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Derivatives of valproic acid are active against pentetrazol-induced seizures in immature rats

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Summary Propylisopropyl acetamide (PID) and valnoctamide (VCD) are two CNS-active constitutional isomers of valproic acid (VPA) corresponding amide (and prodrug) valpromide. VPA is a major antiepileptic drug (AED) used also in children. Consequently, the purpose of the current study was to see if PID, VCD and two of VCD stereoisomers are active also in juvenile anticonvulsant animal seizure models.

Rat pups 7, 12, 18 and 25 days old were pretreated with PID, VCD or the VCD stereoisomers (2S,3S)-VCD, and (2R,3S)-VCD and 30 min later pentetrazol (100 mg/kg s.c.) was administered. The incidence of seizures, their expression pattern and their latencies were registered and the severity was expressed by means of a five-point scale.

All four tested compounds exhibited anticonvulsant activity against generalized tonic—clonic seizures. Lower doses suppressed specifically the tonic phase in 7-, 12- and 18-day-old rats, while higher doses abolished both phases of generalized seizures. This effect was most pronounced in 12-day-old rats. Twenty-five-day-old rats exhibited suppression of the entire pattern of generalized seizures. There were no significant differences among the drugs used.

The CNS-active amide derivatives of VPA, VCD (racemate or individual stereoisomers) and PID exhibit potent anticonvulsant activity against generalized convulsive seizures in developing rats. The majority of these developmental effects are quantitative; while a specific selective action on the tonic phase of generalized seizures is the main qualitative change found in our study.

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Introduction

Valproic acid (VPA) is used as a first-line antiepileptic drug, especially against generalized seizures. The potent anticonvulsant action of VPA is compromised by its side effects — teratogenic (Wyszynski et al., 2005) and idiosyncratic hepatotoxicity (Bryant and Dreifuss, 1996). Derivatives of VPA are designed and evaluated with the aim to develop second generation VPA drugs that will be more efficacious than VPA, and that could be devoid of these negative side effects (Bialer and Yagen, 2007). Among these central nervous system (CNS)-active VPA derivatives are propylisopropyl acetamide (PID) and valnoctamide (VCD). They are two chiral constitutional isomers of VPA corresponding amide (and prodrug) valpromide (Isoherranen et al., 2003a). VCD and PID and their stereoisomers are non-teratogenic (mice) and are more potent anticonvulsants than VPA in a number of seizure models in mature animals (Isoherranen et al., 2003a,b; Kaufmann et al., 2010). These characteristics make them potential candidates to become follow-up compounds or second generation drugs to VPA (Bialer and Yagen, 2007; Bialer and White, 2010). Because VPA is frequently used in children, the current study evaluates the activity of VPA derivatives in immature rats against pentetrazol (PTZ)-induced convulsions. A descriptive study of the anticonvulsant effects at multiple stages of postnatal maturation is necessary before analyses of mechanism of action and possible side effects.

PTZ is able to induce, in a dose-dependent manner, the following three different types of epileptic seizures in adult rats: (1) EEG episodes of spike-wave rhythm with minute motor counterparts (model of absence seizures — Snead, 1992), (2) minimal seizures (mS, clonic seizures with negligible tonic component — a model of myoclonic seizures) and (3) generalized tonic—clonic seizures (GTCS, Velíšek et al., 1992). The first two types of seizures are age-dependent and can be elicited by systemic PTZ injection after the second week of postnatal life. Generalized tonic—clonic seizures are present in rats at all developmental stages. The two types of convulsive seizures were used in the present study. An appropriate dose of PTZ is able to elicit minimal clonic seizures initially, and after a longer latency, generalized tonic—clonic seizures in rats more than 2 weeks old. Younger animals display only generalized tonic—clonic seizures.

Some drugs exhibit qualitative or quantitative changes of their anticonvulsant action during postnatal development (Mareš, 1998, 2009). The current study had two objectives. The first objective was to test the anticonvulsant effects of PID, VCD, and two of VCD stereoisomers (2S,3S)-VCD and (2R,3S)-VCD in immature animals. The second objective was to see if the action of these drugs changes during development.

Methods

Animals

Four age groups of male Wistar albino rats were used: 7, 12, 18 and 25 days old. These age groups were chosen because they cover wide extent of development. Seven-day-old rats correspond to human preterm newborn, and 25-day-old rats correspond to prepubertal children (Andersen, 2003, McCutcheon and Marinelli, 2009). Rats were removed from the nests immediately before the experiment and body temperature of animals in the three younger groups was maintained by means of pads electrically heated to $34 \pm 1^{\circ}C$ (i.e. to the temperature of the nest). The experiments were approved by the Animal Care and Use Committee of the Institute of Physiology ASCR and were in accordance with the Animal Protection Law of the Czech Republic, which is fully compatible with the European Community Council directives 86/609/EEC.

PID, VCD and its stereoisomers ((2S,3S)-VCD and (2R,3S)-VCD) (Isoherranen et al., 2003a,b) were dissolved in dimethylsulfoxide (DMSO) at a concentration of 12.5 mg/ml. PTZ (Sigma, St. Louis, MO) was dissolved in distilled water at a concentration of 50 mg/ml. All solutions were prepared immediately before the experiment.

Procedure

Rats were pretreated intraperitoneally 30 min before the subcutaneous injection of PTZ (100 mg/kg) with PID, VCD, (2S,3S)-VCD or (2R,3S)-VCD. The dose of 12.5 mg/kg was used as a starting dose and other doses (6.25; 18.75; 25; 37.5; 50 and 62.5 mg/kg) were added individually in different drug and age groups according to the results. Control animals were pretreated with DMSO in a volume corresponding to the 50-mg/kg dose, i.e. 4 ml/kg. Rats were then observed in individual Plexiglas cages for 30 min after PTZ injection. Incidence and latency of two types of seizures (minimal and generalized tonic—clonic seizures) were recorded. Severity of seizures was measured using a five-point scale (Pohl and Mareš, 1987, Mareš, 2012). To avoid problems with seizure types that are present only at some stages of maturation, 50% effective doses were calculated for generalized seizures, i.e. the seizure type that is present with the same semiology throughout whole development. The individual age, drug, and dose groups were formed by 6—8 animals, and the control DMSO groups by 10—12 rats.

Statistics

The incidence of seizures was evaluated using a Fisher exact test. Latencies and seizure scores were evaluated by means of one way ANOVA with a subsequent multiple pairwise comparison using a Holm—Sidak test. These calculations were made with SigmaStat® software (SPSS). $P < 0.05$ was taken as statistically significant. ED_{50} values and their 95% confidence intervals (CI) were calculated for GTCS, generalized seizures without the tonic phase (GCS) and generalized seizures (GS, i.e. not differentiating between seizures with, or without, the tonic phase) using a FORTRAN probit analysis program (Finney, 1952; White et al., 2002).

Results

The solvent (DMSO), at a dose of 4 ml/kg (i.p), did not influence PTZ-induced seizures in any age group of rat pups. GTCS were observed in all control animals, mS (involving