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Comparison of acute, chronic and post-treatment effects of carbamazepine and vinpocetine on hearing loss and seizures induced by 4-aminopyridine

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ABSTRACT

Objective: To compare the acute, chronic and post-treatment effects of the classic antiepileptic drug carbamazepine (CBZ) and the potential antiepileptic vinpocetine (VPC), successfully used in the treatment of brain vascular origin disorders, on 4-aminopyridine (4-AP)-induced increase in auditory threshold, brain-auditory-evoked-potentials (BAEPs) later waves alterations and epileptiform activity.
Methods: BAEP and EEG recordings before and following 4-AP (3 mg/kg, i.p.) were obtained in guinea pigs. One week after, the animals received a daily injection (i.p.) of vehicle, 3 mg/kg VPC or 17 mg/kg CBZ for 13 days. The acute and chronic effects before and following 4-AP were tested at the 1st and last days, respectively, and the post-treatment effect 1 month after the end of treatment.
Results: CBZ and 4-AP increased BAEPs threshold and BAEPs P4 wave latency. Chronic CBZ inhibited 4-AP-induced increase in P3 amplitude. In the VPC-treated group, all the 4-AP-induced BAEPs changes were prevented. Seizures were prevented in 50% and 75% of the animals by chronic CBZ and VPC, respectively. After acute VPC and after the end of VPC-treatment 4-AP failed to induce seizures in 50% of the animals.
Conclusion: VPC inhibits 4-AP-induced seizures and hearing loss, even after post-treatment, at a concentration about 10 times lower than CBZ.
Significance: The complications in hearing that can accompany epilepsy can be prevented by VPC, indicating its advantage as an alternative antiepileptic.

1. Introduction

Brain-auditory-evoked-potentials (BAEPs) are far field-evoked-potentials that consist of several waves that occur within 10 ms post-stimulus. Abnormalities in the waves of BAEPs and in some cases hearing deficits have been found in patients medicated with classic antiepileptic drugs (AEDs) (Armon et al., 1990; Medaglini et al., 1988; Japaridze et al., 1993; Yuksel et al., 1995; Rysz and Gajkowski, 1996; De la Cruz and Bance, 1999; Verrotti et al., 2000; Wakamoto et al., 2004). In epileptic patients, however, the study of a specific AED effect on the parameters of BAEP waves can be complicated; because epileptic patients are frequently medicated with more than one AED and for variable time periods.

In the guinea pig the long-term administration of carbamazepine (CBZ), phenytoin and valproic acid, that are among the most common old generation antiepileptic drugs, decreased the hearing sensitivity, as indicated by the increase in the auditory threshold (Sitges and Nekrassov, 2007). However, in animal models of epilepsy, changes in the later waves of the BAEPs (P3 and P4) accompanied by increased auditory thresholds are also produced during seizures (Nekrassov and Sitges, 2003), suggesting that hearing sensitivity can be affected by both, the medication and the illness. In line, the increase in the auditory threshold induced by several antiepileptic drugs facilitates the increase in the auditory threshold induced by pentylenetetrazole (PTZ) during seizures (Nekrassov and Sitges, 2006). In contrast, vinpocetine (VCP, ethyl apovincamine-22-oate), a drug with antiepileptic potential that has successfully been used in the treatment of central nervous system disorders of cerebrovascular origin, did not increase the auditory threshold by itself and prevented the rise in the auditory threshold induced by PTZ (Nekrassov and Sitges, 2006).

In addition to the experimental epilepsy induced by decreasing cerebral inhibitory neurotransmission with drugs such as PTZ that antagonizes GABA transmission (Macdonald and Barker, 1977), experimental epilepsy also can be induced by the opposite mechanism, namely, by increasing cerebral excitatory neurotransmission. Glutamate is the most important excitatory neurotransmitter in the brain. 4-aminopyridine (4-AP) is another convulsing agent which particularly increases cerebral glutamate release in vivo

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(Morales-Villagran and Tapia, 1996). Like for the case of PTZ, the epileptic cortical activity induced by 4-AP in the guinea pig in vivo is accompanied by specific alterations of the later BAEP waves and hearing decline (Nekrassov and Sitges, 2003).

In slice preparations in vitro the epileptiform activity induced by 4-AP is reduced by CBZ (Fueta and Avoli, 1992; Yonekawa et al., 1995; Brückner and Heinemann, 2000; Lees et al., 2006), and in cerebral isolated nerve endings the increase in sodium permeability induced by 4-AP is sensitive to CBZ (Ambrosio et al., 2001) as well as to VPC (Sitges et al., 2005). Acute VPC inhibits 4-AP-induced changes in the EEG and hearing (Sitges and Nekrassov, 2004). The effect of CBZ on the above 4-AP-induced changes is unknown.

In this study the effect of CBZ administered acutely and chronically on the changes in hearing induced by 4-AP at a convulsing concentration were investigated and compared with the effects of VPC. The possible maintenance of the changes induced by the long lasting treatment with the above antiepileptic drugs after their withdrawal was also explored here.

2. Methods

2.1. Drugs

4-AP was obtained from Sigma (St. Louis MO) and the antiepileptic drugs tested, namely, VPC and CBZ were a kind gift of Psicofarma S.A. de C.V. (México).

4-AP was dissolved in oxygenated HEPES buffer (composition in mM: 127 NaCl, 1.18 KH_2PO_4, 3.73 KCl, 1 $CaCl_2$, 1.18 $MgSO_4$, 20 HEPES and 5.6 mM dextrose). The dose of 3 mg/kg 4-AP (i.p.) used to carry out the experiments of this study was chosen on the basis of our previous experience in non-anaesthetized guinea pigs injected with increasing doses (range from 1 to 6 mg/kg) of 4-AP (Sitges and Nekrassov, 2004).

VPC was dissolved in saline acidified with HCl and adjusted to pH 4 with NaOH. CBZ was dissolved in dimethylsulfoxide. This CBZ vehicle was injected to control animals, because in a previous study (Sitges and Nekrassov, 2004) we have already shown that the acidified saline used to dissolve VPC has no effect on the parameters studied in this work. VPC was injected at a dose of 3 mg/kg (i.p.) and CBZ at a dose of 17 mg/kg (i.p.).

2.2. Recordings

BAEP recordings were used to evaluate the hearing status of each animal and EEG recordings to evaluate changes in cortical excitability. For BAEP recordings, needle electrodes were placed subcutaneously at the ipsilateral left pinna (reference electrode), the contralateral pinna (ground electrode), and the vertex (active electrode). For EEG recordings, needle electrodes were placed subcutaneous over the left temporal area (active electrode) and over the left frontal area between the midline and the arched portion of the orbital crest (reference electrode). BAEP and EEG recordings were performed in a sound proof room using a Nihon-Kohden Neuropack IV Mini (MEB-5304 K) system (amplifier specifications noise equal to 0.7 μV rms or less from 1 to 10 KHz) following the method that we have previously used in several studies (Nekrassov and Sitges, 2000, 2003, 2004; Sitges and Nekrassov, 2004). Briefly, monaural stimuli of 8 and 4 kHz were delivered by a TDH 39 earphone located 1 cm from the left ear. The right ear was blocked with a special wax plug that substantially reduced the sound level at this ear. Alternating polarity tone bursts (20/s), with 2 ms duration and 0.5 ms rise-fall times were used for evoking the potentials. Responses were amplified and averaged, displayed vertex positive up and saved on disk for off-line analyses. The time interval between the two tone frequencies was about 15 s. To obtain the BAEP thresholds, stimuli of progressively lower intensity in dB (normal hearing level, nHL), starting from a stimulus of very high intensity (100 dB nHL), were delivered. Threshold was defined as the lowest stimulus intensity at which the P3 wave of the BAEP could still be recorded in three consecutive trials (each trial equaling the average response to 500 stimuli). Each trial took 25 s as 20 stimuli were delivered per second. To identify the auditory threshold, stimulus intensity was progressively reduced by steps of 20, 10 and 5 dB nHL. The inter-stimulus interval when reducing the intensity was not longer than 5 s.

2.3. Subjects

In this study 22 pigmented adult male guinea pigs (417 ± 13 g initial weight) were included. First the animals were anaesthetized with ketamine (50 mg/kg/10 mg/kg xylazine, i.p.), implanted with a microchip for later identification and recorded before any treatment was started and following the injection of the convulsing agent, 4-AP. Then they were divided into three groups defined by the substance to be injected. Namely, vehicle, VPC or CBZ. The control group injected with vehicle included eight animals, and the experimental groups, seven animals per group. The acute, chronic and post-treatment effects of the antiepileptic drugs on the 4-AP-induced increase in BAEP threshold and epileptiform activity were tested as follows: The acute effects were tested about 1 week after the first recordings. For the acute recordings the animals were injected with vehicle ($n = 8$), 3 mg/kg VPC ($n = 7$) or 17 mg/kg CBZ ($n = 7$) 1 h before taking the recordings in the absence and then in the presence of 4-AP. For the chronic effects the animals were daily injected (i.p.) with vehicle, VPC or CBZ at the above doses for 13 days, and on day 13 recordings were taken again 1 h after the last injection and then following exposure to 4-AP. For testing the post-treatment effect of the antiepileptic drugs on the 4-AP-induced changes 1 month after the end of treatment, a last set of recordings before and after the injection of 4-AP was obtained.

In all cases 4-AP was injected immediately after obtaining the first recordings. The recordings following 4-AP injection, however, were taken about 1 h after. Because in a previous study we found that the 4-AP-induced changes on BAEPs and EEG are clearly manifested after that period of time (Nekrassov and Sitges, 2003).

For determining amplitude and latency values under the different experimental conditions tested shown in Tables 2–4, BAEP recordings in response to the stimulus of the highest intensity (100 dB nHL) were used.

The Institutional Animal Use and Care Committee approved all experimental procedures.

2.4. Data analysis

Student's *t*-test (paired, as each animal served as its own control) was used for the evaluation of the BAEP threshold difference: (a) between before and following 4-AP-induced seizures under a specific condition; (b) between before start of treatment and after a specific treatment (acute or chronic) or after post-treatment; (c) between 4-AP before or 4-AP after a specific treatment (acute or chronic) or after post-treatment. The criterion for statistical significance for all measures was $P < 0.05$. All data are expressed as means ± standard error of the mean.

3. Results

3.1. Single and combined effects of 4-AP and CBZ or VPC on the auditory threshold

The average auditory threshold in the 22 animals included in this study before any treatment started was 6.8 ± 0.8 dB (nHL) at