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RESEARCH ARTICLE

"EFFECT OF GENERALIZED SEIZURES ON SLEEP PATTERNS [AN ANIMAL MODEL]"

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Abstract

Background

An interaction between epilepsy and sleep has been established since long time ago. However, the Neurophysiological mechanisms responsible of this interaction are not well understood. The exact mechanisms underlying the precise nature of this relation remain still unclear. Comprehension of the complex mechanisms underlying the interrelationship between sleep and epilepsy cannot be fully obtained from studies with humans. Therefore animal models have played a fundamental role in advancing the understanding of basic mechanisms underlying this interrelationship.

Methods and findings

Electrophysiological recordings were done in chronically implanted rats under control conditions and during seizures induced by PTZ administration. Administration of PTZ induced progressively installed motor alterations. During the period of severe status epilepticus, electrical seizures became synchronized in the anterior and posterior recorded cerebral regions that coincided with motor events and both SWS and REM sleep were completely inhibited. When seizures stopped, both sleep phases remained still inhibited followed by a progressive recovery. At the same time, amount of wakefulness progressively decreased. Compensatory rebound of REM sleep was absent.

Conclusion

This animal model of generalized seizures shows significant

alterations of sleep characteristics.

Introduction

The association between epilepsy and sleep has been described since ancient times, documented as a complex interrelationship. Certain epileptic seizures tend to occur predominantly during determined sleep stages. Paroxysmal events of sleep may be related to parasomnias such as night terrors or may correspond to events of nocturnal epilepsy [1]. Sleep disorders in epileptic patients may be originated by epileptic seizures and anti-epileptic drug treatment.

In order to understand the relationship between sleep and epilepsy, it is necessary to considerer their electrographic characteristics. The electrical activity of the brain during wakefulness and REM sleep consists of a low-amplitude, high-frequency wave pattern. While during NREM sleep, there are high-amplitude, low-frequency waves, which facilitates brain excitation that may induce nocturnal epilepsy.

The organization of sleep stages is essential in the development of epileptic seizures since brain excitability varies across the states of vigilance. Certain sleep stages have been shown to differentially affect various types of seizures [2,3], and sleep patterns of patients with epilepsy have often been described as altered by seizures. However, the exact nature of these sleep disturbances has not yet been fully defined. Different studies have yielded different results, due in part to methodological differences.

The presence of sleep disturbances has been well documented in patients with epilepsy. The alterations reported in the literature related to the effect of epilepsy on sleep architecture include sleep fragmentation, a decrease in sleep efficiency, an increase of sleep latency, an increase in the wake time after sleep onset 2 (WASO), increased stage 1 and 2 NREM sleep [light sleep], and a decrement in both stage 3 NREM and REM sleep.

In spite of numerous experimental studies carried out on epilepsy in relation to sleep, few clinical reports deal with the influence of epileptic seizures on sleep stage organization. A transient decrease in rapid eye movement (REM) sleep has been observed following generalized seizures in epileptic patients [4]. Similar findings have been described in animals after amygdaloid or hippocampal kindling, an experimental model of temporal lobe epilepsy with secondarily generalized seizures [5].

On the other hand, it has been shown that systemic injection of the GABAA antagonist PTZ induces primary generalized seizures. At low doses, PTZ has been used as a model for absence seizures and in higher doses it induced generalized convulsive seizures.

The PTZ induces the presence of epileptiform electrophysiological activity [6], associated to histological and biochemical changes similar to those of human beings [7,8], therefore, it has been used as model for generalized seizures [9].This drug affects the whole brain, but particularly acts on low threshold areas like the motor cortex [10], and limbic regions such as the amygdale, hippocampus and dentate gyrus [11]. Seizures induced by brain excitation after PTZ administration is originated by neuronal activation at the reticular formation level [12]. Seizures induced by PTZ and the subsequent neuropathological alterations in the rat have extensively been studied. These seizures bear a high resemblance to generalized seizures in humans, producing typical seizure-related brain damage.

Seizures induced by administration of PTZ have been considered as a model for 3generalized seizures in humans. Therefore this model can be used as a test system for analyzing the effect of generalized seizures on sleep pattern organization, which is the objective of this work.

Methods

According to procedures previously described [13,14], Experiments were done in 10 chronically implanted adult male Wistar rats weighing between 250 and 300 g. Animals were maintained under controlled conditions (24.4 C; 7:00-19:00 light, 19:00-7:00 darkness; food and water ad libitum). All animals were treated according to regulations specified by the Bioethical Committee and the Mexican Standard for the production care and use of laboratory animals NOM-062-Z00-1999].

Under general anesthesia (Sodium pentobarbital, 50 mg/kg ip) and aseptic conditions a longitudinal incision was made in the middle line of the scalp and the skull was exposed. Then two pairs of stainless steel electrodes were epidurally implanted to obtain the electrical activity of the frontal (2 mm anterior to bregma, 2.5 mm lateral to the midline) and occipital (5 mm posterior to bregma, 2.5 mm lateral to the midline) regions of the brain (EEG) for chronic electrophysiological recordings. Electro-oculogram (EOG) was obtained from a pair of electrodes placed on the supraorbital bone of the right eye. Two stainless steel wires were inserted into the nape muscles to obtain the electromyogram (EMG). Flexible wires from the electrodes were soldered to a connector fixed on the skull with acrylic cement. The animals were allowed to recover from surgery for a week. Polygraphic 4 recordings were made with the animals located inside a sound attenuated chamber, electrically shielded and constantly illuminated by a 60 W white bulb to facilitate observation through a one-way glass window in order to correlate animal behaviour with obtained recordings. The temperature inside the recording chamber oscillated between 22 and 26°C. Food and water were constantly available. Control recordings were done on a Grass Model 7 Polygraph during 10 continuous hours (from 09:00 to 19:00 h), followed by 5 similar recordings for 5 consecutive days after administration of a single dose of PTZ (70 mg/kg; ip). Paper speed was 3 mm/s, with some samples at other speeds.

Data analysis

After criteria for defining the different states of vigilance had been established, polygraphic recordings were assessed visually. Recordings were scored in 25-s epochs as wakefulness (W), slow wave sleep (SWS) and rapid eye movement (REM) sleep. Scoring reliability was 90 % by comparing the independent judgments of two scorers.

The total time spent by animals in each state of vigilance during each 10-h recording was obtained, and the percentage within the recordings was calculated. Furthermore, latency, mean duration and the number of REM sleep phases over the 10-h recording periods were obtained. Sleep parameters were expressed as mean ± SD. The results obtained under control conditions were compared with those obtained after PTZ administration by analysis of variance (ANOVA). Discrepancies with $p < 0.05$ were considered statistically significant.

Besides analyzing sleep parameters, characteristics of seizures induced by PTZ administration were also analyzed. 5

Results

Under the control conditions animals exhibited three different states of vigilance: Wakefulness (W), slow wave sleep (SWS) and rapid eye movement (REM) sleep (Figure 1).

During W, rats displayed diverse types of motor behaviors such as eating, drinking or grooming. Electrophysiological brain activity was constituted by low voltage high frequency waves. EOG exhibited artifacts provoked by ocular movements and blinking. Electromyographic activity was constituted by high tone interrupted by bursts produced by motor activity animals.

The sleep onset coincided with behavioral immobility.

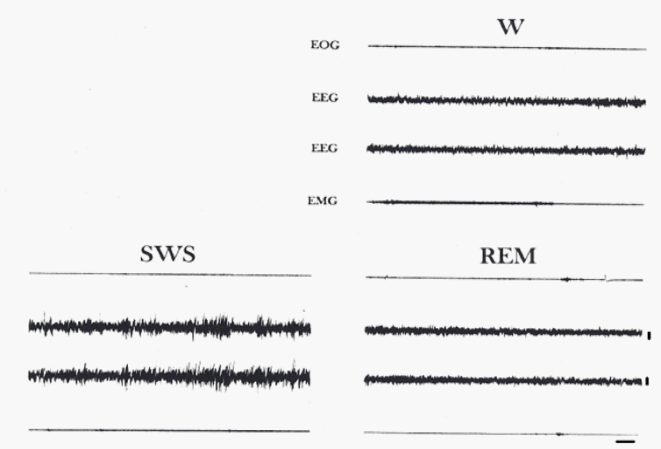

Figure 1: States of vigilance under control conditions. W: Wakefulness; SWS: Slow Wave Sleep; REM: Rapid Eye Movement Sleep; EOG: Electro-Oculogram; EEG: Anterior and Posterior Electrical Brain Activity Respectively; EMG: Electromyogram of Neck Muscles; Calibration: 50 Mv; 5 S.

Animals remained motionless throughout the SWS stage, eyes were closed and ocular movements were absent. High voltage, slow frequency cerebral waves were present across this sleep stage. EMG decreased slightly and ocular movements were absent.

SWS were usually followed by REM sleep. The intermittent apparition of motor activity involving myoclonic jerks of the limbs and ocular movements characterized this sleep phase. Brain activity was similar to that observed in W. Muscular atonia was installed which was interrupted by sporadic muscular discharges.

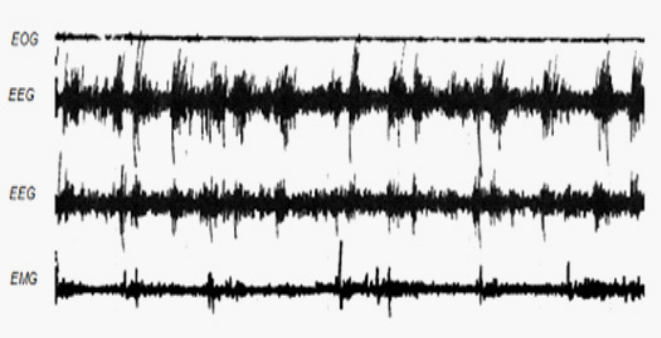

Figure 2: Epileptic Seizure after PTZ administration. EOG: Electro-Oculogram; EEG: Electrical Brain Activity from Anterior and Posterior Cortex Respectively.

Quantitative data

Under control conditions rats spent 214.1± 25.3 min in W of the 10-h recording time, while they remained for 327.6±15.1 min in SWS and 58.3± 12 min in REM sleep. Total sleeping time corresponded to 58% of the 10-h period; 9% was spent in REM sleep and 49% in SWS. REM sleep phases occurred only after several minutes of SWS and were never observed immediately following Wakefulness.

Administration of PTZ

6 100% of the animals administered with the PTZ presented Generalized Tonic-Clonic Seizures (GTCS), characterized by a tonic phase with hind limb extension and a clonic phase with myoclonus of the anterior and posterior limbs. These behaviors correlated with highly synchronized bursting EEG activity and with trains of voltage fluctuations. During the period of severe status epilepticus, electrical seizures became synchronized in the anterior and posterior recorded cerebral regions and coincided with motor events (Figure 2).

Latency to the first myoclonic jerk and GTCS.

Administration of this drug induced progressively installed motor alterations, where head nodding, masticator movements and myoclonic twitches of the face and limbs were observed. The first seizure parameter observed after the PTZ administration was the myoclonic jerk. This parameter was characterized by a strong shaking of the whole body. This characteristic was observed 0.92 ± 0.03 min after PTZ administration.

After PTZ administration, time spent in wakefulness significantly increased even when seizures had stopped ($p< 0.05$ against control and the recovery days values). Afterward, levels of wakefulness similar to control conditions were observed during subsequent recording periods (Figure 3, R1 to R7). In contrast both SWS and REM sleep were significantly reduced ($p < 0.05$ against

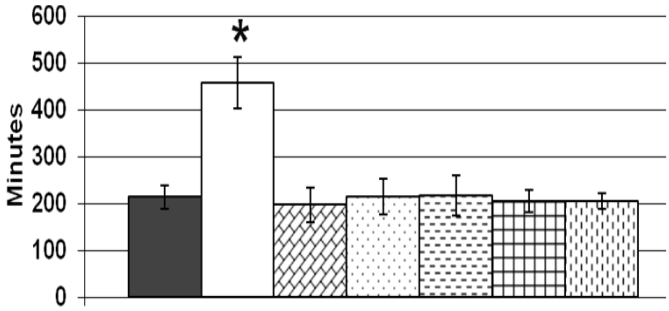

Figure 3: Wakefulness: Amount in minutes under control conditions and after PTZ administration. C, Control recording; E, Administration day; R1,R2, R3, R4 and R7, Recordings Days after PTZ administration. *p< 0.05 against control and the recovery days values

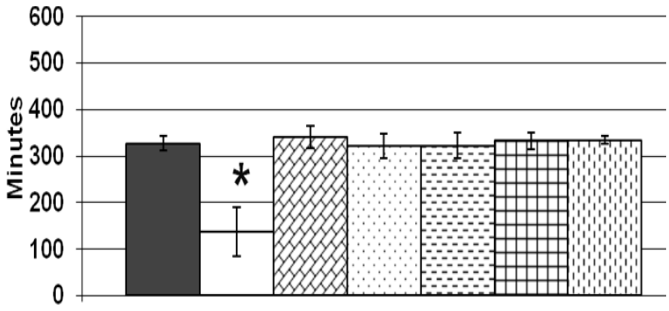

Figure 4: Slow wave sleep: Amount in minutes under control conditions and after PTZ administration. C, Control recording; E, Administration day; R1,R2, R3, R4 and R7, Recordings Days after PTZ administration. *p < 0.05 against control and the recovery days.

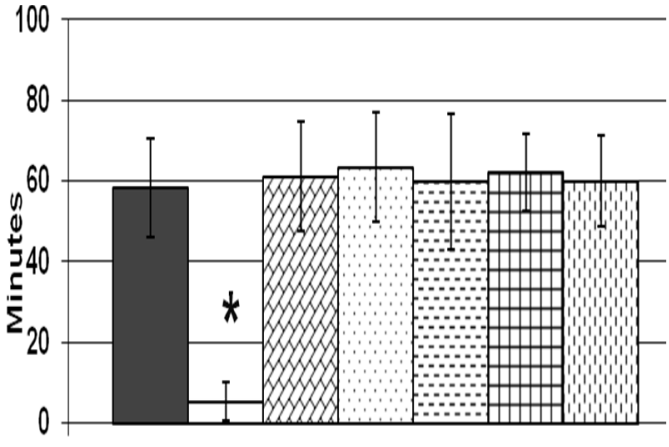

Figure 5: REM sleep: Amount in minutes under control conditions and after PTZ administration. C, Control recording; E, Administration day; R1,R2, R3, R4 and R7, Recordings Days after PTZ administration. *p < 0.05 against control and the recovery days.

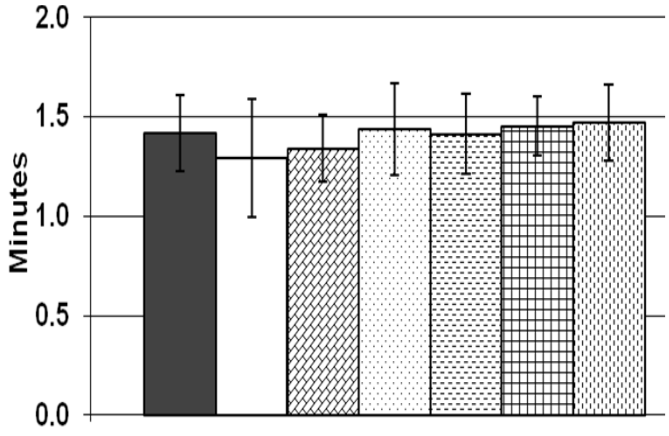

Figure 6: Mean of REM sleep duration. Amount in minutes under control conditions and after PTZ administration. C, Control recording; E, Administration day; R1,R2, R3, R4 and R7, Recordings Days after PTZ administration.

control and the recovery days).

The decrement was markedly higher for REM sleep. SWS diminished from 327.6 ± 15.1 to 137.2 ± 52.2 min. of the 10 hr recording time (Figure 4E), and REM sleep from 58.3 ± 12.1 min to 5.3 ± 4.7 min (Figure 5E). 7 When seizures stopped, the amount of time spent in both sleep phases remained reduced throughout the 10 hr recording period of the PTZ administration day. During subsequent recording days both SWS and REM sleep were recovered since they showed similar values to those of control conditions (R1 to R7).

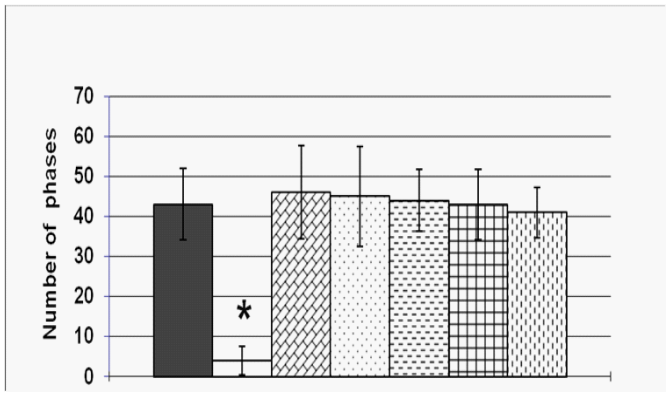

Figure 7: Number of REM sleep episodes. Amount of episodes under control conditions and after PTZ administration. C, Control day; E, Administration day; R1, R2, R3, R4 and R7 days after PTZ administration. *p < 0.05 against control and the recovery days.

Mean duration of REM sleep episodes was 1.29±0.35 min under control conditions. This value did not vary significantly on subsequent recordings after PTZ administration when REM sleep was sporadically present (Figure 6).

Under control conditions average amount of REM sleep episodes was 43 ± 8.9 episodes. This value decreased to 4 ± 3.5 episodes during the recording day after PTZ administration ($p< 0.5$ against control and the recovery days) followed by a complete recuperation on subsequent recording days where values similar to control conditions were observed (Figure 7, R1 to R70.

In conclusion, total time spent in each sleep phase was recovered one day after PTZ administration (R1), reaching similar values to those under control conditions since none of the five subsequent recordings (R1,R2,R3,R4 and R7) presented statistically significant differences. REM sleep rebound was absent since compensatory increment of this sleep stage was no observed.

Discussion

Results show that states of vigilance are significantly altered in this animal model of generalized epilepsy after administration of PTZ. Insomnia was immediately present following PTZ injection preceding the appearance of the first epileptic symptoms. Thus, both SWS and REM sleep were completely inhibited during a relatively long period of time. Generalized seizures observed after administrating 8 PTZ are similar to those previously described after kaínic acid administration [13,15,16]. These seizures show a high resemblance to generalized epilepsy in humans, displaying an acute status epilepticus [17,18]. Some authors have suggested that Pontine Reticular Formation is involved in the generation and maintenance of generalized epileptic seizures [19-21]. Particularly, it has been shown that the Pontis caudalis nucleus [PnC] neurons mediated by NMDA receptors participate in the generation of behaviors related to seizures events [22].

Neurochemical findings have shown that PTZ binds to the picrotoxin site of the GABA receptor complex, disturbing the activity of GABA/BDZ-coupled chloride channel [23], and blocking GABA-mediated postsynaptic inhibition [24]. In addition, it has been described that Pentylenetetrazol-induced seizure is not mediated by benzodiazepine receptors [25]. Considering that GABA modulates BDZ sites [26], the decreased BDZ binding following PTZ can be explained by the impairment of GABA function [27]. Another possibility is that enhanced release of GABA following PTZ treatment causes decreased GABA/BDZ receptor binding [28].

On the other hand, it has been observed that PTZ induced seizures affects the neurotransmitters metabolism incrementing the noradrenaline concentration and at the same time inducing serotonin and dopamine levels in the prefrontal cortex and the striatum but not in the hippocampus [11].

Effects of PTZ are manifested 30 minutes after its administration showing a mean life of 3.8 hr inducing the presence of spike-waves and polyspikes on the EEG and it is associated to biochemical and histological alterations similar to those in humans [7,8], therefore, it has been used as model for generalized seizures [9]. PTZ excites all brain regions, particularly in low threshold areas such as the motor 9 cortex [10], and limbic areas such as amygdale, hippocampus and dentate gyrus [11]. Provoked epilepsy by PTZ administration is related to reticular formation nuclei activation [12], that may be responsible for the significant increment of wakefulness that we evidenced. Although chemical models to induce seizures based on PTZ administration have been widely used, the mechanism by which this drug elicits its action is yet not well understood. Pellmar and Wilson [28] showed that PTZ mainly reduced the chloride conductance and to a minor extent the sodium and potassium conductance. Similarly, Onozuka and Tsujitani [29], showed that PTZ causes neuronal bursting activity by altering the ionic conductance of sodium and potassium channels by

changes in intracellular Ca2+ related processes. At the molecular level, a generally accepted mechanism of PTZ is noncompetitive antagonism of the GABAA receptor complex [30-32].

Inhibition of both sleep phases, particularly REM sleep, may be associated with repetition of complex partial attacks or secondary generalization of seizures. Although reduction in REM sleep may be a facilitating factor for generalization and repetition of seizures, this phenomenon does not appear to be a sufficient condition to explain the development of seizures. In any event, increases in wakefulness, reduced sleep time and repetition or generalization of seizures may be considered as different expressions of a unique underlying disturbed physiological process.

As previously described, there is a relationship between sleep, sleep deprivation, and epilepsy. However, the Neurophysiological mechanisms for this relationship are not completely known. It has been suggested that the relationship is reciprocal. In other words, both sleep and sleep deprivation have an excitatory effect on the genesis and propagation of the seizures [33]. This could explain the 10 inhibition of both REM and slow wave sleep observed immediately after a single convulsive seizure.

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