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Amlodipine enhances the activity of antiepileptic drugs against pentylenetetrazole-induced seizures

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Abstract

Amlodipine (AML), which belongs to the 1,4-dihydropyridine calcium channel antagonists, possesses pharmacological and pharmacokinetic profile that distinguishes it from other agents of this class. Pentylenetetrazole (PTZ)-induced clonic and tonic convulsions in mice were significantly reduced by administration of AML at 10 mg/kg. At this dose AML remained without influence upon the plasma level of PTZ. The ED_{50} value of AML against clonic seizures induced by PTZ was 5.4 mg/kg. This calcium channel antagonist (at 2.5 mg/kg) combined with ethosuximide (ETX), valproate magnesium (VPA) or phenobarbital (PB) significantly reduced their ED_{50} values against clonic phase of PTZ-induced seizures. AML administered alone or in combination with antiepileptic drugs (AEDs) worsened the motor performance of mice in the chimney test. However, these treatments remained without significant influence on the retention time in the passive avoidance test. Plasma levels of antiepileptics remained unchanged in the presence of AML. The results indicate that AML does not seem a good candidate for a combination therapy in epileptic patients because of its adverse potential.

Keywords: Calcium antagonists; Amlodipine; Antiepileptic drugs; Seizures; Pentylenetetrazole

1. Introduction

The pathophysiology of epileptic seizures is complex, but calcium ions (Ca^{2+}) are likely to play a significant role in this regard (Schwartzkroin, 1993). It is well documented that increased intracellular Ca^{2+} level or enhanced Ca^{2+} conductance can be observed during epileptic activity (Tsakiridou et al., 1995; Vreughdenhill and Wadman, 1992). High intracellular Ca^{2+} level may be one of the main factors responsible for neuronal death in *status epilepticus* (Wasterlain et al., 1993). Consequently, animals injected with Ca^{2+} channel opener, Bay k-8644, develop clonic and tonic convulsions (Bolger et al., 1985; De Sarro et al., 1988).

Ca^{2+} channel antagonists posses anticonvulsive potential in many seizure models. Dihydropyridine derivatives (DHPs) were effective against maximal electroshock-induced (Meyer et al., 1986) and amygdala-kindled seizures (Wurpel and Iyer, 1994). Moreover, they were active in various chemically induced seizures e.g. against pentylenetetrazole (PTZ) (Meyer et al., 1987), picrotoxin (Thomas, 1990), *N*-methyl-D-aspartate (Karler et al., 1991) and pilocarpine (Marinho et al., 1997). Based on high efficacy of Ca^{2+} channel antagonists in experimental models some clinical trials were performed. The results of these studies were encouraging, especially in case of flunarizine (Binnie et al., 1985; Moglia et al., 1986). However, DHPs (e.g. nimodipine) appeared to have weak efficacy as add-on treatment in epileptic patients (for review see Czuczwar et al., 1996).

Our earlier studies indicated that Ca^{2+} antagonists enhance the anticonvulsive activity of certain antiepileptic

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drugs (AEDs) in various experimental models of epilepsy (Czuczwar et al., 1996). Generally, DHPs increased the anticonvulsive activity of carbamazepine (CBZ) or diphenylhydantoin (DPH) being without effect on the activity of valproate magnesium (VPA) against maximal electroshock-induced seizures (Czuczwar et al., 1990a, 1992). In addition, the anticonvulsant effects of VPA, ethosuximide (ETX) and phenobarbital (PB) against PTZ-induced seizures were enhanced by various DHPs (Czuczwar et al., 1990b; Gasior et al., 1996b). However, an enhancement of the anticonvulsant activity of AEDs by Ca^{2+} channel inhibitors is not a common feature of all DHPs. Surprisingly, in the case of niguldipine opposite effects were observed and the protective activity of CBZ and PB was actually decreased by this DHP against maximal electroshock-induced seizures in mice (Borowicz et al., 1997).

Amlodipine (AML) was introduced only several years ago (Clavijo et al., 1994), nevertheless nowadays it has a recognized position in therapy of cardiovascular disorders when compared to other Ca^{2+} inhibitors (de Vries et al., 2000; Herman, 1999). It belongs to 1,4-DHPs with chemical structure close to nifedipine. However, AML possesses higher volume of distribution and longer half-life than other 1,4-DHPs (Stopher et al., 1988). It has been documented that AML crossed the blood–brain barrier after peripheral administration (Uchida et al., 1997; Yamada et al., 1994). AML also has unique pharmacological properties that distinguish it from other DHPs (Burges and Moisey, 1994).

There are very few reports suggesting an anticonvulsant potential for AML (Moron et al., 1990; O'Neill and Bolger, 1990). AML given intracerebroventicularly inhibited PTZ-induced spiking in the cortical EEG of rat (Moron et al., 1990). It was more potent than nicardipine and nifedipine in this regard (Moron et al., 1990). AML prolonged also the latency to PTZ-induced seizures (O'Neill and Bolger, 1990). However, AML was without effect upon the electroconvulsive threshold, but enhanced the anticonvulsive activity of some conventional AEDs against maximal electroshock-induced seizures in mice (Kamiński et al., 1999).

Although recent years have brought several new AEDs, therapy of epilepsy is still unsatisfactory. Treatment with only one AED is the rule; however, in refractory epilepsy combinations of two or more drugs are often used (Krämer, 1997). In some cases (20–30%) currently available AEDs are ineffective (Sander, 1993). Even new AEDs, used as add-on therapy, are often without expected effects in terms of better seizure control (Mattson, 1995). Therefore, there is a strong need for new antiepileptic agents or treatment strategies. Optimization of treatment with established AEDs based on their mechanisms of action, so-called rational polytherapy, is also justified (Leach, 1997).

The aim of this study was to assess the anticonvulsant potential of AML given alone or in combination with standard AEDs against PTZ-induced seizures. Behavioral effects of these treatments were also studied in well-recognized experimental models of neurotoxicity and long-term memory. Moreover, plasma levels of AEDs and PTZ were measured in order to exclude possible pharmacokinetic interactions.

2. Method

2.1. Animals

Male Swiss mice weighing 22–27 g were experimental subjects. The animals were housed in standard laboratory conditions and allowed free access to food and water until the time of experiments. Experimental groups consisted of 8–10 mice. The control groups were always tested on the same day as the respective experimental groups. The convulsive and behavioral tests were performed between 10 a.m. and 1 p.m. The experimental procedures listed below were approved by the Bioethical Committee of Lublin Medical University.

2.2. Seizure activity

Mice were injected with PTZ (Sigma, St. Louis, MO, USA) in a dose of 95 mg/kg, which was its CD_{97} (a dose necessary to induce clonic seizures in 97% of animals). Following the injection, mice were put individually into transparent cages and observed for 30 min for the occurrence of clonic (duration of at least 3 s with loss of righting reflex) and tonic seizures (tonic hindlimb extension as the endpoint).

2.3. Drugs

The following AEDs were used: VPA, ETX and PB (all from Polfa, Rzeszów, Poland). AML (Pfizer, Belgium) was suspended in a 1% solution of Tween 80 (Loba-Chemie, Austria), while PTZ and all AEDs were dissolved in sterile saline. All drugs were injected intraperitoneally (ip), except for PTZ, which was given subcutaneously (sc). AEDs were administered 30 min, while AML 120 min before the tests.

2.4. Chimney test

The effects of AEDs alone or combined with AML on motor performance were evaluated in the chimney test (Boissier and Tardy, 1960). In this test, mice had to climb backwards up a vertically positioned plastic tube ("chimney"), which was 25 cm long and 3 cm wide. Animal performance was assumed to be impaired when the task was not completed within 60 s. The results were shown as a percentage of animals which failed to perform the task.

2.5. Passive-avoidance testing

According to Venault et al. (1986), the step-through passive avoidance task is recognized as a measure of long-term memory. The device for passive avoidance testing

consisted of two compartments connected to each other: an illuminated box ($10 \times 13 \times 15$ cm) and a dark one ($25 \times 20 \times 15$ cm). The dark box was equipped with electric grid floor and entrance to this compartment of vehicle- or drug-treated animals was punished by an electric foot-shock (0.6 mA for 2 s; facilitation of acquisition). On the following day (24 h later), the same animals, without any treatment, were placed into the illuminated box and the time of avoiding entrance to the dark compartment (retention time) was measured up to 180 s. Retention times of passive avoidance behavior were expressed as medians with 25 and 75 percentiles.

2.6. PTZ extraction and gas chromatography analysis

PTZ concentrations in plasma were determined by a modification of methods described by Yonekawa et al. (1980) and Dingemanse et al. (1988). The animals were sacrificed by decapitation at the time point, which was 135 min following AML (or vehicle pretreatment) and 15 min after administration of PTZ. At this time, AML showed considerable anticonvulsive activity, while in control groups the occurrence of seizures was remarkable. Blood samples were collected into Eppendorf tubes and centrifuged. Subsequently, plasma was taken for analysis. The analytical procedure consisted of combining 4 ml of *tert*-butyl methyl ether with 200 (μl of plasma, 300 μl of 0.2 M borate buffer (pH 9) and 500 μl of *n*-butyl alcohol. Known amount of 2,5-diphenyloxazole was added as an internal standard (all compounds from Sigma). The mixture was shaken for 1 min and left for sedimentation for additional 30 min. The organic layer was transferred to a tube and evaporated under atmospheric pressure for 12 h until *n*-butyl alcohol resided.

Analysis was carried out with gas chromatograph (CHROM-5, Czech Republic) equipped with a flame-ionization detector (A-FID). A glass column (1.8 m × 3 mm internal diameter) packed with 10% SE-30 was used. Temperature conditions were as follows: injection port 230°C; detector 250°C; oven 220°C. Purified nitrogen was used as the carrier gas with a flow-rate being 40 ml/min, while hydrogen and air flows were 23 and 380 ml/min, respectively. The retention times were: 2.7 min — PTZ and 4.9 min — 2,5-diphenyloxazole. The mean recovery reached more than 90% and detection limit of PTZ was 2.5 μg/ml. Standard calibration curves constructed by extracting blank plasma with known amounts of PTZ were linear between 5 and 300 μg/ml.

2.7. Determination of free plasma levels of AEDs

Estimation of the plasma levels of AEDs alone or in combination with AML was carried out by immunofluorescence with the use of an Abbott TDx automatic analyzer (Abbott, Irving, TX, USA). At least eight mice were used per group and the plasma levels were expressed in μg/ml as means ± S.D. Blood samples were collected after decapitation of animals at the time scheduled for the convulsive experiments. After centrifugation at 10,000 rpm for 4 min (Abbott centrifuge, Abbott), plasma samples were pipetted into Microcon-30 microconcentrators (Amicon, Danvers, MA, USA) and again centrifuged. The filtrates of 50 μl were analyzed by the Abbott TDx analyzer. Control concentrations of AEDs were put at the beginning and end of each experimental carousel for samples for the verification of calibration.

2.8. Statistics

ED_{50} values (with 95% confidence intervals in parentheses or indicated in figures) and their statistical comparisons were performed by computer probit analysis according to the method of Litchfield and Wilcoxon (1949). The results from chimney test were statistically estimated by Fisher's exact probability test, while results obtained from passive avoidance testing were compared by Mann–Whitney *U* test. Plasma levels of PTZ and AEDs were statistically analyzed by the use of unpaired Student's *t* test.

3. Results

3.1. Influence of AML upon PTZ-induced seizures

PTZ evoked clonic and tonic seizures in a dose-dependent manner and its CD_{97} for the induction of the clonic phase was 95 mg/kg (data not shown). AML (up to 5 mg/kg) remained without significant effect against PTZ given at 95 mg/kg. However, AML (10 mg/kg) significantly decreased the number of clonic (Fig. 1) and tonic convulsions (result not shown in Fig. 1) in comparison to control animals. As can be seen from Fig. 1, AML dose-dependently reduced the number of PTZ-induced seizures and its ED_{50} value against the clonic phase was 5.5 (3.6–8.4) mg/kg.

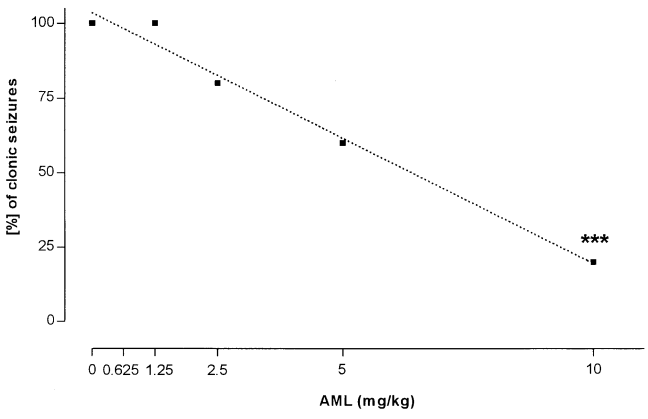

Fig. 1. Effect of AML upon PTZ-induced clonic seizures. AML dose-dependently (r^2=.99) reduced the number of PTZ-induced seizures and its ED_{50} value against clonic phase was 5.5 (3.6–8.4) mg/kg [according to Litchfield and Wilcoxon's (1949) probit analysis]. *** $P < .001$ vs. vehicle-treated animals.

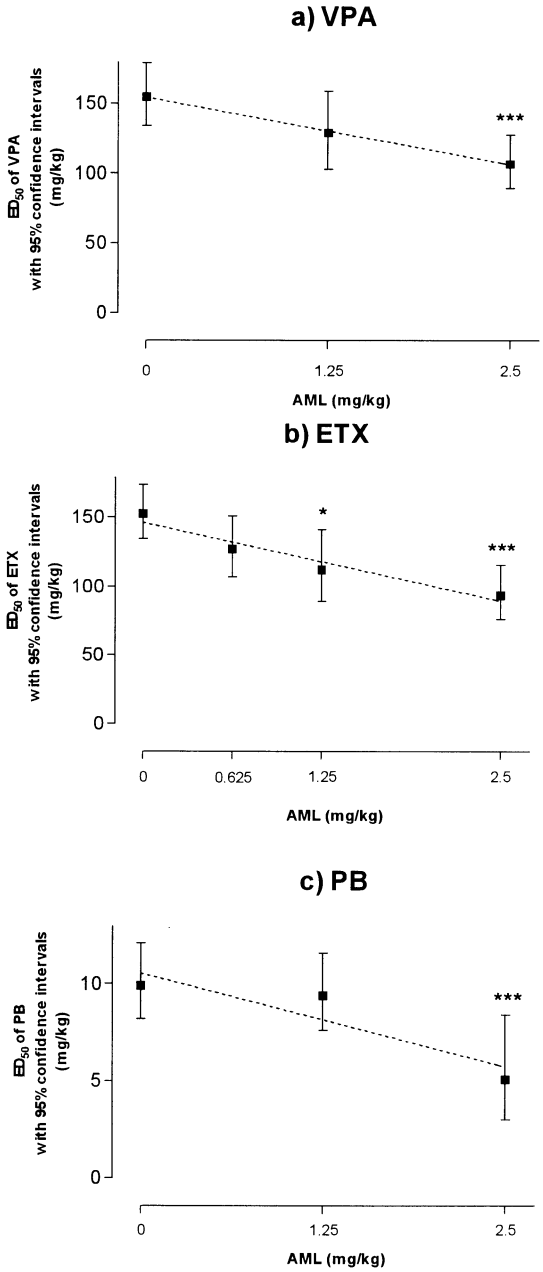

Fig. 2. Effect of AML upon the protective activity of AEDs, expressed in the form of their ED_{50} values with 95% confidence intervals, against PTZ-induced clonic seizures. AML — amlodipine, VPA — valproate magnesium, ETX — ethosuximide, PB — phenobarbital, PTZ — pentylenetetrazole. AML dose-dependently enhanced the protective activity of (a) VPA (r^2=.99), (b) ETX (r^2=.94) and (c) PB (r^2=.83) against clonic phase of PTZ-induced seizures. PTZ was given at its CD_{97} dose (a dose necessary to induce clonic seizures in 97% of animals), which was 95 mg/kg. *$P<.05$ or ***$P<.001$ vs. vehicle treated animals [according to Litchfield and Wilcoxon's (1949) probit analysis].

3.2. Influence of AML upon protective activity of AEDs against PTZ-induced seizures

ED_{50} value of VPA against PTZ-induced clonic seizures was 155 (134–179) mg/kg. Combined treatment with AML (2.5 mg/kg) and VPA significantly reduced this value to 107 (89–127) mg/kg. AML at the dose of 1.25 mg/kg did not affect the ED_{50} of VPA (Fig. 2a).

AML given at doses of 1.25 and 2.5 mg/kg enhanced the protective effects of ETX against PTZ-induced clonic seizures, reducing the ED_{50} value of this antiepileptic from 153 (134.5–174) to 112 (89.2–141) and 93.8 (76.1–116) mg/kg, respectively. At 0.625 mg/kg AML remained without influence upon the protective effects of ETX (Fig. 2b).

AML (2.5 mg/kg) reduced the ED_{50} of PB from 9.9 (8.2–12.1) to 5.1 (3.0–8.4) mg/kg, whilst at the dose of 1.25 mg/kg it was ineffective in this regard (Fig. 2c).

3.3. Influence of AML and AEDs upon the upon the motor performance and memory of mice

AML (up to 2.5 mg/kg) significantly worsened the motor performance of mice in the chimney test (Table 1). However, at this dose range it remained without considerable influence upon the memory of mice in the passive avoidance task (Table 1). Combined treatments with AML and AEDs resulted in significant motor impairment as revealed by the chimney test. AML worsened the motor performance of animals when it was coadministered with VPA since significant differences were observed in comparison to mice treated with VPA alone (Table 1). Interestingly, the combined treatments with AML and AEDs did not affect the performance of mice in the passive avoidance task (Table 1). Only administration of VPA at the dose equal to its ED_{50} against the clonic phase of

Table 1
Effects of AML and AEDs upon the motor performance and passive avoidance task mice

Treatment (mg/kg)[a]	Motor impairment (%)[b]	Retention time (s)[c]
Vehicle	0	180 (160, 180)
VPA (155)	10	68 (20, 140)[¶]
VPA (107)	10	155 (110, 180)
VPA (107)+AML (2.5)	70***,[†]	180 (15, 180)
ETX (153)	10	142 (43, 180)
ETX (112)	20	177 (85, 180)
ETX (112)+AML (1.25)	50*	180 (135, 180)
ETX (94)	30	180 (110, 180)
ETX (94)+AML (2.5)	40*	180 (160, 180)
PB (9.9)	10	130 (21, 180)
PB (5.1)	0	180 (95, 180)
PB (5.1)+AML (2.5)	50*	135 (64, 180)
AML (1.25)	50*	180 (130, 180)
AML (2.5)	50*	150 (63, 180)

AML — amlodipine, VPA — valproate magnesium, ETX — ethosuximide, PB — phenobarbital.

[a] Each experimental group consisted of 10 animals.

[b] Motor performance was tested in the chimney test (see Method).

[c] Long-term memory was tested in the passive avoidance task and expressed as medians and 25 and 75 percentiles (see Method).

* $P<.05$ vs. vehicle-treated group (Fisher's exact probability test).

*** $P<.001$ vs. vehicle-treated group (Fisher's exact probability test).

[¶] $P<.05$ vs. vehicle-treated group (Mann–Whitney U test).

[†] $P<.05$ vs. VPA-treated groups (Fisher's exact probability test).

Table 2
Influence of AML on the free plasma levels of AEDs

Treatment (mg/kg)[a]	Free plasma level (μg/ml)[b]
VPA (107) + vehicle	71.8 ± 7.8
VPA (107) + AML (2.5)	78.9 ± 5.1
ETX (94) + vehicle	52.0 ± 5.9
ETX (94) + AML (2.5)	52.1 ± 7.5
PB (5.1) + vehicle	2.2 ± 0.5
PB (5.1) + AML (2.5)	2.3 ± 0.4

AML — amlodipine, VPA — valproate magnesium, ETX — ethosuximide, PB — phenobarbital.

[a] Each experimental group consisted of eight animals.

[b] Table data are expressed as means ± standard error of the mean (S.E.M.).

PTZ-induced seizures significantly shortened the retention time. However, the combined treatment with AML (2.5 mg/kg) and VPA, which provided a 50% protection against PTZ-induced seizures, was without significant influence upon the retention time (Table 1).

3.4. Influence of AML upon the plasma level of PTZ and AEDs

Plasma level of PTZ was 71.3 ± 1.4 μg/ml when this convulsant was administered at 95 mg/kg. Administration of AML at the anticonvulsive dose of 10 mg/kg did not significantly affect the plasma level of PTZ, which was 76 ± 4.4 μg/ml (data not shown in tables).

AML at 2.5 mg/kg, which enhanced the protective effects of AEDs, did not affect their free plasma levels (Table 2).

4. Discussion

In the present study, AML per se inhibited PTZ-induced convulsions and at subprotective doses enhanced the protective effects of VPA, ETX and PB against PTZ in a way similar to previously examined DHPs. However, this enhancement appeared at much lower doses than in case of the other DHPs, nifedipine, nimodipine and nicardipine (Czuczwar et al., 1990b; Gasior et al., 1996b). The effective dose range for nifedipine and nimodipine was 10–20 and 20–40 mg/kg, respectively. At these doses, combinations of the DHPs with subprotective doses of ethosuximide and valproate resulted in the clear-cut protection against the clonic phase of PTZ-induced convulsions in mice (Czuczwar et al., 1990b; Gasior et al., 1996b). The effective dose of nicardipine for the combined treatment with these antiepileptics was 5 mg/kg, and at higher doses the DHP inhibited per se the clonic seizure activity produced by PTZ (Gasior et al., 1996b). The doses of AML that enhanced the anticonvulsive activity of AEDs against PTZ were also much lower then the respective doses required for maximal electroshock-induced seizures. In this model of experimental epilepsy AML had to be given at 5–10 mg/kg to lower the ED_{50} values of CBZ, phenobarbital and valproate (Kamiński et al., 1999).

Several DHPs were effective against PTZ-induced seizures (Gasior et al., 1996b; Meyer et al., 1987). AML was reported to prolong the latency to PTZ-induced seizures (O'Neill and Bolger, 1990). Moreover, it suppressed spike discharges observed in the EEG after peripheral administration of PTZ (Moron et al., 1990). In this study, AML inhibited PTZ-induced clonic seizures in a dose-dependent manner. It is evident that AML is capable of blocking N-type Ca^{2+} calcium channels (Furukawa et al., 1997). These channels are responsible for glutamate release in the cerebral cortex and hippocampus (Takahashi and Momiyama, 1993). It is noteworthy that Ca^{2+} currents through N-type channel were 20% of total inward Ca^{2+} current in isolated cortical neurons obtained from epileptic patients (Beck et al., 1997; Sayer et al., 1993). These features of AML would probably greatly contribute to its anticonvulsive activity observed in the current study.

VPA, ETX and PB are effective against PTZ-induced seizures; however, only VPA and ETX are useful for the treatment of absence epilepsy in humans (Fisher, 1989; Löscher and Schmidt, 1988). The mechanism of action of these AEDs is not fully understood, but the involvement of calcium channels seems to be a significant factor in their anticonvulsant activity (for review see Stefani et al., 1997). ETX at a therapeutic concentration selectively inhibits T channels (Coulter et al., 1989, 1990) and to a much lesser extent — L channels (Crowder and Brandford, 1987; Kostyuk et al., 1992). VPA is also capable of inhibiting T channels, being almost without effect upon L and N channels. In contrast, barbiturates (e.g. phenobarbital) are able to block only N and L channels (Stefani et al., 1997).

As it was mentioned, AML is characterized by a unique pharmacological and pharmacokinetic profile among other DHPs (Burges and Moisey, 1994; Stopher et al., 1988). AML binds to plasma proteins in more than 95% and is extensively metabolized in the liver (Clavijo et al., 1994; Stopher et al., 1988). Therefore, a likelihood of a pharmacokinetic interaction with other drugs is high. Plasma level of PTZ remained unchanged in the presence of the anticonvulsive dose of AML, thus a pharmacokinetic interaction (in terms of the plasma level of the convulsant), which might be responsible for the protective activity of AML, does not seem probable. In addition, other DHPs did not alter brain concentration of PTZ (Larkin et al., 1992). AML also did not affect the free plasma levels of antiepileptic drugs, so an alternative pharmacokinetic factor, in terms of drug plasma levels at least, can be excluded. However, we previously reported that AML may increase the plasma level of CBZ in mice (Kamiński et al., 1999). It should be underlined that AML is capable of crossing the blood–brain barrier (Uchida et al., 1997; Yamada et al., 1994) and Tween 80 did not affect the permeability of this structure (Larkin et al., 1992). This may indicate that the centrally mediated effects of AML participate in the interaction with

AEDs. Actually, verapamil (a calcium channel antagonist hardly entering the brain) was completely devoid of any influence upon the protective activity of conventional antiepileptics against maximal electroshock-induced convulsions or PTZ-produced seizures in mice (Czuczwar et al., 1990a,b). This also seems to provide evidence that the main peripheral effect of Ca^{2+} channel blockade, hypotension (Burges and Moisey, 1994; Flaim and Kanda, 1982), does not seem a factor responsible for the interaction of AML with AEDs.

PTZ-induced clonic seizures have been recognized as a model of absence epilepsy, since AEDs effective in this model (e.g. ETX and VPA) are also effective in human absence seizures (Fisher, 1989; Löscher and Schmidt, 1988). However, phenobarbital, which is effective against PTZ-induced seizures, may worsen the outcome of absence epilepsy in humans. Therefore, PTZ-induced clonic seizures are more likely to provide an experimental model of myoclonic epilepsy (Löscher and Schmidt, 1988). Mechanism of the convulsive action of PTZ is not fully understood. One of the major components contributing to its seizure activity is a blockade of $GABA_A$ receptor complex (Snead, 1992). However, in view of the fact that $GABA_A$ agonists did not completely inhibit seizures elicited by PTZ administration, other mechanisms were also suggested (Fisher, 1989). PTZ treatment leads to the increase of intracellular Ca^{2+} level (Onozuka et al., 1989; Papp et al., 1990), but the exact mechanism of this action remains to be clarified. Nevertheless, it was reported that PTZ did not affect inward Ca^{2+} currents through T-type channel (Todorovic and Lingle, 1998). Neuronal T-type channels in the thalamus are assumed to be essential for the generation of spike-wave discharges observed in a genetic model of absence epilepsy (Avanzini et al., 1993). The lack of activity of PTZ upon T-type calcium channel may support the idea that PTZ-induced seizures are not a good model of absence epilepsy, but rather the model of myoclonic epilepsy.

In the current study, AML (1.25–2.5 mg/kg) given alone or combined with AEDs produced a significant motor impairment in mice as revealed by the chimney test. Both peripheral and central actions of AML might contribute to this effect. A significant blood pressure reduction was noted in experimental animals after administration of AML (Burges and Moisey, 1994) and apparently this effect of Ca^{2+} channel antagonists may result in motor performance impairment and sedation (Pucilowski, 1992). When taking into account the central effects of Ca^{2+} channel antagonists it should be underlined that these compounds may affect various neurotransmitter pathways in the central nervous system (CNS). Dopamine is widely appreciated as the most important endogenous factor mediating motor activity processes in CNS. Some calcium channel antagonists may interact with brain dopamine receptors (Pucilowski, 1992). Indeed, AML administered peripherally at a relatively high dose of 20 mg/kg was reported to be a weak blocker of dopamine D_1 receptor and evoked catalepsy in mice (Haraguchi et al., 1998). However, AML at a lower dose (2.5 mg/kg) was without effect upon spontaneous motor activity as well as apomorphine- and morphine-induced locomotor stimulation (Dogrul and Yesilyurt, 1999). On the contrary, Ca^{2+} channel antagonists are capable of blocking amphetamine-induced locomotor activity (Grebb, 1986). Interestingly, the data obtained in our laboratory provide an evidence that another DHP derivative, nimodipine (40 mg/kg), similarly to AML, considerably impaired the performance of mice in the chimney test — 75% of the animals were unable to perform this test (Gasior et al., 1996b). Also, the combined treatment of nicardipine with ETX or VPA resulted in the profound impairment of motor coordination in mice (Gasior et al., 1996b). However, nicardipine did not affect this parameter when either given alone (up to 15 mg/kg; Gasior et al., 1996a, 1997) or combined (at 5 mg/kg) with these AEDs (Gasior et al., 1996b). Consequently, one may assume that impairment of motor performance is not a general feature of all DHP derivatives, at least in doses necessary to potentiate the protective activity of AEDs.

Epileptic patients may experience cognitive and memory deficits and these problems are likely due to epileptic process or/and AEDs treatment (for review see Perrine and Kiolbasa, 1999). There is a great body of evidence suggesting that DPHs are capable of enhancing memory processes — nimodipine in aging rabbits (Deyo et al., 1989) and AML in mice (Quartermain et al., 1993). AML was reported to increase the retention time in passive avoidance paradigm when given immediately posttraining or shortly before testing in mice (Quartermain et al., 1993). In addition, AML appeared to be an effective memory consolidation and retrieval enhancer when some other behavioral tests were used (Quartermain et al., 1993). However, these effects were not observed when AML was tested in other species (Zupan et al., 1996). In the present study, AML did not change the performance of animals in the passive avoidance test — similar results were obtained in our previous study with higher doses of AML (Kamiński et al., 1999). It should be underlined that valproate administered at its ED_{50} against PTZ-induced clonic seizures significantly shortened the retention time in passive avoidance test. However, the retention remained unchanged when valproate was coadministered at a lower dose with AML, which provided the same degree of protection as valproate alone against PTZ-induced seizures. This indicates that the combined treatment of valproate and AML, providing the same degree of protection against PTZ as valproate alone, is in fact superior to valproate alone in terms of long-term memory. However, other DHPs, nifedipine (15 mg/kg) and nimodipine (15 mg/kg) alone worsened long-term memory in mice (Gasior et al., 1996a, 1997).

It is evident that potential AEDs combinations should be tested in animal models before introduction to clinical trials. Only certain drug combinations that show increased efficacy against seizures and decreased side effects might be used in further preclinical or clinical studies (Löscher and Wauquier,

1996). In this light, AML appears to be not a good candidate for use as an adjuvant therapy in epileptic patients. Even though it enhanced the protective effects of several AEDs against maximal electroshock- (Kamiński et al., 1999) and PTZ-induced seizures (current study), these effects were accompanied by impaired motor performance in mice. If there is a need to use AML in epileptic patients for other than epilepsy reasons (for instance, cardiovascular diseases), no impaired protective activity of AEDs or memory processes may be expected at least. Actually, the anticonvulsant potency of antiepileptics may be enhanced and their memory-depressing effect reduced by AML.

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