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SHORT COMMUNICATION

Anticonvulsant Effect of SL 75 102 in Adult and Immature Rats

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Summary

The anticonvulsant action of SL 75 102, a metabolite of Progabide, was studied in a model of pentylenetetrazol-induced motor seizures in adult and 12-day-old rats. SL 75 102 suppressed generalized tonic-clonic seizures in adult rats and restricted the tonic phase of these seizures in rat pups. SL 75 102 was less effective than Progabide. In addition, some minor differences in anticonvulsant actions of these two drugs were observed.

Key words

Rat – Motor seizures – Development – Pentylenetetrazol – SL 75 102 – GABA receptor agonist

Augmentation of GABAergic inhibition is one of the possible ways of inducing an anticonvulsant effect (Meldrum 1975, 1989, Mutani *et al.* 1991). Progabide was introduced into the treatment of epilepsies in the eighties as a precursor of gamma-aminobutyric acid passing through the blood-brain barrier (for review see Morselli *et al.* 1986, 1989). Metabolic studies demonstrated that only a small part of Progabide is changed to GABA. Progabide is extensively metabolized by hydroxylation leading to ortho-dihydroxy compounds and by hydrolysis of the amide group to an active acidic metabolite SL 75 102 (Ferrandes *et al.* 1985). This metabolite exhibits the same profile of anticonvulsant action as Progabide (Worms *et al.* 1982). It is more potent in inhibiting [^{3}H]GABA binding in rat and human brain membranes than Progabide (Lloyd *et al.* 1982). Recently, we have demonstrated that Progabide exhibited anticonvulsant action against motor seizures elicited by pentylenetetrazol changes during ontogenetic development. A specific action against the tonic phase of generalized tonic-clonic seizures was observed in the first three weeks of life in rats, whereas adult animals exhibited a tendency to suppression of tonic-clonic seizures as a whole (Staňková *et al.* 1997). To ascertain, whether this qualitative change is a specific property of Progabide or whether this feature is also shared by its active metabolite SL 75 102, a study of the effects of SL 75 102 against metrazol-induced seizures in adult and young rats was initiated.

Experiments were performed on male albino Wistar rats (specific pathogen-free breeding). Only two age groups were studied: adult and 12-day-old rats. Twelve-day-old rat pups were chosen as representatives of immature animals on the basis of the rate of brain development (level of maturation corresponding to early postnatal period in infants – Dobbing 1970) as well as of the results with Progabide (Staňková *et al.* 1997). Body temperature of rat pups was maintained by means of a heating pad. The animals were pretreated by SL 75 102 (a generous gift of Synthelabo) freshly dissolved in dimethylsulfoxide (50 or 100 mg/ml). Doses of 75, 150 or 300 mg/kg were injected i.p. and metrazol (pentamethylenetetrazol, PTZ, Sigma, 10 % water solution) was administered s.c. 30 min later, in a dose of 100 mg/kg. All dose and age groups consisted of 8–10 animals, but the action of the 300 mg/kg was tested in only four 12-day-old rat pups. The remaining four animals died before PTZ administration. The same two control groups as in the preceding paper

(Staňková *et al.* 1997) were used: animals injected with PTZ only and rats pretreated with dimethylsulfoxide (DMSO, solvent) in a volume of 1 or 3 ml/kg i.p. 30 min before PTZ. The two volumes of the solvent yielded identical results and these were consequently pooled in one solvent group in the figures. Both control groups consisted of data published previously, where a few rats were added during the studies of SL 75102 and Progabide. The rats were observed in isolation for 30 min when the latency and incidence of motor seizures and all behavioural phenomena were recorded. The severity of seizures was quantified by means of a five-point scale where the main points were represented by isolated myoclonic jerks [1], minimal, i.e. predominantly clonic seizures [3], and generalized tonic-clonic (major) seizures with a loss of righting ability [5] (Pohl and Mareš 1987). After the end of the experiments the animals were sacrificed by an overdose of ether anaesthesia.

The incidence of minimal, predominantly clonic and major, i.e. generalized tonic-clonic seizures, was compared by means of Fisher's exact test (four-pole table), the latency by means of ANOVA with subsequent comparison according to Holm (1979) and the severity of seizures by means of the non-parametric Kruskal-Wallis test. The level of statistical significance was set at 5 %.

The effects of SL 75 102 before PTZ administration were similar to those of Progabide but less expressed especially in 12-day-old rats. Adult animals survived even the highest dose, but they were heavily sedated; the 150 mg/kg dose did not result in apparent motor impairment. In immature animals, the 150 mg/kg dose led to sedation, but none of these pups died. Only the highest dose (300 mg/kg) was lethal for 50 % of the young rats.

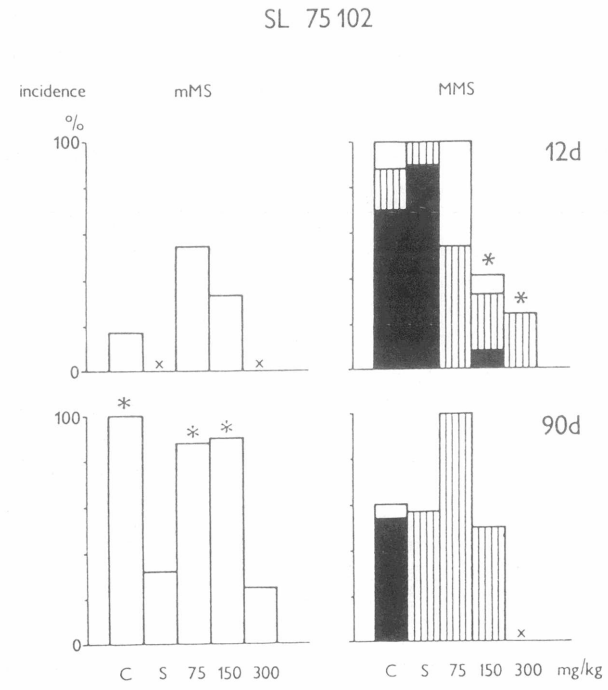

Fig. 1. *Incidence of PTZ-induced seizures in 12-day-old (upper part) and adult (lower part) rats. Left graphs (mMS) – incidence of minimal, clonic seizures; right graphs (MMS) – incidence of major seizures. Black parts of the columns denote complete generalized tonic-clonic seizures, hatched parts – generalized seizures where the tonic phase was restricted to forelimbs and white parts – generalized clonic seizures. x means that this type of seizures was not observed. Abscissae – doses of SL 75 102, C – controls given only PTZ, S – controls pretreated with solvent; ordinates – percentage of animals exhibiting seizures. Asterisks denote significant differences in comparison with the solvent group.*

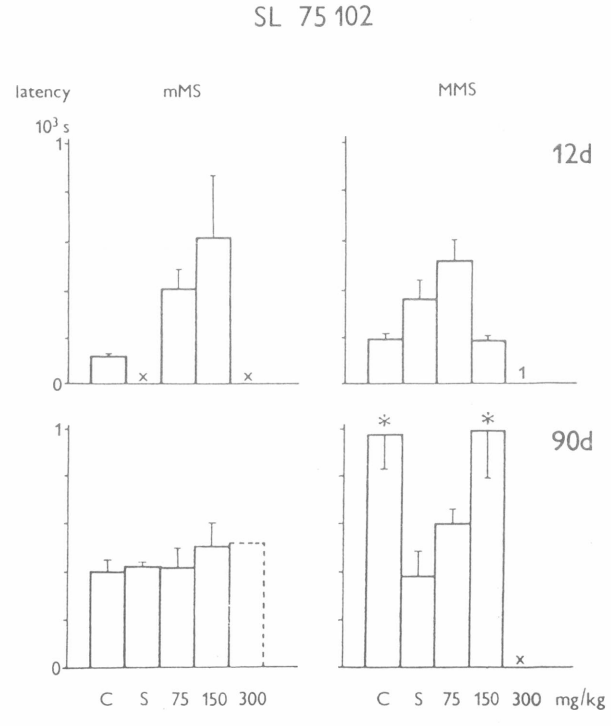

Fig. 2. *Latencies of PTZ-induced seizures (mean + S.E.M.). Details as in Fig. 1, only ordinates represent the latency in thousands of seconds; 1 means that seizures were observed in one rat only; dashed column represents the mean of two values.*

Minimal seizures (Figs 1 and 2): The solvent diminished the incidence of minimal seizures in adult rats in comparison with animals given only PTZ. The two lower doses of SL 75 102 returned the incidence to the value found in rats injected with PTZ only; the highest dose of SL 75 102 did not counteract the effect

of DMSO. The incidence of minimal seizures in 12-day-old rat pups was low under both control and drug conditions. The latencies of minimal seizures tended to increase in a dose-dependent manner in adult rats and were significantly prolonged in immature animals.

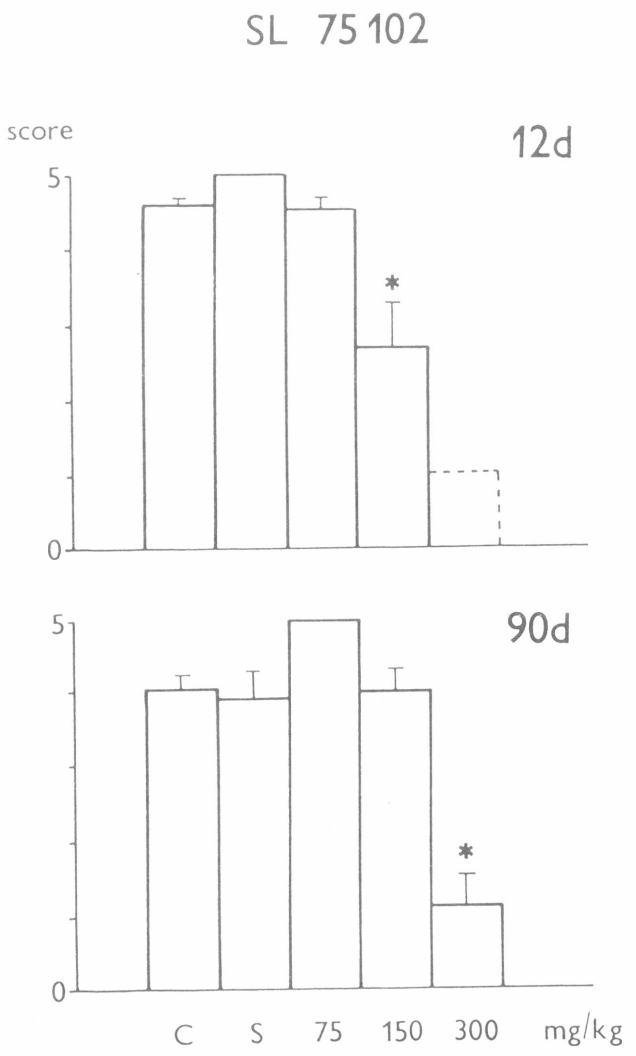

Fig. 3
Severity of PTZ-induced seizures. Details as in Fig. 1, with the exception of ordinates – a five-point scale for scoring the seizures. Dashed column represents the mean from four rats.

Major, generalized tonic-clonic seizures (Figs 1 and 2) were abolished by the 300 mg/kg dose of SL 75 102 in adult animals. The solvent and both lower doses of SL 75 102 abolished the tonic phase of hindlimbs leaving the tonic phase of forelimbs unaffected. The solvent did not exhibit such an action in rat pups, but SL 75 102 was effective in restricting the tonic phase to forelimbs which was significant after all three doses of the anticonvulsant. The 150 and 300 mg/kg doses diminished the incidence of major seizures. The latencies of major seizures were significantly shortened by the solvent in adult rats, the value for the lowest dose of SL 75 102 did not significantly differ from the solvent value. The 150 mg/kg dose returned the mean latency to the value found in animals which had only received PTZ. On the contrary both solvent and the lowest dose prolonged the latencies significantly in rat pups, but again these two values did not differ from each other.

The severity of seizures (Fig. 3) was significantly diminished only by the 300 mg/kg dose in adult animals and by the 150 mg/kg dose in 12-day-old rats. Data for the highest dose in rat pups could not be evaluated because of the insufficient number of animals. The low average value was due to the a fact that only one of the four rats exhibited major seizures, other three remained motionless.

Summarizing our results, the acidic metabolite of Progabide exhibited anticonvulsant action qualitatively similar to that of Progabide: suppression of major seizures in adult rats and restrictive action on the tonic phase in 12-day-old pups. In addition, there was a tendency to an overall suppression of major seizures by SL 75 102 in immature rats. The effective doses of Progabide were lower than those of SL 75 102 (Staňková *et al.* 1997); the difference in molecular weights of both compounds is negligible – approximately 7 %). It is in contradiction with the finding that SL 75 102 binds to $GABA_A$ receptors with a higher affinity than Progabide (Lloyd *et al.* 1982). Similarly, the lower incidence of undesirable side effects (sedation) and lethality was observed in rat pups after SL 75 102 than after Progabide when the same doses were compared. More doses of SL 75 102 should be used to make an exact comparison possible but this quantitative difference might be due either to pharmacokinetic reasons or to different subtypes of $GABA_A$ receptors serving as targets for these two agonists. Minor differences in the action of Progabide and its metabolite SL 75 102 in immature rats might be due to an uneven development of different types of $GABA_A$ binding sites (Moshé and Garant 1993) or individual subunits of the $GABA_A$ receptor, as may be hypothesized from data on the development of messenger RNAs for these subunits (Gambarana *et al.* 1991, Laurie *et al.* 1992).

Both Progabide and SL 75 102 exhibited almost the same profile of action against motor seizures elicited by pentylenetetrazol as primidone (Kubová and Mareš 1991). This similarity should be analysed in further experiments.

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References

DOBBING J.C.: Undernutrition and the developing brain. In: *Developmental Neurobiology,* W.A. HIMWICH (ed), Charles C. Thomas), Springfield, Illinois 1970, pp. 241–261.

FERRANDES B., DURAND A., PADOVANI P., BURKE J.T., GARRIGOU D., FRAISSE-ANDRÉ J., HERMANN P., ALLEN J.: Metabolism of Progabide in four animal species and in man. In: *Epilepsy and GABA Receptor Agonists – Basic and Therapeutic Research.* LERS Vol. 3, G. BARTHOLINI, L. BOSSI, K.G. LLOYD, P.L. MORSELLI (eds), Raven Press, New York 1985, pp. 217–229.

GAMBARANA C., BEATTIE C.E., RODRIGUEZ Z.R., SIEGEL R.E.: Region-specific expression of messenger RNAs encoding $GABA_A$ receptor subunits in the developing rat brain. *Neuroscience* **45:** 423–432, 1991.

HOLM S.: A simple sequentially rejective multiple test procedure. *Scand. J. Statist.* **6:** 56–70, 1979.

KUBOVÁ H., MAREŠ P.: Anticonvulsant effects of phenobarbital and primidone during ontogenesis in rats. *Epilepsy Res.* **10:** 148–155, 1991.

LAURIE D.J., WISDEN W., SEEBURG P.H.: The distribution of thirteen $GABA_A$ receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *J. Neurosci.* **12:** 4151–4172, 1992.

LLOYD K.G., ARBILLA S., BEAUMONT K., BRILEY M., DE MONTIS G., SCATTON B., LANGER S.Z., BARTHOLINI G.: Gamma-aminobutyric acid (GABA) receptor stimulation. II. Specificity of Progabide (SL 76 005) and SL 75 102 for the GABA receptor. *J. Pharmacol. Exp. Ther.* **220:** 672–677, 1982.

MELDRUM B.S.: Epilepsy and gamma-aminobutyric acid mediated inhibition. *Int. Rev. Neurobiol.* **17:** 1–36, 1975.

MELDRUM B.S.: GABAergic mechanisms in the pathogenesis and treatment of epilepsy. *Br. J. Clin. Pharmacol.* **27:** (Suppl. 1): 3S–11S, 1989.

MORSELLI P.L., BARTHOLINI G., LLOYD K.G.: Progabide. In: *New Anticonvulsant Drugs,* B.S. MELDRUM, R.J. PORTER (eds), John Libbey, London 1986, pp. 237–252.

MORSELLI P.L., PALMINTERI R.: Progabide. In: *Antiepileptic Drugs.* Third Edition, R.H. LEVY, F.E. DREIFUSS, R.H. MATTSON, B.S. MELDRUM, J.K. PENRY (eds), Raven Press, New York 1989, pp. 887–904.

MOSHÉ S.L., GARANT D.S. The substantia nigra GABA-sensitive system can mediate either anticonvulsant or proconvulsant effects. *Abstracts of Workshop on Neurobiology of Epilepsy,* Troms, 1993.

MUTANI R., CANTELLO R., GIANELLI M., BETTUCCI D.: Rational basis for the development of new antiepileptic drugs. *Epilepsy Res.* (Suppl. 3): 23–28, 1991.

POHL M., MAREŠ P.: Flunarizine influences metrazol-induced seizures in developing rats. *Epilepsy Res.* **1:** 302–305, 1987.

STAŇKOVÁ L., KOŽUCHOVÁ A., MAREŠ P.: Anticonvulsant effect of progabide in rats during ontogenesis. *Physiol. Res.* **46:**47–52, 1997.

WORMS P., DEPOORTERE H., DURAND A., MORSELLI P.L., LLOYD K.G., BARTHOLINI G.: Gamma-aminobutyric acid (GABA) receptor stimulation. I. Neuropharmacological profiles of Progabide (SL 76 002) and SL 75 102, with emphasis on their anticonvulsant spectra. *J. Pharmacol. Exp. Ther.* **220:** 660–671, 1982.

Reprint requests

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