

Does Vigabatrin Possess an Anticonvulsant Action Against Pentylenetetrazol-Induced Seizures in Developing Rats?

R. HAUGVICOVÁ, H. KUBOVÁ, P. MAREŠ

Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

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Summary

Anticonvulsant action of vigabatrin (300, 600, 900 and/or 1200 mg/kg i.p.), an inhibitor of GABA-transaminase, was studied in a model of motor seizures elicited by pentylenetetrazol. Five age groups of rats (7, 12, 18, 25 and 90 days old) received a s.c. injection of pentylenetetrazol 4, 6 and/or 24 hours after vigabatrin administration. The incidence of minimal, predominantly clonic seizures was not changed in any age group, but their latencies were prolonged in 18- and 25-day-old rats. Generalized tonic-clonic seizures were influenced in a more complex manner. Incidence of these seizures was decreased in 7-day-old rat pups 24 hours after vigabatrin administration. Higher doses of vigabatrin exhibited a similar effect in adult rats at all intervals studied. Specific suppression or at least restriction of the tonic phase was observed in all groups of immature rats, the effect was more marked 24 hours after vigabatrin than at shorter intervals. The anticonvulsant action of vigabatrin, which could be demonstrated mainly against generalized tonic-clonic seizures, varies markedly during development.

Key words

Vigabatrin • Pentylenetetrazol • Motor seizures • Ontogeny • Rat

Introduction

Only two tests are obligatory in routine preclinical testing of potential antiepileptic drugs – minimal (clonic) metrazol seizures and maximal electroshock seizures in rodents. Maximal electroshock seizures represent an adequate model of human generalized seizures of the "grand mal" type (Löscher and Schmidt 1988). On the contrary, there is no agreement about minimal metrazol seizures. Serious doubts have been cast on the original hypothesis that they may be used as a model of human absence seizures and they more probably represent a model of myoclonic seizures (Löscher and Schmidt 1988, Mareš and Zouhar 1988).

There are two ways how to improve this inadequate preclinical testing of antiepileptic drugs (Löscher and Schmidt 1988, Mareš and Zouhar 1988, Swinyard *et al.* 1989). First, which is time and money consuming, is to add some additional models (at least models of simple and complex partial seizures and a model of absences) to the two models mentioned above. At present, it is common to add one more model, namely amygdalar kindling. Another possibility is to increase the informational content of the common tests by more detailed evaluation. We are using motor seizures induced by pentylenetetrazol in a modification allowing us to have at our disposal models of two different types of epileptic seizures at the same time, namely minimal

metrazol and generalized tonic-clonic seizures (Kubová and Mareš 1989, 1991, Velišek *et al.* 1992). This model may provide more information than the routinely performed minimal metrazol seizures not only because of the second type of elicited seizures but also due to the possibility of different actions of drugs against individual phases of tonic-clonic seizures. We therefore decided to use this for testing the anticonvulsant action of vigabatrin, an antiepileptic drug, which was demonstrated to have only moderate action against minimal, clonic pentylenetetrazol-induced seizures (Bernasconi *et al.* 1988).

Vigabatrin (γ -vinylGABA) is an irreversible inhibitor of GABA-transaminase (Lippert *et al.* 1977). It increases GABA levels at those sites, where GABA is physiologically present (for review see Meldrum 1989). It is effective against various pharmacologically-induced seizures (strychnine, picrotoxin, isoniazid, 3-mercaptopropionic acid) as well as against maximal electroshock seizures in animals (Bernasconi *et al.* 1988, Bonhaus and McNamara 1988, Sarhan and Seiler 1989). It is clinically effective against complex partial seizures (for review see Browne *et al.* 1989, Gram 1991, Grant and Heel 1991) and also against one of the age-dependent epilepsies, the Lennox-Gastaut syndrome (Gram *et al.* 1992).

The efficacy in this age-dependent epileptic syndrome was found in spite of the fact that preclinical testing is always performed only in adult animals. Our laboratory studies the action of antiepileptic drugs in immature rats systematically and we have shown not only quantitative (benzodiazepines - Kubová and Mareš 1989, Haugvicová *et al.* 1999) but also qualitative changes (ethosuximide - Mareš *et al.* 1981, Mareš 1998, primidone - Kubová and Mareš 1991, progabide - Staňková *et al.* 1997, topiramate - Haugvicová *et al.* 2000a, tiagabine - Haugvicová *et al.* 2000b) in the action of antiepileptic drugs during development. We therefore extended our study to four age groups of rat pups.

Methods

Experiments were performed in albino rats of the Wistar strain in five age groups: 7, 12, 18, 25 days old and adult (approximately 90 days old) animals. The day of birth was counted as zero. Animals were maintained under standard conditions (temperature 22 ± 1 °C, humidity 50 %, 12:12 h light-dark cycle). All experiments were performed in accordance with Animal Protection Law of the Czech Republic and were approved

by the Animal Care and Use Committee of the Institute of Physiology of the Academy of Sciences of the Czech Republic.

Vigabatrin (a generous gift of Hoechst Marion Roussel - now Aventis) was administered intraperitoneally to 468 rats. A fresh water solution (300 mg/ml) was given in doses of 300, 600 or 900 mg/kg either four, six or 24 hours before pentylenetetrazol (PTZ, Sigma) so that 45 groups were thus formed. The effects of the highest dose of vigabatrin (1200 mg/kg) were studied in all age groups only 24 hours after administration so that five additional groups were examined. Each group consisted of 8-13 animals according to age and the dose. Rat pups were returned to their mothers and taken away again immediately before the PTZ injection. PTZ was administered subcutaneously as a freshly prepared 10 % water solution in a dose of 100 mg/kg in all age groups with the exception of 18-day-old rats where a 90 mg/kg dose was injected because of the higher sensitivity of this age group (Velišek *et al.* 1992). After this injection, the animals were observed in isolation for 30 min, body temperature of pups was maintained by means of a pad heated to 34 °C, i.e. to the temperature in the nest. Control groups which received injections of PTZ only consisted of 23 to 29 rats and were followed during ongoing studies of various anticonvulsants in the pentylenetetrazol seizure test.

Table 1. Scale for scoring seizure severity

0	animals motionless
1	isolated myoclonic jerks
2	atypical minimal seizures, i.e. only some elements present
3	minimal seizures, i.e. clonic seizures involving head and forelimb muscles; tonic component, if present, is represented by rearing or torsion of the trunk; righting ability is preserved
4	generalized clonic seizures, tonic phase is absent, righting reflexes lost
5	complete generalized tonic-clonic seizures with a loss of righting reflexes

The incidence, pattern and latency of the minimal as well as generalized tonic-clonic seizures were recorded for subsequent statistical evaluation. The incidence of seizures was compared with the controls by means of Fisher's exact test (four pole table), ANOVA (BMDP program) was used for evaluation of latencies

with subsequent multiple comparison according to Holm (1979). Seizure severity quantified according to the five-point scale, see Table 1 (Pohl and Mareš 1987) was compared by means of the Kruskal-Wallis nonparametric test. The level of statistical significance was set at $p < 0.05$.

Results

Minimal, clonic seizures

The incidence of this type of seizures (data not shown) under control conditions was age-dependent; they rarely appeared in 7- and 12-day-old rat pups (in 6 and 21 % of animals, respectively) being common in older

age groups (in 92, 93 and 100 % of rats, respectively). Vigabatrin did not influence their incidence in 7-day-old rats, but it tended to increase the incidence in 12-day-old rat pups at the 4-hour interval, especially in the group with the 600 mg/kg dose in which 60 % of rat pups exhibited this type of seizures. No significant changes were found in 18- and 25-day-old and adult animals 4 and 6 hours after vigabatrin. At the 24-hour interval, two significant changes occurred. The incidence of minimal seizures was reduced after the 1200 mg/kg dose in 18-day-old rats and they were almost completely suppressed after the 900 mg/kg dose in adult animals (only one out of eight rats exhibited this type of seizures).

Table 2. Latencies of minimal and generalized tonic-clonic seizures (M±S.E.M.)

Age	Dose (mg/kg)	Minimal seizures			GTCS		
		Time after vigabatrin administration					
		4 h	6 h	24 h	4 h	6 h	24 h
7 days	control	410±210			230±20		
	300	-	-	-	279±65	245±16	590±208* s
	600	-	-	-	234±22	266±11	966±307*
	900	-	-	-	289±35	235±10	-
	1200	n.d.	n.d.	335±120	n.d.	n.d.	-
12 days	control	204±89			188±17		
	300	197±38	-	-	274±53	276±39*	268±36
	600	200±49	176±23	-	283±40*	248±19	234±20
	900	190±49	148±21	189±32	237±61	198±21	389±83*
	1200	n.d.	n.d.	194±49	n.d.	n.d.	265±41*
18 days	control	82±10			178±22		
	300	281±40*	281±62*	123±7*	353±42*	378±81*	199±20
	600	866±184*	266±17*	243±30*	924±167	318±17*	319±31*
	900	303±42*	338±66*	178±21*	422±44*	503±124*	374±49*
	1200	n.d.	n.d.	118±24*	n.d.	n.d.	208±37
25 days	control	177±34			377±38		
	300	516±76*	689±200*	258±30	1090±152	820±156	547±65
	600	568±174*	447±75*	372±132	713±158	789±131	493±68
	900	536±97*	326±44*	263±31	1144±220	465±44	556±121
	1200	n.d.	n.d.	715±84*	n.d.	n.d.	807±111*
90 days	control	396±36			774±98		
	300	243±39*	494±137	257±46*	524±113	506±135	722±68
	600	-	416±92	520±135	-	494±91*	793±213
	900	417±69	794±91*	-	-	-	532±145
	1200	n.d.	n.d.	485±64	n.d.	n.d.	-

Asterisks denote significant difference in comparison with the age-matched controls, n.d. means that the dose was not tested at that time interval.

The latency of minimal seizures (Table 2) was systematically changed in two age groups only. Eighteen-day-old rats exhibited a markedly delayed onset of these seizures in all cases, while 25-day-old animals after all doses at the four and six hour intervals. Only the 1200 mg/kg dose led to a significant increase of latency 24 hours after vigabatrin; changes after the other doses

did not reach the level of statistical significance. Significant prolongation of latency was seen in adult rats only after the 900 mg/kg dose at the interval of six hours. On the contrary, the lowest dose led to paradoxical shortening of latencies four and twenty-four hours after the administration.

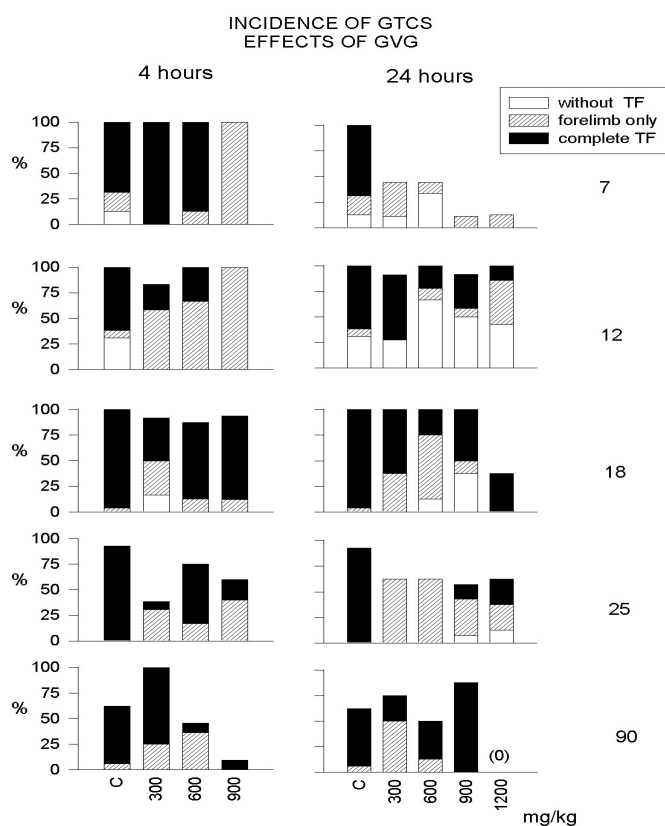


Fig. 1. Incidence of generalized tonic-clonic seizures at three time intervals after vigabatrin administration in five age groups of rats. On the left – an interval of 4 hours between vigabatrin and PTZ administration; in the middle – 6 hours, on the right – 24 hours. From top to bottom: rats 7, 12, 18, 25 and 90 days old. Abscissa – doses of vigabatrin, C controls which had received an injection of PTZ only; ordinates – percentage of rats exhibiting seizures. Black parts of columns – complete generalized tonic-clonic seizures with a tonic phase involving forelimbs as well as hindlimbs; hatched parts of columns – rats in which the tonic phase was restricted to the forelimbs; white parts of columns – generalized clonic seizures, i.e. abolition of the tonic phase. Asterisks denote a significant difference in the incidence of generalized seizures, crosses – a significant difference in the incidence of the complete tonic phase (black parts of the columns) in comparison with age-matched control groups.

Generalized tonic-clonic seizures (GTCS)

These seizures were elicited reliably in all age groups of control rats (Fig.1). There were no significant changes in the incidence of GTCS in rat pups 7, 12 and 18 days old at four and six hours after vigabatrin administration, whereas 25-day-old rats were protected at these intervals by all doses (with the exception of 600 mg/kg at the four-hour interval). Adult animals were significantly protected only by the 900mg/kg dose at the two short intervals. As concerns the 24-hour interval, all doses of vigabatrin decreased the incidence of GTCS in 7-day-old rats. The same was true for 12-day-old rat pups (with the exception of the 1200 mg/kg dose). A general tendency to a decreased incidence found in 25-day-old animals reached the level of statistical significance only after the 900 mg/kg dose of vigabatrin. In 18-day-old and

adult rats the incidence of GTCS was significantly suppressed after the highest dose of vigabatrin only.

The tonic phase of generalized seizures was a constant component of these seizures in control rats during the whole period of observation. The majority of animals exhibited the tonic phase of all four limbs, a small part of rats had tonic seizures of the forelimbs only. Vigabatrin suppressed the tonic phase or at least restricted it to the forelimbs in all groups of rat pups. This effect was best expressed in 12-day-old rat pups in which tonic phase failed to appear in a majority of animals. Comparing the time course of vigabatrin action, the suppression or restriction of the tonic phase was more marked 24 hours after administration than at shorter intervals, the 1200 mg/kg dose led to significant results in all age groups. At four- and six-hour intervals the changes reached the level of statistical significance less

frequently than at the longest interval (Fig. 1). A similar specific action on the tonic phase was only exceptionally found in adult rats.

Seven-day-old rat pups exhibited increased latencies of the onset of these seizures only at the longest interval (Table 2). Latencies generally tended to be longer in experimental 12-day-old rats than in the age-matched controls, the level of statistical significance was attained in some cases. Eighteen- and 25-day-old animals exhibited a significant prolongation of latencies in nearly all cases, however, this tendency did not reach the level of statistical significance only exceptionally. Paradoxically the latencies tended to be shorter in adult rats given vigabatrin than in the controls, but the level of statistical significance was only attained exceptionally (Table 2).

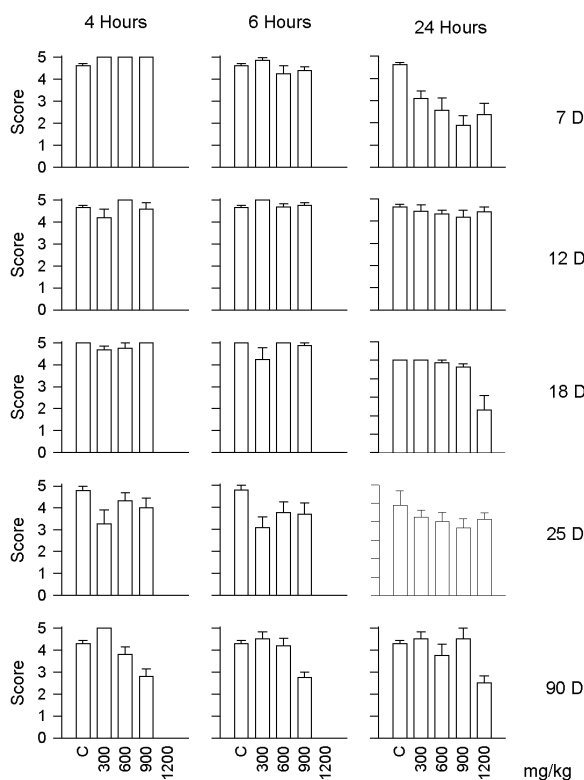


Fig. 2. Influence of vigabatrin on seizure severity (average score \pm S.E.M.). Details as in Fig. 1, only ordinates – a five-point scale. Asterisks denote a significant difference in comparison with age-matched controls.

Seizure severity

Control groups exhibited average scores between four and five (Fig. 2) due to a common pattern of complete generalized seizures (including the tonic phase).

Seizures in 7-day-old rat pups were of lower intensity after all four doses of vigabatrin 24 hours after injection. Twelve-day-old rats exhibited only a tendency to decreased seizure intensity, whereas the level of statistical significance was reached 24 hours after the 1200 mg/kg dose in 18-day-old animals. Seizures were ameliorated in 25-day-old rats after all doses at intervals of six and 24 hours (with the exception of the 1200 mg/kg dose; in this group for $p=0.068$), meanwhile adult animals exhibited a decreased seizure intensity only after the 900 mg/kg dose at four and six hours and 24 hours after the 1200 mg/kg dose.

Discussion

The time course of GABA-transaminase (GABA-T) inhibition and brain GABA levels after a single injection of vigabatrin were described in adult rats (Valdizan *et al.* 1999). Data from both mice and rats did not demonstrate a direct correlation between brain GABA levels and seizure protection (Schechter *et al.* 1977, Bernasconi *et al.* 1988, Löscher *et al.* 1989), because substantially increased brain GABA levels were found even one and two hours after vigabatrin administration when the seizure protection is low. It has to be accepted that the total brain GABA levels are not decisive for seizure suppression, but changes of this inhibitory transmitter in specific structures play a primary role (Löscher *et al.* 1989, Gale 1992). Anticonvulsant effect of vigabatrin was observed at all three time intervals studied. A possible initial proconvulsant effect of vigabatrin (Löscher *et al.* 1989), seen also 4 hours after vigabatrin administration in another study from our laboratory (Mareš and Šlamberová – submitted), was not found in this model. A tendency to increased incidence and decreased latencies of GTCS four hours after vigabatrin administration was outweighed by a clear anticonvulsant effect of the 600 mg/kg dose at the same time interval.

No data are available about the action of vigabatrin on GABA-T in the immature brain and hence it is difficult to draw any parallels. The activity of brain GABA-T in rats increased during the postnatal development, at first starting with a low slope, then followed by an abrupt change in the rate between postnatal days 11 and 12 and a plateau between days 25 and 30. Even at the age of 30 days the activity of GABA-T is lower than in the mothers of the pups studied (Sims *et al.* 1968). Similarly, Sherif *et al.* (1991) mentioned

very low GABA-T activity in one-week-old rat pups with a marked increase up to the age of two weeks. The relatively low activity of GABA-T in rat pups under control conditions might have led to the suggestion that vigabatrin action is more marked at early postnatal stages. Some of our data support this hypothesis, namely that the efficacy of all doses of vigabatrin 24 hours after administration in 7-day-old rats nearly completely blocked generalized tonic-clonic seizures and the tonic phase of GTCS was markedly suppressed in 12-day-old rats. Unfortunately, the situation is much more complicated because the activity of glutamate decarboxylase (GAD) and thus the rate of GABA synthesis also increased during development. The activity of GAD in the whole brain of 12-day-old rat pups is about 40% of the adult levels (Coyle and Enna 1976, Netopilová *et al.* 1995). Furthermore, the properties of this enzyme (e.g. time course of the inhibition induced by 3-mercaptopyruvic acid, see Netopilová *et al.* 1997) are also different from those in the adult brain. No definite conclusions can thus be made until the developmental data on the effects of vigabatrin on GABA-T activity and GABA levels in different brain structures are available. In addition, we cannot exclude a possible action of vigabatrin itself as suggested by Jackson *et al.* (2000).

There are marked differences among the effects of antiepileptic drugs potentiating GABAergic inhibition in PTZ-induced motor seizures. In adult rats, the 900 mg/kg dose of vigabatrin suppressed generalized tonic-clonic seizures four and six hours after administration, whereas only the highest dose (1200 mg/kg) was active after 24 hours. Minimal seizures were blocked only exceptionally. Similar activity, i.e. specific suppression of GTCS exhibited only progabide and its metabolite SL 75102 (Staňková *et al.* 1997, Kubová *et al.* 1997). Valproate, phenobarbital and benzodiazepines were found to block both GTCS and minimal seizures, GTCS being always more sensitive (Mareš *et al.* 1981, Kubová and Mareš 1989, 1991). Similar results were obtained with GABA uptake blockers NNC 711 and tiagabine in adult rats, only the doses suppressing minimal seizures had to be markedly

higher than the effective doses for GTCS (Kubová *et al.* 1998, Haugvicová *et al.* 1999).

Developmental data for some drugs potentiating the GABAergic system, e.g. valproate, phenobarbital and benzodiazepines (Mareš *et al.* 1981, Kubová and Mareš 1989, 1991), exhibit only small quantitative differences in comparison with those for adult rats. In contrast to these drugs, progabide, SL 75102, NNC 711 and tiagabine specifically suppressed the tonic phase of GTCS in immature rats (Staňková *et al.* 1997, Kubová *et al.* 1997, 1998, Haugvicová *et al.* 1999). These four drugs and vigabatrin might also exhibit their anticonvulsant action not only through GABA_A receptors, but GABA_B receptors might also be involved. The involvement of GABA_B autoreceptors in the action of vigabatrin was suggested by Jackson *et al.* (2000) and demonstrated in a completely different model (cocaine-induced increase of dopamine in the nucleus accumbens – Ashby *et al.* 1999). In addition to the above mentioned similar effect on the tonic phase of GTCS, NNC 711 and tiagabine exhibit extremely strong action against minimal seizures in 18- and 25-day-old rats, i.e. in those age groups in which these seizures could be reliably induced by PTZ (Kubová *et al.* 1998, Haugvicová *et al.* 1999). Such an action was never seen after vigabatrin, progabide or SL 75102. A marked difference between the effects of vigabatrin and tiagabine is in agreement with the results of Sills *et al.* (1999). The comparison of the effects of various drugs potentiating GABAergic inhibition leads to the conclusion that in addition to the affected structure (Gale 1992) the developmental stage plays an important role in the action of drugs which exhibit their anticonvulsant effect through the GABAergic inhibitory system.

The question posed in the title of this study has to be answered positively. Furthermore, the marked developmental changes have been found in the anticonvulsant action of vigabatrin.

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Reprint requests

P. Mareš, M.D., D. Sc. Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ-142 20 Prague 4, Czech Republic. E-mail: maresp@biomed.cas.cz