

Anticonvulsant Effect of Progabide in Rats during Ontogenesis

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Received July 13, 1995

Accepted August 28, 1996

Summary

The action of progabide against motor seizures elicited by pentylenetetrazol was studied in 7-, 12-, 18-, 25-day-old and adult rats. Progabide (dissolved in dimethylsulfoxide) was injected in doses from 12.5 to 150 mg/kg i.p. 30 min before pentylenetetrazol. Minimal seizures were not affected by solvent or progabide pretreatment. The action of progabide against major, i.e. generalized tonic-clonic seizures, changed with age: adult rats exhibited a tendency to suppression of whole major seizures, whereas specific suppression of the tonic phase was observed in rat pups during the first three weeks of life. The only effect seen in 25-day-old animals was prolongation of the latency of major seizures after the highest dose of progabide.

Key words

Motor seizures – Rat – Ontogenesis – Progabide – Pentylenetetrazol

Introduction

Progabide (PGB, 4-{{4-(chlorophenyl)(5-fluoro-2-hydroxyphenyl)methylene}amino}butanamide see Fig. 1) is an anticonvulsant drug introduced into clinical in the eighties (for review see Morselli *et al.* 1986, Morselli and Palminteri 1989, Loiseau and Duché 1990). Biochemical and pharmacological experiments have demonstrated that PGB as well as its metabolite SL 75102 (Fig. 1) exhibit GABA-mimetic effects by direct stimulation of the GABA receptors (Lloyd *et al.* 1982, Langer *et al.* 1985). PGB has been shown to be active against experimental seizures related to impairment of GABAergic transmission (picrotoxin, bicuculline and allylglycine models, pentylenetetrazol clonic convulsions and cortical penicillin foci) and also against models not directly related to impairment of GABAergic system – maximal electroshock and audiogenic seizures, kainate- and strychnine-induced convulsions and amygdala kindling (Worms *et al.* 1982, Joy *et al.* 1984, Zivkovic *et al.* 1985, Morselli and Palminteri 1989). The anticonvulsant profile of PGB in these tests was similar to that of valproate but it was more potent than valproate in most of these tests (Morselli and Palminteri 1989).

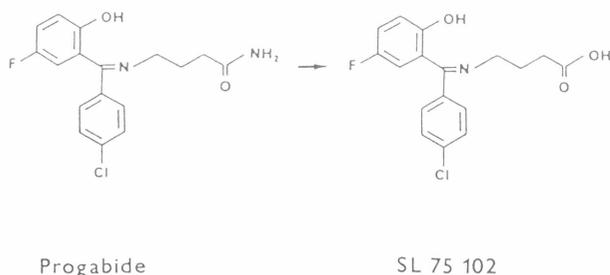


Fig. 1. Chemical formula of progabide (molecular weight = 334.78) and SL 75 102 (molecular weight = 357.75)

All these data were obtained in adult animals. In spite of the fact that PGB has also been introduced for the treatment of childhood epilepsies (for review Loiseau and Duché 1990), the developmental data from animal experiments are scarce (Mecarelli *et al.* 1988). These studies may be important because some antiepileptic drugs change their effects during ontogenesis either qualitatively (phenytoin – Marešová

and Mareš 1983, ethosuximide – Mareš and Velíšek 1983) or quantitatively (valproate – Mareš *et al.* 1989, clonazepam – Kubová and Mareš 1989, carbamazepine – Kubová and Mareš 1993, lamotrigine – Staňková *et al.* 1992).

In addition, we have developmental data on the anticonvulsant effects of various drugs facilitating GABAergic inhibition in different ways (benzodiazepines clonazepam – Kubová and Mareš 1989, midazolam – Kubová and Mareš 1992, and bretazenil – Brabcová *et al.* 1993, valproate – Mareš *et al.* 1989, phenobarbital – Kubová and Mareš 1991) obtained in the same model, at the same ages and in the same rat strain and breeding. Therefore, it might be interesting to know if different modes of activation of GABAergic system yield the same results. Pentylenetetrazol-induced motor seizures (PTZ, metrazol) were chosen as the first model to study the anticonvulsant action of PGB during ontogenetic development. The advantage of this model is that PTZ in an appropriate dose can induce two types of motor seizures: minimal (mMS) and generalized major tonic-clonic (MMS). The clinical correlation is uncertain in the case of mMS – they could be taken as a model of myoclonic seizures (Löscher and Schmidt 1988); major seizures represent a model of generalized tonic-clonic seizures (Snead 1983, Mareš and Zouhar 1988). We can thus evaluate the action of PGB against two different models in one test.

Methods

Male albino rats of the Wistar strain, specific pathogen free breeding ($n = 164$), aged 7, 12, 18, 25 and 90 days were used. Progabide (a generous gift of Synthelabo) was always freshly dissolved in dimethylsulfoxide (DMSO) in a concentration of 25 (two lowest doses) or 50 mg/ml. PGB was administered intraperitoneally in doses of 25, 50, 75 and 150 mg/kg. Ten minutes after the administration of PGB, PTZ was injected subcutaneously in a dose of 100 mg/kg in all age groups with the exception of 18-day-old rats where the 90 mg/kg dose was given because of the higher sensitivity of this age group (Velíšek *et al.* 1992). Three control groups were used: rats receiving only PTZ (this group was taken from the ontogenetic study of convulsant action of PTZ – Velíšek *et al.* 1992 – with several animals added) and rats injected with DMSO in the volume of 1 or 3 ml/kg followed by PTZ. The 1 ml/kg dose was compared to the three lowest doses of PGB, the 3 ml/kg dose to the remaining three doses. These two DMSO control groups were taken from the previous study (Staňková and Mareš 1992) where DMSO (up to 10 ml/kg) was found to possess no anticonvulsant activity against metrazol-induced seizures. The two solvent groups were therefore presented together in the figures. Again, a few rats were added to each solvent group in

this experimental series. Animals were observed in isolation for 30 min after PTZ administration. The body temperature of rat pups was maintained by means of an electrically heated pad.

Each age and dose group was formed by 8–10 animals and the same number was in the DMSO control groups. The PTZ controls comprised from 23 to 27 rats.

The following phenomena were recorded: abnormal behaviour, isolated myoclonic jerks, minimal seizures (mMS, predominantly clonic seizures of facial and forelimb muscles with preserved righting reflexes), generalized tonic-clonic (major) seizures (MMS) formed by three phases: wild running, tonic and clonic seizures. The righting ability was lost at the beginning of the tonic phase.

The incidence of minimal seizures, tonic-clonic seizures, tonic phase of MMS (TP) and especially of hindlimb tonic seizures as the most severe phenomenon in the tonic-clonic seizures category, the latencies of mMS and MMS were evaluated. To quantify the severity of seizures, the following scale was used (Pohl and Mareš 1987) and each animal was scored according to the most severe phenomenon present:

- 0 – no changes,
- 0.5 – abnormal behaviour (e.g. orienting reaction in the home cage, excessive scratching),
- 1 – isolated myoclonic jerks,
- 2 – atypical minimal seizures (only some elements present)
- 3 – minimal metrazol seizures,
- 4 – major seizures without the tonic phase,
- 5 – complete generalized tonic-clonic seizures.

The incidence was statistically evaluated by Fisher's exact test. Latencies were compared by ANOVA; individual comparisons were performed by Tukey's multiple range method. Score of seizure severity was evaluated by the non-parametric Kruskal-Wallis test. The level of statistical significance was set at 5 %.

Results

Major seizures were elicited by PTZ in all age groups, minimal seizures only from the age of 18 days. Motor patterns of these two types of seizures did not substantially change during postnatal ontogenesis (Velíšek *et al.* 1992).

Effects of Progabide on behaviour

PGB in the doses used did not affect spontaneous behaviour in 18-day-old and older rats. On the contrary, the 150 mg/kg dose of PGB was too high for 7- and 12-day-old rat pups. A quarter of these animals died between the 10th and 15th min after PGB administration; half of the pups grew torpid, their hindlimbs were slack and some of these animals lost

their righting ability. These phenomena were never seen after DMSO alone, even if the 10-ml/kg dose was used (Staňková and Mareš 1992). The remaining 25% of 7- and 12-day-old rats did not exhibit any alteration in motor behaviour. No toxic side effects were observed in these age groups after the lower doses of PGB.

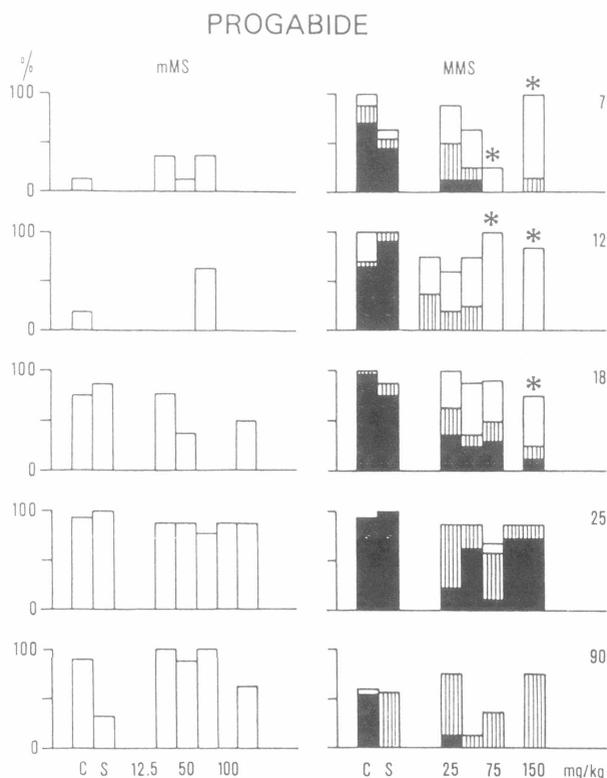


Fig. 2. Incidence of minimal (mMS, left) and major, generalized tonic-clonic (MMS, right) seizures in rats 7, 12, 18 and 25 days old and adult animals (from top to bottom). Abscissae: doses of progabide, C denotes animals injected only with pentylenetetrazol, S animals pretreated with solvent; half of the doses is described in the left part, the other half in the right part. Ordinates: percentage of rats exhibiting seizures. In the graphs on the right, black parts of columns denote animals exhibiting the tonic phase of all four limbs, hatched parts animals with the tonic phase on forelimbs only and white columns rats with purely generalized clonic seizures. x denotes absence of seizures in the corresponding age and dose group. Asterisks denote significant difference in comparison with the solvent control.

Effects of Progabide on PTZ-induced phenomena Initial changes

Under control conditions, PTZ elicited locomotor hyperactivity and isolated myoclonic jerks before the first seizures started. DMSO did not change this activity. A combination of PGB and PTZ resulted

in 7- and 12-day-old animals in pivoting or circling behaviour with their tail becoming erected before the first seizures. This stereotyped behaviour was better expressed with the doses of PGB of 50 and 75 mg/kg; it was never seen after the 150 mg/kg dose. Similar rotation appeared sporadically in 18-day-old rats, but never in older animals.

Minimal metrazol seizures (Figs 2 and 3)

Minimal seizures could be reliably elicited by PTZ in 18-day-old and older control rats. The solvent did not induce any change in mMS. Pretreatment with PGB did not exhibit a consistent effect on the incidence and latencies of mMS throughout the developmental period studied. Similarly, no change in the motor pattern of mMS was observed after PGB.

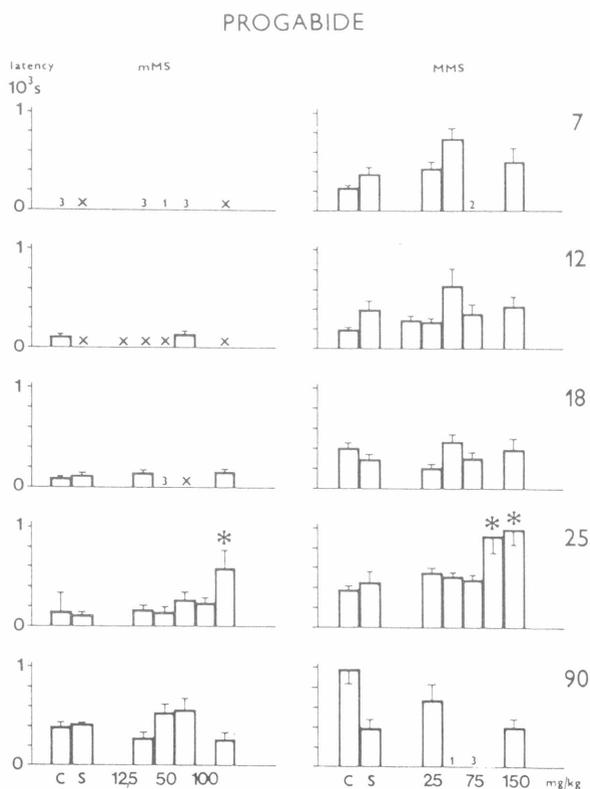


Fig. 3. Latencies of minimal and major generalized tonic-clonic seizures in five age groups of rats. Details as in Fig. 2, only ordinates – latency in seconds.

Generalized tonic-clonic seizures (Figs 2 and 3)

All animals in the three control groups (PTZ only and two doses of DMSO and PTZ) exhibited MMS with the exception of adult rats where the incidence of MMS was 67%. DMSO was without a significant effect on MMS with the exception of restriction of tonic seizures in adult rats to the forelimbs only. Age-dependent changes of PGB effect were observed: significant suppression of the tonic phase of generalized tonic-clonic (major) seizures was

found in 7-, 12-, and 18-day-old rats, the incidence of these seizures remained unchanged in 25-day-old animals and a decreased incidence of tonic-clonic seizures was recorded in adult rats. Due to the 67% incidence of MMS under control conditions, this decrease did not reach the level of statistical significance.

The only effect found in 25-day-old rats was a prolongation of the latencies of major seizures after the highest dose of PGB. This was also the only effect of PGB on the latencies of generalized tonic-clonic seizures in all age groups studied.

doses of DMSO did not significantly change the score in any age group.

Discussion

Our results have demonstrated qualitative changes of the action of progabide during ontogenetic development in the rat. At early developmental stages, progabide specifically suppressed the tonic phase of generalized tonic-clonic seizures, at the age of 25 days its effect was only moderate and in mature animals it exhibited an anticonvulsant action against generalized tonic-clonic seizures without differentiation into individual phases. Such action substantially differs from that of antiepileptic drugs which exhibit an anticonvulsant effect at least partly by influencing the GABAergic system – valproate, benzodiazepines and phenobarbital (MacDonald and McLean 1985). All these drugs were found to suppress both minimal and generalized tonic-clonic motor seizures elicited by PTZ at all maturational stages studied; only quantitative changes of efficacy could be demonstrated (Kubová and Mareš 1989, 1991, 1992, Mareš *et al.* 1989). This difference might signify that drugs acting at different levels of GABAergic inhibition (synthesis, binding at various sites in the supramolecular GABA_A receptor complex, reuptake, catabolism – Meldrum 1985, Mutani *et al.* 1991) might exhibit different profiles of anticonvulsant effects. This possibility has to be tested by means of drugs affecting other components of the GABAergic system (uptake inhibitors; GABA-aminotransferase inhibitors). Data on the absence seizures, where some GABAergic drugs are anticonvulsant (valproate, benzodiazepines – Bourgeois 1989b, Sato 1989) whereas others do not exhibit this effect (phenobarbital, progabide – Morselli and Palminteri 1989, Painter 1989) support this hypothesis. The same difference was shown in models of human absences, where again only valproate and benzodiazepines were effective (Brabcová *et al.* 1993, Marescaux *et al.* 1992). The involvement of the GABAergic system at least in absence seizures, is rather complex (Avanzini and Marescaux 1991).

On the other hand, primidone exhibits the same action against motor seizures elicited by PTZ in developing rats similarly as progabide (Kubová and Mareš 1991). Unfortunately, the exact mechanism of primidone action is not known (Bourgeois 1989a) and there are no data speaking in favour of primidone itself as a GABAergic drug.

The changing action of progabide during maturation, as well as its different anticonvulsant action from that of other antiepileptic drugs potentiating GABAergic inhibition, might be due to the development of individual components of the GABAergic system (Balcar and Johnston 1987, Madtes 1987, Moshé and Garant 1993).

PROGABIDE

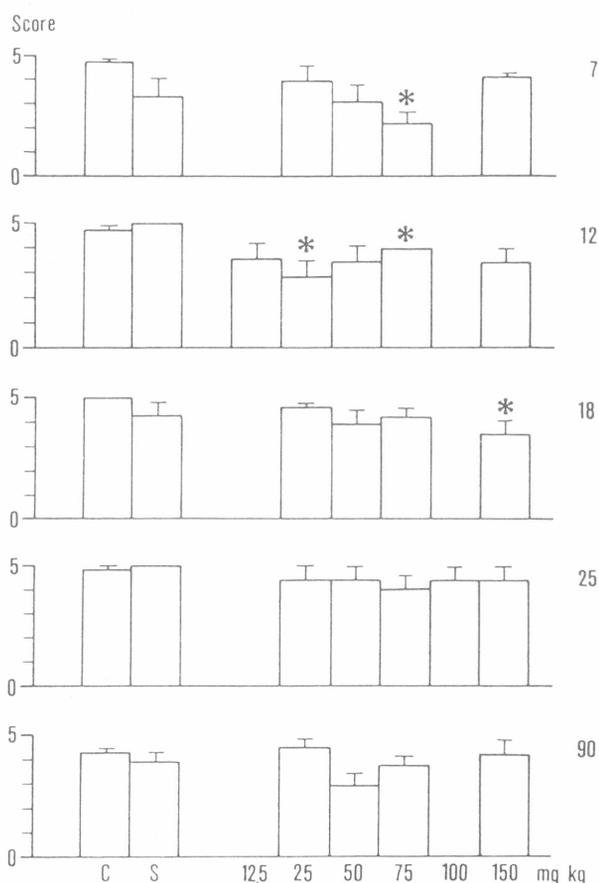


Fig. 4. Seizure severity expressed as an average score (\pm S.E.M.) in individual age and dose groups. Details as in Fig. 2, only ordinates – five-point scale according to Pohl and Mareš (1987).

Seizure severity (Fig. 4)

The score expressing the intensity of seizures was diminished by PGB in the three youngest groups due to the suppression of the tonic phase of generalized seizures. The level of statistical significance was reached with the 75 mg/kg dose in 7-day-old rat pups, with doses from 12.5 to 75 mg/kg in 12-day-old rats and with the 150 mg/kg dose in 18-day-old animals. No significant changes in seizure severity were observed after PGB in rats aged 25–90 days. The two

Acknowledgements

The authors would like to express their thanks to Dr. J. Vorlíček for help in the statistical evaluation of the

results. This research was supported by Grant No. 2668-3 of the Ministry of Health of the Czech Republic.

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