PHT), which are known to decrease their own and each other's steady-state concentrations by this mechanism. Less than 5% of the drug is excreted unchanged in urine and feces, so extensive hepatic metabolism is likely [48].

First clinical evidence for the antiepileptic activity of flunarizine was reported by Declerck and Wauquier [48]. Overweg et al. [49] performed a double-blind placebo-controlled crossover trial. Flunarizine was given as "add-on" therapy in thirty therapy-resistant adult patients in order to evaluate its effect on seizure incidence and on the plasma levels of flunarizine and associated AEDs. A lower seizure incidence was found during the flunarizine period as compared with placebo in 20 patients. Eleven patients showed improvement by 25% or more with flunarizine. Seven patients had a seizure reduction of over 50%. The administration of flunarizine did not increase the plasma levels of the other AEDs.

In other clinical trial of 47 adult patients with intractable epilepsy, flunarizine was added to the existing antiepileptic medication. The dosage over 3-month periods was up to a maximum of 25 mg/day. Three patients became seizure-free, six, however, had a 50% increase in seizure incidence. Overall, 33 patients had less seizures on flunarizine, nine had more, and five were unchanged. Trials that included children showed similar results to those conducted in adult patients. Side effects included dose-related drowsiness occurring in about 30% of patients in the open trials, but appearing less frequently when assessed in a controlled trials. Weight gain was reported in 5% of patients. Extrapyrarnidal symptoms were noted in a small number of patients taking flunarizine [48].

In contrast to the results presented above, Nakane et al. observed a very low efficacy of flunarizine in patients with intractable epilepsy, overall improvement rate reached only 9.4%. No significant side effects were noted after administration of the drug [45].

Nifedipine

Nifedipine is a 1,4-dihydropyridine derivative and is used in the therapy of cardiovascular disorders (angina, hypertension).

Experimental studies

Nifedipine (at 30 mg/kg) showed the anticonvulsant activity against maximal electroshock-induced seizures in mice [62]. The drug enhanced the protective action afforded by MK – 801, LY 235959, CGP 40116, CGP 43487, CBZ, diphenylhydantoin (DPH), phenobarbital (PB) and VPA against electroconvulsions, but was ineffective in modifying the anticonvulsive properties of LY 300164, FBM and GYKI 52466 [10, 20, 21, 24, 62]. Nifedipine did not affect the free plasma concentrations of AEDs and the adverse effects produced by co-administration of nifedipine with antiepileptics were similar to those, which were observed when AEDs were given alone [10, 20, 21, 24, 62].

Nifedipine (20 mg/kg) moderately inhibited PTZ-induced convulsions [13]. It also displayed a protective activity against PTZ-evoked seizures when applied together with otherwise ineffective doses of VPA, PB or ETX. Furthermore, nifedipine significantly increased the plasma levels of PB and VPA, although this increase was not as high as that obtained after doubling the AEDs doses. This indicates that the pharmacokinetic interaction was only partially involved in the improved protection by combined treatment against PTZ-induced seizures [13]. Combinations of nifedipine with VPA, PB or ETX did not produce any signs of toxicity. Interestingly, the Ca²⁺ channel inhibitor did not influence the protective action of diazepam against PTZ-induced seizures [13].

In the experiment carried out by Czuczwar et al. [10], nifedipine at doses up to 40 mg/kg afforded no protection against aminophylline-induced convulsions. This Ca²⁺ channel inhibitor (up to 10 mg/kg) did not modify the anticonvulsive action of VPA and PB against clonic and tonic aminophylline-induced convulsions and, moreover, combination of CBZ, ETX or trimethadione with nifedipine did not protect animals against aminophylline-evoked seizures [10].

The anticonvulsant effects of nifedipine were also evaluated in audiogenic seizure model [37].

Some possible relationships between nifedipine and clorazepate (1,4-benzodiazepine derivative) as anticonvulsant agents against electrically induced seizures and the formation of lipid peroxides (LP) in the rat brain structures were studied by Kulak et al. [37]. The duration of seizures was significantly shortened by nifedipine, clorazepate, and both

drugs given together. The combined treatment enhanced the protective effects against the tonic phase of seizures, yielding a 75% protection. Nifedipine significantly decreased the LP formation in the cortex, hippocampus, striatum, hypothalamus and cerebellum. Moreover, a similar effect was achieved with clorazepate alone or combined with nifedipine. The combined administration did not enhance an inhibition of the formation of LP in the examined structures [37].

The half-life of nifedipine is around 4 h, following a single dose. Nifedipine undergoes extensive first-pass metabolism, mainly by cytochrome P-450 system (P-450 3A4 isoenzymes) in liver [46].

Clinical studies

Larkin et al. [40] in an open study assessed nifedipine efficacy in adult patients with epilepsy. Eight patients completed three-month trial. They had fewer seizures compared with baseline period. Two patients became seizure-free.

In another controlled study [38], nifedipine has failed to confirm its efficacy in epilepsy. Twenty-two adult epileptic patients received nifedipine retard and matched placebo for eight weeks at two doses (20 and 40 mg b.i.d. each for 4 weeks) with a washout period of 8 weeks between treatment phases. In 20 patients who completed the trial, fewer partial seizures were documented during the first two weeks of nifedipine administration. This response was not sustained in the second month of the trial. EEGs suggested a small improvement with nifedipine. More patients reported headache when receiving nifedipine than placebo, but heart rate and blood pressure remained unaffected. The concentrations of nifedipine were low, with mean peak levels at the dose of 40 mg twice daily only reaching 13 ng/ml. In contrast, a therapeutic levels of nifedipine in angina pectoris are in the range of 30–40 ng/ml [38].

A short elimination half-life (only 4 h) is likely to be a major stumbling block to the emergence of nifedipine as a useful AED [39].

Nimodipine

Nimodipine is a Ca²⁺ channel antagonist of the dihydropyridine type which, in addition to its well-documented cerebral vasodilatory effect, also appears to exert a direct neuronal action in the brain [56].

Experimental studies

The anticonvulsant effects of nimodipine have been confirmed in experimental models, bicuculline-, PTZ-, electrocortical shock-, ischemia and kainic acid-induced seizures [33, 44, 56].

However, other studies have revealed that nimodipine (up to 80 mg/kg) administered alone suppressed neither electroconvulsions nor PTZ-induced seizures in mice [11, 22]. According to Czuczwar et al. [11], the drug potentiated the protective activity of CBZ and DPH, but not that of VPA, against MES. The Ca²⁺ channel inhibitor did not affect plasma levels of the antiepileptics, so the pharmacokinetic interactions may be excluded. The combined treatment of nimodipine did not significantly change the motor performance of mice in the chimney test, when compared with AEDs given alone [11].

Nimodipine increased the protective activity of ETX and VPA against PTZ-evoked convulsions, remaining ineffective in case of CLO. The studied Ca²⁺ channel antagonist did not affect the plasma levels of ETX and VPA. Disturbed motor performance in the chimney test was a side effect observed when nimodipine was administered with ETX or VPA [22].

An interesting aspect of nimodipine pharmacokinetics is the fact that its bioavailability following oral ingestion in male volunteers amounted to 16% or less. Nimodipine is metabolized in the liver by two major pathways, neither of which involves the production of an active metabolite [39, 52]. First-pass metabolism seems to play a major role which renders nimodipine particularly vulnerable to the effects of enzyme induction. No accumulation occurs after oral doses of 40 mg three times daily, and peak concentrations remain around 40 ng/ml. Following intravenous infusion until steady-state was attained, no apparent change in elimination half-life occurred [39].

Oral nimodipine has been used successfully following subarachnoid hemorrhage [51] and ischaemic stroke [63].

Clinical studies

Several case reports [5, 26, 33] and clinical trials [15, 50] indicate that nimodipine is effective in patients with seizures refractory to conventional treatment.

Brandt et al. [5] administered nimodipine to two adult patients with continuous focal epileptic seizures not responding to conventional antiepileptic therapy. The nimodipine infusion at 2 mg/h was given for 24 and 28 h. No recurrent seizure activity was observed during this period.

Hans et al. [26] described a younger child who developed generalized epileptic seizures as a result of severe head injury. The onset of epileptic seizures was associated with the occurrence of acute hyponatraemia and hypoosmolality due to excessive desmopressine administration. The seizures resistant to conventional therapy disappeared completely with intravenous nimodipine infusion. No significant fall in blood pressure was observed during the nimodipine infusion. No significant biochemical or haematological changes occurred during the treatment. A reduction in the discharge rate during nimodipine infusion was noted in all the patients.

Three children with partial seizures secondarily generalized, resistant to established AEDs were treated with nimodipine. A reduction in the discharge rate during nimodipine infusion was noted in the patients. The seizures resolved after 36 h of intravenous nimodipine infusion [33].

De Falco et al. [15] used nimodipine at a fixed dosage of 30 mg, t.i.d. in addition to basic AED therapy in 21 patients with intractable epilepsy caused by organic brain lesions. After a 12-week treatment period, 14 patients (67%) showed a decrease in seizure frequency, four patients had no change, and three showed an increase. In eight patients (38%) seizure frequency decreased by more than 40%. No significant modifications in antiepileptic drug or electrolyte serum levels were found. In one patient this dosage caused a lowering of blood pressure.

The first controlled, clinical study of nimodipine in epilepsy has produced disappointing results [41]. Twenty-two patients with refractory epilepsy were admitted into a placebo-controlled crossover study of nimodipine, given over 12 weeks in an escalating dose regimen of 30, 60, and 90 mg all three times daily. Median seizure frequency did not vary between the drug and placebo phases, and a number of patients with a 50% or 25% reduction in seizure numbers from the baseline was not different in patients on nimodipine and on placebo. The failure to demonstrate a beneficial effect with nimodipine in this trial may have been due to in-

adequate dosing. However, 40 mg three times daily has been effective in recovery following ischemic stroke [51]. While the epilepsy study used higher doses than those, the three times daily regimen is less than ideal for a drug with such a short half-life, and many of the successful studies in acute cerebrovascular disease have adopted a four-hourly schedule or used the drug as an intravenous infusion [39]. Furthermore, most epileptic patients also take enzyme-inducing AEDs, which have been shown to accelerate the metabolism of other dihydropyridines. Nimodipine, with its predominantly hepatic first-pass metabolism, may thus suffer a further reduction in bioavailability and shortening of its elimination half-life resulting in substantially lower than predicted steady-state concentrations. Thus, despite high dosing, the epilepsy trial only achieved maximum concentrations approaching 12 ng/ml, while in patients with subarachnoid hemorrhage, the concentrations of 17–42 ng/ml were achieved using an oral nimodipine at 60 mg four times daily [39].

Meyer et al. [45] conducted a double-blind placebo-controlled crossover study in 95 patients with intractable epilepsy. Nimodipine was used as add-on therapy (60 mg four times a day) in a 1-year placebo-controlled crossover study. Seventy-one patients had localized-related epilepsy and 24 of them had generalized seizure disorders. Of the 95 patients, 81 were receiving two or more AEDs. Nimodipine was well tolerated during the study, only two patients were unable to complete the study because of adverse effects. The trial demonstrated no significant crossover effect and no significant effect of nimodipine on either the mean or the median number of seizures or seizure days. The peak median serum nimodipine level was less than 5 ng/ml in the 78 patients who completed the study. This clinical trial found no beneficial effect with use of nimodipine as add-on therapy for intractable epilepsy. The reasons for the absence of efficacy of nimodipine were the inclusion of patients with very refractory epilepsy and the relatively low serum nimodipine concentrations resulting from the pharmacokinetic effect of concurrent antiepileptic medication.

A wide range of doses of the nimodipine (70–360 mg daily) has been used in the clinical trials in patients with subarachnoid hemorrhage [51, 52]. The dosage in epileptic patients remains rather arbitrary, but it seems clear that in those taking con-

comitantly AEDs, inducing hepatic enzymes, doses at least in the upper end of this range will be required to assess whether this drug has any efficacy in the clinical setting. A dose of 30 mg four times a day is a reasonable starting point with increases every 2–4 weeks toward an initial maintenance of 90 mg four times daily [39].

In general, nimodipine is well tolerated. Cardiovascular side effects (headache, flushing, hypotension) were reported [41]. The hypotension may be a problem in younger children [45].

The efficacy and tolerance to intravenous nimodipine therapy of cluster seizures in children have been recently evaluated by Kułak and Sobaniec [36, unpublished data]. Eleven patients, 5 girls and 6 boys, with intractable epilepsy, aged 5 to 15 years were admitted to the study. Six patients had complex partial seizures secondarily generalized and five children had myoclonic astatic seizures. The patients were receiving chronic treatment with at least two AEDs. The initial intravenous nimodipine dose was 0.5 mg/h given over the first 2 h. The nimodipine infusion was then continued at a rate of 1 mg/h for 72 h. None of the patients was excluded from the trial. No significant drop in blood pressure was observed during the nimodipine infusion. A reduction in the discharge rate during nimodipine infusion was noted in all the patients. Computerized spectral analysis of the EEG revealed, an increase in the percentage of alpha and theta waves after 24 and 72 h of nimodipine infusion and a decrease in the percentage of delta. The reduction in seizure frequency within hours after intravenous administration of nimodipine confirms the anticonvulsant effects of nimodipine.

Nicardipine

Nicardipine is a calcium Ca^{2+} antagonist of the dihydropyridine class.

Experimental studies

Nicardipine (up to 30 mg/kg) did not affect the threshold for electroconvulsions in mice [62], but potentiated the efficacy of LY 300164 (AMPA/kainate receptor antagonist), LY 235959, CGP 40116, CGP 43487 (NMDA receptor antagonists) and CBZ, but not that of VPA, against MES [11, 20, 21, 62]. The nicardipine-induced enhancement of the anticonvulsive activity of LY 300164 was not reversed by BAY k-8644, so the involvement of Ca^{2+}

blockade in this effect was excluded. A pharmacokinetic interaction may play a role there, as nicardipine raised the free plasma concentration of LY 300164 [62]. Pharmacokinetic interaction also, at least partially, accounted for the nicardipine-evoked increase in the anticonvulsive efficacy of CBZ [11]. It cannot be totally excluded in case of remaining three AEDs, since the plasma levels of the compounds were not measured. On the other hand, the influence of BAY k-8644 on the anticonvulsant action of CGP 40116 and CGP 43487 suggests that the nicardipine-induced potentiation of their action was due to centrally mediated events of Ca^{2+} channel inhibitor [21]. The combined treatment of nicardipine and AEDs did not significantly change the motor coordination and long-term memory, when compared with antiepileptics given alone [11, 20, 21].

Nicardipine, like other Ca^{2+} channel antagonists, did not modify the protective efficacy and potency of the new antiepileptic drug, FBM, against MES-induced tonic convulsions [24]. It did not potentiate the anticonvulsive potency of MK-801 (a non-competitive NMDA receptor antagonist) and GYKI 52466 (an AMPA/kainate receptor antagonist) in this model of epilepsy either [11].

Nicardipine (10–40 mg/kg) significantly inhibited clonic seizures induced by PTZ [21]. The drug also enhanced the protective activity of ETX and VPA in this test. Pharmacokinetic interactions were excluded. The combinations of the drugs did not produce side effects [22].

Amlodipine

Amlodipine belongs to the 1,4-dihydropyridine class of Ca^{2+} channel antagonists and possesses pharmacologic and pharmacokinetic profiles that distinguish it from other agents of this class. It has a more prolonged half-life (30 h on average) as well as a higher volume of distribution when compared with that of other Ca^{2+} channel inhibitors [1, 6, 41].

Experimental studies

In the experiment carried out by Kamiński et al., amlodipine (up to 10 mg/kg) did not significantly affect the threshold for electroconvulsions [28]. However, this Ca^{2+} channel antagonist enhanced the anticonvulsive activity of CBZ, VPA and PB against MES-induced convulsions in mice. Pharmacokinetic interactions do not account for

this effect in case of VPA and PB, but cannot be excluded in case of CBZ [28].

Amlodipine (at 10 mg/kg) reduced PTZ-induced clonic and tonic convulsions in mice. It also enhanced the anticonvulsant properties of ETX, VPA and PB in this model of epilepsy without changing their plasma levels [29].

The combined treatment with amlodipine and antiepileptics caused motor impairment [27, 28] and disturbed long-term memory [28], therefore, a possible usefulness of this Ca^{2+} channel inhibitor as add-on therapy in epileptic patients may be limited.

Isradipine and niguldipine

Isradipine and niguldipine are two new Ca^{2+} channel antagonists of the dihydropyridine class.

Experimental studies

According to Borowicz et al., isradipine neither suppressed electroconvulsions nor did it modify the anticonvulsive properties of conventional antiepileptics in MES-induced seizures in mice [3].

Niguldipine at the dose of 5 mg/kg significantly raised the electroconvulsive threshold in mice, and at the dose of 7.5 mg/kg, it displayed a potent anticonvulsive action against amygdala-kindled seizures in rats [3, 4]. The drug did not affect the protective activity of DPH and VPA against MES-evoked convulsions [3]. No protective effect against amygdala-kindled seizures was observed when niguldipine (5 mg/kg) was combined with DPH, VPA or CLO at subeffective doses [4]. Unexpectedly, and unlike other Ca^{2+} channel inhibitors, niguldipine impaired the anticonvulsive potency of CBZ and PB against MES and amygdalar kindling. Although the reasons for this effect are poorly understood (involvement of Ca^{2+} channel blockade and pharmacokinetic interactions were excluded), it may be concluded that niguldipine, because of its properties, should not be used in epileptic patients [3, 4].

Diltiazem

Diltiazem belongs to the BZD class of Ca^{2+} channel inhibitors.

Experimental studies

The drug *per se* turned out to provide protection against electroconvulsions and audiogenic seizures in mice, remaining ineffective in aminophylline-

Table 1. Interaction of calcium channel blockers with antiepileptic drugs in the maximal electroshock-induced convulsions in mice

Calcium channel blocker (mg/kg)	Carbamazepine	Diphenylhydantoin	Valproate	Phenobarbital
Amlodipine (10)	↑↑	NS	↑	↑
Diltiazem (1.25)	↑	↑	NS	NS
Flunarizine (20)	↑↑	↑	↑↑	NT
Nifedipine (1.25)	↑↑	↑	NS	NS
Niguldipine (2.5)	↓	NS	NS	↓
Nimodipine (20)	↑↑	↑	NS	NT
Verapamil (10)	NS	NS	NS	NS

The compared data were calcium channel blocker-induced alterations of the ED₅₀ values (in % of the retrospective control group) of carbamazepine (60 min prior to maximal electroshock), diphenylhydantoin (120 min), valproate (30 min), and phenobarbital (120 min). Amlodipine was administered 120 min prior to the test, nifedipine, diltiazem, and flunarizine – 60 min, whilst verapamil and niguldipine – 30 min before the convulsive test. All drugs were given intraperitoneally. Data are from Borowicz et al. [3], Czuczwar et al. [10, 11], and Kamiński et al. [27]. Enhancement of the protective activity by: up to 40% – ↑, more than 40% – ↑↑, reduction of the anticonvulsant action – ↓, NS – not significant, NT – not tested

Table 2. Effects of calcium channel blockers on the protection afforded by antiepileptic drugs against pentetrazole-induced convulsions in mice

Calcium channel blocker (mg/kg)	Ethosuximide	Valproate	Phenobarbital	Clonazepam
Amlodipine ^a (2.5)	↑↑	↑↑	↑↑	NT
Diltiazem (20)	↑↑	NS	NS	NT
Flunarizine (20)	NS	NS	NT	NS
Nifedipine (10)	↑↑↑	↑	↑↑	NT
Nimodipine (20)	↑	↑↑	NT	NS
Verapamil (20)	NS	NS	NS	NT

The compared data represented reduction of the number of mice with clonic seizures expressed in %. Ethosuximide was given at 100 mg/kg when combined with nifedipine, diltiazem, and verapamil or at 50 mg/kg in combination with amlodipine or flunarizine, valproate was administered at 100 mg/kg, phenobarbital at 6.25 mg/kg and clonazepam at 0.01 mg/kg. Combined intraperitoneal treatments with calcium channel blockers were compared with antiepileptics given alone at identical doses. Treatment times: phenobarbital 120 min, clonazepam, ethosuximide, and valproate 30 min, amlodipine 120 min, diltiazem and nifedipine 60 min, nimodipine and flunarizine 45 min, verapamil 30 min prior to pentetrazole. Convulsions were produced by subcutaneous pentetrazole at its CD₉₇ for the induction of clonic seizures. Data are from Czuczwar et al. [13], Gąsior et al. [21], and Kamiński et al. [28]. Treatment more effective: ↑ – by up to 50%; ↑↑ – by more than 50%; ↑↑↑ – by more than 80%; NS – not significant; NT – not tested. The effect of amlodipine upon the protective efficacy of AEDs drugs was quantified as in the case of electroconvulsion (Tab. 1)

and PTZ-induced convulsions [10, 13, 17]. This Ca²⁺ channel antagonist potentiated the anticonvulsant action of CBZ and DPH, but not that of PB and VPA, against electrically evoked seizures in mice. The anti-aminophylline activity of VPA and PB was not enhanced by diltiazem. Moreover, the co-administration of CBZ, ETX or trimethadione with the Ca²⁺ channel blocker afforded no protection against aminophylline-induced convulsions [10]. Diltiazem potentiated the anticonvulsive action of ETX, but not that of VPA, PB and diazepam (DZP) against PTZ-induced seizures [13].

Verapamil

Verapamil represents the group of Ca²⁺ channel antagonists that are derivatives of phenylalkylamine.

Experimental studies

Verapamil *per se* and in combination with conventional AEDs did not display anticonvulsive properties in any animal model of epilepsy [10, 13]. This might result from poor penetration of this Ca²⁺ antagonist through the blood-brain barrier [25], although the pharmacodynamic properties of

this agent could play a role as well. Verapamil administered intracerebroventricularly showed no protection against sound-induced seizures in mice, whereas other Ca^{2+} channel inhibitors were effective [17].

One could assume that this Ca^{2+} channel blocker would also be ineffective in clinical trials.

Table 3. Pharmacokinetic interactions of calcium antagonists (CA) with AEDs

Calcium antagonists	AEDs	Effects on blood levels	
		AEDs	CA
Flunarizine	PHT	none	↓
	PB	none	↓
	CBZ	none	↓
Nifedipine	PHT	↓	↓
	PB	↓	↓
	CBZ	↓	↓
Nimodipine	PHT	↓	↓
	PB	↓	↓
	CBZ	↓	↓
	VPA	↑	↓
	DZP	none	none

AED – antiepileptic drug, PHT – phenytoin, PB – phenobarbital, CBZ – carbamazepine, VPA – valproate, DZP – diazepam. Data are from numerous papers cited in this study

Dantrolene

Dantrolene is an inhibitor of Ca^{2+} release from intracellular stores.

Experimental studies

In the experiment carried out by Świąder et al. [62], dantrolene did not affect the electroconvulsive threshold in mice, but it elevated the protective activity of LY 300164 against MES. It may suggest that the inhibition of Ca^{2+} release from the intracellular stores plays a role in the dantrolene-induced increase in the anticonvulsive action of LY 300164 [62]. On the other hand, dantrolene failed to modify the protective activity of conventional antiepileptics, such as CBZ, PB, DPH or VPA against electrically evoked seizures [3].

Dantrolene seems to be useless in the treatment of epileptic patients not only because of its lack of

effect on the anticonvulsive action of many AEDs, but also because of the considerable adverse effects observed in experimental studies [3, 62].

Concluding remarks

The data presented in this review indicate that generally, Ca^{2+} channel antagonists possess anticonvulsant potential in experimental models of epilepsy and potentiate the protective activity of some AEDs. Among clinical data, there are trials indicating beneficial results with Ca^{2+} channel inhibitors, added to standard anticonvulsant therapy. However, also negative examples exist. Since experimental data indicate that the channel inhibitors may positively interact only with some AEDs, this may be a reason for clinical therapeutic failures. Also, one has to consider that nifedipine may even reduce the protective potential of some AEDs and isradipine or verapamil are completely ineffective when combined with conventional AEDs. So far, other experimental attempts to affect the intraneuronal Ca^{2+} concentration *via* other mechanisms than the blockade of voltage-operated channels (dantrolene) have failed. The most important pharmacodynamic and pharmacokinetic interactions between AEDs and Ca^{2+} blockers are shown in Tables 1–3.

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