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Antipentylenetetrazol Action of Clobazam in Developing Rats

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Summary

Clobazam (0.5 to 7.5 mg/kg i.p.) was tested against motor seizures elicited by pentylenetetrazol in rats 7, 12, 18, 25 and 90 days old. Minimal, predominantly clonic seizures with preserved righting ability were reliably induced by pentylenetetrazol and suppressed by clobazam in rats aged 18 days or more. The incidence of minimal seizures after clobazam pretreatment was not increased in 7- and 12-day-old rat pups. Generalized tonic-clonic seizures were markedly suppressed by clobazam in all age groups. In 18-day-old and older animals clobazam doses suppressing generalized seizures were always lower than those necessary for exerting an effect on minimal seizures. The differences in clobazam action appearing at various levels of maturation are only quantitative.

Key words

Clobazam • Pentylenetetrazol • Seizures • Ontogeny • Rat • Anticonvulsant action

Introduction

One of the basic features of benzodiazepines is their anticonvulsant action which has been described for many classical 1,4-benzodiazepines (for review Haefely 1989) as well as for other drugs that bind to benzodiazepine receptors (Depoortere *et al.* 1986, Haefely *et al.* 1990). This anticonvulsant action is exerted by an allosteric modulation of $GABA_A$ receptors (Haefely 1989, MacDonald 1995). Because the spectrum of activity of 1,5-benzodiazepine clobazam is similar to that of classical 1,4-benzodiazepine diazepam (Shenoy *et al.* 1982, Fielding and Hoffmann 1979), clobazam (7-chloro-1-methyl-5-phenyl-1,5-benzodiazepine-2,4-dione) was introduced into clinical practice as adjunct therapy for the epilepsies (Fischer and Blum 1995, Shorvon 1995). It is used not only in adult patients but also in pediatric epileptology in treating resistant epilepsies of infancy and childhood (Munn *et al.* 1988, Sheth *et al.* 1995). Preclinical data on the anticonvulsant action of clobazam demonstrated its efficacy in various seizure models in adult animals, most markedly in its action against minimal metrazol seizures, i.e. predominantly clonic seizures elicited by pentylenetetrazol (Fielding and Hoffmann 1979, Shenoy *et al.* 1982). Because of quantitative differences in the anticonvulsant action of some 1,4-benzodiazepines during postnatal development (Kubová and Mareš 1989, 1992) we decided to study the action of clobazam in rats at different ages. Developmental changes in the action of clobazam against another model in which this drug is effective – cortical epileptic afterdischarges (Hoogerkamp *et al.* 1996), was recently described in our laboratory (Šlamberová *et al.* 1998). Therefore we started to study clobazam effects against motor seizures induced by pentylenetetrazol. When an appropriate dose of pentylenetetrazol is used, two different seizure patterns may be elicited: at first minimal clonic seizures involving facial and forelimb

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muscles with preserved righting ability (described as threshold seizures by Löscher *et al.* 1991) and, after a longer latency, generalized tonic-clonic seizures. This allows to study two different models of seizures in one experiment. Minimal seizures were taken as a model of human absences (Swinyard 1973), but the analogy is no longer taken for granted so that these seizures more probably represent a model of human myoclonic seizures (Löscher and Schmidt 1988, Mareš and Zouhar 1988). Generalized tonic-clonic seizures are accepted as a model of human generalized convulsive seizures. The ontogenetic development of these seizures was described in detail in our laboratory (Velíšek *et al.* 1992) and the data for clonazepam (Kubová and Mareš 1989) and midazolam (Kubová and Mareš 1992) could be used for comparison.

Methods

Our experiments were performed in 248 male albino rats of the Wistar strain (Charles River Europe) in five age groups: 7, 12, 18, 25 and 90 days old. The animals were maintained under controlled conditions with 12/12 h light/dark cycle, temperature 24 °C. Water and food were accessible *ad libitum*. This experiment was performed in agreement with the guidelines of the Animal Protection Law of the Czech Republic and was approved by the Ethical Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic.

One hundred and ninety animals were injected with clobazam (generous gift of Hoechst Marrion Roussel, freshly dissolved in a mixture of propyleneglykol, ethanol and water in a ratio of 5:2:3) in doses of 0.5, 1, 2.5, or 5 mg/kg i.p. An additional dose of 7.5 mg/kg was used in 25-day-old rats only. Concentration of the clobazam solution varied to keep the injection volume constant (1 ml/kg). Each age and dose group consisted of 8-10 rats. A group of 58 control animals was injected with the solvent in a volume of 1 ml/kg (corresponding to the volume of clobazam injections); individual age groups consisted of 10-14 rats. Ten minutes later, pentylenetetrazol (Sigma, St. Louis, Mo, freshly prepared water solution) was administered subcutaneously in a dose of 100 mg/kg in all age groups with the exception of the 18-day-old animals where a dose of 90 mg/kg was used because of the higher sensitivity of this group (Velíšek *et al.* 1992). The rats were then observed for 30 min in isolation, the body temperature of rat pups being maintained by a heating pad. The incidence and latency of the two seizure types was registered. During the generalized tonic-clonic seizures the presence of the tonic phase and involvement of the forelimbs and hindlimbs were also recorded. Besides this, all other behavioral phenomena were also registered.

To quantify the severity of seizures a five-point scale was used (Pohl and Mareš 1987):

1 - isolated myoclonic jerks
2 - incomplete minimal seizures (only some elements present)
3 - minimal seizures
4 - generalized seizures without a tonic phase
5 - complete generalized tonic-clonic seizures

An average score was then calculated for each age and dose group. Statistical evaluation was performed using SigmaStat (Jandel). Fisher's exact test was used to evaluate the incidence of seizures, whereas ANOVA on Ranks with subsequent comparisons by Dunn's test was used in the cases of latency and score. The level of statistical significance was always set at 5%.

Results

Minimal seizures

These seizures were elicited reliably in control animals aged 18 days or more. In contrast, only a minority of younger pups exhibited this type of seizures. There was no change in 7-day-old rat pups pretreated with clobazam. The same was true for 12-day-old animals with the exception of the lowest dose of clobazam (0.5 mg/kg) after which four out of eight rat pups exhibited this type of seizure. Minimal seizures were suppressed by clobazam in those age groups where they were regularly present (Fig. 1). This action was especially pronounced in 18-day-old rats in which even the 2.5 mg/kg dose significantly diminished the incidence of minimal seizures. On the contrary, the 5 mg/kg dose was ineffective in 25-day-old animals; the level of significance was reached only with the highest dose (7.5 mg/kg). No systematic changes were seen in the latencies of this type of seizure (data not shown).

Generalized tonic-clonic seizures (GTCS)

Under the control conditions GTCS were elicited in all age groups. They consisted of a short intial phase of wild running followed by a tonic phase (usually extension of the forelimbs and flexion of the hindlimbs, tonic extension of the hindlimbs being not common in this

strain of rats). The tonic phase lasted about 10 s and was replaced by a clonic phase which sometimes persisted up to the end of the observation period. Righting reflexes were lost at the beginning of the tonic phase and recovered shortly after the end of the clonic phase. Clobazam suppressed the GTCS in a dose-dependent manner (Fig. 2). In animals where minimal seizures could be regularly elicited (18-day-old and older), the effective doses for GTCS suppression were lower than those necessary for abolishing minimal seizures. The effect against GTCS was most expressed in 12-day-old rat pups (1 mg/kg dose was anticonvulsant), whereas in all other age groups significant changes were observed only after doses of 2.5 mg/kg or higher. Restriction of the tonic phase to forelimbs and/or specific suppression of this phase were seen in the two youngest groups. Comparing the incidence of the tonic phase between age grups after the 1 mg/kg dose, the differences between 7- or 12-day-old pups and any of the older groups were significant.

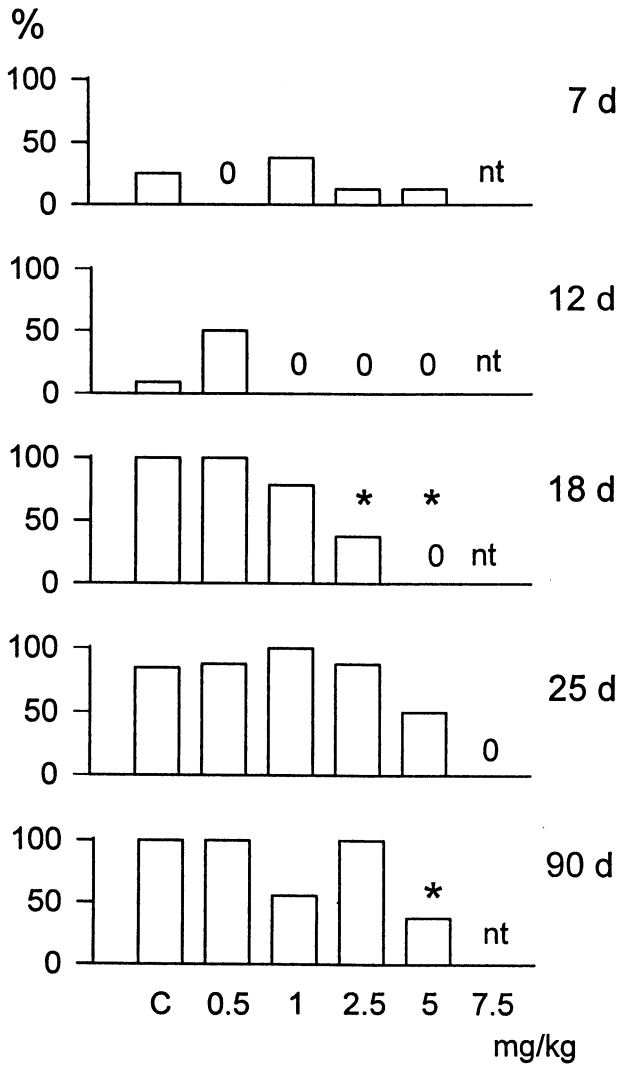

Fig. 1. *Incidence of minimal seizures in 7-, 12-, 18-, 25- and 90-day-old rats (from top to bottom). Abscissa – doses of clobazam (C denotes control group), ordinates – percentage of animals exhibiting seizures. 0 – seizures did not appear, nt – dose was not tested. Significant differences (P<0.05) in comparison with the control group are marked by asterisks.*

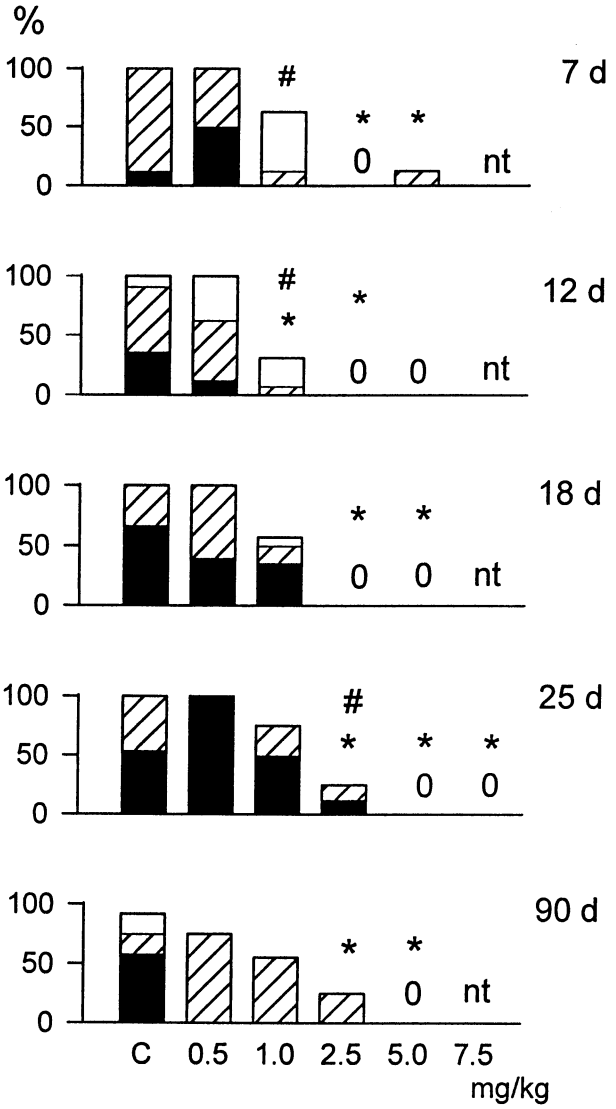

Fig. 2. *Incidence of generalized tonic-clonic seizures (GTCS) in five age groups. Black parts of the columns denote complete GTCS (tonic compl), stripped parts represent GTCS where tonic phase was restricted to forelimbs (tonic fl), white parts indicate generalized clonic seizures (clonic). Asterisks mark significant differences in the incidence of GTCS, # denotes differences in the incidence of the tonic phase. Other details as in Fig. 1.*

Severity of seizures

Scores reflecting seizure severity were equal to 5.0 or very close to this value under the control conditions (Fig. 3). Clobazam led to a dose-dependent decrease in all age groups due to a suppression of GTCS. This effect was more marked in rat pups aged 7, 12 and 18 days than in older animals; intergroup comparison of the effects of the 2.5 mg/kg dose demonstrated significant differences between each of the three younger groups and 25- or 90-day-old animals. Clobazam was especially efficient in 12-day-old pups where even the 1 mg/kg dose significantly decreased the score. In contrast, adult rats exhibited a significant effect only after the dose of 5 mg/kg.

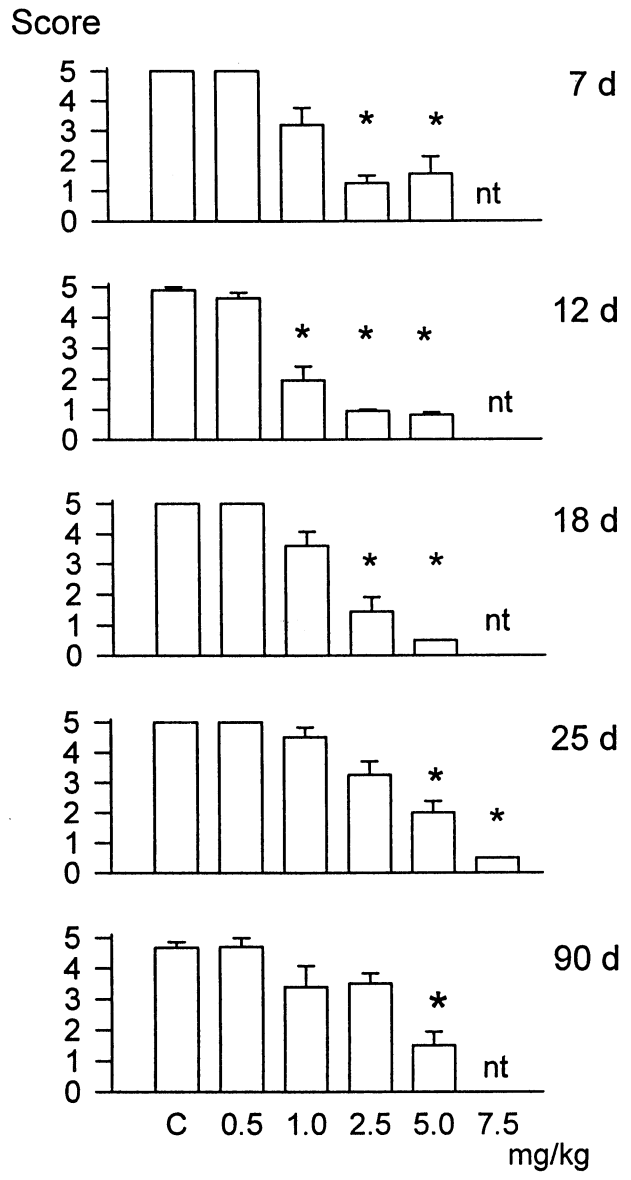

Fig. 3. *Severity of seizures in five age groups expressed as a mean score (± S.E.M.) according to a five-point scale. For other legend see Fig. 1.*

Discussion

Clobazam exhibited a marked anticonvulsant action in all the age groups studied. Similarly to other benzodiazepines, it suppressed both minimal and generalized tonic-clonic seizures, GTCS being more sensitive to clobazam action as it was in the case of clonazepam or midazolam action (Kubová and Mareš 1989, 1992). In contrast to 1,4-benzodiazepines clonazepam and midazolam, clobazam did not systematically increase the incidence of minimal seizures in the two youngest groups. Quantitative differences were found during maturation: clobazam was more efficient in the animals aged 7, 12 and 18 days than in the 25-day-old or adult rats. This ontogenetic development of sensitivity to anticonvulsant action of clobazam differs from that found in our recent study where cortical afterdischarges were used as a model of epileptic seizures (Šlamberová *et al.* 1998). Rats aged 25 days exhibited the highest sensitivity to clobazam. In addition to a suppression of the progressive prolongation of afterdischarges seen in all age groups, 25-day-old rats exhibited a significant shortening of afterdischarges after clobazam. Similarly, the intensity of movements accompanying the stimulation of the sensorimotor cortex (a presumptive measure of excitability of the motor system) as well as the intensity of clonic seizures during the afterdischarges (indicating a spread of epileptic activity from a generator to the motor system) were decreased by clobazam in 25-day-old animals. The difference in the action of clobazam in the two models cannot be due to pharmacokinetic factors. The identical doses were administered intraperitoneally to the same age groups, i.e. 12-, 18- and 25-day-old rat pups from the same animal house maintained under identical conditions. In addition, both series of experiments were performed at the same time. This difference (maximum effects in different age groups) thus suggests an uneven development of sensitivity of the mechanisms and/or structures generating cortical epileptic afterdischarges and the two types of motor seizures induced by pentylenetetrazol. This is especially interesting when cortical epileptic afterdischarges and minimal metrazol seizures are compared because the motor pattern of these seizure models is practically the same. In spite of this similarity a difference in sensitivity between cortical epileptic afterdischarges and minimal metrazol seizures was found with other drugs in adult rats – ketamine suppresses cortical afterdischarges leaving minimal seizures intact (Velíšek *et al.* 1989, Mareš *et al.* 1992),

while ethosuximide is very effective against minimal seizures and has no significant effect on cortical afterdischarges (Mareš *et al.* 1997). Different pharmacological effects were also found in developing rats. Progabide, a mixed $GABA_A/GABA_B$ agonist, is active against cortical epileptic discharges (Polášek *et al.* 1996), whereas minimal seizures are not suppressed by progabide as well as by its metabolite SL 75102 (Staňková *et al.* 1997, Kubová *et al.* 1997). The generator of pentylenetetrazol-induced minimal seizures was localized into the basal forebrain (Browning 1985), but the generator of clonic seizures accompanying cortical afterdischarges is not known. However, the presence of an EEG spike-and-wave rhythm suggests a thalamocortical mechanism (Steriade and Deschenes 1984), although eventually the motor system can be involved. If the generator in the motor system is the same as in the case of minimal metrazol seizures, then the developmental difference may be due to the sensitivity of the triggering structures or systems.

There is an interesting difference between clobazam and 1,4-benzodiazepines found in the two youngest groups: clonazepam and midazolam pretreatment of these animals led to a reliably high incidence of minimal metrazol seizures, i.e. a pattern very uncommon under control conditions (Kubová and Mareš 1989, 1992). In contrast, clobazam did not influence minimal seizures. This finding taken together with excellent efficacy of clobazam against generalized tonic-clonic seizures makes highly improbable one of the possible explanations of the appearance of minimal seizures in the youngest groups after the combination of benzodiazepines and pentylenetetrazol mentioned in our previous papers, i.e. the unmasking of minimal seizures by suppression of GTCS or at least by prolongation of their latency.

The difference between clobazam and 1,4-benzodiazepines might be explained by their affinities to individual subtypes of benzodiazepine receptors. Unfortunately, we have not found any data on clobazam binding to specific recombinant receptors. Ontogenetic studies of benzodiazepine receptors demonstrated their presence in the rat brain from the first postnatal days (Braestrup and Nielsen 1978, Candy and Martin 1979). Two basic types, which were classified on the basis of sensitivity to some ligands (Klepner *et al.* 1979) exhibited a different ontogeny. Type 1 receptors (abundant in the mature cerebellum) represent only a small percentage at birth and their number starts to increase in the third postnatal week, whereas type 2 receptors (typical for the hippocampus) predominate at birth and increase rapidly during the first postnatal week (Lippa *et al.* 1981). More detailed data may only be found for the neocortex but not for structures generating the two types of motor seizures elicited by pentylenetetrazol – basal forebrain (generator of minimal clonic seizures) (Browning 1985) and brainstem (generator of GTCS) (Browning and Nelson 1986).

An alternative explanation of the difference between clobazam and 1,4-benzodiazepines might be found in the partially agonistic properties of clobazam. N-desmethylclobazam, a metabolite of clobazam, was demonstrated to exhibit such properties (Muller *et al.* 1986). Unfortunately, N-desmethylclobazam is not a major metabolite of clobazam in rats (in contrast to mice) (Caccia *et al.* 1980b), its concentration in rat brain is very low (Caccia *et al.* 1980a) and we were unable to find analogous data for clobazam.

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References

BRAESTRUP C, NIELSEN M: Ontogenetic development of benzodiazepine receptors in the rat brain. *Brain Res* **147:** 170-173, 1978.

BROWNING RA: Role of brain-stem reticular formation in tonic-clonic seizures: lesion and pharmacologic studies. *Fed Proc* **44:** 2425-2431, 1985.

BROWNING RA, NELSON DK: Modification of electroshock and pentylenetetrazol seizure patterns in rats after precollicular transections. *Exp Neurol* **93:** 546-556, 1986.

CACCIA S, GUISO G, GARATTINI S: Brain concentrations of clobazam and N-desmethylclobazam and antileptazol activity. *J Pharm Pharmacol* **32:** 295-296, 1980a.

CACCIA S, GUISO G, SAMANIN R, GARATTINI S: Species differences in clobazam metabolism and antileptazol effect. *J Pharm Pharmacol* **32:** 101-103, 1980b.

CANDY JM, MARTIN IL: The postnatal development of benzodiazepine receptor in the cerebral cortex and cerebellum of the rat. *J Neurochem* **32:** 655-658, 1979.

DEPOORTERE H, ZIVKOVIC B, LLOYD KG, SANGER DJ, PERRAULT G, LANGER SZ, BARTHOLINI G: Zolpidem, a novel non-benzodiazepine hypnotic. I. Neuropharmacological and behavioral effects. J *Pharmacol Exp Ther* **237:** 649-658, 1986.

FIELDING S, HOFFMANN I: Pharmacology of anti-anxiety drugs with special reference to clobazam. *Br J Clin Pharmacol* **7:** 7S-15S, 1979.

FISCHER RS, BLUM D: Clobazam, oxcarbazepine, tiagabine, topiramate, and other new antiepileptic drugs. *Epilepsia* **36** (Suppl 2): S105-S114,1995.

HAEFELY W: Benzodiazepines: mechanism of action. In: *Antiepileptic Drugs.* Third Edition. RH LEVY, FE DREIFUSS, RH MATTSON, BS MELDRUM, JK PENRY (eds), Raven Press, New York, 1989, pp 721-734.

HAEFELY W, MARTIN JR, SCHOCH P: Novel anxiolytics that act as partial agonists at benzodiazepine receptors. *Trend Pharmacol Sci* **11:** 452-456, 1990.

HOOGERKAMP A, ARENDS RH, BOMERS AM, MANDEMA JW, VOSKUYL RA, DANHOF M: Pharmacokinetic/pharmacodynamic relationship of benzodiazepines in the direct cortical stimulation model of anticonvulsant effect. *J Pharmacol Exp Ther* **279:** 803-812,1996.

KLEPNER CA, LIPPA AS, BENSON DI, SANO MC, BEER B: Resolution of two biochemically and pharmacologically distinct benzodiazepine receptors. *Pharmacol Biochem Behav* **11:** 457-462, 1979.

KUBOVÁ H, MAREŠ P: Time course of the anticonvulsant action of clonazepam during ontogenesis in the rat. *Arch Int Pharmacodyn Ther* **298:** 15-24, 1989.

KUBOVÁ H, MAREŠ P: The effect of ontogenetic development on the anticonvulsant activity of midazolam. *Life Sci* **50:** 1655-1672, 1992.

KUBOVÁ H, HAUGVICOVÁ R, MAREŠ P: Anticonvulsant effect of SL 75 102 in adult and immature rats. *Physiol Res* **46:** 73-76, 1997.

LIPPA AS, BEER B, SANO M, VOGEL RA, MEYERSON LR: Differential ontogeny of type 1 and type 2 benzodiazepine receptors. *Life Sci* **28:** 2343-2347, 1981.

LÖSCHER W, SCHMIDT D: Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. *Epilepsy Res* **2:** 145-181, 1988.

LÖSCHER W, HÖNACK D, FASSBENDER CP, NOLTING B: The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentylenetetrazole seizure models. *Epilepsy Res* **8:** 171-189, 1991.

MACDONALD RL: Benzodiazepines: Mechanisms of action. In: *Antiepileptic Drugs.* Fourth Edition. RH LÉVY, RH MATTSON, BS MELDRUM (eds), Raven Press, New York, 1995, pp 695-703.

MAREŠ P, ZOUHAR A: Do we posses adequate models of childhood epilepsies? *Physiol Bohemoslov* **37:** 1-9, 1988.

MAREŠ P, LANŠTIAKOVÁ M, VAŇKOVÁ S, KUBOVÁ H, VELÍŠEK L: Ketamine blocks cortical epileptic afterdischarges but not paired-pulse and frequency potentiation. *Neuroscience* **50:** 339-344, 1992.

MAREŠ P, KUBOVÁ H, MOCKOVÁ M: Two models of epileptic spike-and-wave rhythm in rats are differently influenced by ethosuximide. *Physiol Res* **46:** 397-402, 1997.

MULLER WE, GROH B, BUB O, HOFMANN HP, KREISKOTT H: In vitro and in vivo studies of the mechanism of action of arfendazam, a novel 1,5-benzodiazepine. *Pharmacopsychiatry* **19:** 314-315, 1986.

MUNN R, CAMFIELD P, CAMFIELD C, DOOLEY J: Clobazam for refractory childhood seizure disorders – a valuable supplementary drug. *Can J Neurol Sci* **15:** 406-408, 1988.

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

POLÁŠEK R, KUBOVÁ H, ŠLAMBEROVÁ R, MAREŠ P, VORLÍČEK J: Suppression of cortical epileptic afterdischarges in developing rats by anticonvulsants increasing GABAergic inhibition. *Epilepsy Res* **25:** 177-184, 1996.

SHENOY AK, MIYAHARA JT, SWINYARD EA, KUPFERBERG HJ: Comparative anticonvulsant activity and neurotoxicity of clobazam, diazepam, phenobarbital, and valproate in mice and rats. *Epilepsia* **23:** 399-408, 1982.

SHETH RD, RONEN GM, GOULDEN KJ, PENNY S, BODENSTEINER JB: Clobazam for intractable pediatric epilepsy. *J Child Neurol* **10:** 205-208, 1995.

SHORVON SD. Benzodiazepines: clobazam. In: *Antiepileptic Drugs*. Fourth Edition. RH LÉVY, RH MATTSON, BS MELDRUM (eds), Raven Press, New York, 1995, pp 763-777.

ŠLAMBEROVÁ R, MAREŠ P, VORLÍČEK J: Clobazam exerts an anticonvulsant action in immature rats. *Physiol Res* **47:** 301-305, 1998.

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

STERIADE M, DESCHENES M: The thalamus as a neuronal oscillator. *Brain Res Rev* **8:** 1-62, 1984.

SWINYARD EA: Assay of antiepileptic drug activity in experimental animals: standard tests. In: *Antiepileptic Drugs,* Vol 1, J MERCIER (ed), *Int Encycl Pharmacol Ther*, Sect 19, Pergamon Press, Oxford, 1973, pp 47-65.

VELÍŠEK L, MIKOLÁŠOVÁ R, BLANKOVÁ-VAŇKOVÁ S, MAREŠ P: Effects of ketamine on metrazol-induced seizures during ontogenesis in rats. *Pharmacol Biochem Behav* **32:** 405-410, 1989.

VELÍŠEK L, KUBOVÁ H, POHL M, STAŇKOVÁ L, MAREŠ P, SCHICKEROVÁ R: Pentylenetetrazol-induced seizures in rats: an ontogenetic study. *Naunyn-Schmiedeberg's Arch Pharmacol* **346:** 588-591, 1992.

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