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Influence of some β-adrenoceptor antagonists on the anticonvulsant potency of antiepileptic drugs against audiogenic seizures in DBA/2 mice

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Abstract

The two enantiomers of propranolol antagonize generalized tonic–clonic seizures in DBA/2 mice with the (−)-enantiomer being about 1.5 times more potent than the (+)-enantiomer. Metoprolol was less active and atenolol was unable to affect audiogenic seizures. In combination with conventional antiepileptic drugs, both propranolol enantiomers tested in doses not affecting the occurrence of audiogenic seizures increased the anticonvulsant activity of diazepam, phenobarbital, valproate and lamotrigine and tended to increase that of carbamazepine and phenytoin. The effect was more pronounced with the (−)-enantiomer. This increase was associated with an enhancement of motor impairment, however, the therapeutic index of combined treatment of the antiepileptic drugs with both propranolol enantiomers was more favourable than the combination with saline alone. Metoprolol was also able to decrease the ED_{50} values of the antiepileptic drugs, whereas atenolol showed no effects. Since neither enantiomer of propranolol significantly influenced the total and free plasma levels of the antiepileptics, pharmacokinetic interactions are not likely. In addition, (+)- and (−)-propranolol did not significantly affect the hypothermic effects of the antiepileptics tested. In conclusion, both enantiomers of propranolol and metoprolol showed an additive anticonvulsant effect when co-administered with some conventional antiepileptic drugs, most notably diazepam, phenobarbital, lamotrigine and valproate, implicating a possible therapeutic relevance of such drug combinations.

Keywords: Epilepsy; β-Adrenoceptor antagonist; Antiepileptic drug; Carbamazepine; Anticonvulsant potency; Audiogenic seizure; DBA/2 mouse

1. Introduction

In recent years, major progress in understanding of the mechanisms associated with epileptiform events and anticonvulsant activity of antiepileptic drugs has led to a clear improvement in the treatment of human epilepsies. However, approximately 20–30% of epileptic patients are refractory to the standard therapy (McNamara, 1994; Rogawski, 1998).

There are a number of reports concerning the involvement of noradrenergic mechanisms in seizure susceptibility and epileptogenesis (Russell et al., 1979; Przegaliński, 1985; Tsuda et al., 1990; Jimenez-Rivera and Waterhouse, 1991; Pericic et al., 2000; Joye, 2000). The hippocampus is known for its low seizure threshold and dominant role in the propagation of seizures (McNamara, 1994). Hippocampal density of β_1/β_2-adrenoceptors is the highest among brain structures (Reznikoff et al., 1986). However, the role of β-adrenoceptors-mediated neurotransmission in epileptic phenomena is not clear. Both pro- and anti-convulsant effects were demonstrated following stimulation of β-adrenoceptors. Exacerbation of chemically induced seizures by (±)-propranolol was previously reported (Kilian and Frey, 1973; Madan and Barar, 1974).

β-Adrenoceptor activation increased the rate of spontaneous epileptiform discharges in hippocampal or limbic slices (Mueller and Dunwiddie, 1983; Rutecki, 1995; Stoop et al., 2000) or the epileptiform abnormalities occurring in slices of pyriform cortex obtained from kindled animals (McIntyre and Wong, 1986). β-Adrenoceptor agonists were also shown to augment the inhibitory synaptic events in cerebral cortex (Waterhouse et al., 1982).

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In vivo studies have previously revealed the anticonvulsant effects of propranolol in various seizure models of experimental epilepsy (Murmann et al., 1966; Singh et al., 1971; Anlezark et al., 1979; Jaeger et al., 1979; Louis et al., 1982; Fischer et al., 1985; Lints and Nyquist-Battie, 1985; Khanna et al., 1989; Raju et al., 1998; Fischer, in press). There is only limited information available about the interactions between β-adrenoceptor antagonists and conventional antiepileptic drugs. In one study, propranolol, pindolol and alprenolol were shown to enhance the anticonvulsant activity of phenobarbital against maximal electroshock-induced seizures in mice (Fischer and Müller, 1988). Thus, it is conceivable that the use of such β-adrenoceptor antagonists might influence the efficacy of some antiepileptic drugs.

The aim of the present study was to investigate the efficacy of conventional antiepileptic drugs such as carbamazepine, diazepam, phenytoin, felbamate, lamotrigine, phenobarbital and valproate administered in combination with (+)- and (−)-propranolol, metoprolol and atenolol against audiogenic seizures in DBA/2 mice. The β-adrenoceptor antagonists used are commonly administered in humans and display different pharmacological profiles including β-adrenoceptor selectivity, lipophilicity and membrane-stabilizing properties.

2. Materials and methods

2.1. Animals

Male and female DBA/2 mice weighing 8–12 g (22–26 days old) or 20–28 g (48–56 days old) were used (Charles River, Calco, Como, Italy) The animals were housed in groups of 8–10 under a 12-h light/dark cycle (lights on at 7: 00 a.m.) with food and water available ad libitum. Experimental groups, consisting of 10 animals were assigned according to a randomised schedule, and each mouse was used only once. Control animals were always tested on the same day as the respective experimental groups. Procedures involving animals and their care were conducted in conformity with international and national laws and policies.

2.2. Experimental design

DBA/2 mice were exposed to auditory stimulation, 60 min following intraperitoneal (i.p.) administration of the β-adrenoceptor antagonists (1–50 mg/kg) or saline and 45 min following i.p. injection of the antiepileptic drugs studied. In a previous manuscript, we observed that all antiepileptic drugs tested showed good anticonvulsant activity when administered 45 min before the auditory convulsant test (De Sarro et al., 1998, 2000a,b). Each mouse was placed under a hemispheric Perspex dome (diameter 58 cm) and 1 min was allowed for habituation and assessment of locomotor activity. Auditory stimulation (12–16 kHz, 109 dB) was applied for 1 min or until tonic extension occurred. As previously reported, the seizure response (De Sarro et al., 1984) was assessed using the following scale: 0 = no response, 1 = wild running, 2 = clonus, 3 = tonus, 4 = respiratory arrest. The maximum response was recorded for each animal. Rectal temperature was recorded immediately prior to auditory testing using a digital thermometer. Behavioural changes were observed during the period between drug administration and auditory testing.

2.3. Determination of the plasma levels of the antiepileptic drugs

DBA/2 mice (20–28 g) were given i.p. either saline and one antiepileptic drug or one β-adrenoceptor antagonist and one antiepileptic drug. The same protocol was used for behavioural and pharmacokinetic studies. Older DBA/2 mice were used for pharmacokinetic studies because is very difficult to collect blood from younger DBA/2 mice. No changes in pharmacokinetics were reported between 21–26 and 48–56 days old mice (De Sarro et al., 1998, 2000a,b). The animals were lightly anaesthetised with ethyl ether and killed by decapitation at appropriate times and blood samples of approximately 1 ml were collected into Eppendorf tubes. The felbamate and lamotrigine assay was carried out by high-performance liquid chromatography (HPLC) (Rizzo et al., 1997). Blood samples were centrifuged at 2000 rpm for 15 min for carbamazepine, diazepam, phenytoin and phenobarbital determination. The plasma was put into a system, MPS-1 (Amicon, Danvers, MA, USA), for the separation of free from protein-bound microsolutes. Plasma samples of 60 μl were transferred to special sample cups and inserted into an Automatic Clinical Analyser (ACA II, du Pont, Wilmington, DE, USA) which uses a method based on the homogenous enzyme immunoassay technique. For the valproate assay, a serum sample of 50 μl was diluted twice with Tris buffer and analysed with the same method. Control drug solutions were put before and after the respective antiepileptic experimental samples.

2.4. Effects on motor movements

Behavioural changes and their onset and duration were recorded after drug injection until the time of the rotarod test. In particular, two independent observers followed gross behavioural changes consisting of locomotor activity, ataxia, squatting posture and possible piloerection. These behavioural changes were noted but not analysed statistically. Groups of 10 DBA/2 mice, 8–12 g (22–26 days old), were trained to do coordinated motor movements continuously for 2 min on a 3-cm diameter rotarod turning at 8 rev min^{-1} (U. Basile, Comerio, Varese, Italy). Impairment of coordinated motor movements was defined as inability of the mice to remain on the rotarod for a 2-min test period (Dunham and Miya, 1957). The ability of the mice to remain on the rotarod was tested 45 min after the i.p. administration of saline and one of the conventional antiepileptics or after the

combined treatment with one of β-adrenoceptor antagonist and one of the antiepileptic drugs.

2.5. Statistical analysis

Statistical comparisons among groups of control and drug-treated animals were made using Fisher's exact probability test (incidence of the seizure phases and influence on motor performance) or analysis of variance (ANOVA) and Dunnett's test (rectal temperature). The percent incidence of each phase per dose of the administered compound and the dose–response curves were fitted using linear regression analysis. ED_{50} values (± 95% confidence limits) for each compound and each phase of seizure response were estimated using a computer program for the method of Litchfield and Wilcoxon (1949); the relative anticonvulsant activities were determined by comparison of respective ED_{50} values. The lines of best fit of conventional antiepileptic drug plus saline or in association with one β-adrenoceptor antagonist were compared using a χ^2-test, with results expressed for position, parallelism and heterogeneity. TD_{50} values (± 95% confidence limits) for each compound were estimated using the method of Litchfield and Wilcoxon (1949). The plasma levels of the drugs are expressed as means ± S.E.M. of at least eight determinations and Student's *t*-test was used for statistical comparisons.

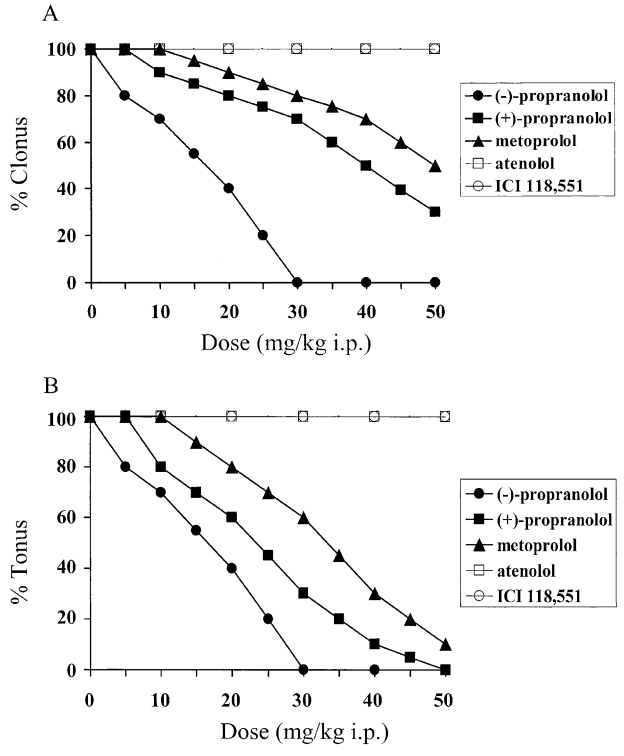

Fig. 1. The dose–response curves of the anticonvulsant effects of some β-adrenoceptor antagonists administered i.p. 1–50 mg/kg at 60 min (●–●) (–)-propranolol, (■–■) (+)-propranolol, (▲–▲) metoprolol, (□–□) atenolol and (○–○) ICI 118,551. Abscissa shows the doses, ordinate shows (A) percentage of clonic seizures, (B) percentage of tonic seizures.

Table 1
ED_{50} values (± 95% confidence limits) for (–)- and (+)-propranolol and other β-adrenoceptor antagonists on audiogenic seizures in DBA/2 mice 60 min after drug administration

Treatment	Seizure phase		
	Wild running	Clonus	Tonus
(–)-Propranolol	33.27 (24.55–45.08)	21.42 (14.36–31.94)	12.45 (7.24–21.39)
(+)-Propranolol	44.76 (37.22–53.84)	37.60 (26.64–53.07)	19.42 (13.71–27.51)
Metoprolol	50.63 (40.62–63.11)	48.95 (36.35–65.94)	37.9 (28.48–50.44)
Atenolol	NA	NA	NA
ICI 118,551	NA	NA	NA

All data above are expressed in mg/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). NA = not active until 50 mg/kg.

2.6. Drugs

The sources of the drugs used were: diazepam (Hoffmann La Roche, Basel, Switzerland), carbamazepine (Novartis, Basel, Switzerland), felbamate (Schering Plough,

Table 2
ED_{50} values (± 95% confidence limits) for saline plus the antiepileptic drugs or in combination with (–)- and (+)-propranolol (both 2.5 mg/kg, i.p.) against audiogenic seizures in DBA/2 mice

Seizure phase	Drug + saline	Drug+ (–)-propranolol	Drug+ (+)-propranolol
Wild running			
Carbamazepine	10.6 (8.1–13.8)	8.4 (6.5–10.9)	10.1 (7.6–13.4)
Diazepam	0.49 (0.34–0.71)	0.27 (0.19–0.38)[a]	0.32 (0.25–0.41)[b]
Felbamate	115 (92–143)	89 (62–128)	101 (86–119)
Lamotrigine	6.1 (4.6–8.1)	3.6 (2.4–5.4)[b]	4.2 (3.1–5.7)[b]
Phenobarbital	7.1 (5.6–9.0)	3.9 (2.6–5.8)[a]	4.3 (3.4–5.4)[a]
Phenytoin	4.3 (3.1–6.0)	3.9 (2.6–5.8)	3.9 (2.6–5.8)
Valproate	84 (63–114)	51 (36–72)[a]	59 (44–80)[b]
Clonus			
Carbamazepine	4.4 (3.6–5.4)	3.4 (1.9–6.1)	3.7 (2.5–5.6)
Diazepam	0.28 (0.20–0.39)	0.15 (0.11–0.20)[a]	0.17 (0.13–0.22)[a]
Felbamate	48.8 (35.4–67.2)	37.6 (26.9–52.6)	40.6 (29.4–56.1)
Lamotrigine	3.5 (2.4–5.1)	2.1 (1.6–2.7)[b]	2.3 (1.9–2.8)[b]
Phenobarbital	3.4 (2.3–5.0)	1.7 (1.2–2.4)[a]	2.0 (1.4–2.8)[a]
Phenytoin	2.5 (1.8–3.5)	2.1 (1.7–2.6)	2.2 (1.8–2.7)
Valproate	43 (33–56)	25.2 (23.1–27.5)[a]	27.7 (23.4–32.8)[a]
Tonus			
Carbamazepine	3.0 (2.6–3.8)	2.6 (2.1–3.2)	2.7 (2.1–3.3)
Diazepam	0.24 (0.15–0.39)	0.13 (0.09–0.19)[a]	0.15 (0.11–0.20)[a]
Felbamate	23.1 (12.1–44)	16.2 (8.9–29.5)[b]	17.8 (14.2–22.4)[b]
Lamotrigine	1.1 (0.7–1.8)	0.7 (0.5–1.0)[a]	0.8 (0.5–1.3)[b]
Phenobarbital	2.4 (1.7–3.4)	1.2 (0.8–1.8)[a]	1.4 (1.1–1.9)[a]
Phenytoin	2.0 (1.6–2.5)	1.7 (1.4–2.1)	1.9 (1.4–2.4)
Valproate	31 (22–43)	18.2 (14.5–22.9)[a]	20.0 (15.4–26)[a]

All data above are expressed in mg/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). Significant differences in the ED_{50} values among concurrent groups of saline plus antiepileptic drug and propranolol plus antiepileptic drug-treated groups are denoted by $^{b}P < 0.05$ and $^{a}P < 0.01$.

Table 3
ED_{50} values (± 95% confidence limits) for saline plus the antiepileptic drugs or in combination with metoprolol (5 mg/kg, i.p.) and atenolol (10 mg/kg, i.p.) against audiogenic seizures in DBA/2 mice

Seizure phase	Drug + saline	Drug + metoprolol	Drug + atenolol
Wild running			
Carbamazepine	10.6 (8.1–13.8)	10.1 (8.1–12.6)	10.6 (8.1–13.8)
Diazepam	0.49 (0.34–0.71)	0.33 (0.26–0.42)[b]	0.45 (0.33–0.61)
Felbamate	115 (92–143)	100 (82–122)	107 (91–127)
Lamotrigine	6.1 (4.6–8.1)	4.6 (3.4–6.2)[b]	5.7 (4.1–7.9)
Phenobarbital	7.1 (5.6–9.0)	4.5 (3.6–5.6)[b]	6.9 (5.3–9.0)
Phenytoin	4.3 (3.1–6.0)	4.1 (3.0–5.8)	4.2 (3.0–5.9)
Valproate	84 (63–114)	61 (48–77)[b]	79 (61–103)
Clonus			
Carbamazepine	4.4 (3.6–5.4)	3.7 (2.3–5.9)	4.5 (3.5–5.8)
Diazepam	0.28 (0.2–0.39)	0.15 (0.11–0.20)[b]	0.24 (0.18–0.32)
Felbamate	48.8 (35–67)	41 (28–60)	45 (30–69)
Lamotrigine	3.5 (2.4–5.1)	2.4 (2.1–2.8)[b]	3.3 (2.7–4.0)
Phenobarbital	3.4 (2.3–5.0)	2.0 (1.6–2.6)[b]	3.2 (2.3–4.5)
Phenytoin	2.5 (1.8–3.5)	2.2 (1.8–2.7)	2.6 (1.9–3.6)
Valproate	43 (33–56)	28.1 (23.2–34.0)[a]	39.7 (31.2–50.5)
Tonus			
Carbamazepine	3.0 (2.6–3.8)	2.7 (2.1–3.5)	2.9 (2.2–3.8)
Diazepam	0.24 (0.15–0.39)	0.15 (0.12–0.19)[b]	0.21 (0.17–0.26)
Felbamate	23.1 (12.1–44)	17.2 (10.9–27.1)[b]	0.21 (0.16–0.28)
Lamotrigine	1.1 (0.7–1.8)	0.8 (0.6–1.1)[a]	1.2 (0.9–1.6)
Phenobarbital	2.4 (1.7–3.4)	1.3 (0.9–1.9)[a]	2.1 (1.5–2.9)
Phenytoin	2.0 (1.6–2.5)	2.1 (1.7–2.6)	2.3 (1.8–3.3)
Valproate	31 (22–43)	19.6 (14.9–23.8)[a]	23.3 (21.7–39.6)

All data above are expressed in mg/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). Significant differences in the ED_{50} values among concurrent groups of saline plus antiepileptic drug and metoprolol plus antiepileptic drug or atenolol plus antiepileptic drug-treated groups are denoted by [b]$P<0.05$ and [a]$P<0.01$.

Milano, Italy), metoprolol tartrate (Sigma, St. Louis, MO, USA) and atenolol (RBI, Natick, USA) which were suspended in a 1% solution of Tween 80. Valproate (Mg^{2+} salt) (Sigma Tau, Pomezia, Italy), lamotrigine (Glaxo-Wellcome, Verona, Italy), phenobarbital (Bracco, Milano, Italy), phenytoin (Na^{+} salt, Recordati, Milano, Italy), ICI 118,551 (±)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxyl]-3-[(1-methylethyl)amino]-2-butanol) hydrochloride (Tocris Cookson, Bristol, UK) and (–)- and (+)-propranolol hydrochloride (Isis-Chemie, Zwickau, Germany) were dissolved in sterile saline. All drugs were administered i.p., 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 test.

3. Results

3.1. Anticonvulsant properties of β-adrenoceptor antagonists in DBA/2 mice

To allow better evaluation of β-adrenoceptor antagonists in DBA/2 mice, we exposed the animals to the auditory test 60 min after drug administration. In fact, following preliminary experiments carried out in this strain of mice we observed that this was an optimal pretreatment time before to assess possible anticonvulsant activity of β-adrenoceptor antagonists. (–)-Propranolol, at the doses of 30, 40 and 50 mg/kg i.p. produced significant protection ($P<0.01$) against the clonic or tonic phase of the audiogenic seizure response (Fig. 1). Only at the doses of 40, and 50 mg/kg i.p. was significant protection against the wild running phase observed (data not shown). No significant anticonvulsant activity or behavioural changes were observed following the doses of 1–10 mg/kg, i.p. Parallel investigations with (+)-propranolol showed a significant protection against the clonic and tonic phases of seizures at 40 and 50 mg/kg, i.p. (Fig. 1). Only at the dose of 50 mg/kg was (+)-propranolol able to significantly protect against the wild running phase of the audiogenic seizures. No significant anticonvulsant activity or behavioural changes were observed with lower doses. ED_{50} values (± 95% confidence limits) of both enantiomers are reported in Table 1. After metoprolol administration (50 mg/kg, i.p.), the clonic, tonic and wild running phases of the audiogenic seizure were significantly antagonized in DBA/2 mice (Fig. 1). The 40 mg/kg i.p. dose significantly reduced the incidence of the tonic phase, whereas no protection was observed after metoprolol 1–30 mg/kg i.p. ED_{50} values (± 95% confidence limits) of

Table 4
TD_{50} values (with 95% confidence limits) for various antiepileptic drugs plus saline or in combination with (–)- or (+)-propranolol (50 mg/kg, i.p.) obtained with the rotarod test

Treatment	TD_{50} locomotor deficit	TD_{50}/ED_{50}
Saline + Carbamazepine	46.5 (37.9–57)	15.5
(–)-Propranolol + Carbamazepine	41.2 (28.4–59.7)	17.6
(+)-Propranolol + Carbamazepine	40.8 (29.1–57.2)	10.9
Saline + Diazepam	3.8 (3.0–4.8)	13.5
(–)-Propranolol + Diazepam	2.2 (1.4–3.5)	14.7
(+)-Propranolol + Diazepam	2.4 (1.6–3.6)	14.1
Saline + Felbamate	816 (590–1024)	16.7
(–)-Propranolol + Felbamate	646 (521–700)	17.2
(+)-Propranolol + Felbamate	668 (527–847)	16.5
Saline + Phenytoin	48.3 (34.1–68.4)	19.3
(–)-Propranolol + Phenytoin	35.1 (28.3–43.5)	16.7
(+)-Propranolol + Phenytoin	34.3 (27.5–42.8)	15.6
Saline + Lamotrigine	81 (55–118)	23.1
(–)-Propranolol + Lamotrigine	69 (53–85)	31.9
(+)-Propranolol + Lamotrigine	65 (52–85.2)	31.9
Saline + Phenobarbital	139 (115–168)	40.9
(–)-Propranolol + Phenobarbital	87 (61–124)	51.5
(+)-Propranolol + Phenobarbital	84 (62–114)	52.4
Saline + Valproate	290 (240–251)	7.3
(–)-Propranolol + Valproate	265 (211–333)	10.5
(+)-Propranolol + Valproate	258 (207–321)	9.3

All data are expressed as mg/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). TD_{50}/ED_{50} = therapeutic index which represents the ratio between TD_{50} and ED_{50} from the clonic phase of the audiogenic seizures. No significant differences were observed between concurrent groups.

metoprolol are reported in Table 1. Since (–)- and (+)-propranolol, at the dose level of 2.5 mg/kg i.p. and metoprolol, at the dose level of 5 mg/kg i.p. did not affect audiogenic seizures per se, these doses were selected for the following next studies.

Atenolol (1–50 mg/kg, i.p.) and ICI 118,551 (1–50 mg/kg, i.p.) showed no anticonvulsant activity at 60 min (Table 1), and a dose of 10 mg/kg, i.p. was used for both compounds. In addition, according to previous studies, all the conventional antiepileptics were administered 45 min before auditory stimulation (De Sarro et al., 1996, 1998, 2000a,b). The doses of (–)- and (+)-propranolol, metoprolol, atenolol and ICI 118.551 used in the present study did not reduce locomotor activity or produce ataxia or a fall in rectal temperatures.

3.2. Influence of (–)-propranolol and (+)-propranolol upon the anticonvulsant activity of conventional antiepileptic drugs against audiogenic seizures

The influence of (–)-propranolol upon the activity of the conventional antiepileptic drugs on the audiogenic seizure response varied according to the different classes. As shown in Table 2, carbamazepine, diazepam, felbamate, lamotrigine, phenobarbital, phenytoin and valproate exhibited anticonvulsant activity in the audiogenic seizure model of DBA/2 mice. Pretreatment with (–)-propranolol (2.5 mg kg^{-1}, i.p.) was able to produce a consistent shift to the left of the dose–response curves and a significant reduction of ED_{50} values of conventional antiepileptics with some exceptions for carbamazepine or phenytoin compared with concurrent groups, suggesting an increase in anticonvulsant activity. All dose–response curves were parallel except those for carbamazepine plus (–)-propranolol or phenytoin plus (–)-propranolol. Also (+)-propranolol was able to produce a significant reduction of ED_{50} values for the antiepileptic drugs studied. The increased anticonvulsant effects induced by (+)-propranolol varied among the antiepileptic drugs tested, being greatest for diazepam, phenobarbital and valproate, less for lamotrigine and felbamate, and least for carbamazepine and phenytoin (Table 2). The (–)-enantiomer was more potent than the (+)-propranolol enantiomer to enhance the anticonvulsant activity of the conventional antiepileptic drugs on the audio genic seizure response, but this effect varied according to the different classes.

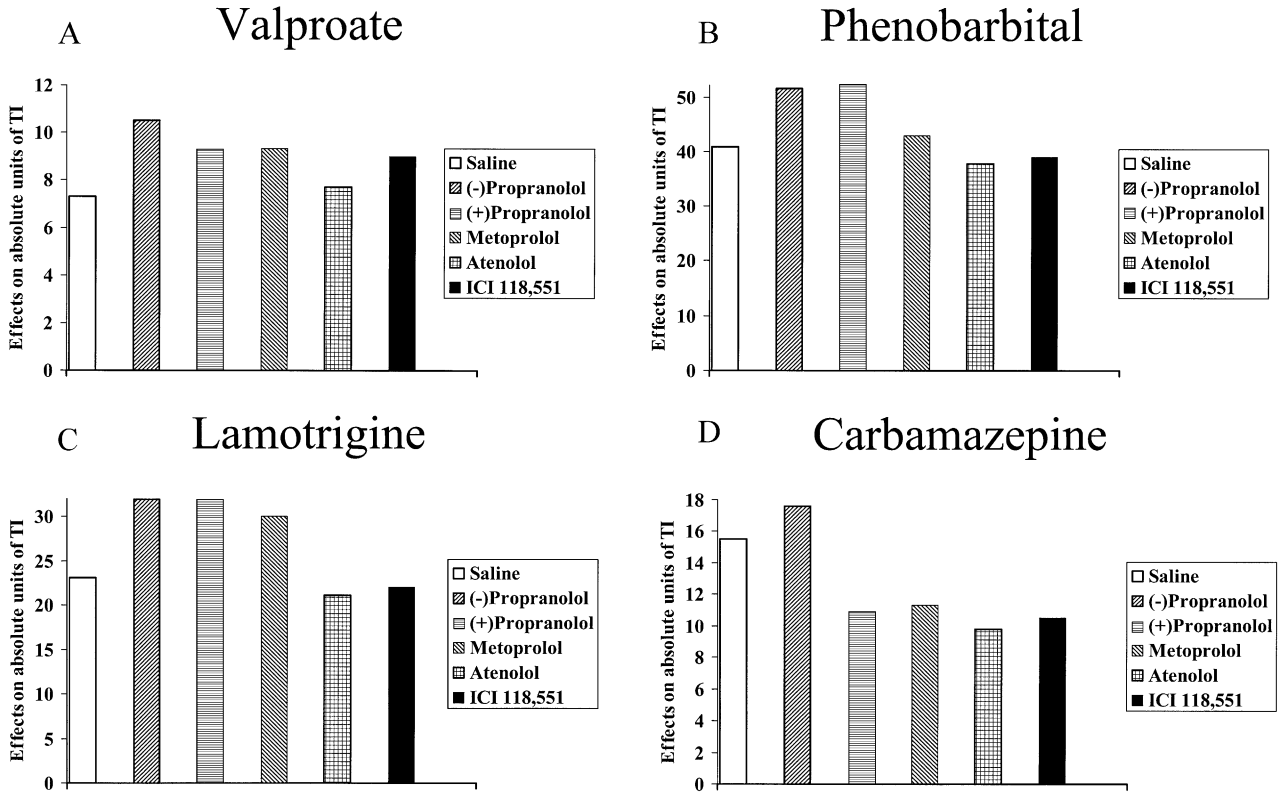

Fig. 2. Effects of a single administration of β-adrenoceptor antagonists in combination with some antiepileptic drugs on absolute units of therapeutic index (TI). Note that the combined administration of (–)-propranolol (2.5 mg/kg, i.p.) with lamotrigine, phenobarbital, valproate and carbamazepine resulted in a favourable therapeutic index, whereas the combination of (+)-propranolol with lamotrigine, phenobarbital and valproate resulted in a favourable therapeutic index. Metoprolol caused an increase of motor impairment following combined treatment with carbamazepine. Atenolol and ICI 118,551 caused an increase of motor impairment following combined treatment with carbamazepine, lamotrigine and phenobarbital.

3.3. Influence of metoprolol, atenolol and ICI 118,551 upon the anticonvulsant activity of conventional antiepileptic drugs against audiogenic seizures

The anticonvulsant activity of diazepam, felbamate, lamotrigine, phenobarbital and valproate against audiogenic seizures was slightly increased by metoprolol but not by atenolol and ICI 118,551. In particular, metoprolol lowered the ED_{50} value of diazepam for clonus from 0.28 to 0.15 mg/kg and that of phenobarbital from 3.4 to 2.03 mg/kg ($P < 0.005$), respectively (Table 3). Metoprolol, but not atenolol, enhanced the protective efficacy of felbamate, lamotrigine and valproate, lowering their ED_{50} values. Metoprolol and atenolol did not affect the anticonvulsant activity of carbamazepine and phenytoin (Table 3). The β_2-adrenoceptor selective antagonist ICI 118,551 (10 mg/kg, i.p.) was unable to affect the antiseizure properties of any of the antiepileptics studied (data not shown).

3.4. Influence of (−)-propranolol and (+)-propranolol upon the motor impairment induced by antiepileptic drugs

No behavioural changes and neurological deficits were detected at any dose of (−)-propranolol and (+)-propranolol alone (up to 50 mg/kg). When applied at doses equal to their ED_{50} values against the clonic phase of the audiogenic seizures, carbamazepine (4.4 mg/kg), diazepam (0.28 mg/kg), felbamate (48.8 mg/kg), lamotrigine (3.5 mg/kg), phenytoin (2.5 mg/kg), phenobarbital (3.4 mg/kg) and valproate (43 mg/kg) did not influence the motor performance of DBA/2 mice. Higher doses were necessary to produce motor impairment (Table 4). (−)-Propranolol or (+)-propranolol at dose levels up to 50 mg/kg, did not significantly affect locomotor performance. The concomitant treatment using (−)- or (+)-propranolol with carbamazepine, diazepam, felbamate, phenobarbital, lamotrigine, phenytoin or valproate resulted in a decrease of motor impairment (Table 4). The therapeutic index of these antiepileptic drugs (exception phenytoin) with (−)-propranolol was more favourable than the combinations with saline alone (Fig. 2 and Table 4).

3.5. Effects of combined administration of β-adrenoceptor antagonists with antiepileptic drugs on body temperature

Hypothermic effects were observed only after administration of saline plus the highest doses of carbamazepine (20, 30 and 50 mg/kg, i.p.), diazepam (3 and 5 mg/kg, i.p.) and valproate (100, 200 and 300 mg/kg, i.p.). In particular, we observed a dose-dependent reduction in body temperature of 2–4 °C only after administration of these drugs. No significant differences among groups treated with saline plus felbamate, lamotrigine, phenytoin, phenobarbital or low doses of carbamazepine, diazepam or valproate were evident (data not shown). In addition, groups treated with propranolol enantiomers (2.5 mg/kg, i.p.), metoprolol (5 mg/kg, i.p.), ICI 118,551 or atenolol (10 mg/kg, i.p.) in combination with different antiepileptic drugs showed no significant changes of hypothermic effects when compared with saline-treated animals (data not shown).

3.6. Influence of metoprolol and atenolol upon the impairment induced by antiepileptic drugs

Metoprolol and atenolol, given at the doses used for further concomitant application with antiepileptic drugs, i.e. 5 and 10 mg/kg i.p. did not themselves alter the locomotor activity of animals. Metoprolol administered at dose levels up to 50 mg/kg, did not significantly affect locomotor performance. The concomitant administration of metoprolol (5 mg/kg) with phenobarbital, lamotrigine, diazepam and valproate resulted in a decrease of motor impairment (Table 5). In fact, the therapeutic index of these antiepileptic drugs co-administered with metoprolol was more favourable than that of the combination with saline alone (Table 5). On the other hand, atenolol (10 mg/kg) did not affect the therapeutic index of antiepileptic drugs (Table 5).

3.7. Influence of β-adrenoceptor antagonists on the total and free plasma levels of antiepileptic drugs

Blood concentrations of carbamazepine, diazepam, felbamate, lamotrigine, phenytoin, phenobarbital and valproate are presented in Table 6. The doses of (−)- and (+)-

Table 5
TD_{50} values (with 95% confidence limits) for various antiepileptic drugs plus saline or in combination with metoprolol (5 mg/kg, i.p.) or atenolol (10 mg/kg, i.p.) obtained with the rotarod test

Treatment	TD_{50} locomotor deficit	TD_{50}/ED_{50}
Saline + Carbamazepine	46.5 (37.9–57.0)	15.5
Metoprolol + Carbamazepine	41.8 (28.7–60.9)	11.3
Atenolol + Carbamazepine	44.2 (29.1–67.1)	9.8
Saline + Diazepam	3.8 (3.0–4.8)	13.5
Metoprolol + Diazepam	2.7 (1.8–4.1)	15.0
Atenolol + Diazepam	4.1 (3.3–5.1)	17.0
Saline + Felbamate	816 (590–1024)	16.7
Metoprolol + Felbamate	662 (529–828)	16.1
Atenolol + Felbamate	724 (582–901)	16.0
Saline + Phenytoin	48.3 (34.1–68.4)	19.3
Metoprolol + Phenytoin	37.5 (29.7–47.3)	17.0
Atenolol + Phenytoin	45.3 (37.5–54.7)	17.4
Saline + Lamotrigine	81 (55–118)	23.1
Metoprolol + Lamotrigine	72 (59–88)	30.0
Atenolol + Lamotrigine	70 (57–86)	21.1
Saline + Phenobarbital	139 (115–168)	40.9
Metoprolol + Phenobarbital	87 (61–124)	42.9
Atenolol + Phenobarbital	121 (89–165)	37.8
Saline + Valproate	290 (240–251)	7.3
Metoprolol + Valproate	261 (219–311)	9.3
Atenolol + Valproate	305 (257–361)	7.7

All data are expressed as mg/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). TD_{50}/ED_{50} = therapeutic index which represents the ratio between TD_{50} and ED_{50} from the clonic phase of the audiogenic seizures. No significant differences were observed between concurrent groups.

Table 6
Influence of (–)-propranolol or (+)-propranolol on total and free plasma levels of some antiepileptic drugs (carbamazepine, diazepam, phenytoin, phenobarbital, valproate, felbamate and lamotrigine) in DBA/2 mice

Treatment (time) (dose mg/kg)	Saline + antiepileptic		(–)-Propranolol + antiepileptic		(+)-Propranolol + antiepileptic	
	Total	Free	Total	Free	Total	Free
Carbamazepine (45 min) (15 mg/kg)	6.5 ± 0.9	0.77 ± 0.20	6.4 ± 0.7	0.79 ± 0.19	6.4 ± 0.7	0.79 ± 0.18
Diazepam (45 min) (5 mg/kg)	2.8 ± 0.3	0.19 ± 0.07	2.7 ± 0.3	0.19 ± 0.06	2.7 ± 0.2	0.20 ± 0.05
Phenytoin (45 min) (10 mg/kg)	2.7 ± 0.6	0.30 ± 0.03	2.8 ± 0.3	0.28 ± 0.03	2.8 ± 0.3	0.28 ± 0.03
Phenobarbital (45 min) (20 mg/kg)	39.2 ± 3.5	4.9 ± 0.3	38.9 ± 3.5	4.8 ± 0.4	39.1 ± 3.6	4.8 ± 0.4
Valproate (45 min) (200 mg/kg)	276 ± 24	44.2 ± 4.2	278 ± 26	43.8 ± 4.1	277 ± 27	44.1 ± 4.1
Felbamate (45 min) (100 mg/kg)	4.6 ± 0.4	3.0 ± 0.3	4.5 ± 0.4	2.9 ± 0.3	4.6 ± 0.4	2.9 ± 0.3
Lamotrigine (45 min) (10 mg/kg)	1.8 ± 0.2	0.67 ± 0.07	1.9 ± 0.2	0.69 ± 0.10	1.9 ± 0.20	0.68 ± 0.10

Drugs were administered i.p. Saline or (–)-propranolol (2.5 mg/kg, i.p.) 60 min before plus one antiepileptic drug 45 min before drawing the blood sample. Values are means (μg/ml) of at least eight determinations ± S.E.M. Student's *t*-test was used for statistical analysis of the data.

propranolol (2.5 mg/kg, i.p.) did not significantly modify the plasma levels of any of the antiepileptics studied. In addition, metoprolol (5 mg/kg, i.p.) and atenolol (10 mg/kg, i.p.) were also unable to modify significantly the plasma levels of the antiepileptic drugs (data not shown).

4. Discussion

The present data indicate that the protective action of diazepam, felbamate, lamotrigine, phenobarbital and valproate against audiogenic seizures is enhanced by co-administration of the mixed β_1/β_2-adrenoceptor antagonist, (–)-propranolol, as well as of the non-receptor-active (+)-enantiomer and the selective β_1-adrenoceptor antagonist, metoprolol, applied at doses that did not affect the occurrence of audiogenic seizures. On the contrary, no significant changes were observed when atenolol was co-administered with any of the antiepileptic drugs. In addition, (–)-propranolol was 1.34–1.75 more potent than (+)-propranolol to antagonize generalized tonic–clonic seizures and to enhance the anticonvulsant activity of the antiepileptic drugs. In contrast, the potency of carbamazepine and phenytoin was not significantly modified by either enantiomer of propranolol or metoprolol. The mechanisms causing the observed augmentation of the effectiveness of the conventional antiepileptics mentioned are most probably not related to a pharmacokinetic interaction, as the plasma level of all compounds studied remained unchanged in the presence of β-adrenoceptor antagonists.

Both enantiomers of propranolol and metoprolol are lipophilic agents that cross the blood–brain barrier (Giacomini and Thoden, 1985; Sproat and Lopez, 1991; Kendall, 1997). Atenolol, a hydrophilic drug, was unable to affect either audiogenic seizures or the potency of the antiepileptic drugs. This might indicate that the consequences of propranolol and metoprolol administration on effects of antiepileptic drugs now observed are mediated at the central level. On the other hand, the anticonvulsant activity of (–)- and (+)-propranolol and metoprolol itself was observed in a dose range, at which a reduction in heart rate and a decrease in mean arterial blood pressure must be assumed (Kittner et al., 1991). The possibility, however, that the haemodynamic effects could be of relevance for the observed anticonvulsant properties of these β-adrenoceptor antagonists seem very unlikely. For example, atenolol, which can be expected to produce similar changes of cardiovascular parameters at adequate doses, showed no anticonvulsant activity. None of the β-adrenoceptor antagonists themselves influenced the locomotor activity of animals, as revealed by the rotarod test. The present data suggest that peripheral effects may not contribute to the observed enhancement of anticonvulsant activity displayed by these drugs.

An important question that must be discussed is whether the anticonvulsant effects of both enantiomers of propranolol and metoprolol could be related to blockade of central β-adrenoceptors or more so to the Na^+ channel blocking properties of these drugs. In DBA/2 mice, a model for generalized tonic–clonic seizures (Kellogg, 1976), there was greater anticonvulsant activity for the (–)-enantiomer than for the non-receptor-active (+)-enantiomer of propranolol (Anlezark et al., 1979). It should be mentioned that this genetically audiogenic seizure-susceptible strain at the time of highest seizure susceptibility has been reported to have an increase of β-adrenoceptor density (Lints and Nyquist-Battie, 1985) as well as a decrease of α_1- and/or α_2-adrenoceptor binding sites (Graillot et al., 1985). Thus, it seems possible that β-adrenoceptor blockade may be of some advantage in reducing the pronounced seizure susceptibility, but the exact mechanisms of action are not clear so that there is little to discuss. In many other models of experimental epilepsy, however, both enantiomers of propranolol were equieffective or the (+)-enantiomer with practically negligible β-adrenoceptor blocking activity but similar lipophilic and local anaesthetic properties (Barrett and Cullum, 1968) was even more potent than the (–)-enantiomer (Jaeger et al., 1979; Fischer et al., 1985). Indeed, in the present study, some interesting findings argue against a dominant role of the blockade of central β-adrenoceptors in the anticonvulsant activity of the β-adrenoceptor antagonists investigated. (i) Although the β-adrenolytic potency

of metoprolol and atenolol is comparable to that of propranolol (Giacomini and Thoden, 1985), these drugs exhibit a smaller or absent anticonvulsant effect. (ii) The non-receptor-active (+)-enantiomer of propranolol was more effective than the β_1 adrenoceptor-selective antagonists metoprolol. (iii) The doses of (–)- and (+)-propranolol or metoprolol causing anticonvulsant effects are much higher than those necessary for adequate β-adrenoceptor blockade. On the other hand, it is well documented that propranolol exhibits strong Na^+ channel blocking (''membrane stabilizing'') activity (Matthews and Baker, 1982; Chidlow et al., 2000; Fischer, in press), whereas metoprolol and atenolol show only weak or no such properties (Giacomini and Thoden, 1985; Doggrell, 1989; Boucher et al., 1992).

Considering all the arguments, it can be suggested that the Na^+ channel blocking properties of the β-adrenoceptor antagonists studied may play a greater role in the anticonvulsant activity than the β-adrenolytic action. Under our experimental conditions, the anticonvulsant potency of diazepam, felbamate, phenobarbital, valproate and lamotrigine but not that of phenytoin or carbamazepine was significantly increased by (–)- and (+)-propranolol and metoprolol. What might account for such a selective action? A local anesthetic activity of β-adrenoceptor antagonists was implicated as one of the major mechanisms behind their anticonvulsant action (Madan and Barar, 1974; Fischer et al., 1985; Fischer and Müller, 1988; Khanna et al., 1989). Assuming that the observed additive anticonvulsant actions are related to the Na^+ channel blocking effects exerted by some β-adrenoceptor antagonists, one can hypothesise that these drugs should not enhance the antiepileptic action of Na^+ channel blockers, such as carbamazepine or phenytoin. This was the case, which might argue for role of Na^+ channel blocking activity in the interaction observed.

An alternative explanation can also be suggested for the different mechanisms of action underlying the antiseizure activity of the antiepileptic drugs studied. Diazepam, phenobarbital, lamotrigine, felbamate and valproate, in contrast to phenytoin and carbamazepine, act mainly via other specific mechanisms (augmentation of γ-aminobutyric acid (GABA)-ergic transmission, action on NMDA-receptors and on voltage-dependent Ca^{2+} channels) (White, 1997, 1999; Meldrum, 1997; Calabresi et al., 1999; Gareri et al., 1999).

In summary, both propranolol enantiomers and metoprolol significantly enhance the anticonvulsant activity of diazepam, felbamate, phenobarbital and valproate against audiogenic seizures in DBA/2 mice, whereas the action of phenytoin and carbamazepine is not changed by the coadministration of these β-adrenoceptor antagonists. It can be suggested that, first, the Na^+ channel blocking activity accounts for this action and maybe, to a lesser degree, the β-adrenolytic properties also, but possible peripheral effects are unlikely candidates in this seizure model. Although experimental data may be hard to extrapolate to clinical practice, further investigations are warranted in order to address the possibility that drugs acting as β-adrenoceptor antagonists could be potentially useful in the human therapy of some types of pharmacoresistant epilepsies.

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