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Propranolol and metoprolol enhance the anticonvulsant action of valproate and diazepam against maximal electroshock

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Abstract

The anticonvulsive potential of classical antiepileptics co-administered with β-adrenergic receptor antagonists against generalized tonic–clonic seizures was evaluated in the model of maximal electroshock (MES)-induced convulsions. Propranolol, acebutolol, metoprolol and atenolol were tested in the doses not affecting the electroconvulsive threshold. Propranolol and metoprolol lowered the ED_{50} of valproate and diazepam. Acebutolol reduced valproate's but not diazepam's ED_{50} value. In contrast, hydrophilic atenolol, not penetrating via blood–brain barrier, affected neither the action of valproate nor diazepam. None of the studied drugs changed the protective activity of carbamazepine and phenytoin against MES. β-blockers *per se* did not alter the motor performance of mice. Moreover, propranolol and metoprolol did not influence diazepam-evoked impairment of locomotor activity. The free plasma and brain levels of antiepileptic drugs were not affected by β-blockers. In conclusion, the use of certain β-adrenoceptor antagonists, such as propranolol and metoprolol, might improve the antiepileptic potential of valproate and diazepam.

Keywords: β-Adrenergic receptor antagonist; Propranolol; Metoprolol; Anticonvulsant drugs; Valproate; Diazepam; Epilepsy; Maximal electroshock-induced seizures

1. Introduction

The contribution of noradrenergic neurotransmission to the seizure susceptibility and epileptogenesis is gaining more attention recently. Hippocampus, known for its low seizure threshold and the involvement in propagation of seizures (McNamara, 1994), receives substantial noradrenergic input originating primarily in the locus coeruleus (Loy et al., 1980). Hippocampal density of β_1/β_2 receptors is the highest among brain structures (Reznikoff et al., 1986). However, the role of β-receptor mediated neurotransmission in epileptic phenomena is not equivocal. Both pro- and anticonvulsant effects were ascribed to the stimulation of β-adrenergic receptor. β-adrenoceptor agonists were demonstrated to potentiate the epileptiform abnormalities occurring in slices of pyriform cortex obtained from kindled animals (McIntyre and Wong, 1986). Similarly, β-receptor activation increased the rate of spontaneous epileptiform discharges in hippocampal slices (Rutecki, 1995). In contrast, stimulation of locus coeruleus was found to reduce the hippocampal epileptiform discharges in rats, mainly via the activation of β-adrenergic receptors (Ferraro et al., 1994). The noradrenergic system was demonstrated to participate in the occurrence of seizures in epileptic EL mice and to increase epileptiform discharges in rat limbic system via β-adrenergic receptor stimulation (Tsuda et al., 1990; Stoop et al., 2000).

Propranolol displays anticonvulsant effects against audiogenic, pentylenetetrazol-or maximal electroshock (MES)-induced seizures (Fischer et al., 1985; Khanna et al., 1989; Lints and Nyquist-Battie, 1984; Louis et al., 1982). Some β-adrenoceptor antagonists were demonstrated to enhance the antiepileptic activity of swim stress in the model of convul-

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Table 1
Influence of β-adrenergic receptor antagonists on the electroconvulsive threshold (CS_{50})

Treatment (mg/kg)	CS_{50} (mA)
Saline	6.3 [5.4–7.4]
Propranolol (5)	6.8 [6.1–7.5]
Saline	6.7 [6.3–7.1]
Metoprolol (50)	6.7 [6.2–7.2]
Saline	6.0 [5.5–6.5]
Acebutolol (100)	6.5 [6.1–6.8]
Saline	6.6 [6.0–7.1]
Atenolol (10)	6.7 [6.1–7.3]

Data are presented as the CS_{50} values, i.e., the current strength (in mA, with 95% confidence limits) necessary to induce tonic hind limb extension in 50% of mice tested. The calculations of CS_{50} values and statistical comparisons were performed using the computerized linear regression analysis, based on the method of Litchfield and Wilcoxon (1949).

sions generated by picrotoxin administration (Pericic et al., 2000). There is, however, very limited information on the efficacy of anticonvulsant drugs against generalized tonic–clonic seizures when used simultaneously with β-adrenergic antagonists. In one study, propranolol, pindolol and alprenolol were shown to enhance the anticonvulsant activity of phenobarbital in MES test (Fischer and Muller, 1988). Thus, it is conceivable to assume that the use of β-adrenergic antagonists might also influence the efficacy of other than phenobarbital anticonvulsants.

The aim of this study was to investigate the efficacy of classical antiepileptic drugs, such as valproate, diazepam, phenytoin and carbamazepine, administered together with β-adrenergic receptor antagonists, against generalized tonic–clonic seizures in mice. Studied β-blockers are commonly given in clinic and display different pharmacological profile including their β-adrenoceptor selectivity, lipophilicity and sodium channel blockade.

2. Materials and methods

2.1. Animals

The experiments were carried out on male Albino Swiss mice, weighing 20–25 g and kept in colony cages at room

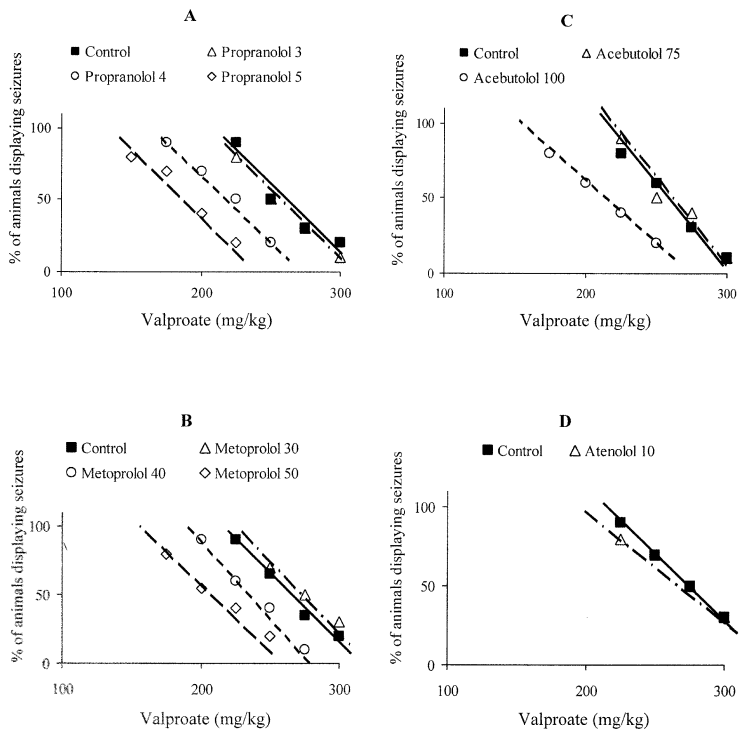

Fig. 1. Influence of β-adrenergic receptor antagonists on the protective efficacy of valproate against MES-induced seizures. Data are presented as percentage of animals displaying seizures following administration of various doses of valproate with saline (control) or with the respective β-adrenoceptor antagonist. The calculations of ED_{50} values and statistical analyses of the data were performed according to the method of Litchfield and Wilcoxon (1949). Dose regression curves were calculated using GraphPAD software. (A) Control: ED_{50}: 255.4 [236.4–274.8] ($y=-0.92x+289$, $r=-.96$); propranolol 3 mg/kg: ED_{50}: 247.2 [230.1–262.7] ($y=-0.92x+284$, $r=-.99$); propranolol 4 mg/kg: ED_{50}: 213.7 [191.7–238.1] ($y=-92.0x+253$, $r=-.99$) ($P<.01$); propranolol 5 mg/kg: ED_{50}: 183.5 [152.7–215.7] ($y=-0.84x+210$, $r=-.98$) ($P<.001$). (B) Control: ED_{50}: 265.2 [248.8–282.7] ($y=-0.96x+304.5$, $r=-.99$); metoprolol 30 mg/kg: ED_{50}: 261.4 [234.9–285.3] ($y=-0.8x+270$, $r=-1.0$); metoprolol 40 mg/kg: ED_{50}: 230.8 [216.1–246.5] ($y=-1.04x+297$, $r=-.99$) ($P<.01$); metoprolol 50 mg/kg: ED_{50}: 215.2 [196.3–235.6] ($y=-0.78x+214.5$, $r=-.99$) ($P<.001$). (C) Control: ED_{50}: 261.7 [241.0–283.6] ($y=-0.96x+297$, $r=-.99$); acebutolol 75 mg/kg: ED_{50}: 259.0 [240.5–279.0] ($y=-x+310$, $r=-.98$); acebutolol 100 mg/kg: ED_{50}: 211.4 [191.4–233.6] ($y=-0.8x+220$, $r=-1.0$) ($P<.01$). (D) Control: ED_{50}: 259.8 [239.4–276.7] ($y=-0.8x+270$, $r=-1.0$); atenolol 10 mg/kg: ED_{50}: 257.4 [236.1–277.3] ($y=-0.68x+236$, $r=-.99$).

temperature, under a natural light–dark cycle. The animals were housed with a free access to food pellets and tap water. Experimental groups, consisting of 7–12 animals, were assigned according to a randomised schedule, and each mouse was used only once. Control animals were always tested on the same day with respective experimental groups. Experimental procedures have been approved by the local Ethical Committee and are in agreement with European Communities Council Directive.

2.2. Drugs

Diazepam (Polfa, Warsaw, Poland), carbamazepine (Sigma, St. Louis, MO, USA), phenytoin (Polfa), metoprolol tartrate (Polpharma, Starogard Gdanski, Poland) and atenolol (RBI, Natick, MA, USA) were suspended in a 1% solution of Tween 80. Valproate (sodium salt) (Sigma), propranolol (Sigma) and acebutolol (Polfa, Grodzisk, Poland) were dissolved in sterile saline. All drugs were administered intraperitoneally: diazepam, valproate, propranolol, metoprolol and acebutolol 30 min, carbamazepine and atenolol 60 min, phenytoin 120 min prior to the test. The injection volume was always 0.1 ml/10 g of body weight. Control animals received equivalent volumes of the solvent at the respective times before the tests.

2.3. Electroconvulsions

Seizure threshold and MES-induced seizures were investigated according to Swinyard et al. (1952). The electroshock generated by Hugo-Sachs stimulator (Type 221, Freiburg, Germany) was applied via ear-clip electrodes separately to each mouse, given saline or respective drug(s) at appropriate times. The stimulus duration was 0.2 s and the current frequency 50 Hz. The animals were observed for the occurrence of tonic hind limb extension within 60 s following the stimulus. To evaluate the convulsive threshold (CS_{50}), i.e., the current strength (in mA) necessary to induce tonic hind limb extension in 50%

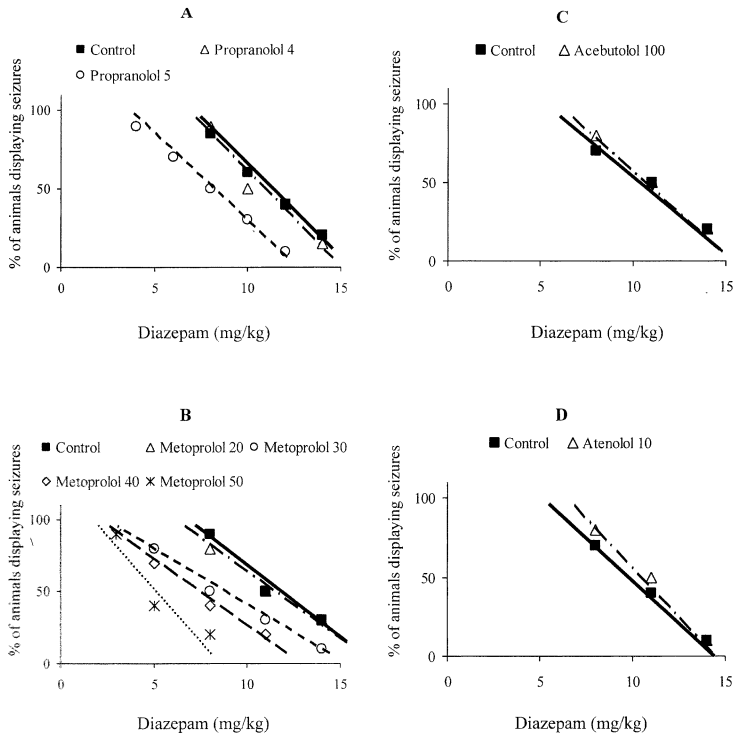

Fig. 2. Influence of β-adrenergic receptor antagonists on the protective efficacy of diazepam against MES-induced seizures. Data are presented as percentage of animals displaying seizures following administration of various doses of diazepam with saline (control) or with the respective β-adrenoceptor antagonist. The calculations of ED_{50} values and statistical analyses of the data were performed according to the method of Litchfield and Wilcoxon (1949). Dose regression curves were calculated using GraphPAD software. (A) Control: ED_{50}: 10.5 [8.7–12.7] ($y=-10.75x+169.5$, $r=-.99$); propranolol 4 mg/kg: ED_{50}: 10.7 [9.1–12.7] ($y=-11.75x+178$, $r=-.97$); propranolol 5 mg/kg: ED_{50}: 7.3 [5.7–9.2] ($y=-10x+130$, $r=-1.0$) ($P<.05$). (B) Control: ED_{50}: 10.5 [8.9–12.8] ($y=-8.3x+145$, $r=-.99$); metoprolol 20 mg/kg: ED_{50}: 10.5 [8.9–12.8] ($y=-8.3x+145$, $r=-.99$); metoprolol 30 mg/kg: ED_{50}: 7.1 [5.6–9.0] ($y=-7.7x+115.3$, $r=-.99$) ($P<.05$); metoprolol 40 mg/kg: ED_{50}: 6.4 [4.9–8.3] ($y=-8.3x+110$, $r=-.99$) ($P<.01$); metoprolol 50 mg/kg: ED_{50}: 4.7 [3.3–6.6] ($y=-13.4x+121.6$, $r=-.94$) ($P<.001$). (C) Control: ED_{50}: 9.9 [7.1–14.7] ($y=-8.3x+138.3$, $r=-.99$); acebutolol 100 mg/kg: ED_{50}: 9.2 [6.9–12.2] ($y=-10x+160$, $r=-1.0$). (D) Control: ED_{50}: 10.1 [8.3–12.5] ($y=-10x+150$, $r=-1.0$); atenolol 10 mg/kg: ED_{50}: 10.9 [9.1–13.0] ($y=-11.7x+175$, $r=-.99$).

of mice tested, at least 3 groups of mice, consisting of 10–12 animals, were challenged with electrical shocks of various intensities. MES-induced seizures were evoked with the current of 25 mA intensity. The prevention of hind limb tonic extensor component was considered as fully protective action of anticonvulsant. The anticonvulsant activity of antiepileptic drugs was expressed as the effective dose (ED_{50}), i.e., the dose of drug (in mg/kg) required to protect 50% of mice against MES-induced tonic hind limb extension.

2.4. Chimney test

The influence of β-adrenoceptor antagonists on the motor performance was evaluated according to Boissier et al. (1960). Motor impairment was indicated by the inability of animals to climb up backwards in the plastic tube (3 cm inner diameter, 25 cm length) within 60 s. TD_{50} value, i.e., dose of the antiepileptic drug (in mg/kg) causing motor impairment in 50% of tested animals, was evaluated using at least three groups of mice, given different doses of drug.

2.5. Plasma and brain level of anticonvulsants

For the estimation of blood and brain levels of antiepileptic drugs mice were injected with either the antiepileptic drug and solvent, or the antiepileptic drug and β-adrenergic receptor antagonist. Animals were killed by decapitation at times scheduled for the convulsive test. Samples of blood of approximately 1 ml and brains of animals were collected. Brains (without cerebellum) were homogenized on ice, 1:1 (w/v) in TDx buffer (Abbott, Irving, TX, USA). Samples of blood and brain homogenates were centrifuged at 11,000 rpm for 5 min. Plasma samples of 100 μl were transferred into MPS-1 system (Amicon, Danvers, USA) for separation of free from protein-bound microsolutes, and centrifuged at 11,000 rpm for 10 min. The levels of antiepileptic drug in serum filtrate (free plasma level) and in brain supernatant (brain level) were estimated by the immunofluorescence method, using an Abbott TDx Analyser (Abbott, Irving, TX, USA). The plasma and brain levels of antiepileptic drugs were expressed in μg/ml of plasma or μg/g of wet brain tissue, respectively, and are presented as means ± S.D. of at least seven determinations.

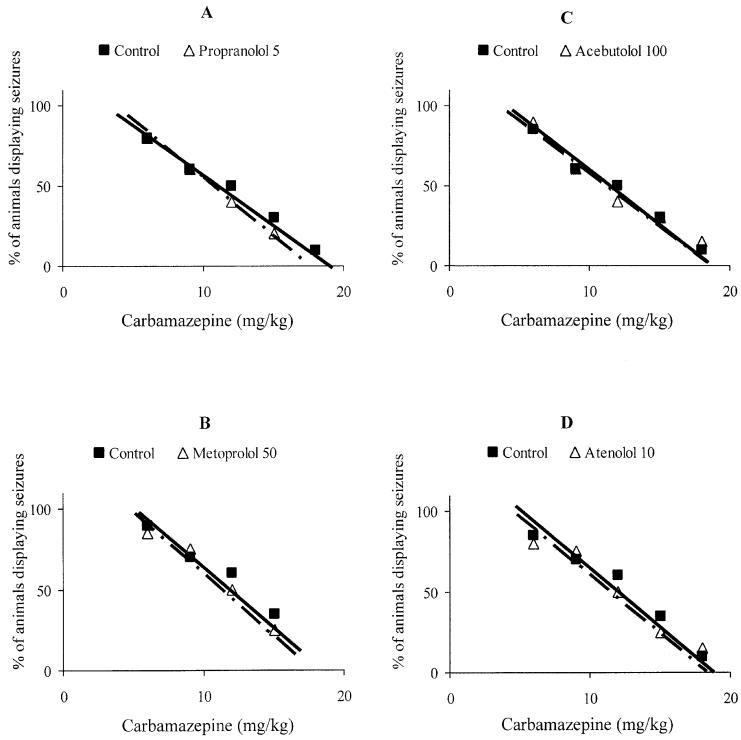

Fig. 3. Influence of β-adrenergic receptor antagonists on the protective efficacy of carbamazepine against MES-induced seizures. Data are presented as percentage of animals displaying seizures following administration of various doses of carbamazepine with saline (control) or with the respective β-adrenoceptor antagonist. The calculations of ED_{50} values and statistical analyses of the data were performed according to the method of Litchfield and Wilcoxon (1949). Dose regression curves were calculated using GraphPAD software. (A) Control: ED_{50}: 11.9 [9.6–14.2] ($y = -5.67x + 114$, $r = -.99$); propranolol 5 mg/kg: ED_{50}: 10.1 [8.0–12.0] ($y = -6.67x + 120$, $r = -1.0$). (B) Control: ED_{50}: 12.4 [10.1–14.7] ($y = -5.83x + 125$, $r = -.99$); metoprolol 50 mg/kg: ED_{50}: 12.2 [11.0–13.4] ($y = -6.83x + 130.5$, $r = -.98$). (C) Control: ED_{50}: 12.1 [10.6–13.6] ($y = -6.0x + 119$, $r = -.99$); acebutolol 100 mg/kg: ED_{50}: 12.5 [10.8–14.2] ($y = -6.0x + 119$, $r = -.98$). (D) Control: ED_{50}: 12.4 [10.1–15.3] ($y = -6.17x + 126$, $r = -.98$); atenolol 10 mg/kg: ED_{50}: 12.9 [10.4–15.9] ($y = -6.0x + 121$, $r = -.98$).

2.6. Statistics

Calculation of the CS_{50}, ED_{50} and TD_{50} values (with 95% confidence limits) and statistical comparisons of the results, were performed using computerized linear regression analysis, according to the method of Litchfield and Wilcoxon (1949). Plasma and brain levels of the antiepileptic drugs were compared using Student's *t* test. Fisher's exact probability test was used for statistical analysis of the data concerning influence of β-adrenergic receptor antagonists *per se* on motor performance.

3. Results

3.1. Influence of β-adrenergic receptor antagonists on the protective efficacy of antiepileptic drugs

Propranolol, acebutolol, metoprolol and atenolol applied at the doses of 5, 100, 50 and 10 mg/kg ip, respectively, did not influence the CS_{50} value and these doses were used for the further studies (Table 1).

The anticonvulsant activity of valproate against MES-evoked seizures was potentiated by propranolol, acebutolol and metoprolol, but not atenolol, as revealed by valproate's ED_{50} lowered from 255.4, 261.7 and 265.2 to 183.5 ($P<.001$), 211.4 ($P<.01$) and 215.2 mg/kg ($P<.001$), respectively (Fig. 1). Propranolol and metoprolol, but not acebutolol or atenolol, enhanced the protective efficacy of diazepam, lowering its ED_{50} from 10.5 and 10.9 to 7.3 ($P<.05$) and 4.7 mg/kg ($P<.001$), respectively (Fig. 2). The protective activity of carbamazepine or phenytoin was not changed by propranolol, acebutolol, metoprolol or atenolol (Figs. 3 and 4).

3.2. Motor performance

Propranolol, metoprolol, acebutolol and atenolol, given in the doses used for further concomitant application with antiepileptic drugs, i.e., 5, 50, 100 and 10 mg/kg ip, respectively, did not alter the locomotor activity of animals *per se* (Fig. 5). Administration of propranolol together with valproate or diazepam did not influence their TD_{50} values (Fig. 5). Co-administration of metoprolol with diazepam

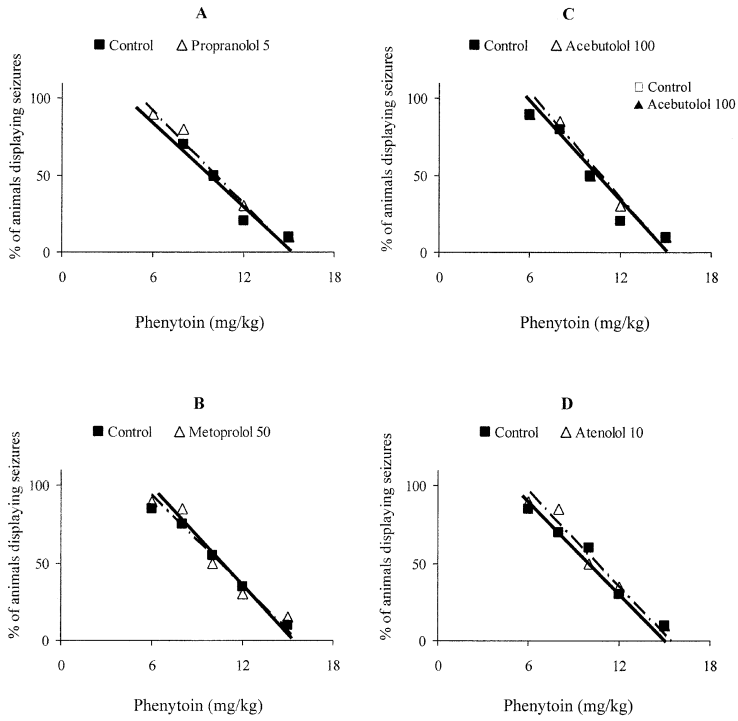

Fig. 4. Influence of β-adrenergic receptor antagonists on the protective efficacy of phenytoin against MES-induced seizures. Data are presented as percentage of animals displaying seizures following administration of various doses of phenytoin with saline (control) or with the respective β-adrenoceptor antagonist. The calculations of ED_{50} values and statistical analyses of the data were performed according to the method of Litchfield and Wilcoxon (1949). Dose regression curves were calculated using GraphPAD software. (A) Control: ED_{50}: 10.3 [8.4–11.7] ($y=-9.47x+148.6$, $r=-.99$); propranolol 5 mg/kg: ED_{50}: 9.5 [7.8–11.3] ($y=-8.88x+137.4$, $r=-.96$). (B) Control: ED_{50}: 9.9 [8.7–11.1] ($y=-8.65x+139$, $r=-.99$); metoprolol 50 mg/kg: ED_{50}: 10.1 [8.9–11.3] ($y=-9.2x+147.9$, $r=-.97$). (C) Control: ED_{50}: 9.6 [8.1–11.3] ($y=-9.84x+150.3$, $r=-.97$); acebutolol 100 mg/kg: ED_{50}: 9.9 [8.3–11.5] ($y=-9.69x+151.9$, $r=-.98$). (D) Control: ED_{50}: 9.9 [8.7–11.1] ($y=-8.65x+139$, $r=-.99$); atenolol 10 mg/kg: ED_{50}: 10.0 [9.0–11.0] ($y=-9.5x+151$, $r=-0.98$).

also did not affect its TD_{50} value. Metoprolol and acebutolol, administered in combination with valproate, significantly decreased its TD_{50} from 366.2 to 301.8 ($P<.01$) and 16.9 mg/kg ($P<.001$), respectively (Fig. 5).

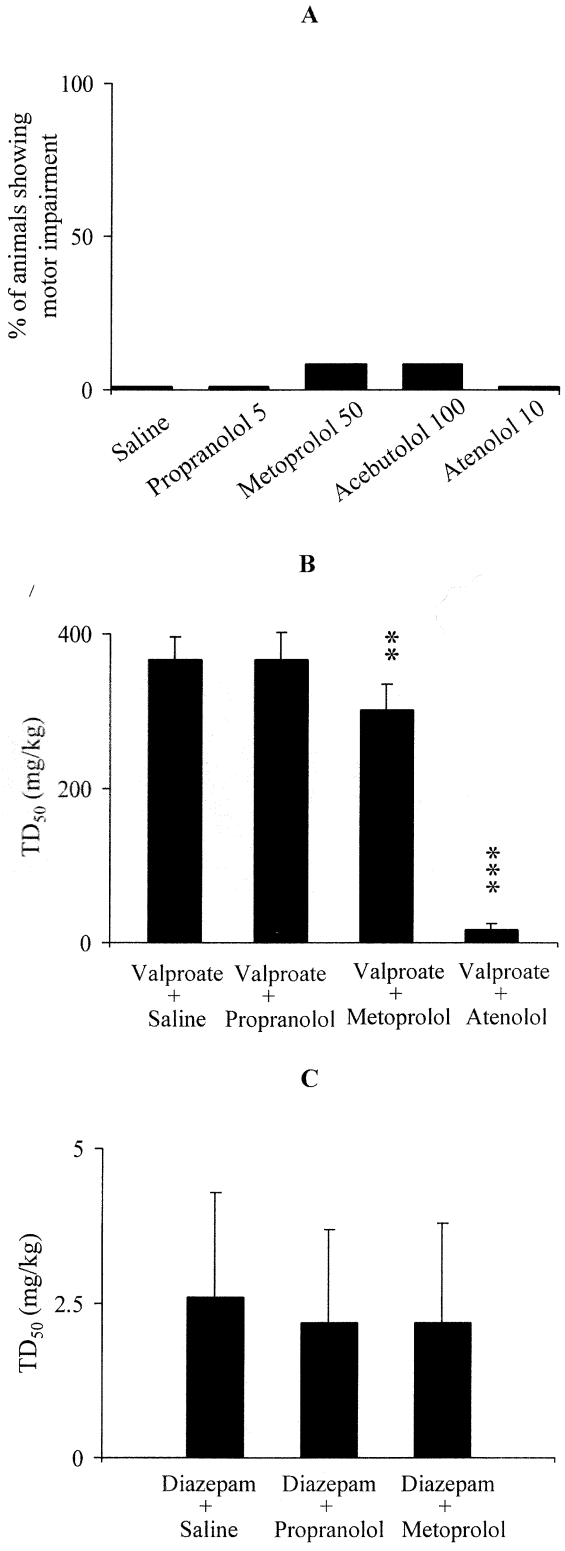

Table 2
Influence of propranolol, metoprolol and acebutolol on the free plasma levels and brain levels of valproate and diazepam in mice

Drugs (mg/kg)		Plasma levels (μg/ml)	Brain levels (μg/g)
Valproate (193)	+ saline	145.3 ± 16.4	74.4 ± 8.2
	+ propranolol (5)	150.9 ± 12.2	73.6 ± 6.5
Valproate (236)	+ saline	203.7 ± 22.5	102.5 ± 9.9
	+ metoprolol (50)	207.4 ± 25.1	104.1 ± 12.3
Valproate (211)	+ saline	173.5 ± 15.8	88.6 ± 9.2
	+ acebutolol (100)	166.6 ± 14.5	87.9 ± 7.3
Diazepam (7.3)	+ saline	0.321 ± 0.025	4.029 ± 0.14
	+ propranolol (5)	0.315 ± 0.028	3.977 ± 0.34
Diazepam (4.7)	+ saline	0.215 ± 0.018	2.689 ± 0.21
	+ metoprolol (50)	0.217 ± 0.015	2.692 ± 0.17

$N=7$ for each determination. The animals were killed by decapitation at appropriate times, and blood samples of approximately 1 ml together with brain specimens were collected. Free plasma and brain levels of antiepileptics were estimated by the immunofluorescence method, using Abbott TDx Analyser (Abbott, Irving, TX, USA). Data are presented as mean ± S.D. (in μg/ml). Student's *t* test was used for statistical comparisons of the data.

3.3. Influence of β-adrenergic receptor antagonists on the free plasma and brain level of valproate and diazepam

Propranolol (5 mg/kg), acebutolol (100 mg/kg) and metoprolol (50 mg/kg) have not changed the free plasma and brain levels of valproate or diazepam (Table 2).

4. Discussion

Presented data indicate that the protective action of valproate and diazepam against MES-induced seizures is enhanced by the mixed β_1/β_2-adrenergic receptor antagonist–propranolol and selective β_1-adrenergic receptor antagonist–metoprolol, applied in the doses not affecting the electroconvulsive threshold *per se*. The anticonvulsant effect of valproate, but not diazepam, is also potentiated by the application of selective β_1-adrenoceptor antagonist–

Fig. 5. (A) Influence of β-adrenergic receptor antagonists on the motor performance in mice. All drugs were administered i.p. 30 min before the test except for atenolol, given 60 min prior to the test. $N=12$ for each group. Data are presented as a percentage of animals which failed to climb up backwards in the plastic tube (3 cm inner diameter, 25 cm length) within 60 sec. Fisher's exact probability test was used in statistical analysis. (B) Influence of propranolol, metoprolol and acebutolol on valproate or (C) diazepam-induced alterations in motor performance of mice. ** $P<0.01$, *** $P<0.001$ vs respective controls. $N=$ at least 8 for each group. Data are presented as the TD_{50} values, i.e. the doses of drugs (in mg/kg, with 95% confidence limits) causing motor impairment in 50% of tested animals, and are based on experiments with at least three different doses of anticonvulsant. Motor impairment was estimated according to Boissier et al. (1960), and indicated by the inability of animals to climb up backwards within the plastic tube (3 cm inner diameter, 25 cm length), during 60 s. The statistical comparisons were performed using the computerized linear regression analysis, based on the method of Litchfield and Wilcoxon (1949).

acebutolol. In contrast, the antiepileptic potential of carbamazepine and phenytoin is not changed by propranolol, metoprolol or acebutolol. Atenolol, the selective β_1-adrenergic receptor blocker, does not affect the anticonvulsive activity of any studied here antiepileptics. Thus, the concomitant use of certain β-adrenergic antagonists with classical anticonvulsants may improve the antiepileptic action of the latter drugs.

The mechanism behind the observed augmentation valproate's and diazepam's anticonvulsive activity is most probably not related to a pharmacokinetic interaction, as the free plasma level of valproate and diazepam remained unchanged in the presence of β-adrenergic receptor antagonists. The range of anticonvulsants' plasma levels in mice corresponds with previous data (Borowicz et al., 1999). Moreover, the brain level of these anticonvulsants, estimated at the time point when MES is induced, was also not altered. Also studies in humans revealed the lack of pharmacokinetic or pharmacodynamic interactions between propranolol and metoprolol used with diazepam or valproate (Nemire et al., 1996; Klotz and Reimann, 1984).

Propranolol and metoprolol are highly lipophilic agents, easily penetrating to the brain, whereas acebutolol crosses the blood–brain barrier at a moderate degree (Kendall, 1997; Sproat and Lopez, 1991). Atenolol, known for its hydrophilic properties, very poorly enters the brain (Kendall, 1997; Sproat and Lopez, 1991). Consistently, atenolol did not influence the activity of anticonvulsants in the MES test. This might indicate that the observed effect of propranolol, metoprolol and acebutolol's administration is mediated at the central level.

Used here, effective doses of propranolol do not seem to disturb peripheral circulation parameters such as arterial blood pressure in animals (Brenner et al., 1984; Lee et al., 1983; Moreau et al., 1997). In fact, none of the β-adrenoceptor blockers *per se* influenced the motor activity of animals, moreover propranolol did not potentiate the effects evoked by anticonvulsants, as revealed by the chimney test. Therefore, it seems that peripheral effects do not contribute to the exerted by propranolol, metoprolol and acebutolol potentiation of antiepileptic activity.

Under studied conditions, the antiepileptic potential of diazepam and valproate, but not this of phenytoin or carbamazepine, was enhanced by β_1-adrenergic antagonists. The nature of this selectivity is not quite clear. The so-called "membrane stabilizing" activity of β-adrenergic antagonists related to the blockade of sodium channels, was implicated as one of the major mechanisms behind their anticonvulsant action (Fischer and Muller, 1988; Fischer et al., 1985; Khanna et al., 1989). It is well documented that propranolol and acebutolol display, respectively, strong and week membrane stabilizing properties (van Zwieten and Timmermans, 1983). As regards metoprolol, the majority of studies indicate that it lacks such activity (Kendall, 1997; Takeo et al., 1990). Only limited data show that, when used in higher doses, metoprolol might block sodium channels (Boucher et al., 1992). Providing that the augmentation of antiepileptic potential observed in our study is related exclusively to the blockade of sodium channels exerted by β_1-adrenergic antagonists, the action of carbamazepine or phenytoin, drugs acting via blockade of sodium channels, should be also enhanced. However, it was not the case. Different conclusion comes from the study in which clenbuterol, a lipophilic β_2-adrenergic agonist potently blocking sodium channels, enhanced the antiepileptic effects of not only valproate but also carbamazepine indicating that sodium channels blockade and not the β_2-receptors modulation contributes to the effect (Fischer et al., 2001). This contrasts with our study in which propranolol, strongly blocking sodium channels, has not affected the action of carbamazepine. The discrepancy between these two studies might result from the use of different tool substances (β-antagonists vs. β-agonists) and different animal species.

Alternative explanation can be sought in the mechanisms underlying the anticonvulsive potential of antiepileptic drugs. Diazepam and valproate, in contrast to phenytoin and carbamazepine, act mainly via the augmentation of GABA-ergic transmission (White, 1997). The interaction between blockade of β-adrenergic receptors and GABA-mediated inhibition could occur. However, it seems quite unlikely in the view of the data demonstrating that the inhibitory effects of GABA in cerebral and cerebellar cortices are facilitated by the β-adrenergic receptors activation, and not by their blockade (Parfitt et al., 1990).

Adenylyl cyclases regulate a number of intracellular processes initiated by extracellular or intracellular signals including neurotransmitters and calcium (Cooper et al., 1995). Increasing body of evidence indicate that cAMP formation may contribute to the pathogenesis of seizures (Ferrendelli, 1986). Enhanced norepinephrine-sensitive accumulation of cAMP was observed in cortical slices with iron-induced epileptic activity and the elevation in levels of both cyclic GMP and cyclic AMP was demonstrated to correlate with the development of kindled seizures (Moriwaki et al., 1988; Wasterlain and Farber, 1986). Moreover, β-receptor mediated increases in cAMP levels potentiate glutamatergic transmission (Herrero and Sanchez-Prieto, 1996). There are also data indicating that antiepileptic drugs may modify the central levels of cAMP. Carbamazepine and phenytoin were demonstrated to diminish cAMP levels in vivo and to block norepinephrine-or depolarization-induced increases in cAMP content in cerebral cortex (Ferrendelli and Kinscherf, 1979; Palmer, 1979; Palmer et al., 1979). In contrast, valproate does not alter the concentration of cAMP, either basal or depolarization-induced (Ferrendelli and Kinscherf, 1979), and diazepam even elevates the basal level of cyclic nucleotides, possibly due to its inhibitory effects on phosphodiesterase activity (Collado et al., 1998). Since β-adrenergic blockade leads to the reduced formation of cAMP, it might be hypothesized that β-adrenoceptor antagonists potentiate the activity of antiepileptic drugs that do not diminish the cAMP levels *per se*, such as valproate and

diazepam. Indeed, we have observed the selective enhancement of the anticonvulsive effects of valproate and diazepam, but not of phenytoin or carbamazepine, by β-adrenergic antagonists.

In summary, certain β-blockers significantly enhance the antiepileptic activity of valproate and diazepam against generalized tonic–clonic seizures, whereas the action of phenytoin and carbamazepine is not changed by the co-administration of propranolol, metoprolol and acebutolol. As much as experimental data can be converted into clinical practice, one can hypothesize that the use of certain drugs antagonizing the function of β-adrenergic receptors might not only be safe in the course of antiepileptic therapy, but could also be potentially useful in the polytherapy of seizures.

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