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Original article

Circadian variation in anticonvulsant activity of valproic acid in mice

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ABSTRACT

Purpose: This study aims to investigate whether valproic acid (VPA) anticonvulsant activity varied according to circadian dosing-time in mice.
Methods: VPA was administered to mice at four circadian stages (1, 7, 13 and 19 h after light onset, (HALO)). The controls received a saline injection followed by a *s.c.* injection of pentylenetetrazol (PTZ) 30 min later. In pretreated animals, VPA was administered 30 min before PTZ administration.
Results: The results obtained showed that VPA-induced anticonvulsant activity depends on circadian dosing-time in mice. VPA has significantly increased the latency of the first clonic seizure at all circadian stages. This increase varied depending on the time of VPA pre-treatment, the highest and the lowest increases were recorded respectively at 7 and 19 HALO. The Cosinor analysis has also validated a circadian rhythm of this increase. The intensity of seizures in pretreated mice varied significantly according to circadian stage. The highest seizure intensity was recorded at 19 HALO. A circadian rhythm of the seizure intensity in VPA pretreated mice was detected, with an acrophase located at the middle of the dark span (Ø = 18.09 HALO ± 2.27 h).
Conclusion: The present findings provide evidence for a pronounced anticonvulsant effect of VPA when administered in the 2nd middle of the light-rest span in mice. These results might probably be due to the circadian variation of VPA pharmacokinetic since our previous studies showed that the optimal tolerance corresponded to the middle of the rest span, the time which induces the highest total plasma clearance.

1. Introduction

Chronobiology is the study of biological rhythms and the mechanisms of biological time keeping. This daily variation in biological functions is thought to affect the efficacy and/or toxicity of drugs. Moreover, the dosing of a medication at the proper biological time with reference to circadian rhythms can result in modulation of its efficiency or toxicity [1,2]. Dosing time-dependent differences in the therapeutic effects of drugs are, at least in part, due to circadian-related changes in drug disposition. The biological responses to various drugs follow circadian rhythms in experimental animals as well as in human beings [3]. Rhythm identification in animal models helps to provide an optimal dosing-time and to suggest guidelines for a potential chronotherapy [4]. The chronopharmacological studies of antiepileptic drugs are of considerable importance in optimizing therapeutic tolerance in patients and reducing these drugs' important adverse effects.

Valproic acid (VPA) is the most commonly used antiepileptic drug in the treatment of generalized epilepsy, and it is also effective in partial epilepsy [5]. It is a short-chained branched fatty acid and its structure differs from all other clinically used antiepileptic drugs since it has no cyclic moieties [6]. Several studies aims to both improving therapeutic activity and reducing side effects of this drug by synthesizing and testing derivatives of VPA to obtain new CNS-active compounds that could potentially become second-generation VPA drugs [7,8]. VPA action mechanism is probably due to a combination of several effects in the central nervous system because of its wide spectrum of activity against different types of seizures [6]. VPA increases the activity of the neurotransmitter Gamma Amino Butyrate (GABA) through several mechanisms, including inhibition of GABA degradation, inhibition of GABA transaminase, increased GABA synthesis, and decreased turnover [5,6]. VPA also attenuates N-Methyl-D-Aspartate-mediated excitation and blocks Na^+ channels, Ca^{2+} channels and voltage-gated K^+ channels [5].

The objective of this study was to investigate the time-dependent anticonvulsant activity of VPA after an intraperitoneal (*i.p.*) administration at four circadian times 1, 7, 13 and 19 h After

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Light Onset (HALO) using mice as an animal model. Pentylenetetrazol (PTZ) was used to experimentally induce seizures in animals; it is the most frequently used chemical convulsant for experimentally evaluate the effectiveness of a molecule against seizures [9]. PTZ acts predominantly by antagonizing GABA-ergic inhibition via an effect on the $GABA_A$ receptor [10]; the seizures induced by PTZ result from an increase in glutamatergic excitatory activity; this enhancement is a consequence of GABA-A receptors blockage because PTZ is a blocker of the picrotoxin site of the chloride ionophore of the GABAA receptor complex [11]. Several studies showed that an appropriate PTZ dose can elicit minimal clonic seizures initially, and after a longer latency, generalized tonic-clonic seizures in rodents [11,12].

2. Material and methods

2.1. Animals and experimental conditions

A total of 80 ten-week-old male Swiss albino mice (SIPHAT, Tunisia) were used throughout this study. All mice were synchronized for at least 3 weeks prior to the beginning of experiments. During this period, the mice were entrained in two air-conditioned rooms specially designed for chronobiological investigations under a lighting regimen consisting of an alternation of 12 h of light (L) and 12 h of darkness (D) (LD12:12). The light-dark regimen was inversed between the 2 rooms (Room1: L from 7 to 19; Room 2: L from 19 to 7) in order to allow the exploration of circadian times during the day [13]. The room temperature was maintained at $22 \pm 2\,^{\circ}C$ and the relative humidity was about 50–60%. During all experiments, a standard diet (Purina Rat Chow; SICO, Sfax, Tunisia) and water were provided ad libitum. In the present study, the entrainment was assessed by the circadian rhythmicity in rectal temperature, the acrophase (peak time) was used as a marker rhythm index and the rectal temperature was measured with a digital thermometer (OMRON Ecosmart, Holt 55005). All experiments were performed according to the guidelines of care and use of laboratory animals [14].

2.2. Drugs

The used compounds were kindly provided by Medis laboratories (Nabeul, Tunisia). The VPA solution was freshly prepared by adding an adequate volume of sterilized physiological saline with a few drops of Tween 80. Each dose was administered to mice by intraperitoneal (*i.p.*) route in a fixed fluid volume (10 ml/kg, body weight). PTZ was dissolved in 0.9% NaCl.

2.3. Study design

A preliminary study was conducted to determine the convulsive dose 97 (CD_{97}) (the dose inducing 97% of clonic seizures in mice). Different doses of PTZ (inducing from 0 to 100% clonic seizures) were prepared following a geometric progression with a ratio of 1.2 between each two successive doses (50, 60, 72, 86.4 and 103.6 mg/kg). 75 mice were used in and the drug was subcutaneous (*s.c.*) administrated at a single fixed time (11:00 h local time, i.e., 4 HALO) during the diurnal (rest) span to minimize the influence of circadian changes. Following PTZ injection, the mice were placed separately in transparent plexiglass cages ($25 \times 15 \times 10$ cm) and observed for 30 min for the occurrence of clonic seizures. The Probit method was used to estimate the PTZ activity and the CD_{97} was determined from curves expressed as percentage (%) of clonic seizures (converted to a probit scale) against the log-dosage of PTZ

Another preliminary study was conducted to determine the VPA effective dose 50 (ED_{50}) (the dose inducing 50% of the activity in mice). Different therapeutic doses of VPA were prepared (100, 200, 300, 400 and 500 mg/kg). A total of 60 mice were used for this preliminary study (10 mice for the control group and 10 mice for each VPA tested dose). The control group received a saline injection (NaCl 0.9%) by *i.p.* route followed, 30 min later, by a *s.c.* injection of PTZ (97 mg/kg). The drug was *i.p.* administrated (30 min before the *s.c.* PTZ injection) at a single fixed time (11:00 h local time, i.e., 4 HALO) during the diurnal (rest) span to minimize the influence of circadian changes. The Probit method was used and the ED_{50} value was estimated from curves expressed as percentage (%) of protection against clonic seizures (converted to a probit scale) as a function of the log-dosage of VPA.

The animals were randomly assigned to one of four groups of 20 mice in order to explore four circadian stages (1, 7, 13 and 19 HALO). The 40 control mice (10 mice/circadian stage) received a saline injection (NaCl 0.9%) by *i.p.* route followed by a *s.c.* injection of the PTZ CD97 previously determined (97 mg/kg). The 40 pretreated mice (10 mice/circadian stage) were *i.p.* injected with ED_{50} (182.6 mg/kg) of VPA 30 min before the *s.c.* PTZ (97 mg/kg) administration.

Following PTZ injection, the mice were placed separately in transparent plexiglass cages ($25 \times 15 \times 10$ cm) and observed for 30 min for the occurrence of clonic and tonic seizures. A clonic seizure activity was defined as clonus of the whole body and lasting over 3 s accompanied with a loss of righting reflex, whereas the tonic extension of the hind limbs was considered as the endpoint for tonic seizures.

Animal response to PTZ administration was evaluated using the following criteria: latency to first clonic seizure, latency to the generalized tonic-clonic seizure, protection percentage against clonic and/or tonic seizure, protection percentage against lethality and seizure intensity. A threshold convulsion was an episode of clonic spasms lasting for at least 5 s. The absence of convulsion for 30 min indicated a full protection. The number of animals protected in each group was recorded and the percentage of protection was calculated. Seizure intensity was measured according to a modified six-point Racine scale [15], which has also been used by numerous other authors [16,17]: Stage 0: no response; stage 1: hyperactivity, vibrissae twitching; stage 2: head nodding, head clonus and myoclonic jerk; stage 3: unilateral forelimb clonus; stage 4: generalized clonic convulsions; stage 5: generalized convulsions with a tonic extension episode and stage 6: mortality.

2.4. Statistical analysis

The means and standard deviation of the mean (S.D.) were computed for each circadian dosing-time. The two-way factorial analysis of variance (ANOVA) was used to test the significance of differences over time. Both the CD_{97} and ED_{50} values with their 95% confidence limits were calculated by computer log-probit analysis [18].

Animal synchronization and time series were analyzed for 24-h rhythm with the Cosinor method based on the least-squares method. For a given period, a rhythm was characterized by three parameters: the mesor (M) is the 24 h rhythm adjusted mean, the amplitude (A) is the half of the difference between maximal and minimal values of the derived cosine curve and the acrophase (Ø) is the peak time (with light onset 0 HALO used as phase reference) [19]. If a statistically significant rhythm was detected, the three parameters were computed with their respective 95% confidence limits.

All rhythm characteristics were obtained with their 95% confidence limits. In principle, a rhythm is detected when A is different from zero (non-null amplitude as verified by F test of the variance accounted by the fit of the time series data to cosine curve of a given period versus that accounted for by a straight line fit to