Anticonvulsant action of a new analogue of allopregnanolone in immature rats

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Summary

Neuroactive steroids represent potential antiepileptic drugs. We tested a newly synthesized analogue of allopregnanolone 3α-hydroxy-21ξ,22-oxido-21-homo-5α-pregnan-20-on (HOHP) against two types of pentylenetetrazol-induced seizures (100 mg/kg s.c.) in 12- and 25-day-old rats. Ganaxolone, a neuroactive steroid in clinical trials, served as a reference drug. Pretreatment with either steroid suppressed generalized tonic-clonic seizures in both age groups, their efficacy was comparable. HOHP as well as ganaxolone were more active in 12- than in 25-day-old rats (effective doses were 40 and 60 mg/kg, respectively). Minimal clonic seizures, which can be elicited only in 25-day-old rats were not influenced by any drug. Very short duration of anticonvulsant action of HPOP demonstrated in 12-day-old animals indicates that this drug might be used only in acute treatment in epileptology.

Key words: neuroactive steroids – ganaxolone – convulsions – pentylenetetrazol - developing rats

Reason for a search for new antiepileptic drugs is pharmacoresistance present in approximately 30% of epileptic patients (Kwan and Sander 2004). Research is focused mainly on known mechanisms of anticonvulsant action. One of these mechanisms is potentiation of GABAergic inhibition (Rogawski and Löscher 2004). Neuroactive steroids represent a group of potential anticonvulsant drugs with a specific effect on GABA-A receptors – positive allosteric modulation (for review Rogawski and Reddy 2004, Reddy – in press). Their anticonvulsant action is due to a prolongation of opening time of chloride channel (Akk et al. 2004) and is markedly expressed if GABA-A receptors contain a delta subunit (Wohlfarth et al. 2002). Neurosteroids exhibit good anticonvulsant action in various seizure models in adult rodents (for review Reddy and Woodward 2004). A disadvantage of natural neurosteroids (e.g. allopregnanolone) is their short biological half-life, therefore some derivatives are synthesized with the aim to have a drug which is not quickly metabolized. One of these neuroactive steroids with biological half-life in order of hours, ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one, Gasior et al. 2000) is now in early clinical trials (Reddy and Woodward 2004). We started to study the anticonvulsant action of neuroactive steroids against pentylenetetrazol-induced seizures in immature rats. Studies in immature animals are important because of a fact that many epilepsies start in infancy and childhood (Hauser 1998). A good anticonvulsant action of a newly synthesized steroid THDOC conjugate (triethylammonium 3 –hydroxy-20-oxo-5 pregnan-21yl hydrogensuccinate), pregnanolone and allopregnanolone as a reference drug was found at all developmental stages studied but the action of all three steroids was short especially in 25-day-old, i.e. prepubertal rats (Mareš et al. 2006a,b). In an effort to find a molecule with long duration of action various homologues of allopregnanolone were synthesized in the Institute of Organic Chemistry and Biochemistry of the Academy of Sciences (Kasal et al. – in preparation). According to binding studies 3α-hydroxy-21ξ,22-oxido-21-homo-5α-pregnan-20-on (HOHP) seemed to be perspective and therefore we started to study its anticonvulsant action. Experiments performed in two age groups of Wistar albino rats (12 and 25 days old) were approved by Animal Care and Use Committee of the Institute of Physiology to be in agreement with Animal Protection Law of the Czech Republic and

ECC directives 86/609/EEC. The choice of these age groups was based on our previous results (Mareš et al. 2006a,b).

An anticonvulsant action of HOHP was tested against motor seizures elicited by pentetrazol. As a reference drug served ganaxolone (Sigma, MO). Either steroid was suspended using a drop of Tween 80. The concentration of the drugs was 5 mg/ml. Animals were taken from their mothers and immediately injected intraperitoneally with HOHP or ganaxolone in doses of 10, 20, 40 or 60 mg/kg. Younger age group was kept on a pad electrically heated to 34^{o}C (i.e.temperature in the nest). Twenty minutes later pentetrazol (Sigma, MO) was administered subcutaneously in a dose of 100 mg/kg. Rats were then observed in isolation for 30 minutes, incidence and pattern as well as latencies of seizures were registered. Severity of seizures was scored according to the 5-point scale (Pohl and Mareš 1987). Each age and dose group consisted from 6-8 animals, control groups were substantially bigger (18 and 16 rats for the two age groups) because they were formed by rats from more experiments performed in the last two years. ANOVA with subsequent pairwise comparison by Bonferroni test (SigmaStat SYSTAT) was used for statistical evaluation with 5% as a level of statistical significance.

Control rats exhibited a 100% incidence of generalized tonic-clonic seizures (GTCS) in both age groups. Minimal seizures could be elicited only in 25-day-old animals (7 out of 8 control rats exhibited this type of seizures). The highest dose of both ganaxolone and HPOP significantly decreased the incidence of GTCS in either age group. In addition, the 40-mg/kg dose of both steroids decreased the incidence of GTCS in 12-day-old rats. The outlined tendency in 25-day-old animals (with 40-mg/kg dose of either drug GTCS were suppressed in 4 out of 8 rats) did not reach the level of significance ($p=0.077$). Incidence of minimal clonic seizures remained untouched by the two neuroactive steroids.

Seizure severity reflected presence or failure of GTCS. It was significantly decreased by 40- and 60-mg/kg doses of HOHP (Fig.1) as well as ganaxolone (Fig.2) in 12-day-old rats and only by the 60-mg/kg dose of either drug in 25-day-old animals.

Latencies of GTCS were not changed by ganaxolone in 12-day-old rats whereas the 40-mg/kg dose of HOHP resulted in a significant prolongation in this age group. Older age group exhibited an increase of these latencies only after the 10-mg/kg dose of

ganaxolone, other doses did not lead to significant changes (Fig.2). HOHP exhibited a dose-dependent tendency to prolongation of GTCS latencies but due to high variability of results the level of significance was not reached (Fig.1). Minimal clonic seizures were elicited only in 25-day-old rats and their latencies were also prolonged but level of statistical significance was reached only with some doses (Figs.1,2).

Anticonvulsant action of the 60-mg/kg dose of HOHP in 12-day-old rats was present 60 (only 3 out of 6 rats exhibited GTCS) but not 120 and 180 min after the administration.

Newly synthesized neuroactive steroid HPOP exhibited similar action as ganaxolone, i.e. it suppressed GTCS and did not change minimal clonic seizures. Even the effective doses were the same, there was a tendency of HOHP to be a little more efficient than ganaxolone but there was no statistically significant difference. Allopregnanolone, pregnanolone and THDOC exhibit the same spectrum of action but in contrast to the two drugs studied in present experiments they are able to suppress GTCS at lower doses (20, 10 and 10 mg/kg for these three steroids, respectively). Changes in the molecule did not fulfill our expectations that this new homologue of allopregnanolone will not be quickly catabolized.

Our present study also demonstrated age differences in the action of both drugs. Anticonvulsant effect was more expressed in 12- than in 25-day-old rats; even the 40-mg/kg dose of either drug resulted in a significant change in the younger group. Again, similar results were obtained with ganaxolone in flurothyl seizures (anticonvulsant action was stronger in 15-day-old rats than in older animals - Liptáková et al. 2000) and with allopregnanolone, pregnanolone and THDOC in PTZ-induced seizures (Mareš et al. 2006a,b) as well as in cortical epileptic afterdischarges (Mareš 2005).

A serious drawback of neuroactive steroids is a short duration of their action. It was demonstrated with our previous drug THDOC and therefore very short duration of anticonvulsant effect of HOHP even in 12-day-old rats will surely prevent its possible clinical use. An open question to be answered is an acute use of neuroactive steroids in status epilepticus. Such a possibility is indicated by efficacy of neuroactive steroids in kainate and pilocarpine models of status in adult mice (Kokate et al. 1996).

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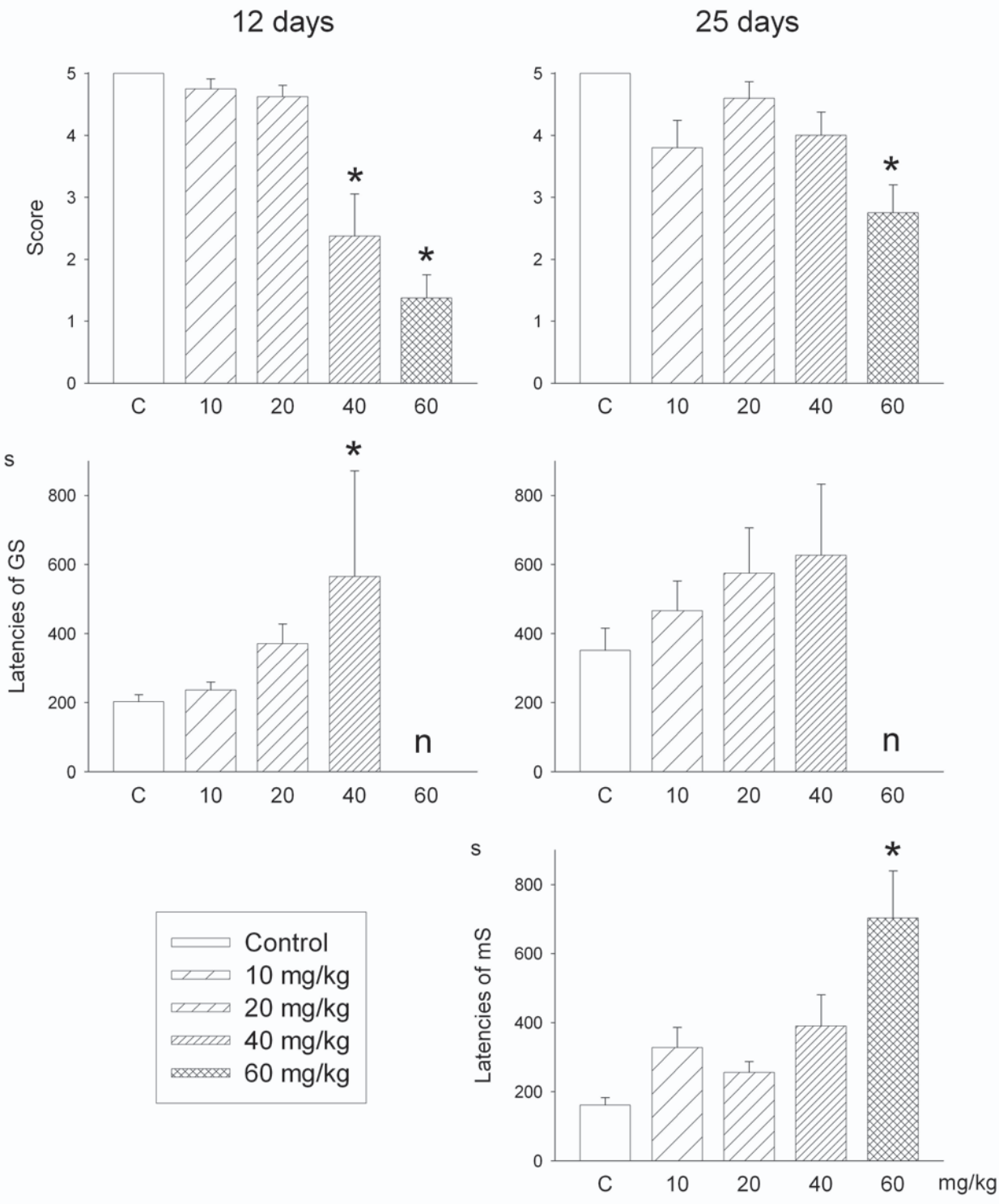
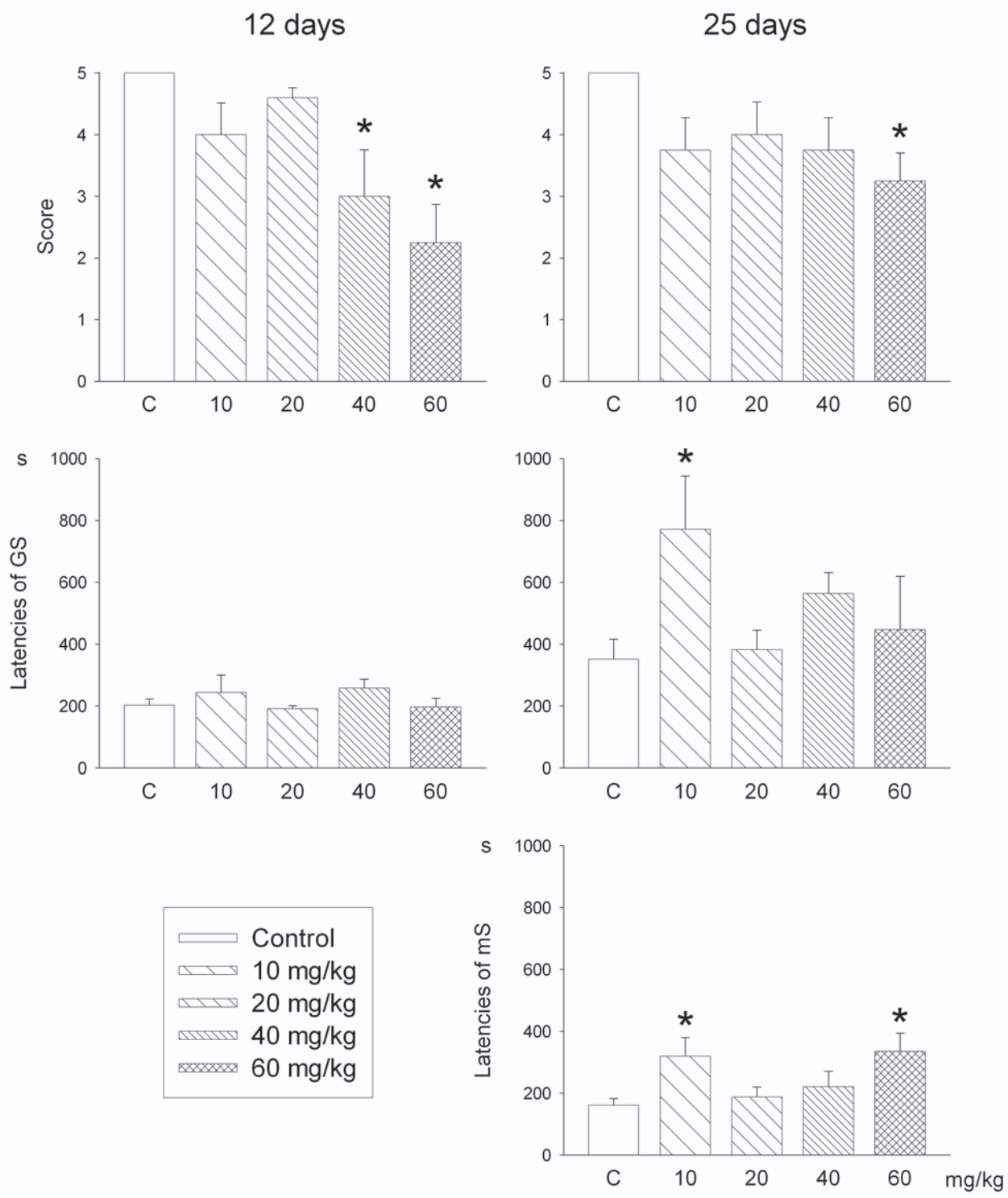
Text to Figures:

1. Effects of 3α-hydroxy-21ξ,22-oxido-21-homo-5α-pregnan-20-on (HOHP) on convulsions elicited by pentylenetetrazol in 12- (left column) and 25-day-old (right column) rats. The upper two graphs demonstrate severity of seizures (five-point scale), the middle row shows latencies of generalized tonic-clonic seizures and the lowest graph demonstrates latencies of minimal clonic seizures (this type of seizures cannot be elicited by pentylenetetrazol in 12-day-old rats). Abscissae in all graphs: doses of the steroid, C means a control group; ordinates in the top row – score of seizure severity, in the remaining three graphs – latencies in seconds. Asterisks denote a significant difference in comparison with appropriate control group, n means that generalized seizures were not present in the group.
2. Effects of ganaxolone. All details as in Fig.1.

PTZ-induced seizures - HOHP




PTZ-induced seizures - Ganaxolon
12 days
25 days
Score
Latencies of GS
Latencies of mS
s
C
10
20
40
60
mg/kg
Control
10 mg/kg
20 mg/kg
40 mg/kg
60 mg/kg