FRENTE DIAGNÓSTICA E TERAPÊUTICA NA NEUROLOGIA 2

BENEDITO RODRIGUES DA SILVA NETO
(ORGANIZADOR)

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APRESENTAÇÃO

Apresentamos o segundo volume do livro “Frente Diagnóstica e Terapêutica na Neurologia”, um material rico e direcionado à todos acadêmicos e docentes da área da saúde com interesse em neurologia e áreas afins.

A especialidade médica responsável por trabalhar e analisar os distúrbios estruturais do sistema nervoso é denominada como neurologia. Do diagnóstico à terapêutica, todas as enfermidades que envolvem o sistema nervoso central, periférico, autônomo, simpático e parassimpático, são estudadas pelos profissionais com especializaçãoo em neurologia. Além das doenças neuropscicopatológicas, o CID divide as patologias do sistema nervoso em dez grupos com fins de análise epidemiológica.

Assim abordamos aqui assuntos relativos aos avanços e dados científicos aplicados aos estudos de base diagnóstica e terapêutica nesse reamo tão interessante da medicina, oferecendo um breve panorama daquilo que tem sido feito no país. Neste segundo volume o leitor poderá se aprofundar em temas relacionados ao Alzheimer, Hospitalização, Atenção Primária à Saúde, Apraxia, Demencia, Cognição, Neuropsicologia, Esclerose lateral amiotrófica, VIH tipo I, Parkinson, Epidemiologia, Indicadores de Morbimortalidade, Melanoma, Metástase, Neurossarcoidose, Endocardite bacteriana, Oligodendroglioma, Epilepsia Refratária, Tumor Cerebral Primário, Lobectomia Temporal Anterior e Doenças Neurodegenerativas como um todo.

Esperamos que o conteúdo deste material possa somar de maneira significativa ao conhecimento dos profissionais e acadêmicos, influenciando e estimulando cada vez mais a pesquisa nesta área em nosso país. Parabenizamos cada autor pela teoria bem fundamentada aliada à resultados promissores, e principalmente à Atena Editora por permitir que o conhecimento seja difundido em todo território nacional.

Desejo à todos uma ótima leitura!

Benedito Rodrigues da Silva Neto

SUMÁRIO

CAPÍTULO 8

EFFECTS OF ANTIEPILEPTIC DRUGS ON SPREADING DEPRESSION IN THE CHICK RETINA: IMPLICATIONS FOR MIGRAINE PROPHYLAXIS

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ABSTRACT: Background: The Spreading Depression (SD) is an answer of the nervous tissue to a different type of local stimulus. It was described initially in the cortex of anesthetized rabbit and characterizes by a depression of the spontaneous electric and provoked activity, which initiate in the stimulated point and propagates, at the rate of 3.0-5.0 mm.min-1, for adjacent regions of the cerebral cortex. The eletrocorticographyc period of activity depression belongs to 2-5 min and the re-establishment of the normal activity occurs in 15-20 min after the beginning of the reaction. The gear of this wave does not respect the limits of different vascular territories in the cortex and neither citoarchitectural differences nor functional differences The knolled of this phenomenon is fundamental for the correct knowledge and treatment migraine (MG). Methods: In the present study, we analyze the effects of antiepileptic drugs (AED), also used in migraine (MG) prophylaxis, on the spreading depression (SD) in isolated retina of chick' (Gallus gallus domesicus). We studied five drugs with proven effect on the modulation of GABAergic transmission: Topiramate (TP), Valproate semisodium (VS), Gabapentin (GP), Lamotrigine (LT) and Levetiracetam (LV). Chicks´ retinas were kept at 30-31°C in superfusion chamber, with a flow of 1,0 – 1,8 ml/min. of a Ringer's reference solution, and the reaction was evoked by mechanical and chemical stimulus, every 15 minutes. Using this model, we first measured the speed (mm/min.), the amplitude (mV), the deflagration threshold (after chemical stimulus with KCl-) and the absolute refractory period (sec.) of the SD, with and without the drugs used in the study. Subsequently, the speed and amplitude parameters, also with and without the drugs in study, were analyzed in an in vivo model. In

addition, the GABA-transaminase enzyme (GABA-T) activity was determined, with and without the drugs in study. Analysis of variance was used to determine the activity of GABA-transaminase (GABA-T). Results: We verified that all the drugs, particularly Topiramate (TP), reduce the speed and amplitude in a dose-dependent and reversed manner, in vitro as well as in vivo. All the drugs also increase, in a reversible form, the deflagration threshold for the SD, after chemical stimulus with KC-, in specific concentrations. It was also verified, that all the drugs increase, in a reversible form, the absolute refractory period. Topiramate (TP) was considered the most effective drug in the context of the proposed parameters. Levetiracetam (LV), in spite of its sui generis mechanism of action, was considered the less effective drug. The enzyme GABA-transaminase (GABA-T) displayed slight decrease activity, in the presence of Topiramate (TP), Valproate semisodium (VS) and Gabapentin (GP).
KEYWORDS: Migraine, Spreading Depression, Antiepileptic drugs, Neuropharmacology, Retina

CONCLUSIONS

These results reinforce the notion that SD is a subjacent and relevant factor for the pathophysiology of migraine (MG), the treatment of this pathology must emphasizes the use of antiepileptic drugs (AED), in special Topiramate (TP).

Antiepileptic drugs, and more especially Topiramate, can significantly affect both the threshold and the propagation of SD in a dose-dependent manner. Our results reinforce the notion that SD may underlie the physiopathology of migraine and that, although further investigations on cortical SD are needed, our model may be a useful tool for the test of new prophylactic drugs.

Cortical spreading depression was first described in 1943 by the Brazilian neurophysiologist Aristides Leão, during his PhD fellowship in physiology at Harvard University. In 1944, Leão published a paper entitled “Spreading depression of activity in the cerebral cortex”, subsequently known internationally as Leão´s spreading depression (Leão, 1944a).

Several studies indicate that spreading depression (SD) is fundamentally related to seizures marches and to aura of classical migraine. Others more recent studies, moreover call attention to its possible relevance in clinical disturbance associated with brain ischemia, trauma and hypoglycemia (Avoli and cols., 1991; Strong and cols., 2002). However, studies concerning the effect of antiepileptic drugs (AED) on the reaction are scarce. The importance of such studies was first shown by Woodbury (Woodbury, D.M., 1978) who pointed out their interest not only in terms of developing a model for evaluation of antiepileptic drugs but also in terms of their heuristic value in SD.

SD is defined as a slowly moving depression of cortical activity, is characterized

by a transient, slowly propagating depression of electroencephalographic activity (EEG), a marked reduction of the sensory and other evoked potentials, a negative slow potential change, a rise in tissue electrical impedance and some translocations of ion and water between the intra and extracellular compartments. Typically propagated at 2-3 mm per minute and, if conditions are appropriate and uniform in surrounding cortex, it proceeds in all directions from the point of origin (Do Carmo,R.J. and Martins-Ferreira,H,1984; Hansen,A.J. and Olsen,C.E.,1980; Mori,S.,Miller,W.H.andTomita,T.,1976 ; Nicholson,C.,Bruggencate,G.,Stockle,H. And Steinberg R., 1978; Marshall, 1959). The propagation of the Leão´s wave does not respect the limits of different vascular territories in the cortex, neither different citoarchitectural nor functional variances of the cortex (Somjen, 2001; Gorji, 2001).

The fact that a lot of animal species are susceptible to SD may have a significance explained by the phylogenetic conservation of the phenomenon (Bûres, 1974).This considerations, in addition to the other characteristics of the phenomenon; allow us to considerate the SD as a property inherent of the nervous system.

The main characteristics of spreading depression (SD) are a decrease of spontaneous electrical activity, slow negative potential changes, trans membrane ion translocations and an increase in tissue lactate (Leão, 1947; Martins-Ferreira, H.1994).

The velocity of propagation has been measured and some physical and chemical factors underlying the spread of the electrophysiological depression have been identified however, some experimental studies have shown outstanding local reversible features of SD: 1. Decrease of “spontaneous” and evoked electrical cortical responses; 2. Extracellular negative slow-voltage variations (SVV); 3. Overflow of K+ into the extracellular space concomitant with depletion of Ca^{2} and 4. Increase of intracellular Na+, Cl- and water (Kaig & Nicholson, 1978; Lauritzen, 1978; Leão, 1987; Van Harreveld, 1959; Vysckocil e cols. 1972).

The first hypothesis of the mechanism of propagation of SD was made by B.Grafstein in 1956 who postulate that the liberation of K+ from cortical neurons as a result of intense activity or some other humoral or traumatic stimuli caused an increase of these ions in the extracellular space sufficient to depolarize adjacent neurons which, being thus stimulated to activity liberated a further quantity of potassium (Grafstein, 1963). In 1959, Van Harreveld suggests the glutamaergic hypothesis of propagation of SD. When applied for a short time, L-glutamate may trigger light scattering areas but there is no limit between excitatory and toxic effects (Van Harreveld and Fifková, 1970). This fact have been recently re-examined by Scheller e cols.in 1993 (Scheller and cols., 1993) using a microdialysis technique to measure amino acids release during cortical SD in anesthetized rats. Based on their observations and on literature on NMDA receptors, they concluded that glutamate release has indeed occurred in

the site where KCl- elicited the SD but, on the other regions of the cortex, it has has indeed occurred but could not be detected by the technique employed (Scheller and cols.,1993).

Retinal spreading depression is a very useful model for this phenomenon, has been extensively studied in terms of its optical, electrical and mechanical components. Ionic changes in the extracellular microenvironment have also been assessed and chemical substances liberated from tissue have been detected (Gouras, P, 1958; Martins-Ferreira, H.1962).

Two conspicuous characteristics make the avian retina in vitro a preparation of choice for experimental studies of the basic aspects of SD: 1. the anatomical localization of its cellular elements permits the isolation of a slice of neural tissue with less damage than if using any other from the Central Nervous System (CNS), 2. Due to the “inverted” position of its receptor cells, the pars nervosa has to be relatively transparent to visible light. These properties, in special the transparency, are a simple and useful method for judging the health of the retina preparation and studying the SD. Mechanical and chemical (KCl- solution) stimulus in the fragment of retina are easily detected. With the appropriate Ringer solution using in the preservation of the retina in healthy conditions, the SD could be seen with the unaided eye or under a low-power microscope as an enlarging milk circle with its center at the site of stimulation. This milky wave is due to changes of intensity of the light scattered in the invaded tissue. These properties allow us to study the Leão´s wave with this retinal preparation (Oliveira-Castro & Martins-Ferreira, 1977).

Under defined physical and chemical conditions, an experimental baseline “state” of the retina was chosen as reference, in which the velocity of propagation of SD, after a mechanical or chemical stimuli, was shown to be 3.7 ± 0.2 mm\min, at 300. Near this value, the velocity of propagation is a function of appropriately balance between physical and chemical factors in such a way that the spreading rate can be controlled by well-poised interplay of the components of the standard solution (Ringer), temperature, chemical factors and some drugs like the AEDs used in this work.

For the propagation study in his “in vitro” isolated retina, Martins-Ferreira and cols., (1974) developed a preparation in which SD keeps up circulating, arrested in a retina ring, since some variables like temperature and the superfusion flow are controlled. The phenomenon also keeps walking inside the circular track with uniform speed, during several hours. Where in the chemical composition of the Ringer basic solution used up for the superfusion had been established, with the method of “attempt and mistake”, for the exclusive purpose of obtaining the reaction in isolated retinas, at the work cited, the authors make a systematic study of the influence of each one of the solution components, in the propagation speed of the circulating wave of Leão,

study this that can be used, as well, for the non-circulation SD. Varying at a time the concentration of one of the components, inside a band that included the one used in the basic solution, we can evaluate the individual influence of each one of these components. We can see consequently, that is very clear the antagonistic action among ions potassium and magnesium, as well as an antagonistic relation among ions chloride and the propagation speed, inside the bands of explored concentration. It becomes clear, as well, the effect of the increase in the temperature, however there is a maximum (about 35°C) above of which the speed of the reaction starts to decrease. Martins-Ferreira and cols. (1970) opted, in this work, by the following composition for a Ringer standard (mM): NaCl 100, Kcl- 6, $CaCl^2$ 1, MgSO4 1, NaH^2PO4 1 and glucose 30. Finally was related the solution osmolarity influence, whose variation is inversely related to propagation speed of the SD. Using the Ringer's Solution standard to 270 mosmol and keeping itself the temperature around 30°C, the propagation speed of the SD belonged to 3,71+/- 0,21 mm/min. Keeping up these experimental terms, this speed is obtained routinely. We ask for the attention to the fact that this standard Ringer, experimentally developed, owns its peculiar electrochemistry balance that keeps up the isolated retina viable for many hours. Fig 1.

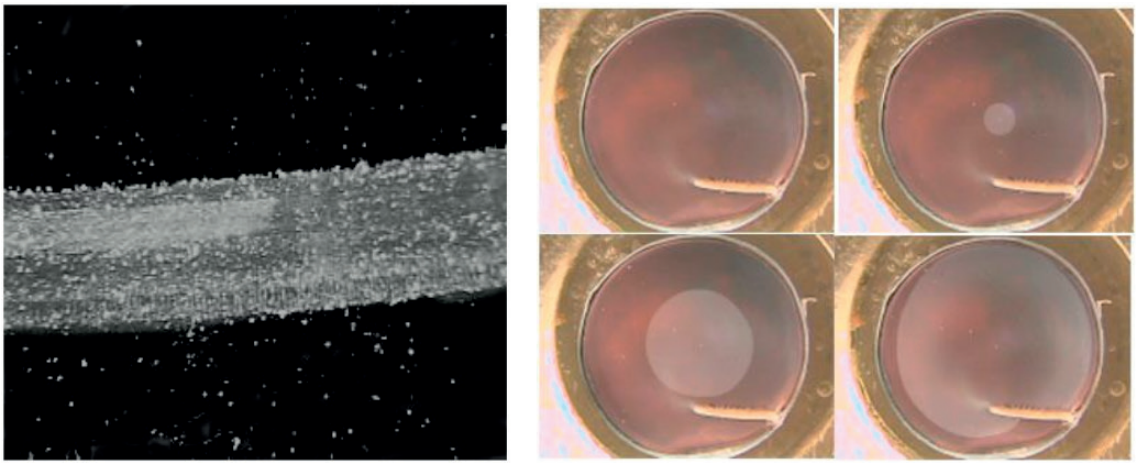


Fig 1: We can see the light scattered (spreading depression) after a mechanical stimulus in the chick' retina and the propagation of the Leão´s wave in a sagittal cut of retina also after a mechanical stimulus.

1 I PHYSIOPATHOLOGY OF MIGRAINE

The vascular and neuronal theories could, therefore, be associated, if we consider the fact that the initial SD of the cortex, responsible for the focal neurological signals at the beginning of migraine crisis could depolarize some trigeminal sensitive fibers related to the pain sensation around of the pia-arachnoid blood vessels, on the level of the ganglion of Grasser and the ventral surface of the brain (Hardebo, 1991). This activation of the trigeminovascular system (TVS) would induce neurogenic inflammation, with increase of the vascular permeability, promoting vasodilatation and

swelling of the vascular wall (Moskowitz, 1984). At the same time, some inflammatory substances could be released from the sensitive fibers, for example: calcitonin gene-related peptide (CGRP), substance P (SP), and others cytokines as the alpha tumor necrosis factor (ATNF), leucotriens and some interferon's. Moreover, some local mastocytes would be activated. Other vasoactive neurotransmitters, like intestinal vasoactive peptide (IVP) and neuropeptide Y (NPY) could be released reflexively by some sympatic and parasympatic fibers, respectively. The orthodromic conduction of trigeminal pulses would carry on the pain perception. Some vasoactive peptides like neurokin A (NKA), somatostatin, IVP and dinorfin were already identified in afferent primary neurons. SP, CGRP and NKA were confirmed as neurotransmitters of the trigeminovascular system (Limmroty and cols. 1996). It was demonstrated that the cortical SD provokes the protoncogen expression" c-fos" in the cortex (Herrera, and cols., 1993) and in the trigeminal nucleus (Moskowitz and cols., 1993), suggesting a connection between Lion's wave and the activation of the TVS. This "protoncogen", a nonspecific marker of the neural activity, is well characterized as marker of cellular activation after harmful stimulus and expresses him at the level of the cytoarquitectural blade I and II, which wisely contains primary nonmielinizated terminations (Limmroth and cols., 1996). Finally, it is considered that this cascade of events', specifically TVS's activation comes to provoke an arterial extracranial vasodilatation and an intracranial modification of the arteriolar vascularization, mostly in the pia-arachinoid circulation level, that could explain the vascular alterations observed during migraine attacks (Moskowitz and cols., 1993; Moskowitz, 1993).

SD in humans and the Lion´s phenomenon relation with the migraine aura.

About 20% of the patients with migraine present visual manifestations that precede the pain crises (Ropper, 2005), the visual manifestations are characterized by scintillations that are propagated slowly by the patient's visual field (fortification spectrums) that are followed by a scotoma. Lashley (1941) analyzed the progression of its own visual aura, that consisted of a scotoma with a scintillate edge, that propagated slowly by his visual field. He proposed that scotoma resulted from a region with depressed neuronal activity in the cerebral cortex, and the scintillations were the resultant of a region with intense neuronal activity in the periphery of this depressed region. This phenomenon would be propagated in the cerebral cortex, according to their calculations, in a speed of 3 mm.min-1 (Lashley, 1941). Lashley's hypothesis obtained an "experimental" support in 1956, when Grafstein demonstrated in the cerebral cortex of rabbits, that the wave front of the SD, was marked by a discharge of high frequency potentials, while in the cortical region affected by the SD, the neuronal activity were decreased (Grafstein, 1956).

The visual aura is the most frequent type of aura, and it has its origin in the

primary visual cortex (area 17), who is the cortex region with the biggest neurons density. Leão and Morrison (1945) were the first to suggest that the SD might explain the scintillate scotomas of migraine aura. Milner (1959) observed the likeness between the propagation speed of the SD in the rabbit cortex (3 mm.min-1) and the velocity of progression of the scintillate scotomas described by Lashley (1940) in their migraine own crises.

Migraine crises are accompanied by a market reduction of the cerebral blood flow (CBF) initially in the occipital polar region, with propagation for the previous regions of the brain in a speed of 3 mm/min. These CBF's Alterations were already measured in patients with a migraine crisis, using a positron emission tomography (Woods and cols, 1994). The maxim reduction of the CBF is about 30-40%, very smaller than the CBF reduction necessary to carry on an ischaemia of the cerebral cortex (Pietrobon & Striessnig, 2003). This fact suggests that the visual manifestations of the aura are secondary of a cerebral cortical ischaemia of the cerebral cortex.

AEDs utilizad in migraine prophylaxis.

Migraine is one of the commoner forms of headache. Between 6 to 8% of men and 12 to 14 % and of women suffer from migraine (Lipton and cols., 2002; Rasmussen and cols., 1991; Sher and cols., 1998; Silberstein, 1996). The prevalence during life in women and men is greater than 25%; before the puberty this is between 4 and 5 %. The peak of incidence of migraine attacks occurs between 35 and 45 years of life. In this phase the women are affected three times more than the men (Olsen 2004). As a fact, the frequents attacks of migraine carries an important alteration in the patients quality of life. As a consequence of this fact the World Health Organization (WHO) defined migraine as one of the 20 life limitation larger causes (disability) in the world (Maytal and cols., 1997). Migraine prophylaxis main goal is the reduction of its frequency, severity, duration of the “attacks” and the prevention concerning the use of high doses of abortive drugs. Migraine prophylaxis is considered effective in case of reduction in at last 50% of the frequency of the “attacks” (Diener, 2007).

A recent study with the use of mice as an experimental model suggests that the cortical SD is a therapeutic target for migraine prophylaxis with the use of AEDs (Ayata and cols., 2006). In this study mice were cares for some weeks (on an average of three), with AEDs (Topiramate, Divalproate semisodium and Gabapentin). The treatment impact is measured through the events frequency of SVV provoked with the use of chemical stimulus (Kcl-) or mechanical stimulus. The continuous use of AEDs suppressed SDs´ frequency provoked in 40 to 80 %.

Divalproate of Sodium (DV) is made up of Valproate of Sodium and Acid Valproic acid in a molar reason of 1:1. The ion Valproate is the acid circulation form Valproic acid in the blood. DV has several action mechanisms in the Central Nervous System (CNS), who include the acid synthesis increase δ-amino butyric acid (GABA) and

the reduction of the degradation of GABA, because it inhibits the enzyme GABA transaminase (GABA-T) that degrades the GABA. Beyond of these effects, the drug also blocks voltage dependent Na+ channels and increases the conductance of channels of K+ / Ca2+ dependent (Cutrer and cols., 1997). Their main side effects are weight gain, mostly at the beginning of the treatment besides hiperamonmia and moderated alterations of the tests of renal function. (Victor & Ropper, 2005)

Gabapentin (GP) (Acid 1-amino-metilciclohexanoacétic) is a new aminoacid created from the addition of a radical ciclohexano to the structure of the GABA. Gabapentin in concentrations of 100 μM interacts with the α2β subunit of the Ca2+ channels type T, reducing the current for these channels (Rogawski & Löscher, 2004). Gabapentin also increases the concentration of GABA in the brain (Yacubian, 1998). Can be associate to leucopenia and anemia (Victor & Ropper, 2005).

Topiramate is a derivate sulfanate-substituted of the natural monosaccharide D-frutose. It owns multiple mechanisms of action, that include blockade of Na+ channels, voltage-dependents, potencialization of the GABAa current mediated by an action in a different location from benzodiazepinc action site, block of glutamaergic receptors AMPA and blockade of Ca2+ channels type L (White, 1997; White and cols., 1997). Is associate to the glaucoma and hypercholesterolemia. (Victor & Ropper, 2005).

Lamotrigine (LT) besides inhibiting voltage-dependent Na+ channels stabilizes the neuronal membranes and acts as a pre-synaptic modulator for the release of excitatory aminoacids (glutamate) and inhibitory (GABA) (Wang and cols., 1996; Steiner and cols., 1997). The main limitation for the clinical use of this drug is a cutaneous rash in about 1% of the patients; rare cases of reversible chorea have been reported (Victor & Ropper,2005).

Levetiracetan (LV), besides the partial inhibition of the Ca2+ type N channels, acts in the opposition activity of some negative GABAergic modulators. It seems that the site of ligation of the drug is the synaptic protein SV2A. Through of this connection the drug modulates the release of some excitatory neurotransmitters and promote a facilitation of the GABAergic effect (Lynch and cols., 2004). It is associate to sleepiness and vertigos (Victor & Ropper, 2005).

2 I OBJECTIVES

1. Verify the effect of these drugs at tissue “in vivo”;

2. Based on clinical observation that the five drugs in study are effective in migraine treatment with and without aura and, as well, in the epilepsy, verify the GABAergic transmission involvement in the SD. Like a parameter of GABAergic activity, it will be measured the GABA-t activity “in vitro”, in isolated retinas of

chick´(Gallus gallus) submitted by a mechanical stimulus to the Lion´s phenomenon (SD), isolated and in the presence of the main drugs in study;

3. Determine the minimum, maximum and average dose of the drugs in study, without producing irreversible lesions, also enabling relative power verification of these drugs at tissue in this experimental model;

4. Verify the effects of Gabapentin, Valproate semisodium, Topiramate, Lamotrigine and Levetiracetan in the following parameters:

- Speed propagation;
- SVV´s amplitude;
- Deflagration threshold;
- Absolute refractory period.

3 I MATERIALS & METHODS

3.1 Animals

In this work were used chicks´ Gallus gallus domesicus (White Leghorn) females and males, with age between 10 to 20 days, age considered ideal for the study of their retinas.

3.2 Prepare of the retina fragments and solutions

The animals, with age of 10-20 day and weight of 50-90 g. were sacrificed by beheading, enucleated and the eyeball sectioned in its equatorial region to remove the vitreous humor. Soon after, the posterior hubcap was sectioned in rectangular fragments of 0,5 x 3,0 cm, and the retina is separated of the others ocular layers with a dissection tweezers.

Each animal was used to a unique experience.

The retina was put in Petri's Plate contend Ringer's reference solution (Rref), with the following composition (in mM): NaCl 100; Kcl- 3; MgSO4 1; NaHCO3 30; NaH2PO4 1; CaCl2 1; Glucose 15. In this work, this Ringer's Composition was defined as ideal after several pilots' experiments (about 30) done with and without some drugs in study, whose goal went to the obtainment of a Ringer composition effective for the study in other words, with a susceptibility average for elicitation of the phenomenon. The tissue was put on a paper of percolate black and carried to a chamber of acrylic, with close volume of 0,2 ml, in one circuit with total volume of 4.0 ml, which was superfunded with Rref to a flow of 1,0-1,8 ml.min-1 using a Harvard peristaltic bomb. The temperature was kept in 30-310C, and the Rref saturated, along all the experience, with carbogenic (95% O2- 5% CO2), the pH kept in 7.4-7.6.

The model was used not only for visual inspection of the variations of spread

light (light-scattering), as for the voltage slow variation record, deflagration absolute refractory period and deflagration threshold. The superfusion chamber was made starting from a block of acrylic, having in the superior compartment a hemispheric or rectangular receptacle, to accommodate respectively a fragment of retina or the posterior hubcap of the eyeball in study. That compartment was bathed by Ringer's Solution standard (Rs), led through a polystyrene tube and impelled by means of an infusion bomb (Harvard Apparatus CO.), by a constant flow of 1.2 ml/min.

That polystyrene tube, when entering in the chamber, is coiled about himself and accommodated in a compartment at the bottom, before of being addressed to the retina location in study. That inferior compartment was endowed of a flow of heated water, supplied by a thermostatic bath (Form Scientific Inc.), what came to enable the solution heating of Ringer standard during his course by the reeled of the polystyrene tube, before reach the retina compartment, where the temperature was kept to 30°C, in bath adjustment function. The solution conductive polystyrene tube of Ringer standard was intercepted, between infusion bomb and the chamber, for a system of valves through which was possible to introduce the solution test (drugs in study) in Ringer's Chain. In the superfusion solution exit were positioned the electrodes of record.

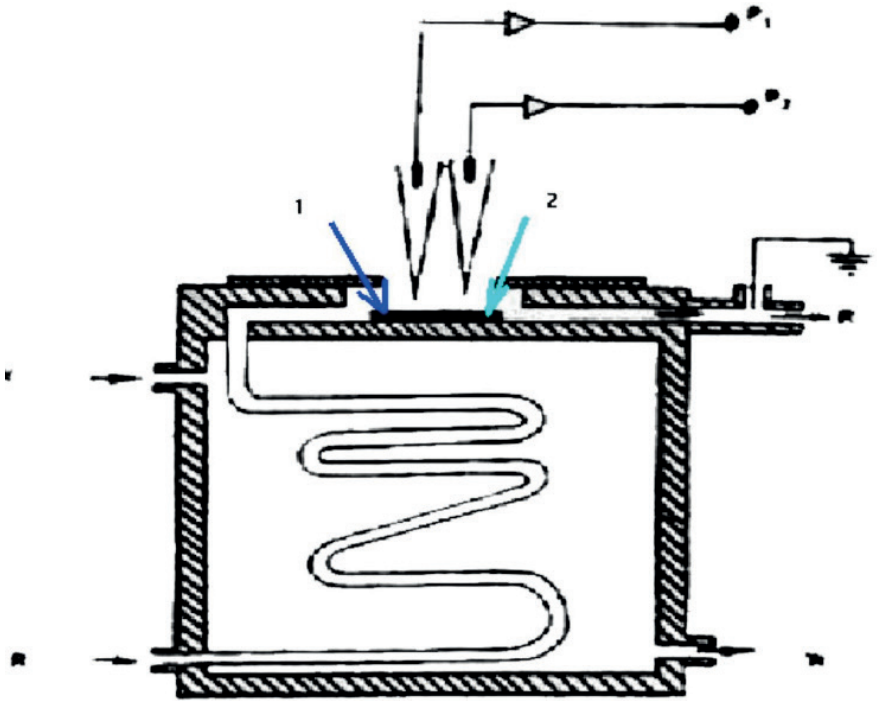


Fig 2) Superfusion chamber outline where will be kept the retina fragment. The numbers 1 and 2 represent both local used to stimulate the tissue. W- bath water; R- solution of Ringer; P1 and P2- Electrodes of record put about the “retina confetti”.

3.3 Microelectrodes

They will be used microelectrodes of glass (capillary with filament, 1,5 x 0,86 mm, Systems, Inc.) with external diameter of tip between 1 and 3 mm, stretched in puller

NARISHIGE (MOD. PE-2) and filled out by a solution of NaCl's 150 mM. The tip of the microelectrode will be observed by the dissection microscope until touch the vitreous surface of the retina. The glass tubes were filled out through its non-capillary extremity, with the help of a sharp glass tube, with tip diameter around 70μm, connected to a syringe. Finally, both microelectrode tubes received each one of them, an Ag+/AgCl thread. These threads were linked to the entrance of an electrometer of two channels (Electromiter model FD 223 World Precision Instruments), whose exit was connected to a differential amplifier. The quality of amplification of the system was tried through pulses of electric chain, generated by a battery put in the circuit, in series. The checked values were registered in a polygraph Grass, model 5D (Grass Instrument CO., It uses).

With the help of a micromanipulator (Narishige Scientific Instrument Lab. Japan) the microelectrode tip was put in the internal plexiform layer of the retina, in other words, in the location where were verified the biggest manifestation of the Lion phenomenon. Thus was possible to check the voltage variation during the reaction.

The experimental montage was provided by a reference electrode, landed, with Ag+/AgCl2, whose metallic thread was inserted in the light of a glass tube performed with Agar, in solution of NaNO3 1 M. The inferior extremity of this tube was obstructed by a small disk of porous pottery, propitiating contact with the superfusion solution, in the point in which she was being drained of the chamber. (Fig.2)

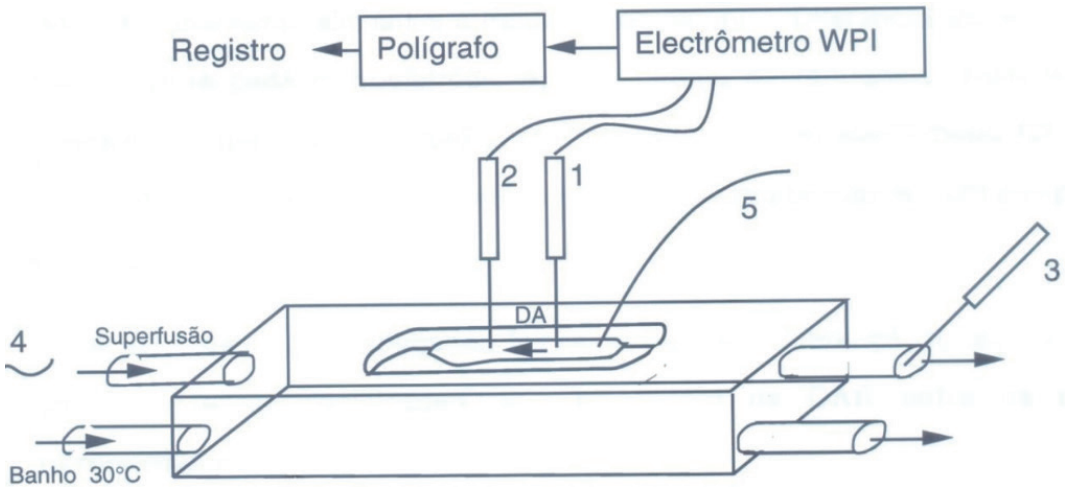


Fig.3) Preparation outline of isolated retina: Plexiglas chamber. 1 and 2: record electrodes. 3: indifferent electrode. 4: Superfusion bomb. 5: Tungsten probe.

3.4 Drugs in study

At present work were used the next AEDs: DV, GP, TP, LV and LT. The Semisodium Divalproate used were the one of presentation in capsules of 125mg. The content of the same will be dissolved in Rs. and the resultant solution percolated in a "Millipore filter". Was used concentrations between 0, 07 and 7 mM, having as reference to concentration of 0, 7 mM, which is the therapeutic concentration of

the drug in human beings serum (Yasui-Furukori and cols, 2007; Nikitina and cols, 2006; Pucci and cols, 2003). Gabapentin is commercialized in capsules of 300 mg, whose content was dissolved in Rs. and afterwards filtrate. Was used concentrations situated between 6 and 600 μM, once the therapeutic concentration of this drug in the human plasma it situates around 60 μM. (Joetta and cols, 2003; Belal and cols, 2002; Ratnaraj and cols, 1998). Topiramate was used in the form of 25 mg capsules. The capsules was macerated and solubilized in Rs., and the percolated resultant solution was used in a concentrations situated between 3 and 300 μM, once Topiramate therapeutic concentration in the human plasma it situates next of 30 μM. (Bahrami & Mahammadi, 2007; Johannessen & Tomson, 2006). Lamotrigine was, as well, used in the form of 25 mg capsules; it was macerated and solubilized in Rs. for posterior filtration. Was used concentrations situated between 1 and 500 μM, once Lamotrigine therapeutic concentration in the human plasma it situates near to 20 μM (Lampl and cols., 2005). Finally, Levetiracetan was used in the form of capsules of 500mg that were macerated and solubilize in Rs. For posterior filtration it was used concentrations situated between 10 and 500 μM, since the therapeutic concentration of the drug in the human plasma it situates near to 100 μM (Coupez and cols., 2003; Patsalos and cols, 2000). We opt by the use of the available medication in the pharmaceutical market, with the goal of approach the real terms available to the users in day by day. It was done, however, an effective research among laboratories that make the available drugs for the survey.

3.5 Lion wave deflagrations

It denominates spontaneous SD (SDexp.) to that which arises without the retina being submitted to any stimulus. In all experiences, except in cases where is accomplished the deflagration threshold measure of the SD (chemical stimulus), the reaction was elicited by means of “mechanical stimulation” of the retina using a tungsten thread with the tip affiliated in about 100 mM. The stimulated location presents, frequently, a puncture lesion, observed to the dissection microscope. The tissue lesion in the stimulated location checks to the type of stimulus a character more structural than mechanic, characterizing him as an over threshold, fact that enables the measure of the absolute refractory period.

3.6 Voltage slow variation Records of the voltage slow variation (SVV) of the SD

It was used microelectrodes of glass (capillary with filament, 1,5 x 0,86 mm, Systems, Inc.) with external diameter of tip between 1 and 3 mm, stretched in puller NARISHIGE (MOD. PE-2) and performed with a solution of NaCl- 150 mM, as previous description. Microelectrode tip was observed to the dissection microscope

until touch the vitreous surface of the retina. Microelectrode tip position is given by the amplitude comparison between the amplitude and the form of SVV obtained with previous records (Martins-Ferreira and Do Carmo, 1987) where was made histological demarcation, serving of criteria for the tip location in the internal plexiform. The reading in the scale of a personal computer NARISHIGE manipulator, as the microelectrode tip penetrates inside the woven, serves of additional criterion for the tip location, situating itself between 70 and 120 mm from the point in which electrode touched the vitreous surface of the retina. Electrodes were linked to a WPI (FD 223) electrometer with entrance high impedance (1013 Ohm) and SVV registered in a polygraph Grass (mod.79) with two channels. After the positioning of the electrodes in the internal plexiform layer, the retina remained in the chamber for a period of 15 min. without being submitted for mechanical stimulus, before start SVV´s Measures, speed of the SD, absolute refractory period and threshold.(Fig.3)

3.7 Measurement of the propagation speed of the SD

In the experiences, the reaction was evoked in a retina region near the exit location proximities of the Ringer superfusion chamber. The reaction speed was calculated measuring time expense for the SD to running through the distance (4-5 mm) between two microelectrodes of glass (1-5 micromeres), positioned in the internal plexiform layer of the retina and aligned orthogonally ahead of wave front. Each microelectrode was connected to an electrometer (WPI). It denominates SD propagated (SDprop.) To the SD that, evoked by mechanical stimulus, running through all the distance between both microelectrodes, being detected by each one of the two microelectrodes in sequence (illustration). The speed was measure in retinas superfunded for Rref (control) and in retinas superfunded for 15 min. with Ringer's Solution contend different physiologic concentrations of each one of the five drugs: DV, GP, TP, LV and LT. The mechanical stimulus (prick) was done with the use of a Tungsten filament (100 tip diameter micrometers) in the period of time of 15 minutes.

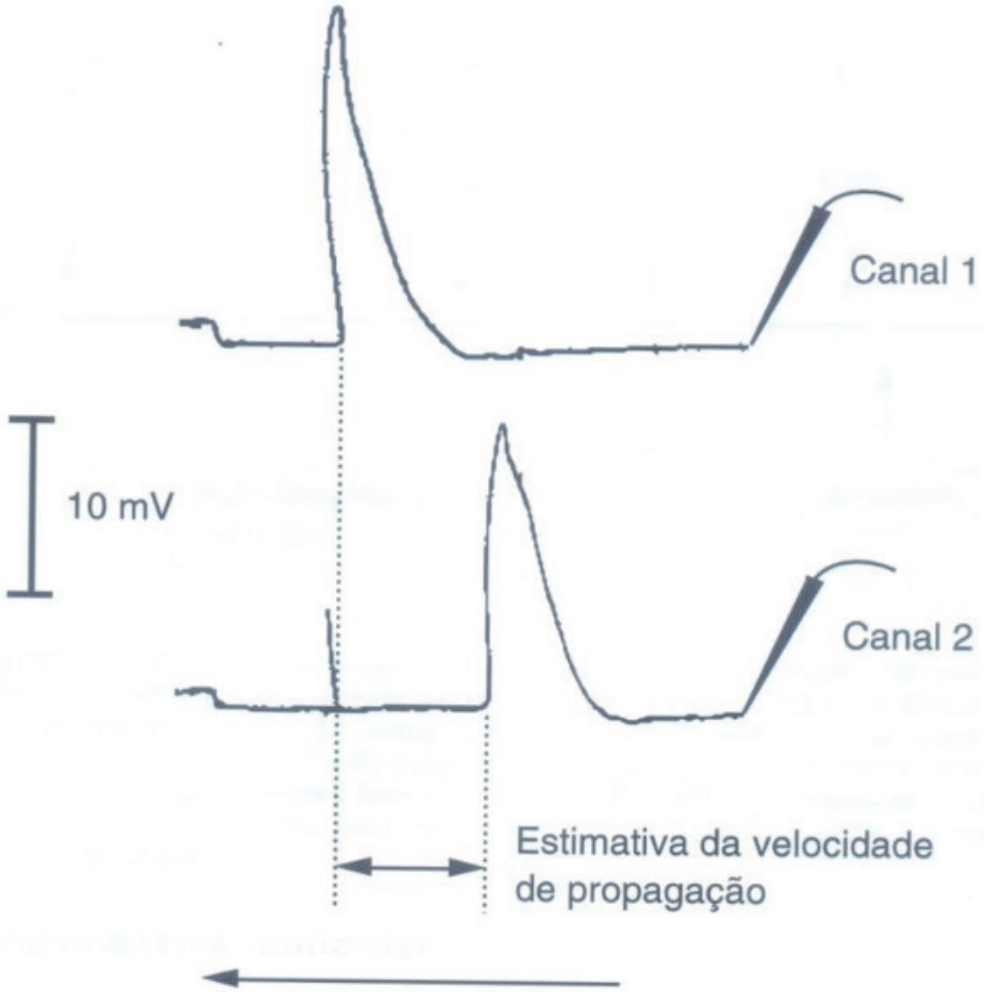


Fig.4) Voltage slow variation representative tracing (SVV) registered by two successive electrodes. The propagation speed of the SD is estimate from the distance among records in the two channels, knowing the paper displacement speed that is constant during the whole experiment. Speed calculation of the SD (propagation): Space runninged through the paper by the SD under drugs action divided by 25 mm/min (paper speed); after, the value of the average distance between both electrodes is divided by the value found after the first operation. One retina “confetti” (biconvex cut of retina) is used.

In each retina preparation was verified the effect of a certain drug concentration in study. After a short period of balance in the Ringer's Solution, it was elicited SD with mechanical stimulus and, to follow, introduced into the superfusion circuit, a determinated substance concentration of one drug in study. From three to four SD was, so, eliciaded with interval between these of 15 minutes and its corresponding speeds and amplitudes was registered. Next to the final, the retina is superfunded with a Ringer's Solution without the drug (Rbi), one SD of control was obtained (DAref.).

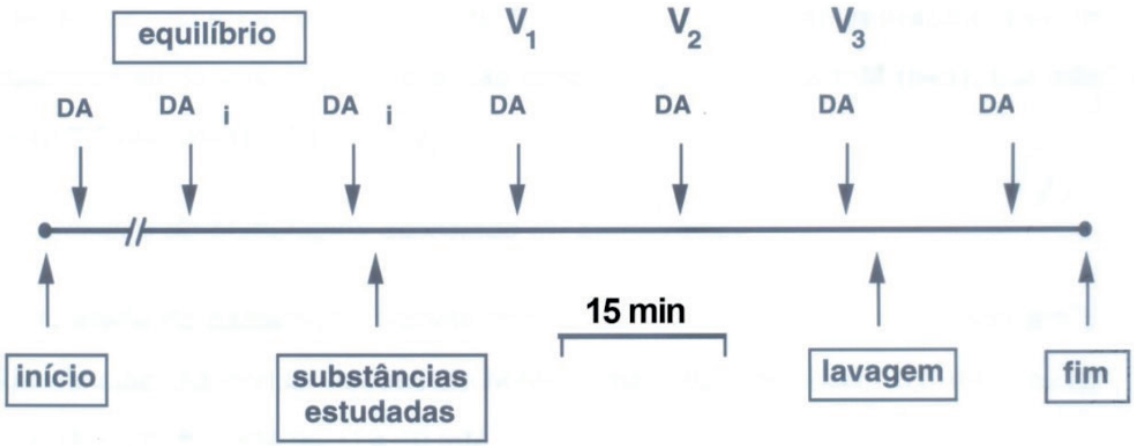


Fig.5) Methodology for the evaluation of the reduction propagation speed effect of the SD in isolated retinas of chick´. After the obtainment of two successive SDs with speeds of propagation constant (SDi.). The substance, in the concentration to be studied, is added. Three successive SDs are afterwards obtained. The corresponding speeds are registered (V1, V2 and V3, respectively). After wash with the solution standard (RBi.), one SD to be a final control is obtained. Mechanical stimulation in made by the use of a tungsten thread with tip sharpened to the about 100 micromeres, every 15 minutes.

Measured of the absolute refractory period (Γr).

Starting from a first mechanical stimulus that results in one SD propagated (effective stimulus), several stimulus were applied in sequence, with time's intervals of 30 seg. between each stimulus, until a new SD propagated was obtained. With this procedure, it is measured the minor interval of time possible between a pair of SDs propagated (Γr) evoked by the mechanical stimulus. Time was measured with a chronometer, that was activated when is applied the first stimulus that results in one SD propagated in the retina. Besides the Γr, also were measured the speed of the first and the speed of the second SD (V1 and V2, respectively) and the voltage slow variation amplitude of the first and of the second SD (SVV1 and SVV2, respectively). In the experiences in which it was used this protocol, the mechanical stimulus was applied in a region of the retina near to Ringer's entrance in the chamber, the SD is propagated in the direction of the Ringer's Flow.

3.8 Measured of the deflagration threshold of the SD

The deflagration threshold of the SD was measured using a protocol in which Ringer's solutions contend different concentrations of K+ (starting from 8 mM) will be applied in the retina surface using a micropipette connected to an eject bomb. The location where was applied Ringer's solution with K+ situates near the superfusion chamber exit. The ejection flow was situated in the interval between 20-200 μl/min. and it was considered effective to the concentration of K+ in the ejected solution that deflagrated the SD in up to 60 seconds (Martins-Ferreira and cols., 1993). The effect of DV,GP, TP, LT and LV on the deflagration threshold of the SD, for some solutions contend several concentrations of K+, was quantified by the use of different

concentrations of K+ applied in retinas previously treated for 15 min with these AEDs.

Experiences with the administration of drugs “in vivo”.

The propagation speed of Lion´s wave (SD), as well as the referred amplitude (SVV), was checked with the techniques used early (described early). In properly prepared retinas as described early. Five animals groups (Chicks´ Gallus) with four elements in each was prepared; the animals age and weight was the same described before; three animals of each group were treated with ideal pharmacological dose for the species, equivalent to the physiologic average dose obtained in experiments made before. According to the group, the animals were treated with TP, GP, DV, LT and LV, for 15 consecutive days, using intraperitonial applications. The forty of each group of animal, was considered as control and, consequently, was not treated. After 15 days the animals were sacrificed and their retinas prepared for the study of the SD parameters, as indicated previously.

3.9 Activity dosage of the GABA-T in control retinas and after multiple waves with and without the drugs in study

Nowadays it is estimate that about 20 to 50% of the synapses of SNC, especially the located in the retina, use the GABA as your neurotransmitter, depending on the studied region (Young and Chu, 1990). The GABA is degraded by the GABA-T; enzyme that catalyzes the grouping amino transfer of the GABA for the acid α-cetoglutaric, coming to supply intermediary metabolites for the cycle of acid tricarboxilic (Krebs) aiming the oxidative metabolism (shunt of the GABA). The GABA-T removes the GABA, converting it in the succinic-semi aldehyde through a transamination with α-cetoglutarate, rebuilding glutamate molecule. Succinic semi-aldehyde is oxidized by NAD and by semi-aldehyde succinc dehydrogenase in NADH+ and succinate that, then, comes in again to the Krebs' Cycle (Illustration). The GABA liberated in the synaptic rifts is entailed to the pre-synaptic stimulation of GABAergic neurons; its action about receivers target in the post-synaptic surface is finished with the reuptake of this by the nervous pre-synaptic terminals and by glia. This transportation that is mediated by the plasmalemma transportation system, besides being bi-directional is dependent to the temperature and to the extracellular ions Na+ and Cl-. In ideal situations, the reason between GABA intracellular and extracellular is of about 200 times; in this way, the responsible force for this reuptake process is supplied by the movement of the Na+ against your gradient (Martin & Olsen, 2000; Chen and cols., 2004). The reuptake GABA by the nervous terminations is then, reused or enters into the called “GABA shunt”, already described (Cooper and cols., 1991; Jung, 1978). The increased activity of the GABA-T in CNS, decreases the concentration of GABA carrying convulsive crisis, coma and death in mammalians (Kang and cols., 2001; There be and cols., 1996); inversely, is expected that antiepileptic and /or antimigraine

drugs (AEDs) , come to increase the concentration of GABA. This fact might be the case of the drugs in study (TP, DV, LT GP and LV); their pharmacological actions may come to interfere and, maybe, decrease the activity of the GABA-T or come to block directly this enzyme, as the mechanism of action of Vigabatrine (Metacalf, 1979; Sherif & Oreland, 1995). To test the AEDs effectiveness in study , we check the activity of the GABA-T with a method based on kinetic analysis of the activity of the GABA-T with the use of spectrophotometry. (Nascimento and cols., 2007).

Chicks' with about 14 days of life, with close weight of 200 grams and free access the food and water, were separated in an specific place with temperature close of 25° C, and with normal light\dark regime. There was separated about 50 animals for this experimental phase.

The drugs and the equipment used in this phase were the next: I-Acid n-Gamma-Amino-Butyric-GABA (A-2129); II-Tetrazolium Nitro Blue "NBT" (N-6876); III-Nicotinamide Dinucleotide-NAD (N-0632); IV-Dimethyl Sulfoxide (D-8418),V-Acetic Acid (A-4508); VI-Tris (hydroxymethyl) amino-methane (T-1378) and VII-α-Cetoglutaric Acid (4210); obtained from the Sigma (St. Louis, MO, USA). Sulfuric Acid (Vitek) was given way by the laboratory of Neurobiology of IBCCF/UFRJ. It also was used an ultrasonic homogenized (HD2070, Bandelin, Berlim,); a refrigerated centrifuge (3K 30, Sigma, St. Louis, MO, USA), and a spectrophotometer (SPEKOL 1100, ZEISS).

The enzyme preparation was made with the own recipient used for the standard preparation used for the experiments described previously. Before the specific preparation, these retinas were submitted to the deflagration of a SD with mechanical stimulus, as already described protocol, without and with previous treatment for 15 minutes with three drugs in study (TP, GP and DV), in average dose already used in the previous phases also described, according to the animals group selected for control and for each drug. After this phase, the retina was "washed" with Ringer and the pigmented part of this retreat manually of the remaining. The retinas were then triturated with the use of an ultrasonic homogenater. The homogenate was centrifuged with a tampon solution of 4ml of Tris-HCl's to 50 mM (pH close of 8,4) for 1000g for 10 minutes to 4°C. The precipitated was then discarded and the overlie recentrifugated to 14000g for 20 minutes to 4°C. The "pellet" was treated again 4 ml of tampon (as already described) and used directly for the activity determination of the GABA-T.

The protein determination (protein dosage) of homogenate was determined according to Bradford's method (Bradford,1976).

The activity of the GABA-T is determined by the average incubation of 0,05 ml of 50mM of Tris-HCl (pH 8,4), 0.05 ml of substratum (contend it GABA, acid α-cetoglutaric, NAD and NBT) and 0,1 ml of homogenate (0,1mg of protein). The

enzymatic reaction occurs for 20 minutes to 37°C. After this period the reaction is interrupted with 1,7 ml of sulfuric acid to 100mM. The reaction product is checked with the use of a spectrophotometer to 540nm.

Finally, we determine the curve that demonstrates the concentration effect of GABA and of the acid α-cetoglutaric on the activity of the GABA-T, in function of the substratum. The reason between GABA and the acid α-cetoglutaric was always kept in 1/0,7. The concentrations used of GABA went of 1mM, 10mM, 24mM and 97mM, respectively. Coordinated of the curves in: X-(S)mM (concentration of GABA) and Y-H/PTN.-Mg/U(activity of the GABA-T). The concentration effect curve of protein in function of the activity of the GABA-T also was made, following the protein concentrations of 50, 100, 150 and 200 μg. Coordinated of the curve in: X - μg PTN and Y - H/U.

The used enzymatic unit is arbitrary. ”When we have the purified enzyme becomes easy to define itself as the enzymatic unit; when we have a tissue, the unit is defined as arbitrary unit. Therefore we have to dose the protein and normalize her for 1 hour, since the reaction occurs for 20 minutes.” (Nascimento and cols,2007.).

The curve that demonstrates the concentration effect of the GABA and of the acid α-cetoglutaric on the activity of the GABA-T in function of the substratum was made according to the following methodology: Average reaction contend 50mM of Tris-HCl (pH 8,4); 2,9 NAD's mM; 1,2mM of NBT diluted in 20 μ1 of Dimethyl Sulfoxide (DMSO) and different concentrations of GABA and acid α-cetoglutaric, namely - GABA (1, 10, 24 and 97 mM), acid α-cetoglutaric (0,7, 7, 17 and 68mM). As already exposed, the reason between GABA and the acid α-cetoglutaric was always kept in 1/0,7 in other words: For 1mM of GABA → 0,7 mM of acid α-cetoglutaric was used. In the reactions in which the average doses were tried, the drugs in study, these drugs, in average effective doses, as already described previously, were added to the substratum.

The resultant curves were analyzed with the use of the program “GRAPHPAD PRISM” version 5.0. The statistical analysis was made, as well, with the help of GRAPHPAD PRISM version 5.0. It was used Kruskal-Wallis' Tests - one-way ANOVA - followed by the Dunn's Test´s for multiple comparisons “versus” group control and the Friedman's Test, considering the controls values, in each experiment group, equal to 100% with the goal of the avoidance of variations of non-identifiable causes in the results bands of the different experiments.

4 I RESULTS

Determination of the speed and amplitude of the SD in mM/min and mV:

The results obtained can be analyzed with an objective form with the help of the

table below (table 1), where are represented the obtained data, in percentages, after analysis of all the experiments of this phase.

Table 1:

Drogas:	V(mm/min)	VLV (Mv)	Concentração Máxima
Levetiracetam	21,6%	11,6%	10 mM
Lamotrigina	41,2%	11,3%	1 mM
Divalproato	47,1%	23,7%	6 mM
Gabapentina	35,6%	28,4%	1.12 mM
Topiramato	67,9%	21,1%	600 mM

Table 1: Percentage of speed reduction in mm/min. and of the amplitude in mV of the slow variation of voltage, regarding the SDs initials. All of these concentrations variations are significant regarding the first stimulus (initials SDs).

The propagation speed variations of the SD, as well as of the amplitude of SVV, regarding the drugs used in the study can be visualized, with clearness, in the graphics below. During this phase of work phase were done, for parameter, four mechanical stimuli, with tungsten probe and with time's interval of 15 minute between these, initiating by the control, solution of Ringer “basic” (RB) and progressing, most of the time, for six phases, with the drugs concentration increasing progressively, according to the pharmacological concentrations of the drugs, already described previously. At the end of each sequence, the retina is “washed” with RB, obtaining a Ringer's Solution after wash (Rbi.), During this process, another four mechanical stimulus are done (Fig.); during this experiments phase, the speed and amplitude go inexorably up, but do not reach the standard observed initial before the drugs introduction. It is possible that, if the experiences could be prolonged by a period greater than 6 hours (average of time's used for each experiment) until, at least, 24 hours the parameters evaluated by drug can come to come back to the normality; This procedure showed, however, improper in function of the time's availability lack to the same and, as well, in function of the fact that the retinas kept in the preparation at issue tend to die after 12 utilization hours.

The doses used in the experiments were based on pharmacological doses used to the prophylactic treatment of migraine treatment.

Determination of the deflagration Threshold:

In this phase were checked, for every drug in study, the deflagration threshold measures regarding a chemical stimulus done with Kcl-, in different concentrations, starting from 8 mM. The characteristics and concentrations of each drug, as well as the physical characteristics of the environement in study, are described before each experiments sequence. For each substance ten experiments were done. The deflagration threshold was checked, for drug; regarding the concentration of Kcl- (mM) minimum that was effective in generate SD, for each drug in study. A

comparative graph was done to the final of this phase. The drugs concentration in study was obtained by the median of the pharmacological concentrations used in the phase II (determination of the speed and amplitude) with 50% of this concentration more than the average concentration. Ejection flow of KCl-.: 100 μl/min.

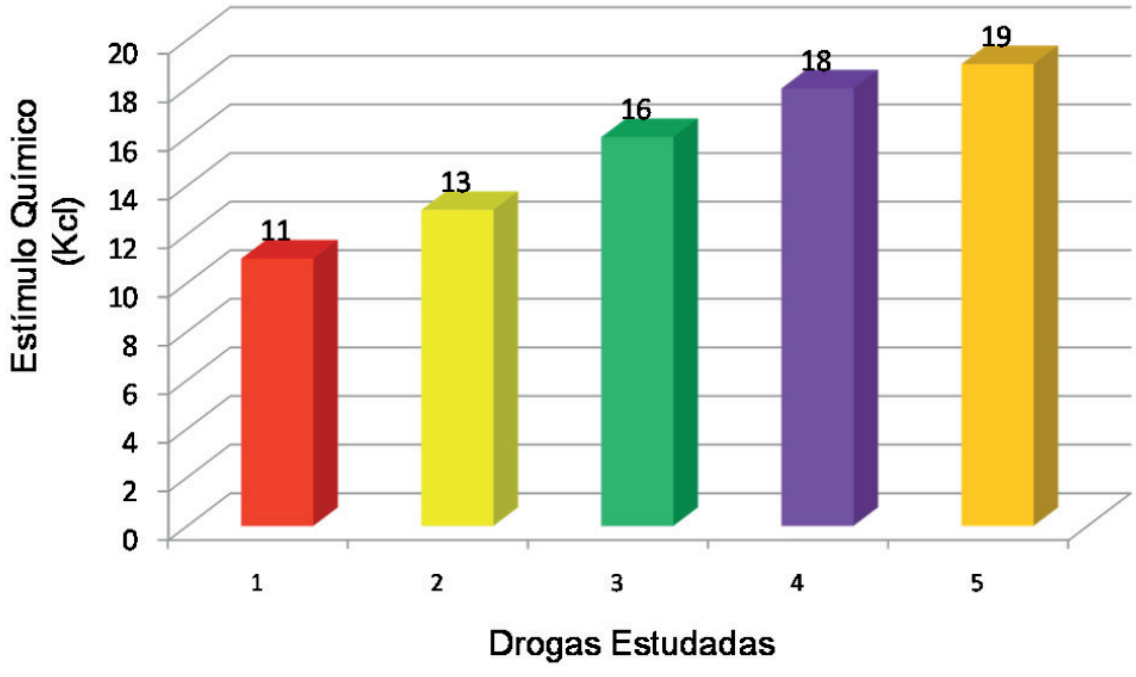
The analysis of the obtained data in this work phase, we can easily verify that Topiramate was the drug that determined a largest deflagration threshold for the phenomenon. It was necessary a chemical stimulus of 19mM of Kcl- for deflagrating the SD with the use of this drug. This data reinforces the fact described in before in this work.

Table 2: Comparison of the effects five drugs regarding the deflagration threshold for a chemical stimulus (Kcl-).

1- Levetiracetan	2,3 mM	11 mM/K^+
2 - Lamotrigine	320 µM	13 mM/K^+
3 - Divalproate	3,5 mM	16 mM/K^+
4 - Gabapentin	365 µM	18 mM/K^+
5 - Topiramate	350 µM	19 mM/K^+

Table 2: We verify that Topiramate was the drug in which the deflagration threshold of the SD was larger (larger concentration of K+). Levetiracetan was the drug in which the deflagration threshold of the SD was smaller (smaller concentration of K+)

Histogram revealing an effects comparison of the five drugs regarding the deflagration threshold for chemical stimulus (Kcl-).



Graphic 1: Histogram revealing the results described in the table 7.

Determination of the absolute refractory period (Γ).

In this phase were checked, for every drug in study, the measures of the absolute refractory period (in units of time's) regarding a mechanical stimulus, done without any drug, after the use of each drug in study, in effective average concentration, already determined by the simple average of the concentrations of each drug determined in the Phase II of the study, after “wash” with Rbi. The characteristics and concentrations of each drug in study, as well as the physical characteristics of the environment in study, are described before each sequence of experiences sequence. For each drug five experiments were done; after each one of these experiments, it was calculated the simple average regarding the data of each experiment. These comparative data, for drug, were used as standard for comparison. A comparative graphic was done in the final of this phase.

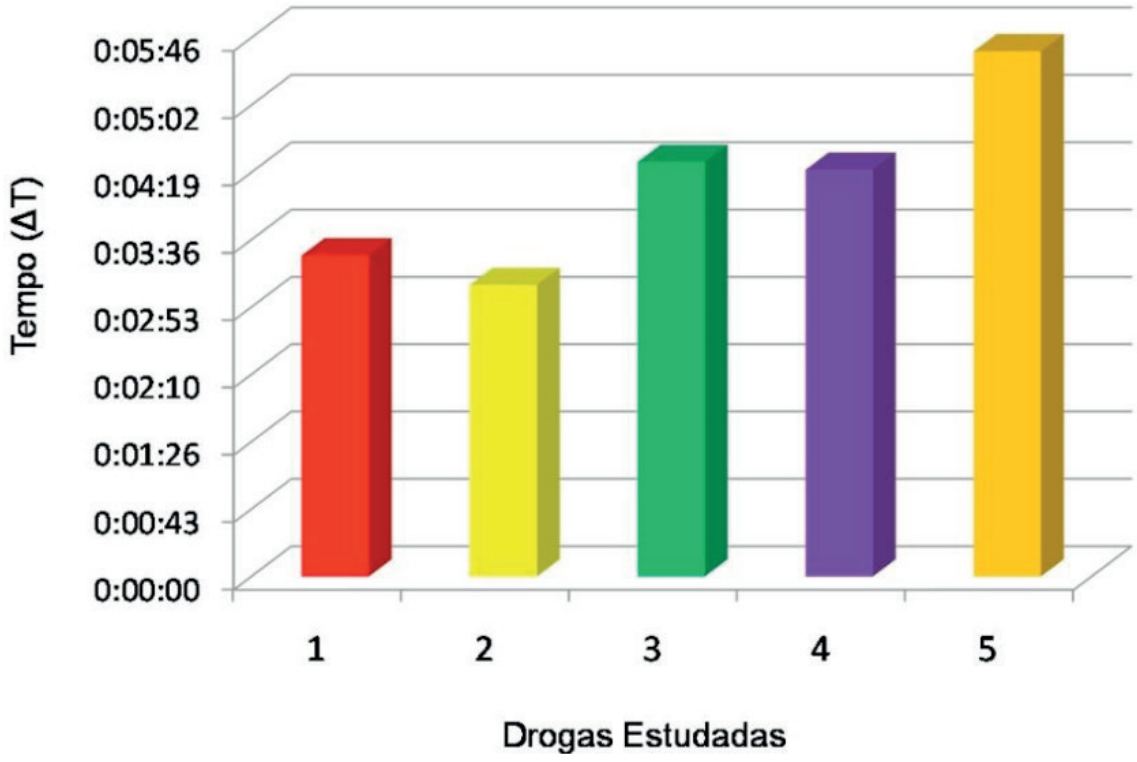
By the analysis of the data obtained work phase, we can easily also verify that Topiramate was to the drug that determined the largest absolute refractory period for the phenomenon. It was verified an absolute refractory period of five minutes and thirty-seven seconds for the deflagration of SD with the use of this drug. This data reinforces the observations of the phase II and III of this work.

Table 3: Comparison between the effects five drugs regarding the absolute refractory period for a mechanical stimulus.

1 - Levetiracetan	0:03:26
2 - Lamotrigine	0:03:07
3 - Divalproate	0:04:26
4 - Gabapentin	0:04:21
5 - Topiramate	0:05:37

Table 3: Comparative - Absolute refractory period, we verify that Topiramate was the drug in which the absolute refractory period was larger (0:05:37) and Lamotrigine was the drug in which absolute refractory period was smaller (0:03:07).

Graphic 2: Histogram revealing the comparison of the effects of the five drugs regarding the absolute refractory period for mechanical stimulus.



Graphic 2: Histogram showing the results described in the table13.

Experiences “in vivo”.

In this phase were checked, for each drug in study, the speed measures (mm/min) and of the slow variation of voltage (mV) in chick´ retinas, with average age of 15 days treated “in vivo”, for fifteen days with TP, GP, DV, LT or LV in effective average concentration and average dose adequate to the middleweight of each animal, with the use of intraperitonial injections use daily. The animals remained in environment closed under heating for lamps of 60 watts, After 15 days the animals were sacrificed by beheading and the retina fragments superfunded with Rbref.. Mechanical stimulus were done every 15 minutes, For each drug were studied 4 animals, and 1 between these was considered control, not receiving, consequently the “treatment” with the drugs in study, After all the experiments, was calculated the simple average regarding the speed (mm/min.) and the voltage of the SVV amplitude (mV), These comparative data, for drug, were used as standard for comparison, two comparative graphs were done, in the final of this phase.

Of the analysis of the obtained data in this work phase, we can verify that all the studied drugs, in pharmacological concentrations corrected for weight and corporal mass of the animals treaties, did not provoke side effects and/or relevant toxicity that has carried to the death of some animal. All the drugs indeed crossed the hamate-retina barrier and probably also the barrier hamate-encephalic barrier. Topiramate was considered as well, in this work phase, the most effective drug and Levetiracetan the least effective regarding the studied drugs. This data reinforces the observations of the phase II, III and IV of this work.

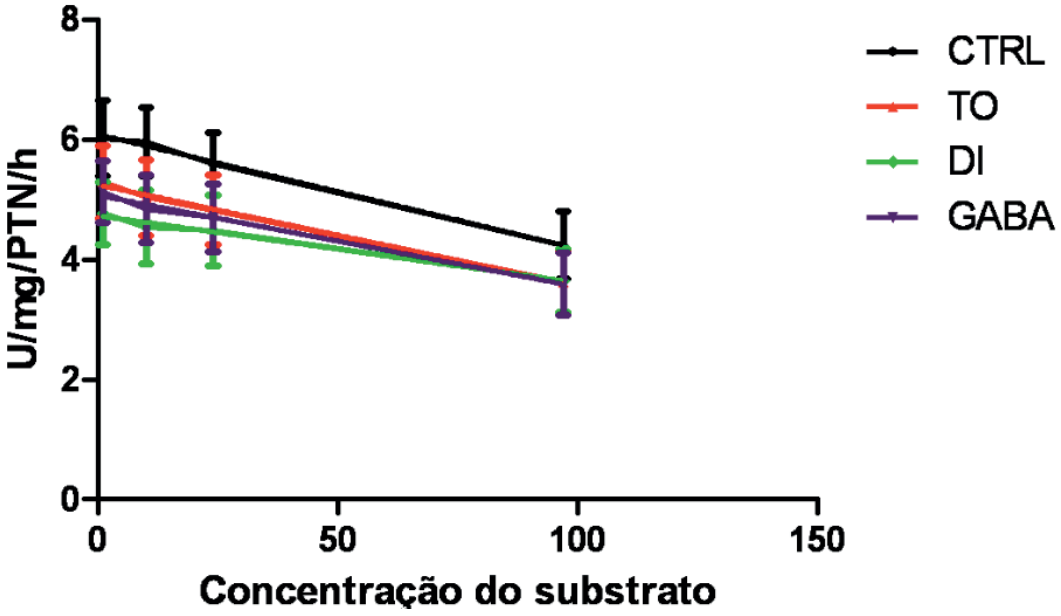
Table 4: Effects comparison of the 5 drugs regarding propagation speed in mm/min and the amplitude in mV. “in vivo”.

	V(mm/min)	VLV (mV)
1 - Levetiracetan (2,3 mM,)	4,7	19,4
2 - Lamotrigine (320 µM)	4,3	18,6
3 - Divalproate (3,5 mM,)	4,1	18,0
4 - Gabapentin (365 µM)	4,6	17,4
5 - Topiramate (350 µM)	3,0	18,1

able 4: We verify that Topiramate was the drug in which speed was smaller (larger effect about this variable), Gabapentin was the drug in which the amplitude was smaller and Levetiracetan was the drug in which not only the speed but also the amplitude were larger (smaller effect about these variable). These important results are similar to obtained in the Phase II of this work.

Dosage of the GABA-T activity

With the use of three drugs in study: Topiramate, Gabapentin and Divalproate, besides control without the drug use, the band of used protein was, rigorously, inside the linearity. Six experiments in this phase were done. The doses of the used drugs in this phase corresponded rigorously to the effective average dose observed before in this work, in other words, 350μM of Topiramate, 365μM of Gabapentin and 3,5 mM of Divalproate. We could note that, after the data analysis obtained in the six experiments done with the drugs used, there is a clear tendency for the enzyme activity decrease. This fact suggests that, somehow these drugs act or directly or indirectly about the activity of this enzyme. This fact was proved, in this study, by the made statistical analysis of this work phase, where they were obtained statistically important results with the use of the proposed statistical tests (Kruskal-Wallis - one-way ANO-VA - followed by the Dunn's Test for multiple comparisons “versus” group control and Friedman's Test).



Atividade da GABA- T (Curvas Comparativos)

	Controle		Topiramato		Divalproato		Gabapentina	
	Média	DP +/-	Média	DP +/-	Média	DP +/-	Média	DP +/-
1	6,02	0,63	5,29	0,6	4,77	0,52	5,13	0,52
10	5,97	0,57	5,03	0,63	4,54	0,61	4,84	0,56
24	5,6	0,52	4,82	0,58	4,49	0,59	4,7	0,56
97	4,24	0,56	3,63	0,54	3,65	0,53	3,6	0,52

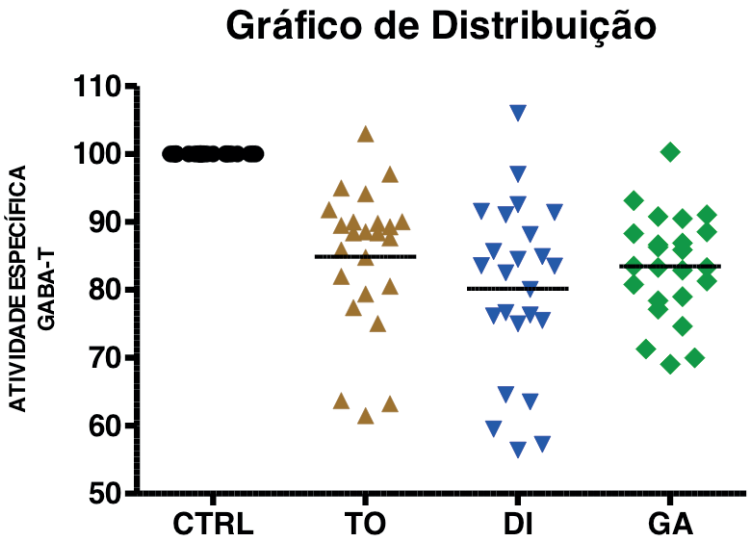
Graphic 3: Dosage of GABA-T with three drugs in study (Topiramate , Divalproate and Gabapentin). Comparative curves.

Activity satatistical data analysis of GABA-T:

Were used Kruskal-Wallis' Tests - one-way ANOVA - followed by the Dunn's Test for multiple comparisons “versus” group control and Friedman's Test. Due to cause variations not identified in the results bands in some experiments was made a transformation considering the values controls for each data sequence, inside the same concentration of GABA, used to the obtainment of the specific activity of the GABA-T, in each experiment group, equal to 100%.

Data analysis for all concentrations of GABA (1 , 10 , 24 and 97 mMs.) :

- able with data in perceptual, 100% - control.



Graph 4: Dosage of the activity of GABA-T with three drugs in study (Topiramate, Divalproate and Gabapentin). Distribution graph for all studied concentrations of GABA.

Kruskal-Wallis' Test:

P < 0, 0001, gaussian approach. (P < 0,05).

Multiple comparative test of Dunn´s:

CTRL X TO: 38,75 (P < 0,05).

CTRL X DV: 48,58 (P < 0,05).

CTRL X GP: 44,67 (P < 0,05).

Friedman's Test:

P < 0, 0001, gaussian approach. (P < 0,05).

CTRL X TO: 37,00 (P < 0,05).

CTRL X DV: 50,00 (P < 0,05).

CTRL X GP: 45,00 (P < 0,05).

5 I DISCUSSION

This work suggests that basically, in this model and clinically effective concentrations, antiepileptic drugs preferably with a modulator of GABAergic mechanism of action phenomena (MGF) (Wiedemann et al, 1996; Calabresi et al, 2007), which have been used for the chronic treatment of migraine, may interfere with the Lion phenomenon, concentration-dependent manner. This interference can lead to a decrease not only the speed of propagation of AD (in mm / min) but also its amplitude (in mV), triggering threshold and absolute refractory period.

Migraine is a multifactorial inherited disorder in the brain involves a dysfunction of subcortical structures that modulate the afferent sensory stimulation. Currently, migraine is explained not only by a vascular mechanism but also by a heterogenic channelopathy related to some ions, resulting in the phenomenon of spreading

depression. The development of drugs that may provide a reduction in cortical hypersensitivity states (action on ion channels) seems like a good way for the prophylactic treatment and for acute migraine. Based on this point of view, the use of GABAergic drugs has been approved for the prophylactic treatment of migraine from 2003. (Puppe & Limmroth, 2007).

The mechanism of action of drugs that act in the prevention of migraine are many, but it is postulated that these will converge on two targets: inhibition of cortical excitation and control of nociceptive demodulation. It seems that the lack of proper modulation of GABAergic inhibition system in some areas of the brain have to have a key role in the pathogenesis of the disease (Calabresi et al, 2007). Antiepileptic drugs FGM, mainly topiramate, divalproex, gabapentin, levetiracetam and lamotrigine, which act as a Lion Wave inhibitors, are clear examples of drugs that reduce the cortical heperexcitabilidade. Furthermore, modulators of the cholinergic and serotonergic appear to have also a role for the prevention of migraine since it can restore the nociceptive descending inhibition (Ramadan, 2007).

A number of experimental works by using in vivo models show the effectiveness of FGM drugs for inhibition of spreading depression and migraine prophylaxis consequent (Sanchez-Del-Rio et al, 2006, Akerman & Goadsby 2005). According to Ayata et al. (2006), chronic daily administration of these drugs, dose-dependent decreases the frequency of cortical of 40 to 80%, and increase the stimulation threshold for obtaining them. This work demonstrates, above all, that the model developed by Martins-Ferreira et al, FGM blocking drugs can come concentration-dependent way, even an avascular structure.

By virtue of the connection between a putative DA and migraine (Lion, 1944). Previous studies used the model of isolated retinal paint under the effect of a classic anti epileptic drug - diphenylhydantoin (HFD) - Chebabo & Do Carmo, 1991 on spreading depression induced by mechanical stimulation and / or químico.Observou up, in the study, the DFH not only increases the KCL concentration threshold for the outbreak of the phenomenon as also decreases the speed of propagation of AD. It was not done, however, a "link" between the observed and the prophylaxis of migraine, perhaps by the fact that, to date, we do not know the correct the DFH mechanism of action. In fact, the literature makes no reference to the prophylactic use of DAP in migraine, although several drugs used in this prophylaxis are anti epileptics.

Martins-Ferreira and Ribeiro effected preliminary experiments topical application in the cerebral cortex of rabbits (1 mM) and injection (6 mM) of Sumatriptan, a drug used clinically as "abortion" in acute migraine phase. With this method did neither blocking nor reducing the speed of VLV. However, blockade of DA was obtained Sumatriptan, serotonin and / or Ergotamine in the range of 0.5 - 5.0 mM, isolated chick retina (Maranhao-Son, 1997). Moreover, Wiedemann et al, 1996, obtained

similar results with Sumatriptan, but not with Ergotamine.

In 1993, Martins-Ferreira et al, and check the action of various channel blockers, compared the speed reduction caused by serotonin and by Sumatriptan. It was concluded that serotonin in 3 mM concentration caused a dramatic reduction in propagation speed of AD, followed by complete blockage of current DA, Sumatriptan while, at the same concentration, was more effective in blocking the DA 2 -5 min.

In 2005, Akerman and Goadsby, studied the effect of topiramate on the cortex in the cat and mouse and found that the drug, at a dose of 30 mg / kg, can inhibit DA 90% of the animals. Margineanu and Klitgaard, 2009, also studying rat cortex models, significant results obtained with regard to the inhibition of the use of Brivaracetam (UCB-34714), experimental drugs with high binding affinity for synaptic protein SV2A and close relative, but more powerful, of levetiracetam. This paper presents more complete results than those described above because, in that dosages were used "clinics" of FGM drugs used for the prophylactic treatment of migraine. In this event, the model used in the work (chick retinas) seems to be more reproducible than those seen studies that used the chick retina structure is avascular, been allowing the occurrence of a "neural effect - on neurons and / or glia - "drug under study.

Furthermore, all of our results with the drugs tested both in vitro models as in vivo models were effective; independent of this case leaving to consider the passage of the drug through the blood-brain barrier.

We observed initially, after completion of the Phase II study all drugs that interact in some way with the phenomenon described within the context of the proposed experimental (isolated chick retina). There is a decrease in different proportions from both the propagation velocity in mm / min. as the amplitude of AD mV after mechanical stimulus in all the preparations according to the proposed drug effect. This reinforces the concept of the GABAergic transmission, and is an important phenomenon for Spreading Depression (both generation and propagation) is central to the pathophysiology of migraine.

Initial experimental results appear to be in agreement with the reviewed medical literature (migraine prophylaxis). The drug, which according to experiments done in this work, presents the best result in the context of decreased DA velocity is topiramate (decrease of 67.9% in the rate of spread). This fact, in addition to being relevant, is in line with most of the work described in the medical literature.

Recently, topiramate was considered by the European Neurological Society the drug of choice, within the group of antiepileptic drugs for the prophylactic treatment of migraine. The second drug with better performance in this study is the Divalproex (decrease of 47.1% in the rate of spread), a fact that is also in line with the main studies reviewed. Today Divalproex is the second most used drug in Europe, within the group of antiepileptic drugs for the prophylaxis of migraine and the first option,

within this group, USA. Gabapentin was reduced by 35.6% in the rate of spread of DA; this fact reveals only a partial reasonable result for the drug in question. The literature review for Gabapentin reveals that this drug was considered at the end of the last decade, very promising for the prophylaxis of migraine. However, the main clinical studies at the time did not present encouraging results, a fact which, added to various side effects of the drug, was of relevance to the placement of this drug in the background, in the prevention of migraine with drug use antiepileptic.

In this experimental study, lamotrigine decreased by 41.2% in the rate of spread of AD This fact, in addition to being consistent with the medical literature, is quite encouraging, given that we are dealing with a new drug. The Lamotrigine has been the focus of important clinical studies in the past five years, all with encouraging results. Several therapeutic approaches have emerged for the use of this drug in migraine prevention protocols in Europe; however, because it is a relatively new drug, new studies should be developed. In this experimental work, Levetiracetam fell by 21.6% in the speed of AD, a fact considered very encouraging and consistent with little medical literature The Levetiracetam, drug walking newer than Lamotrigine, has been the target of some clinical studies isolated for migraine prophylaxis, due to its innovative mechanism of action, and its positive results in the context of control of complex epileptic frames. The results of these tests have been very encouraging, however, still make up needed more clinical studies (more significance level) for a complete evaluation of the potential of this drug for the prophylaxis of migraine. We draw attention, however, to the fact that the Brivaracetam (ucb-34714), more powerful experimental analogue that Levetiracetam, seems to have the best effect on the AD than the latter. (Margineanu and Klitgaard, 2009).

All drugs studied had, in varying proportions, a decrease in the amplitude of the DC. The drug with the highest percentage reduction in this variable mV was Gabapentin (28.4%). Topiramate Despite having the largest percentage decrease in speed of propagation, showed only a reduction of 22.1% in the amplitude of DC. We believe it is important in the experimental context, verificarmos a decrease in the amplitude of DC when studying drugs that modulate the Lion phenomenon. However, further studies are needed in this area because they have not mastered in full, the biochemical and physiological phenomena involved in "phases" of the call Lion Wave.

Finally, we would like to emphasize that all results obtained in this phase of the study were reversible with time. In all experiments done after we get the maximum proposed dose for each drug, an increase in speed and amplitude of the DC when the retina (cutting) was again washed only with Ringer, after the removal of each drug in Bath study .

In phase III of this work, the firing threshold of in relation to a chemical stimulus done at high KCl concentrations, in mM, was measured based on the model proposed

by Martins-Ferreira (Ferreira-Martins et al., 1993). From the concentration of 8 mM, chemical stimulus was increased gradually until the measurement of the concentration limit for the firing of DA, for each drug in estudo.A analysis of the results obtained in this phase shows that the threshold of outbreak obtained with higher concentrations of KCl occurred with topiramate, with a concentration of 19 mM KCl. This fact is consistent with the results obtained so far and also to the journal literature. We can, directly, stating that among the five drugs under study, topiramate was shown to be the most potent in blocking or decrease the DA.

Gabapentin presented an outbreak threshold in 18 mM KCl, a fact that can also be considered effective seen that the drug in question has shown results in many experiments, however, has been in the background in relation to Topiramate and Divalproex. The Divalproex presented, in turn, one outbreak in 16 mM threshold, threshold that puts you at an intermediate position in relation to the drugs under study. The drug showed lower firing threshold was Levetiracetam, this drug has not shown encouraging results among the antiepileptic drugs for the prophylaxis of migraine. May be of relevance to the study of his most potent analog, Brivaracetam (ucb-34714).

We can only draw attention to the fact that, in this work phase, the concentrations of the drugs under study, used in the experiments represented the median of pharmacological concentrations studied in phase II as well, a concentration greater than 1.5 this median.

In phase IV study was measured absolute refractory period of study drug, in units of time, for an effective mechanical stimulus for triggering AD. At this stage, too, topiramate was found to be more potent compared to other drugs being studied. The absolute refractory period for Topiramate was 5 minutes and thirty seven seconds followed by obtained for Divalproex in 4 minutes and twenty-six seconds. The lower refractory period was measured Levetiracetam, in 3 minutes and twenty-six seconds. These results are in agreement with the literature reviewed and the findings of the phase II and III of this work.

In vivo experiments in which pharmacological concentrations of the drugs under study were used, ie the median of concentrations used in the experiments of Phase I was found that in all study drugs, changes, even if minimum, speed, and amplitude of the obtained retina of animals after the same treatment for 15 days and sacrifice by decapitation. Again, at this stage the drug showed that topiramate was most effective (measured speed of 3.0 mm / min and amplitude of VLV, measured to be 18.1 mV) followed by Divalproex (measured speed of 4.1mm / min and amplitude of VLV, measured 18 mV). The drug is the least effective was, again, the Levetiracetam (speed measured at 4.7 mm / min and amplitude of VLV, measured in 19.4mV). These findings are in strict accordance with literature findings and phases I, II and III of the

work in question. It is important to note that all drugs, at physiological concentrations, crossed the blood-brain barrier and have provided some effect on the DA, in animal studies; this fact is also seen according to the literature, all drugs studied seem, MGF by mechanisms and possibly other more or less effective in the prophylaxis of migraine. This fact does not seem to happen for Sumatriptan seen that, in an experiment similar to this work, Maranhão-Filho (1997) found that the required dose of this drug in effective concentration, so that it had some effect on the Lion Wave, should be in the order of 100 g / kg dose this extremely high and toxic for use in vivo.

In VI phase of this work we chose the dosage of GABA-T activity, following the birth protocol et al (2007), for measuring the activity of this enzyme. In this study, we used the chick retina without the use of these drugs and without mechanical stimulus to the elicitation of AD and chick retinas with three drugs studied (topiramate, gabapentin and Divalproex) and without the drugs under study (control), with DA elicitation by mechanical stimulation (tungsten pin). The point of this phase was to verify that GABA-T activity, protein concentrations within the linear range of the standard curve of protein in all six experiments, with the use of three drugs under study came to decrease, U / mg STP / h compared to control, significant $P < 0.05$ - Kruskal-Wallis and Friedman -. However, in all experiments, the decline of the GABA-T activity was very small relative to that observed with the use of vigabatrin - anticonvulsant drug that works by blocking the GABA-T - (Graeme, 2003; Nascimento et al, 2007) . This suggests that inhibition of GABA-T, there seems to be neither the only nor the main mechanism through which the electro-physiological effects are manifested. Antiepileptic drug / antimigranosas that have been studied seem not act exclusively on the inhibition of GABA-T activity. Another aspect that we can only focus is the fact that the GABA-T seems to have a different mechanism of inactivation in the retina compared to that observed in the brain; This fact provides a "limited" increased GABA concentration in the retina compared to that observed in the brain (Rando et al, 1982).

In the cerebellum, the values of the enzymatic reaction of GABA transaminase in units are around 200 U / mgPTN / H (Nascimento et al, 2007). In the retina the values found in this study are about 5 to 6 U / mgPTN / M, or 30 to 40 times lower than that found in that organ. This difference could be related to retinal features, ie: if the expression is neuronal or glial, moves with the development, it is affected by the environment, especially by light, is affected by hormones and / or extracellular factors and is modulated by GABA itself.

The release of GABA and acetylcholine in AD is reproducible, as the voltage changes, potassium, chloride and water movement (Martins Oliveira-Ferreira and Castro, 1966; Nicholson and Kraig, 1981; Martins- Ferreira and Do Carmo, 1984). The reduction to 0.5 mM calcium and magnesium to increase 2.0-4.0 mM in

the extracellular medium decreases both the phenomenon of "light-scattering" as increasing the concentration of potassium and acetylcholine and GABA release during AD. This phenomenon and suggests that at least in part, the mechanism of release of GABA and acetylcholine in AD, either from synaptic mechanisms. Despite this fact, it is not possible to be established whether the release of GABA and ACh are essential for the occurrence of the phenomenon or if it occurs concurrently with this (Rodrigues et al, 1988). It is known, however, that much GABA and anti-physiologic high concentrations (about 3.0 mM) can come to lock the retina in a short period of time (Rodrigues et al, 1988). In 1987, Roberts et al demonstrated that the use of eserine (cholinergic agonist) is able to decrease GABA release during AD. This suggests that the DA in different CNS regions, at least in part, there is a direct influence conjugates mechanisms between at least two neurotransmitters, particularly the retina (Rodrigues et al, 1987).

Finally, we draw attention to the fact that the main focus of this work was to study the spreading depression and the effect of GABAergic drugs used in migraine prophylaxis rises this, not the study of the effect of these compounds on the GABA-T kinetics . It is suggested for future experiments assessing the effect of these compounds vigabatrin more brivaracetam and, depending on the kinetics of this enzyme. On this occasion should be made "bows" over "spots" and with higher concentrations of the compounds, a fact that would be desirable in order to obtain a more reliable analysis of the kinetics of GABA-T. We believe that in the context of this paper, to obtain a correlation between the effect of compounds on enzyme kinetics, even if a trend is rich.

6 I CONCLUSIONS

1. antiepileptic drugs and / or antimigranosas with modulatory action on GABAergic transmission mechanism, that is, GABAergic drugs such as topiramate, gabapentin, divalproex, and lamotrigine Levetiracetam reversibly reduce the rate of AD in mm / min, and the amplitude of AD in mV in isolated chick retinas, subjected to mechanical stimulation, concentration-dependent manner.

2. topiramate, gabapentin, Divalproex, Lamotrigine and Levetiracetam increase reversibly both the ignition threshold for AD as the refractory period of AD, in units of time, in isolated retinas subjected chicks, respectively, to chemical stimuli with KCl in specific concentrations and mechanical stimuli, such concentration-dependent.

3. Topiramate is most effective drugs of which were studied, with regard to the reduction of velocity, amplitude increase in the absolute refractory period and triggering threshold of isolated chick retinas, reversibly and so the concentration-dependent.

4. Levetiracetam is less effective drug, of which were studied, with regard to the reduction of velocity, amplitude increase in the absolute refractory period and triggering threshold of isolated chick retinas, reversibly and so the concentration-dependent.

5. All the drugs studied (topiramate, gabapentin, Divalproex, Lamotrigine and Levetiracetam) appear in vivo cross the blood-brain barrier.

6. Topiramate is most effective in vivo drug of which have been studied in regard to deceleration.

7. Levetiracetam is less effective in vivo drug of which have been studied in regard to reducing the speed and amplitude of AD.

8. Both gabapentin and topiramate as divalproex decrease GABA-T enzyme activity in the experimental model under study, and possibly some modest important in functional terms, suggesting that inhibition of GABA-T is neither the one, or the main mechanism through which electro-physiological effects of the drugs studied were manifested.

9. The present study suggests that antiepileptic drugs that act on the GABAergic transmission, especially topiramate, may prevent migraine and interfere with the generation and propagation of spreading depression.

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