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Gabapentin potentiates the antiseizure activity of certain anticonvulsants in DBA/2 mice

Giovambattista De Sarro [a], Cinzia Spagnolo [a], Pietro Gareri [a], Luca Gallelli [a], Angela De Sarro [b,*]

[a] *Chair of Pharmacology, Department of Experimental and Clinical Medicine, Faculty of Medicine and Surgery, University of Catanzaro, Policlinico Mater Domini, Via T. Campanella, 88100 Catanzaro, Italy*

[b] *Chair of Chemotherapy, Institute of Pharmacology, Faculty of Medicine and Surgery, University of Messina, Messina Italy*

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Abstract

Gabapentin (1–50 mg/kg, intraperitoneally (i.p.)) was able to antagonize audiogenic seizures in Dilute Brown Agouti DBA2J (DBA/2) mice in a dose-dependent manner. Gabapentin at dose of 2.5 mg/kg i.p., which per se did not significantly affect the occurrence of audiogenic seizures in DBA/2 mice, potentiated the anticonvulsant activity of carbamazepine, diazepam, felbamate, lamotrigine, phenytoin, phenobarbital and valproate against sound-induced seizures in DBA/2 mice. The potentiation induced by gabapentin was greatest for diazepam, phenobarbital and valproate, less for felbamate and phenytoin and least for carbamazepine and lamotrigine. The increase in anticonvulsant activity was associated with a comparable increase in motor impairment. However, the therapeutic index of combined treatment of the above drugs + gabapentin was more favourable than that of the same drugs + saline. Since gabapentin did not significantly influence the total and free plasma levels of the anticonvulsant drugs studied, we suggest that pharmacokinetic interactions, in terms of total or free plasma levels, are not probable. However, the possibility that gabapentin can modify the clearance from the brain of the anticonvulsant drugs studied can not be excluded. In addition, gabapentin did not significantly affect the hypothermic effects of the anticonvulsants tested. In conclusion, gabapentin showed an additive effect when administered in combination with certain classical anticonvulsants, most notably diazepam, phenobarbital, felbamate, phenytoin and valproate.

Keywords: Epilepsy; Gabapentin; Carbamazepine; Phenytoin; Valproate; Felbamate; Anticonvulsants; Seizures audiogenic; (DBA/2 mouse)

1. Introduction

The mechanisms of action of conventional antiepileptic drugs are complex. With regard to mechanisms that may lead to seizure activity, much attention has been focused on the existence of inhibitory and excitatory amino acid neurotransmitters in the central nervous system. Impairment of γ-aminobutyric acid (GABA) neurotransmission by a variety of drugs may result in focal or generalized seizures, whilst enhancement of GABAergic inhibition may prevent seizures in several animal models of epilepsy (Macdonald and Meldrum, 1995; Upton, 1994). Some common anticonvulsant drugs, among them benzodiazepines, phenobarbital and primidone, enhance this inhibitory action of GABA (Meldrum, 1984; McLean and MacDonald, 1986).

Gabapentin has been shown to act as an anticonvulsant drug in different models of chemically and electrically induced seizures in rodents, but its mechanism of action appears to be different from that of the conventional antiepileptics (Bartoszyk et al., 1986; Loscher et al., 1991; Stewart et al., 1993; Suman-Chauhan et al., 1993; Taylor et al., 1992a,b; Taylor, 1995). Originally, it was synthesized as a structural analogue of the inhibitory neurotransmitter GABA, with the idea that it would mimic the physiological actions of GABA in the brain. Gabapentin consists of an amino acid backbone similar to that of GABA, except for incorporation of a cyclohexyl ring. This carbohydrate bulk permits gabapentin to penetrate the

* Corresponding author. Tel.: +39-961-712323; fax: 39-961-774424; e-mail: desarro@unicz.thebrain.net

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blood–brain barrier, whilst GABA fails to cross the blood–brain barrier because of the absence of this lipophilic ring. Gabapentin does not interact with $GABA_A$ or $GABA_B$ receptors, it is not converted metabolically into GABA or a GABA receptor agonist and it is not an inhibitor of GABA uptake or of GABA degradation, thus at relevant concentrations it is not considered a GABA mimetic drug (Vollmer et al., 1986). However very recent studies of epileptic patients have demonstrated that gabapentin is able to increase brain GABA levels (Petroff et al., 1996). Neurochemical studies on the possible mechanisms of action of gabapentin show that it does not act at voltage-sensitive Na^+ channels so as to stabilize neuronal membranes, thus distinguishing it from phenytoin, carbamazepine and lamotrigine, and neither does it inhibit the release of excitatory amino acids (Rock et al., 1993). Gabapentin appears to move across the gut and into the blood and to penetrate into the brain by a saturable transport mechanism competitively inhibited by L-leucine (Stewart et al., 1993). In addition, recent studies have demonstrated that this anticonvulsant drug possesses specific binding sites in the brain which are involved in epileptogenesis (Taylor, 1995). It has been shown that gabapentin binding to its specific site is not affected by carbamazepine, diazepam, phenytoin, phenobarbital, valproate and other neuroactive compounds (Suman-Chauhan et al., 1993), suggesting that gabapentin may possess a novel and interesting anticonvulsant pharmacologic profile. In particular, the gabapentin binding sites are located in the superficial layers of neocortex and dendritic layers of hippocampus (Hill et al., 1993). Thus, gabapentin binding sites appear quite dense in areas rich in glutamatergic synapses. In particular, the distribution of [^{3}H]gabapentin binding sites bears a striking resemblance to those of [^{3}H]α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and [^{3}H]6-cyano-7-nitroquinoxaline-2-dione (CNQX) sites (Nielsen et al., 1990). However, the significance of the similarity in binding localization of [^{3}H]gabapentin and [^{3}H]AMPA remains unclear, since the AMPA ligands showed very low affinity for displacing [^{3}H]gabapentin binding from its specific binding site (Suman-Chauhan et al., 1993). Indeed, gabapentin fails to modulate excitatory postsynaptic potentials that depend on AMPA-type glutamate receptors (Taylor et al., 1988) or other glutamate receptors (Taylor, 1995). Very recently it has been shown that the anticonvulsant properties of gabapentin are due to an interaction with an $\alpha_2\delta$ subunit of a voltage-dependent Ca^{2+} channel (Gee et al., 1996).

The aim of the present study was to investigate the effects of a pretreatment with gabapentin on the anticonvulsant properties of carbamazepine, diazepam, felbamate, lamotrigine, phenytoin, phenobarbital and valproate against audiogenic seizures in Dilute Brown Agouti DBA2J (DBA/2) mice. Moreover, the effects of the combined treatment of gabapentin with the above reported anticonvulsant drugs on rotarod performance, body temperature and total and free plasma levels of antiepileptics were studied.

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. Procedures involving animals and their care were conducted in conformity with international and national law and policies.

2.2. Experimental design

DBA/2 mice were exposed to auditory stimulation 45, 120 or 180 min following intraperitoneal (i.p.) administration of gabapentin (2.5–50 mg/kg) or saline and 45 min following i.p. injection of certain antiepileptics. Groups of 10 DBA/2 mice were used for each dose of drug studied. 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 an Elektrolaboratoriet thermometer type T.E.3. Behavioural changes were observed during the period between drug administration and auditory testing.

2.3. Determination of the plasma levels of the antiepileptic compounds

DBA/2 mice, 48–56 days old were injected i.p. with either saline + one of the antiepileptic compounds or gabapentin + one of the antiepileptic drugs. The animals were lightly anaesthetized with ethyl ether and killed by decapitation at appropriate times and blood samples of approximately 1 ml were collected into Eppendorf tubes. Felbamate and lamotrigine were assayed was carried by high-performance liquid chromatography (HPLC) (Rizzo et al., 1997).

Blood samples were centrifuged at 2000 r.p.m. for 15 min for carbamazepine, diazepam, phenytoin and phenobarbital determinations. 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 in an automatic clinical analyser (ACA II, du Pont, Wilmington, DE, USA) which uses a method based on the homogenous enzyme immunoassay technique. For the magnesium valproate assay, serum samples of 50 μl were diluted twice with Tris buffer and analysed with the same method. Control drug solutions were put before and after the respective experimental samples.

2.4. Effects on motor movements

Behavioural changes and their onset and duration were recorded after drug injection until the rotarod test was performed. In particular, two independent observers monitored gross behavioural changes consisting of locomotor activity, ataxia, squatting posture and possible piloerection. These behavioural changes were noted but not statistically analysed.

Groups of 10 DBA/2 mice, 8–12 g, 22–26 days old, were trained to perform coordinated motor movements continuously for 2 min on a rotarod of 3-cm diameter turning at 8 rev min^{-1} (U. Basile, Comerio, Varese, Italy). Impairment of coordinated motor movements was defined as the 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 40 min after the i.p. administration of saline + one of the conventional antiepileptics or after the combined treatment with gabapentin + one of the antiepileptic drugs.

2.5. Statistical analysis

Statistical comparisons among groups of control and drug-treated animals were made by using Fisher's exact probability test (incidence of the seizure phases) or analysis of variance (ANOVA) with Dunnett's test (rectal temperature). The percentage incidence of each seizure phase per dose of compound administered and dose–response curves were fitted by using linear regression analysis. ED_{50} values (with 95% confidence limits) for each compound and each phase of seizure response were estimated by using a computerized version of 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 + saline or antiepileptic drug in association with gabapentin were compared by using chi-squared analysis, with results expressed for position, parallelism and heterogeneity. TD_{50} values (with 95% confidence limits) for each compound was estimated by 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. Student's *t*-test was used for such statistical comparisons.

2.6. Drugs

The sources of the drugs used were: carbamazepine, (Ciba–Geigy, Basel, Switzerland), diazepam (Hoffmann–La Roche, Basel, Switzerland), felbamate (Schering–Plough, Milano, Italy), sodium phenobarbital (Bracco, Milano, Italy), sodium phenytoin (Recordati, Milano, Italy), lamotrigine (Glaxo–Wellcome, Verona, Italy) and magnesium valproate (Sigma–Tau, Pomezia, Italy). Gabapentin was extracted with ethyl alcohol from commercial tablets (Neurontin, Parke Davies, Milano, Italy).

3. Results

3.1. Anticonvulsant properties of gabapentin in DBA/2 mice

To allow better evaluation of the anticonvulsant effects of gabapentin, we exposed the animals to the auditory test at different times after gabapentin administration. Gabapentin (30, 40 and 50 mg/kg i.p.), produced a dose-dependent significant protection ($P < 0.01$) against the clonic and tonic phases of the audiogenic seizure response in DBA/2 mice 45 min after its administration (Fig. 1). Significant protection against the wild running phase was

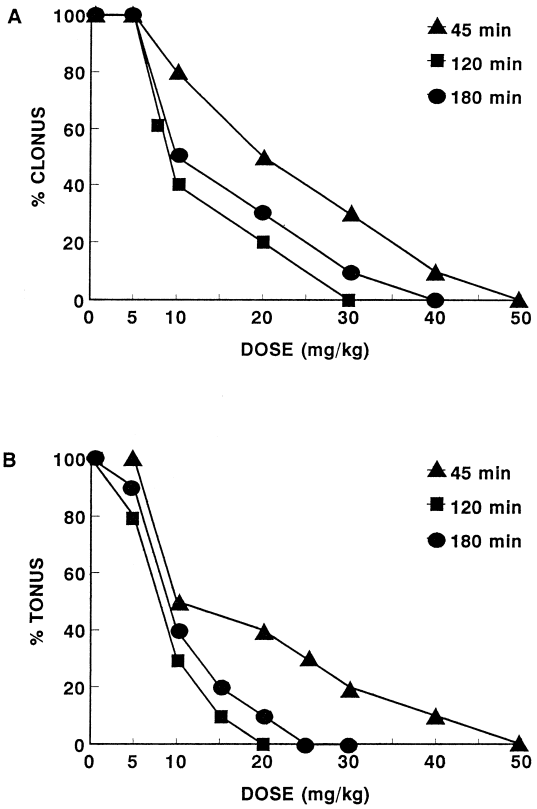

Fig. 1. Dose–response curves for the anticonvulsant effect of gabapentin 2.5–50 mg/kg at (▲) 45 min, (■) 120 min and (●) 180 min after i.p. administration. Abscissae shows the doses, ordinate shows (A) % of clonic seizures, (B) % of tonic seizures. Groups of 10 mice for each dose were pretreated with increasing doses of gabapentin and auditory stimulation was applied at 45, 120 and 180 min after gabapentin administration.

Table 1
ED_{50} values (±95% confidence limits) of gabapentin against audiogenic seizures in DBA/2 mice after various pretreatment times

Pretreatment time (min)	Seizure phase		
	Wild running	Clonus	Tonus
45	38 (16–51)	20.3 (13.7–30.2)	13.9 (8.7–22.3)
120	11.6 (8.3–16.2)	8.1 (6.1–10.8)	5.4 (4.1–7.1)
180	13.6 (10.9–17)	10.1 (6.9–14.7)	6.7 (5.3–8.4)

All data above reported are expressed in mg/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). At least 40 animals were used to calculate each ED_{50} value.

observed following gabapentin 40 and 50 mg/kg i.p. After gabapentin 2.5, 5 and 10 mg/kg i.p., no significant anticonvulsant activity or behavioural changes were observed. ED_{50} values (±95% confidence limits) of gabapentin administered 45 min before the auditory test are reported in Table 1. When the auditory test was carried out 120 min following gabapentin administration (10, 20 and 30 mg/kg i.p.) a significant protection ($P < 0.01$) against the clonic and tonic phases of the audiogenic seizure response was observed in DBA/2 mice. Gabapentin administered 120 min before auditory testing at doses of 20 and 30 mg/kg was able to significantly protect ($P < 0.01$) against the wild running phase of the audiogenic seizures. After gabapentin 2.5 and 5 mg/kg i.p. no significant anticonvulsant activity or behavioural changes were observed. ED_{50} values (±95% confidence limits) of gabapentin administered 120 min before the auditory test are reported in Table 1. When the auditory test was carried out 180 min after the i.p. administration of gabapentin (20, 30 and 40 mg/kg) the clonic, the tonic and the wild running phases of the audiogenic seizure response were significantly ($P > 0.01$) antagonized in DBA/2 mice, whereas no protection was observed after gabapentin 2.5 and 5 mg/kg i.p. ED_{50} values (±95% confidence limits) of gabapentin administered 180 min before the auditory test are reported in Table 1. The doses of gabapentin studied did not reduce locomotor activity or produce ataxia and/or a fall in rectal temperature. Since gabapentin exerted its maximal anticonvulsant activity at 120 min (Table 1), we decided to use this pretreatment time for the following studies. In addition, according to previous studies, all the conventional anticonvulsants were administered 45 min before auditory testing (De Sarro et al., 1992, 1996).

Table 2
ED_{50} values (±95% confidence limits) of saline + certain antiepileptic drugs or gabapentin (2.5 mg/kg i.p.) + antiepileptic drugs against audiogenic seizures in DBA/2 mice

Seizure phase	Drug + saline	Drug + gabapentin
Wild running		
Carbamazepine	10.6 (8.1–13.8)	4.6 (3.3–6.4)[a]
Diazepam	0.49 (0.34–0.71)	0.23 (0.16–0.33)[a]
Felbamate	114.6 (92–142.7)	56.4 (44.5–71.6)[a]
Lamotrigine	6.1 (4.6–8.1)	4.6 (2.7–7.8)
Phenobarbital	7.1 (5.6–9)	3.2 (2.1–4.9)[a]
Phenytoin	4.3 (3.1–6)	2.1 (1.4–3.1)[a]
Valproate	84 (63–114)	41 (25–67)[a]
Clonus		
Carbamazepine	4.4 (3.6–5.4)	3.2 (1.8–5.7)
Diazepam	0.28 (0.2–0.39)	0.11 (0.08–0.15)[a]
Felbamate	48.8 (35.4–67.2)	23.4 (14.9–36.8)[a]
Lamotrigine	3.5 (2.4–5.1)	2.9 (1.8–4.7)
Phenobarbital	3.4 (2.3–5)	1.4 (0.88–2.23)[a]
Phenytoin	2.5 (1.8–3.5)	1.2 (0.91–1.82)[a]
Valproate	43 (33–56)	21.3 (16.5–27.5)[a]
Tonus		
Carbamazepine	3.0 (2.6–3.8)	1.5 (0.8–2.9)[b]
Diazepam	0.24 (0.15–0.39)	0.10 (0.06–0.17)[a]
Felbamate	23.1 (12.1–44)	11.2 (5.9–21.1)[a]
Lamotrigine	1.1 (0.7–1.8)	0.7 (0.5–1.03)
Phenobarbital	2.4 (1.7–3.4)	0.9 (0.6–1.35)[a]
Phenytoin	2.0 (1.6–2.5)	0.6 (0.4–0.9)[a]
Valproate	31 (22–43)	13.9 (10.7–18.1)[a]

All data above reported 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 + antiepileptic drug and gabapentin + antiepileptic groups are denoted by [a]$P < 0.01$ and [b]$P < 0.05$ according to the method of Litchfield and Wilcoxon (1949). At least 40 DBA/2 mice were used to calculate each ED_{50} value.

3.2. Influence of gabapentin on the anticonvulsant activity of conventional antiepileptic drugs against audiogenic seizures

The influence of gabapentin on the activity of the conventional antiepileptic drugs (administered 45 min before testing) against the audiogenic seizure response varied according to the different classes.

Table 3
TD_{50} values (with 95% confidence limits) of saline + various antiepiletics and gabapentin + antiepilepics in the rotarod test

Treatment	TD^{50} Locomotor deficit	TD^{50}/ED^{50}
Saline + carbamazepine	46.5 (37.9–57)	15.5
Gabapentin + carbamazepine	30.1 (18.6–48.7)	11.9
Saline + diazepam	3.8 (3.0–4.8)	13.5
Gabapentin + diazepam	2.0(1.6–2.5)	18.2.
Saline + felbamate	816 (590–1,024)	16.7
Gabapentin + felbamate	466 (249–871)	19.9
Saline + phenytoin	48.3 (50.9–68.4)	19.3
Gabapentin + phenytoin	26.8 (22.1–32.5)	22.3
Saline + lamotrigine	81 (55–118)	23.1
Gabapentin + lamotrigine	74 (55–99.6)	25.5
Saline + phenobarbital	139 (115–168)	40.9
Gabapentin + phenobarbital	78 (60–101.4)	55.7
Saline + valproate	290 (240–351)	7.3
Gabapentin + valproate	178 (133–238)	8.4

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 the TD_{50} and the ED_{50} for the clonic phase of the audiogenic seizures. No significant differences were observed between concurrent groups. At least 32 animals were used to calculate each TD_{50} value.

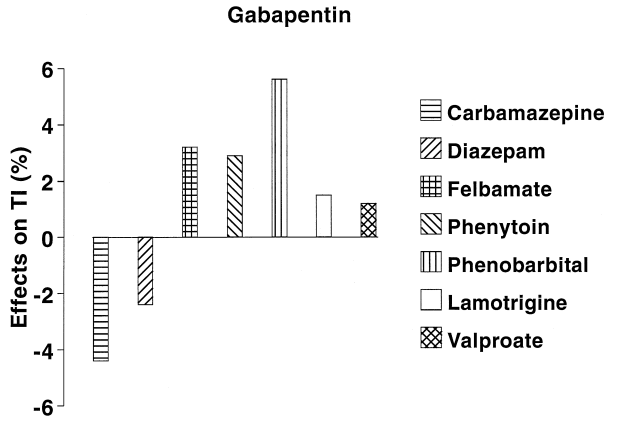

Fig. 2. Effects of a single administration of gabapentin (2.5 mg/kg, i.p.) in combination with certain antiepileptics on percent change in the therapeutic index (TI). Note that the combined treatment with gabapentin (2.5 mg/kg, i.p.) and felbamate, lamotrigine, phenobarbital, phenytoin or valproate resulted in a favourable therapeutic index, whereas the combination of gabapentin with carbamazepine or diazepam caused an increase in motor impairment.

As shown in Table 2, diazepam, carbamazepine, felbamate, lamotrigine, phenobarbital, phenytoin and valproate exhibited anticonvulsant activity in the audiogenic seizure model of DBA/2 mice. Pretreatment (75 min before anticonvulsant administration) with gabapentin (2.5 mg/kg i.p.) was able to produce a consistent shift to the left of the dose–response curves of conventional antiepileptics, with the exception of lamotrigine compared with concurrent groups, suggesting an increase in anticonvulsant activity. All dose–response curves were parallel except those for carbamazepine or lamotrigine plus gabapentin. There was no significant heterogeneity, i.e., any residual variation was consistent with binomial sampling. The potentiation by gabapentin varied among the anticonvulsant drugs, being greatest for diazepam, phenobarbital and valproate, less for felbamate and phenytoin, and least for carbamazepine and lamotrigine.

3.3. Influence of gabapentin on the motor impairment induced by antiepileptic drugs.

When applied in 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 motor performance of DBA/2 mice. Larger doses were necessary to produce motor impairment (Table 3). Gabapentin administered at doses up to 100 mg/kg did not significantly affect locomotor performance. Concomitant treatment with carbamazepine and gabapentin or diazepam and gabapentin resulted in an increase in motor impairment, whereas considerable impairment of locomotor performance was not observed when gabapentin was administered with felbamate, lamotrigine, phenytoin, phenobarbital or valproate (Table 3). In fact, the therapeutic index of combined treatment of felbamate + gabapentin, lamotrigine + gabapentin, phenobarbital + gabapentin, phenytoin + gabapentin or valproate + gabapentin was more favourable than that for felbamate + saline, lamotrigine + saline, phenobarbital + saline, phenytoin + saline or valproate + saline (Fig. 2).

3.4. Effects of combined treatment of gabapentin with antiepileptic compounds on body temperature

The body temperature was recorded in animals treated with saline + anticonvulsant drugs or gabapentin + anticonvulsant drugs. We observed hypothermic effects only after administration of saline + the highest doses of carbamazepine (20, 30 and 50 mg/kg i.p.), diazepam (3 and 5 mg/kg i.p.) or valproate (100, 200 and 300 mg/kg i.p.). No significant differences among groups treated with saline + felbamate, lamotrigine, phenytoin, phenobarbital or low doses of carbamazepine, diazepam or valproate were evident (data not shown). Groups treated with

Table 4
Influence of gabapentin on total and free plasma levels of some antiepileptic compounds (carbamazepine, diazepam, felbamate, lamotrigine, phenytoin, phenobarbital, and valproate) in DBA/2 mice

Treatment (time) (dose, mg/kg)	Saline + compound		Gabapentin + compound	
	Total	Free	Total	Free
Carbamazepine (60 min) (15 mg/kg)	5.2 ± 0.7	0.62 + 0.2	5.2 ± 0.6	0.61 + 0.2
Diazepam (60 min) (5 mg/kg)	2.1 ± 0.2	0.15 ± 0.05	2.1 ± 0.3	0.16 ± 0.05
Phenytoin (120 min) (10 mg/kg)	8.8 ± 1.8	0.9 ± 0.1	8.9 ± 2.1	0.8 ± 0.1
Phenobarbital (60 min) (20 mg/kg)	35.3 ± 3.1	4.4 ± 0.3	35.2 ± 3.4	4.5 ± 0.5
Phenobarbital (120 min) (20 mg/kg)	22.4 ± 2.5	3.1 ± 0.3	22.3 ± 2.4	3.0 ± 0.3
Valproate (30 min) (200 mg/kg)	251 ± 22	40.2 ± 3.9	252 ± 24	40.5 ± 3.9
Valproate (60 min) (200 mg/kg)	309 ± 29	49.4 ± 4.1	310 ± 32	49.8 ± 4.2
Felbamate (60 min) (100 mg/kg)	4.2 ± 0.3	3.1 ± 0.3	4.1 ± 0.3	3.0 ± 0.3
Lamotrigine (45 min) (10 mg/kg)	1.8 ± 0.2	0.67 ± 0.07	1.9 ± 0.2	0.7 ± 0.1

Drugs were administered i.p. Saline or gabapentin (2.5 mg/kg i.p.) + lamotrigine, 45 min; carbamazepine, diazepam, and felbamate, 60 min; phenobarbital, 60 and 120 min; phenytoin, 120 min; and valproate, 30 and 60 min before blood samples were collected. Values are means (μg/ml) of at least 8 determinations + S.E.M. Student's *t*-test was used for statistical analysis of the data.

gabapentin (2.5 mg/kg i.p.) + different antiepileptic drugs showed no significant changes in hypothermic effects when compared with groups treated with saline + antiepileptic drugs (data not shown).

3.5. Influence of gabapentin 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 4. The doses of gabapentin studied did not significantly modify the plasma levels of carbamazepine (15 mg/kg, i.p.), felbamate (100 mg/kg, i.p.), lamotrigine (10 mg/kg, i.p.), phenytoin (10 mg/kg, i.p), phenobarbital (20 mg/kg, i.p), valproate (200 mg/kg, i.p.) and diazepam (5 mg/kg, i.p.).

4. Discussion

The present results clearly demonstrate that gabapentin, at doses which did not significantly affect or slightly influence the audiogenic seizures in DBA/2 mice, markedly enhanced the anticonvulsant properties of diazepam, felbamate, phenytoin, phenobarbital and valproate in this strain of audiogenic seizure-sensitive mice.

A pharmacokinetic interaction does not seem to be responsible for the potentiation by gabapentin of the antiseizure effects of the anticonvulsant drugs studied. In fact, it has been demonstrated in humans, following single as well as repeated administration of gabapentin, that this compound does not affect the plasma concentrations of conventional antiepileptics (see McLean, 1994, 1995). Gabapentin shows desirable pharmacokinetic properties: it does not bind to plasma proteins, it is not metabolized and does not induce or inhibit hepatic enzymes (Richens, 1993; Graves et al., 1989, 1990; Hooper et al., 1991; Basim et al., 1990; Radulovic et al., 1994). Conversely, conventional antiepileptics do not affect the pharmacokinetics of gabapentin (see McLean, 1995).

The present study clearly shows that, in the absence of pharmacokinetic interactions, gabapentin increased the anticonvulsant potency of certain conventional antiepileptics. These data are in agreement with those of a human multicenter clinical study (Anhut et al., 1994). In addition, we clearly demonstrated that gabapentin did not affect the total or free plasma levels of carbamazepine, diazepam, felbamate, lamotrigine, phenytoin, phenobarbital and valproate. However, the present data do not exclude the possibility that gabapentin modified the time course of the anticonvulsants which penetrate the brain or their clearance from the cerebral area. The first hypothesis appears unlikely since gabapentin did not significantly modify the changes in body temperature induced by the antiepileptic drugs studied. Furthermore, gabapentin 2.5 mg/kg in combination with valproate, phenobarbital, phenytoin or felbamate caused some motor impairment but still showed a favourable therapeutic index (Table 3 and Fig. 2). In contrast, a combination of gabapentin and carbamazepine or diazepam caused an increase in motor impairment.

Gabapentin shows a new and different mechanism of action compared to that of the conventional antiepileptic drugs used in the present study. In particular, it appears to possess specific binding sites in the brain (Hill et al., 1993), it interacts with at least three cytosolic enzymes (i.e., branched-chain amino acid aminotransferase, glutamate dehydrogenase, GABA-transaminase) involved with amino acid metabolism and, as recently demonstrated, it interacts with an $\alpha_2\delta$ subunit of a voltage-dependent Ca^{2+} channel (Gee et al., 1996).

The effects of gabapentin on the antiseizure activity of conventional anticonvulsant drugs appear similar to those already described in our laboratory for dihydropyridines, compounds which act as antagonists at L-type Ca^{2+} channels (De Sarro et al., 1992). It is also possible that gabapentin, like the dihydropyridines, enhances the anticonvulsant activity of the compounds studied by diminishing synaptic Ca^{2+} entry. Thus the anticonvulsant properties of gabapentin might result from alterations in the concentration or metabolism of brain amino acids (Taylor, 1995) and/or a reduction of synaptic Ca^{2+} entry. It could be suggested that the observed increase in antiseizure activity of the antiepileptics might be related to the synergic effects elicited by drugs with different mechanism of actions.

In our opinion, the combination of certain antiepileptics with gabapentin would not only allow the dosage of the former to be decreased, which might be of importance for reduction of their adverse effects, but would also considerably reduce the dosage of gabapentin as well. We performed an acute study, which may have some importance for chronic therapy even if different effects may occur under these different conditions. Our experimental data, showing a potentiation of the effects of certain conventional antiepileptic agents induced by gabapentin in DBA/2 mice, suggest that further investigations are warranted, particularly in those forms of human epilepsy that are resistant to classical antiepileptic drugs.

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References

The International Gabapentin Study Group, Anhut, H., Ashman, P., Fenerstein, T.J., Sauermann, W., Saunders, M., Schmidt, B., 1994. Gabapentin (neurotonin) as add-on therapy in patients with partial seizures: a double-blind, placebo controlled study. Epilepsia 35, 795–801.

Bartoszyk, G.D., Meyerson, N., Reimann, W., Satzinger, G., vonHodenberg, A., 1986. Gabapentin. In: Meldrum, B.S., Porter, R.J., (Eds.), New Anticonvulsant Drugs. John Libbey, London, pp. 147–163.

Basim, M., Uthman, E.J., Hammond, E.J., Wilder, B.J., 1990. Absence of gabapentin and valproate interaction: an evoked potential and pharmacokinetic study. Epilepsia 31, 645.

De Sarro, G.B., Croucher, M.J., Meldrum, B.S., 1984. Anticonvulsant actions of DS 103–282: Pharmacological studies in rodents and the baboon *Papio papio*. Neuropharmacology 23, 526–530.

De Sarro, G.B., De Sarro, A., Trimarchi, G.R., Nisticò, G., 1992. Effects of some calcium antagonists upon the activity of common antiepileptic compounds on sound-induced seizures in DBA/2 mice. Gen. Pharmacol. 23, 75–82.

De Sarro, G.B., Nava, F., Aguglia, V., De Sarro, A., 1996. Lamotrigine potentiates the antiseizure activity of some anticonvulsants in DBA/2 mice. Neuropharmacology 35, 153–158.

Dunham, N.W., Miya, T.S., 1957. A note on a simple apparatus for detecting neurological deficit in rats and mice. J. Am. Pharm. Assoc. 46, 208–209.

Gee, N.S., Brown, J.P., Dissanayake, V.U.K., Offord, J., Thurlow, R., Woodruff, G.N., 1996. The novel anticonvulsant drug, gabapentin (neurontin), binds to the $\alpha_2\delta$ subunit of a calcium channel. J. Biol. Chem. 271, 5768–5776.

Graves, N.M., Holmes, G.B., Leppik, I.E., Rask, C., Slavin, M., Anhut, H., 1989. Pharmacokinetics of gabapentin in patients treated with phenytoin. Pharmacotherapy 9, 196.

Graves, N.M., Leppik, I.E., Wagner, M.L., Spencer, M.M., Erdman, G.R., 1990. Effect of gabapentin on carbamazepine levels. Epilepsia 31, 644–645.

Hill, D.R., Suman-Chauhan, N., Woodruff, G.N., 1993. Localization of [^{3}H] gabapentin to a novel site in rat brain: autoradiographic studies. Eur. J. Pharmacol. Mol. Pharmacol. 244, 303–309.

Hooper, W.D., Kavanagh, M.C., Herkes, G.K., Eadie, M.J., 1991. Lack of pharmacokinetic interaction between phenobarbital and gabapentin. Br. J. Clin. Pharmacol. 31, 171–174.

Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose–effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

Loscher, W., Honack, D., Taylor, C.P., 1991. Gabapentin increases aminoxyacetic acid-induced GABA accumulation in several regions of rat brain. Neurosci Lett. 128, 150–154.

Macdonald, R.L., Meldrum, B.S., 1995. Principles of antiepileptic drug action. In: Levy, R.H., Mattson, R.H., Meldrum, B.S. (Eds.), Antiepileptic Drugs, 4th edn. Raven Press, New York, 1995, pp. 61–77.

McLean, M.J., 1994. Clinical pharmacokinetics of gabapentin. Neurology 44, S17–S22.

McLean, M.J, 1995. Gabapentin. Chemistry, Absorption, distribution and excretion. In: Levy, R.H., Mattson, R.H., Meldrum, B.S. (Eds.), Antiepileptic Drugs, 4th edn. Raven Press, New York, 1995, pp. 843–849.

McLean, M.J., MacDonald, R.L., 1986. Sodium valproate, but not ethosuximide, produces use and voltage-dependent limitation of high frequency repetitive firing of action potentials of mouse central neurons in cell culture. J. Pharmac. Exp. Ther. 244, 789–795.

Meldrum, B.S., 1984. Amino acid neurotransmitters and new approaches to anticonvulsant drug action. Epilepsia 25, S140–S149.

Nielsen, E.O., Drejer, J., Cha, J-HI, Young, A.B., Honore, T., 1990. Autoradiographic characterization and localization of of quisqualate binding sites in rat brai using the antagonist [^{3}H] 6-cyano-7-nitroquinoxaline-2,3-dione: comparison with (*R,S*)-[^{3}H]alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid binding sites. J. Neurochem. 54, 686–695.

Petroff, O.A.C., Rothman, D.L., Behar, K.L., Lamoureux, D., Mattson, R.H., 1996. The effect of gabapentin on brain gamma-aminobutyric acid in patients with epilepsy. Ann. Neurol. 39, 95–99.

Radulovic, L.L., Wilder, B.J., Leppik, I.E., Boekbrader, H.N., Chang, T., Posvar, E.L., Sedman, A.J., Uthman, B.M., Erdman, G.R., 1994. Lack of interaction of gabapentin with carbamazepine or valproate. Epilepsia 35, 155–161.

Richens, A., 1993. Clinical pharmacokinetics of gabapentin. New trends in epilepsy management: the role of gabapentin. International Congress and Symposium Series, Vol. 198, pp. 41-46

Rizzo, M., Morrone, L., Longo, P., Sinopoli, V.A., Spagnolo, C., Lo Pilato, R., Pelaggi, T., David, E., Rotiroti, D.A., De Sarro, G., 1997. Simultaneous determination of lamotrigine, felbamate and some conventional antiepileptic drugs using high-performance liquid chromatography. Pharmacol. Res. 35, 105.

Rock, D.M., Kelly, K.M., MacDonald, R.L., 1993. Gabapentin actions on ligand and voltage-gated response in cultured rodent neurons. Epilepsy Res. 16, 89–98.

Stewart, B.H., Kugler, A.R., Thompson, P.R., Bockbrader, H.N., 1993. A saturable transport mechanism in the intestinal absorption of gabapentin is the underlying cause of the lack of proportionality between increasing dose and drug levels in plasma. Pharm. Res. 10, 296–301.

Suman-Chauhan, N., Webdale, L., Hill, D.R., Woodruff, G.N., 1993. Characterization of [^{3}H]gabapentin binding to a novel site in rat brain: homogenate binding studies. Eur. J. Pharmacol. Mol. Pharmacol. 244, 293–301.

Taylor, C.P., 1995. Gabapentin: Mechanism of action In: Levy, R.H., Mattson, R.H., Meldrum, B.S. (Eds.) Antiepileptic Drugs, 4th edn. Raven Press, New York, pp. 829–842.

Taylor, C.P., Rock, D.M., Weinkauf, R.J., Ganong, A.H., 1988. In vitro and in vivo electrophysiological effects of the anticonvulsant gabapentin . Soc. Neurosci. Abstr. 14, 866, abstract.

Taylor, C.P., Vartanian, M.G., Andruszkiewiewicz, R., Silverman, R.B., 1992a. 3-Alkyl GABA and 3-alkylglutamic acid analogues: two new classes of anticonvulsant agents. Epilepsy Res. 11, 103–110.

Taylor, C.P., Vartanian, M.G., Yuen, P.W., Bigge, C., Suman-Chauhan, N., Dill, D.R., 1992b. Potent and stereospecific anticonvulsant activity of 3-isobutyl GABA relates to in vitro binding at a novel site labeled by tritiated gabapentin. Epilepsy Res. 14, 11–15.

Upton, N., 1994. Mechanism of action of new antiepileptic drugs: rationale design and serendipitous findings. Trends Neurosci. 15, 456–463.

Vollmer, K.O., von Hodenberg, A., Kolle, E.U., 1986. Pharmacokinetics and metabolism of gabapentin in rat, dog and man. Arzneim.-Forsch./Drug Res. 36, 830–839.