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Antiepileptic Drugs Targeting Cerebral Presynaptic Ion Channels Reduce Cerebral Excitability Decreasing Glutamate Release

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1. Introduction

Ion channel dysfunction has been implicated in several neurological diseases including epilepsy. Cerebral ion channels, and particularly presynaptic channels controlling neurotransmitter release, are among the most important targets of various antiepileptic drugs.
In comparison with other parts of the neuron, in presynaptic nerve endings Na^+ and Ca^{2+} channels controlling neurotransmitter release are particularly abundant. However, most studies directed to test the effect of antiepileptic drugs on ion channels are done in preparations suitable for electrophysiological approaches. Because using those approaches in the small sized cerebral nerve endings is almost impossible.
In the present chapter I describe the strategies that we have used for investigating the effects of several compounds known for their anticonvulsant properties, including several of the most commonly used antiepileptic drugs of the first and second generations, as well as of the new potential antiepileptic drug, vinpocetine on cerebral presynaptic ionic channels. For discriminating the effects of those compounds on presynaptic Na^+ and Ca^{2+} channels, we first used depolarizing strategies, such as veratridine that triggers the entrance of Na^+ by activation of cerebral presynaptic Na^+ channels even when the participation of Ca^{2+} channels is eliminated, or such as a high external concentration of K^+, that activates cerebral pre-synaptic Ca^{2+} channels even when the participation of Na^+ channels is eliminated (Sitges & Galindo, 2005; Sitges et al., 2007a; 2007b). More recently, we also test the effects of antiepileptic drugs in the cerebral nerve endings *in vitro* using 4-aminopyridine as depolarizing strategy. Because 4-aminopyridine exposure may more closely mimic some of the changes that may take place in the epileptic tissue, since in cerebral nerve endings 4-aminopyridine besides increasing the permeability of Na^+ and Ca^{2+} channels, also decreases the permeability of some K^+ channels, and by this mean arrests indirectly the Na^+/K^+ ATPase (Galván & Sitges, 2004), making even more difficult the limitation of neuronal excitability.

2. Effects of antiepileptic drugs on cerebral presynaptic Na^+ channel mediated responses induced with veratridine

Voltage sensitive Na^+ channels are responsible for the initiation and conduction of neuronal action potentials. Therefore, the pharmacological down-modulation of those channels in

situations in which all neurons are firing, such as during epileptic seizures, is likely to be particularly beneficial.

Several of the most effective antiepileptic drugs are believed to stop the paroxysmal neuronal activity acting as Na^+ channel blockers. In comparison with other parts of the neuron, Na^+ channels in presynaptic boutons are particularly abundant (Engel & Jonas, 2005). Nonetheless since the small size of cerebral presynaptic boutons (< 0.3 μm) make electrophysiological approaches very difficult, most of the pioneer as well as the sophisticated and important actual studies directed to test the effect of antiepileptic drugs on Na^+ channels were done in preparations suitable for electrophysiological approaches**.** These preparations include molluscan giant axons, kidney cells and Chinese hamster ovary cells transfect with the alpha subunit (the pore moiety) of the channel, and cells in culture among others (Lipicky et al.,1972; Fohlmeister et al., 1984; Xie et al., 1995; Sun & Lin, 2000; Xie et al., 2001; Huang et al., 2006; Lenkey et al., 2010; Karoly et al., 2010); and there are only few studies in which the effect of antiepileptic drugs on presynaptic ion channels controlling neurotransmitter release in the brain were investigated.

Among the first evidences suggesting an involvement of brain presynaptic Na^+ channel blockade in the mechanism of action of some antiepileptic drugs, was the displacement of ^{3}H-batrachotoxin binding to Na^+ channels in cerebral membranes and brain isolated nerve endings by the antiepileptic drugs carbamazepine, phenytoin and lamotrigine (Willow & Catterall, 1982; Cheung et al., 1992; Deffois et al., 1996; Bonifacio et al., 2001; Santangeli et al., 2002; Lingamaneni & Hemmings, 2003). Batrachotoxin, like veratridine, is a toxin of natural origin that binds to the site 2 (voltage sensor) of the Na^+ channel impeding its inactivation and by this mean increases the rate of Na^+ entry and depolarizes the plasma membrane of cerebral isolated nerve endings (Krueger et al., 1980). With the aid of: SBFI, fura-2 and PBFI, that are selective indicator dyes which change their emission fluorescence in response to the changes in Na^+, Ca^{2+} or K^+ in its vicinity, respectively, the changes in those ion channel permeability can be monitored in cerebral isolated nerve endings. Using cerebral isolated nerve endings preloaded with the Na^+ selective indicator dye, SBFI, we found that veratridine was able to increase the internal concentration of Na^+ independently of the presence of external Ca^{2+} (Sitges et al., 1998). Figure 1 adapted from our previous work (Sitges & Galindo, 2005) shows that in hippocampus isolated nerve endings veratridine is still increasing Na^+ when presynaptic Ca^{2+} channels are blocked by ω-agatoxin-TK but not when Na^+ channels are blocked by tetrodotoxin, a toxin of natural origin that binds irreversible to the external pore of the Na^+ channel (*i.e.* site 1) and by this mean blocks Na^+ entrance into the cytoplasm. The hippocampus is a brain structure particularly involved in seizures.

Cerebral isolated nerve endings (commonly referred to as synaptosomes) preserve many physiological properties of intact nerve terminals, including a tight coupling of neurotransmitter release to ion fluxes during depolarization (Turner et al., 1992; Sitges & Chiu, 1995a; 1995b; Sitges et al., 1998; Galván & Sitges, 2004). Depolarization-evoked neurotransmitter release, including the release of the excitatory amino acid neurotransmitter glutamate, the most abundant neurotransmitter in cerebral isolated nerve endings (Sitges et al., 2000), is composed by two fractions: a Ca^{2+} dependent fraction released by exocytosis and a Na^+ dependent fraction released from the cytoplasm by reversal of the neurotransmitter transporters.

When the internal concentration of Na^+ is substantially elevated with toxins such as veratridine in the absence of external Ca^{2+} brain neurotransmitters can be released from the cytoplasm (Nicholls, 1989; Sitges 1989; Adam-Vizi, 1992; Sitges et al., 1993; 1994; Sitges & Chiu, 1995a; Sitges et al., 1998; Sitges & Galindo 2005). In hippocampus synaptosomes the

release of glutamate induced by veratridine in the absence of external Ca^{2+} was sensitive to the EAAT (excitatory amino acid transporter) inhibitor, TBOA (Fig. 2), indicating that the release of glutamate induced by veratridine originates from the cytoplasm by reversal of the neurotransmitter transporters located at the level of the presynaptic nerve endings.

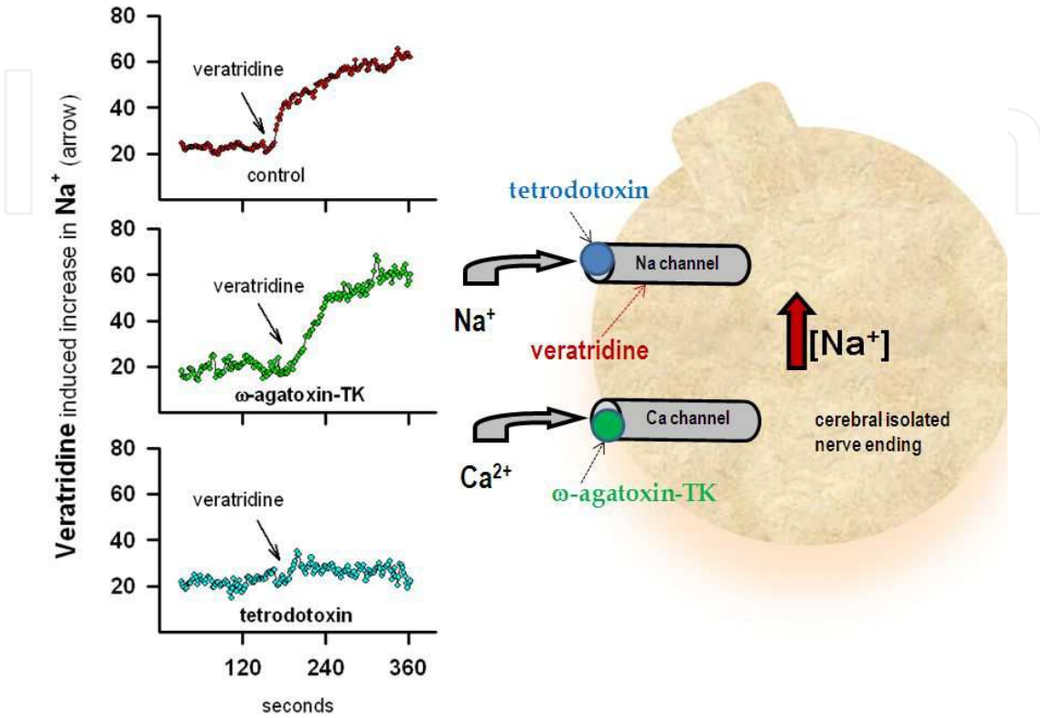

Fig. 1. The elevation of Na^+ (in mM) induced by veratridine is insensitive to ω-agatoxin-TK and completely blocked by tetrodotoxin. In this and the following cartoons channels are represented like tubes, although they are trans-membrane proteins well characterized.

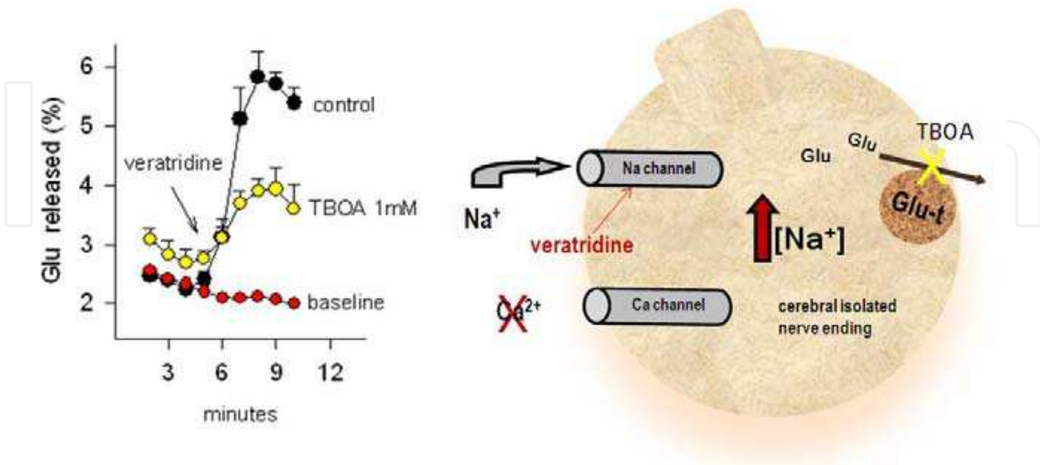

Fig. 2. Inhibition exerted by the EAAT inhibitor, TBOA, on glutamate (Glu) release induced by the Na^+ channel opener veratridine (arrow) via reversal of the glutamate transporter (Glu-t) in hippocampal synaptosomes.

Neurotransmitter release evoked by veratridine in synaptosomes isolated from the whole brain or different brain regions is also highly sensitive to the blockade of Na^+ channels with tetrodotoxin and absolutely dependent on the presence of external Na^+, but is independent of external Ca^{2+} (Sitges, 1989; Sitges & Chiu, 1995a; Galindo & Sitges, 2004; Sitges & Galindo, 2005). This Ca^{2+} independence of veratridine induced responses is particularly valuable as it allows testing the inhibitory effect of compounds on responses selectively mediated by activation of presynaptic voltage sensitive sodium channels in the cerebral isolated nerve endings.

The action of carbamazepine as a brain presynaptic Na^+ channel blocker was first indicated by the sensitivity of the veratridine-induced release of glutamate to that antiepileptic drug (Ambrosio et al., 2001). In a previous study in cerebral nerve endings isolated from the hippocampus we compared the effect of increasing concentrations of several antiepileptic drugs, including carbamazepine, on the release of glutamate induced by veratridine in the absence of external Ca^{2+}. Figure 3, adapted from our previous work (Sitges et al., 2007a; 2007b), shows that the antiepileptic drugs: carbamazepine, phenytoin, lamotrigine and oxcarbazepine, progressively inhibit glutamate release induced by veratridine in a range from 150 to 1500 µM, whereas the antiepileptic drug topiramate only exerted a modest inhibition (20%) at the highest concentration tested (1500 µM). Interestingly, valproate which mechanism of action has been related to the increase in GABAergic transmission (Loscher 2002) was unable to inhibit glutamate release to veratridine at all, although a very large range of valproate concentrations was tested.

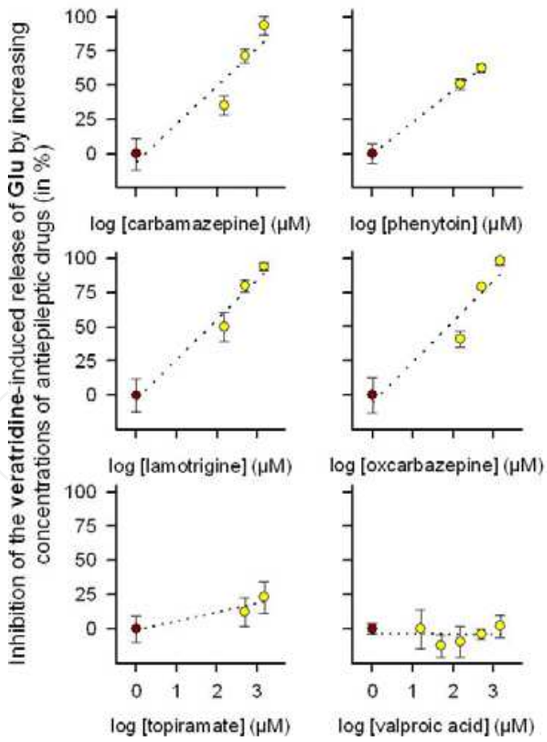

Fig. 3. Inhibition (in percentage of control) exerted by several antiepileptic drugs at increasing concentrations on glutamate (Glu) release induced by veratridine in hippocampus synaptosomes.

Results in figure 3 indicate that blockade of presynaptic Na^+ channels permeability contributes to the anticonvulsive action of carbamazepine, phenytoin, lamotrigine and oxcarbazepine, but not to the anticonvulsive action of topiramate or valproate.

3. Effects of antiepileptic drugs on high K^+ induced responses

On the basis of electrophysiological studies in dissociated cells, neurons in culture or brain slices also a reduction of Ca^{2+} channels permeability by several of the most effective antiepileptic drugs was suggested (Schirrmacher et al., 1993; Lees & Leach, 1993; Wang et al., 1996; Stefani et al., 1996; 1997; Kuzmiski et al., 2005). However, Ca^{2+} currents obtained in those preparations not necessarily reflect the effect of antiepileptic drugs on brain presynaptic Ca^{2+} channels controlling neurotransmitter release. Because in cell bodies, dendrites and nerve endings different types of calcium channels were localized (Timmerman et al., 2001), and Ca^{2+} currents obtained in the above preparations must be mainly somatic.

Again, as for the case of cerebral presynaptic ion Na^+ channels, cerebral presynaptic Ca^{2+} channels cannot be easily approached with electrophysiological techniques because of the small size of cerebral nerve endings. Nevertheless, with the selective Ca^{2+} indicator dye, fura-2, the changes in the internal concentration of Ca^{2+} concomitant to the changes in cerebral presynaptic Ca^{2+} channels permeability can be monitored directly in the cerebral isolated nerve endings. Using this technique it has been shown that among the several types of Ca^{2+} channels present in neurons, those sensitive to ω-agatoxin-IVA and to ω-agatoxin-TK, two peptides isolated from the venom of the spider Agelenopsis aperta, were particularly implicated in neurotransmitter release from cerebral nerve endings (Turner et al., 1992; Sitges & Chiu, 1995a; 1995b; Carvalho et al., 1995; Sitges & Galindo, 2005). P/Q type Ca^{2+} channels are pharmacologically characterized by their sensitivity to the above mentioned ω-agatoxins. In line, the cloned neuronal Ca^{2+} channel α_{1A}subunit encoding Ca^{2+} channels of the P/Q type was localized at a high density in presynaptic nerve terminals of many neurons (Westenbroek et al., 1995).

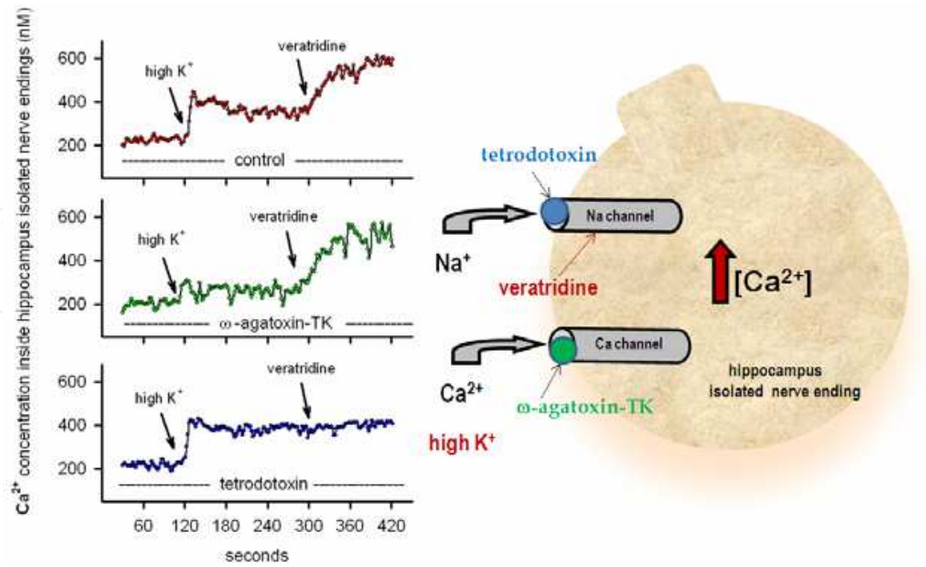

Fig. 4. The rise in Ca^{2+} induced by high K^+ depends on presynaptic Ca^{2+} channels availability and the rise in Ca^{2+} induced by veratridine depends on Na^+ channels availability (this figure was adapted from Sitges & Galindo (2005)

The top graph in figure 4 shows the increase in Ca^{2+} induced by high K^+ depolarization followed by the increase in Ca^{2+} induced by veratridine depolarization in hippocampal synaptosomes under control conditions. The middle graph shows the failure of high K^+ depolarization to increase Ca^{2+} when Ca^{2+} channels are blocked by ω-agatoxin-TK and the failure of this blockade to prevent the veratridine induced increase in Ca^{2+}. Oppositely, the bottom graph shows that when Na^+ channels are blocked by tetrodotoxin, high K^+ depolarization is still increasing Ca^{2+}, but veratridine does not.

It is important to mention that in the presence of external Ca^{2+}, veratridine depolarization also can increase the internal concentration of Ca^{2+} like high K^+ depolarization. Nonetheless, the underlying mechanisms are different. Because while the entrance of external Na^+ via tetrodotoxin sensitive Na^+ channels is strictly required for the increase in Ca^{2+} and the increase in neurotransmitter release induced by veratridine, the increase in Ca^{2+} induced by high K^+ is insensitive to the absence of external Na^+ or to the presence of tetrodotoxin (Sitges & Chiu, 1995a; Sitges et al., 1998; Sitges & Galindo 2005).

In the absence of Na^+ or in the presence of tetrodotoxin, high K^+ depolarization also is still increasing neurotransmitter release. In hippocampus isolated nerve endings the fraction of glutamate release induced by high K^+ depolarization in the absence of external Na^+, however, is completely dependent on external Ca^{2+} and is highly sensitive to nanomolar concentrations of ω-agatoxin-IVA and ω-agatoxin-TK, as shown in figure 5 adapted from Sitges & Galindo (2005). This figure shows that high K^+ depolarization induced responses in the absence of external Na^+ are directly linked to the inhibition of cerebral presynaptic Ca^{2+} channels permeability.

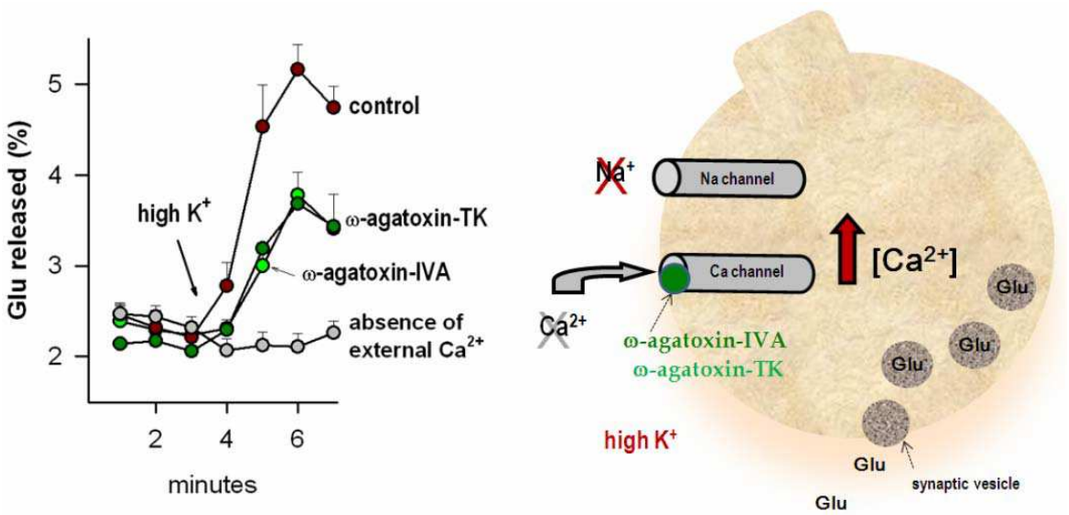

Fig. 5. Glutamate (Glu) release induced by high K^+ depends on the presence of external Ca^{2+} and is sensitive to the P/Q type Ca^{2+} channel blocker toxins. This figure was adapted from Sitges & Galindo (2005).

Since high K^+ can selectively release the Ca^{2+} dependent fraction of neurotransmitter release by exocytosis, for investigating the action of antiepileptic drugs on cerebral presynaptic Ca^{2+} channels permeability, we tested their effects at increasing concentrations on the Ca^{2+} channel-mediated release of glutamate evoked by high K^+ in the absence of external Na^+ in

hippocampus isolated nerve endings. Figure 6, adapted from Sitges et al. (2007b), shows that carbamazepine, phenytoin and oxcarbazepine only reduced in about 30% and 55% glutamate release to high K^+ at concentrations of 500 µM and 1500 µM, respectively; that lamotrigine and topiramate were even less effective, as at the highest concentration tested (1500 µM) they only exerted a mild reduction (about 25%) of glutamate release to high K^+, and that valproate failed to modify the K^+ response at all. These results indicate that only some of the antiepileptic drugs tested, namely carbamazepine, phenytoin and oxcarbazepine, are able to reduce cerebral presynaptic Ca^{2+} channels permeability in some degree at high doses.

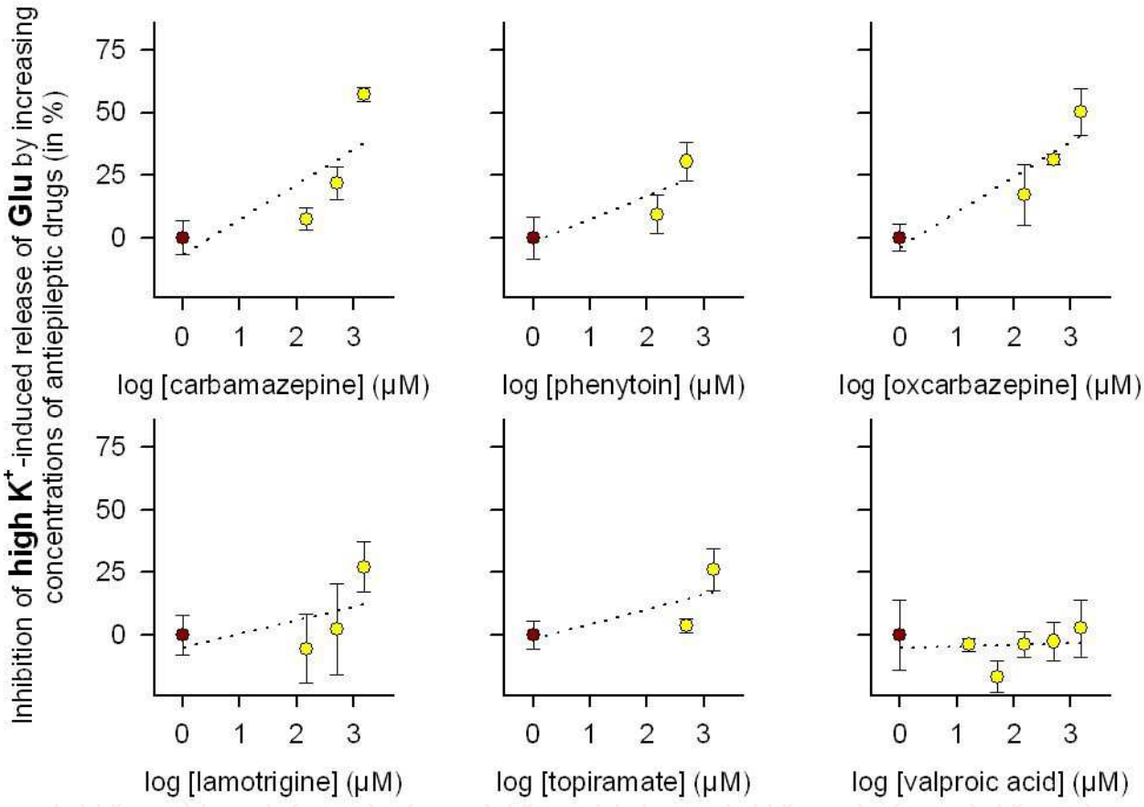

Fig. 6. Inhibition (in percentage of control) exerted by the indicated antiepileptic drug at increasing concentrations on glutamate (Glu) release induced by high K^+ in hippocampus synaptosomes.

4. Effects of antiepileptic drugs on 4-aminopyridine induced responses

4-aminopyridine is a convulsing agent that induces epileptiform activity in brain slices *in vitro* as in animal models of epilepsy *in vivo* (Ives & Jefferys, 1990; Perreault & Avoli, 1991; Yamaguchi & Rogawski, 1992; Psarropoulou & Avoli, 1996; Armand et al., 1999; Nekrassov & Sitges, 2003). 4-aminopyridine increases neurotransmitters release, including glutamate, the most important excitatory amino acid neurotransmitter in the brain, that is by far the most concentrated neurotransmitter in cerebral isolated nerve endings (Sitges et al., 2000).

The action of 4-aminopyridine at the brain presynaptic level is amply documented. Although the rise in the internal concentration of Ca^{2+} induced by 4-aminopyridine was not resolved using $^{45}Ca^{2+}$ (Tapia et al., 1985), it became evident when the more sensitive fura-2 technique was used in cerebral isolated nerve endings. The role of voltage sensitive sodium channels in the mode of action of 4-aminopyridine, first suggested by the sensitivity of the Ca^{2+} response induced by 4-aminopyridine to the Na^+ channel blocker, tetrodotoxin (Tibbs et al., 1989; Heemskerk et al., 1991) was later demonstrated in cerebral isolated nerve endings using the Na^+ selective indicator dye, SBFI (Galván & Sitges 2004). The involvement of K^+ channels in the mode of action of 4-aminopyridine at the presynaptic brain level, first suggested by the changes on $^{86}Rb^+$ fluxes in brain nerve endings (Sitges et al., 1986), was confirmed later using the K^+ selective indicator dye, PBFI (Galindo & Sitges 2004). In summary, in cerebral isolated nerve endings 4-aminopyridine increases Na^+ channels permeability (Galván and Sitges, 2004), Ca^{2+} channels permeability (Tibbs et al., 1989; Heemskerk et al., 1991; Galván & Sitges, 2004; Sitges et al., 2005), and decreases K^+ channels permeability (Sitges et al., 1986; Galván & Sitges, 2004). Therefore, the changes that may occur in cerebral nerve endings under the excitatory conditions that take place during seizures seem to be more closely resembled by 4-aminopyridine; although its mechanism of action is complicated. For instance, in contrast to veratridine, that can increase Na^+ and glutamate release independently of Ca^{2+} channels activation, or in contrast to high K^+ that can increase Ca^{2+} and glutamate release independently of Na^+ channels activation (Sitges & Galindo, 2005; Sitges et al., 2007a; 2007b), 4-aminopyridine is unable to increase Ca^{2+} and to induce glutamate exocytosis, when Na^+ channels are blocked by tetrodotoxin (Tibbs et al., 1989; Heemskerk et al., 1991; Galván & Sitges, 2004; Sitges et al., 2005). Thus, as the tetrodotoxin-sensitive fraction of glutamate release induced by 4-aminopyridine requires the presence of external Ca^{2+} and is sensitive to presynaptic Ca^{2+} channel blockade, the tetrodotoxin-sensitive fraction of glutamate release induced by 4-aminopyridine is expected to be the fraction released from the vesicular pool by exocytosis. This also contrasts with veratridine depolarization, that increases glutamate release in a tetrodotoxin sensitive manner via reversal of the neurotransmitter transporter independently of presynaptic Ca^{2+} channels, and with high K^+ depolarization that increases Ca^{2+} and glutamate exocytosis from the vesicular pool in a tetrodotoxin insensitive manner independently of presynaptic Na^+ channels (Sitges & Galindo, 2005; Sitges et al. 2007a; 2007b). Moreover, in addition to the tetrodotoxin-sensitive increases in Na^+, Ca^{2+} and glutamate exocytosis, 4-aminopyridine also produces an accumulation of Na^+ that is tetrodotoxin insensitive and is accompanied by a decrease in the internal concentration of K^+, due to inhibition of the Na/K-ATPase that restores K^+ (Galván & Sitges, 2004). This tetrodotoxin insensitive accumulation of Na^+, that is independent of presynaptic Na^+ or Ca^{2+} channels activation, is likely to also release the cytoplasm fraction of glutamate by reversal of the glutamate transporter in a tetrodotoxin insensitive manner.

Figure 7 shows that the maximal inhibitory effect on glutamate release to 4-aminopyridine exerted by the antiepileptic drugs: carbamazepine, phenytoin, lamotrigine and oxcarbazepine in a range from 75 to 750 μM in hippocampus isolated nerve endings, that is almost reached with the concentration of 250 μM, is not larger than 50-60%. Similarly 1 μM tetrodotoxin also inhibited glutamate released to 4-aminopyridine only in about 50%; and at

that concentration tetrodotoxin completely abolished the veratridine-induced responses in synaptosomes, indicating that the decrease in glutamate release to 4-aminopyridine exerted by the above antiepileptic drugs, is linked to the blockade of presynaptic Na^+ channels. Figure 7 also shows that topiramate at the highest concentration tested (750 µM) only exerted a modest inhibition of 4-aminopyridine induced glutamate release; further indicating that the anticonvulsant mechanism of action of that antiepileptic drug is unrelated with a reduction in cerebral presynaptic Na^+ channels permeability. In line with this last interpretation previous studies showed that neuronal Na^+ currents were only slightly reduced by topiramate at high doses (Zona et al., 1997; Taverna et al., 1999; Mc. Lean et al., 2000).

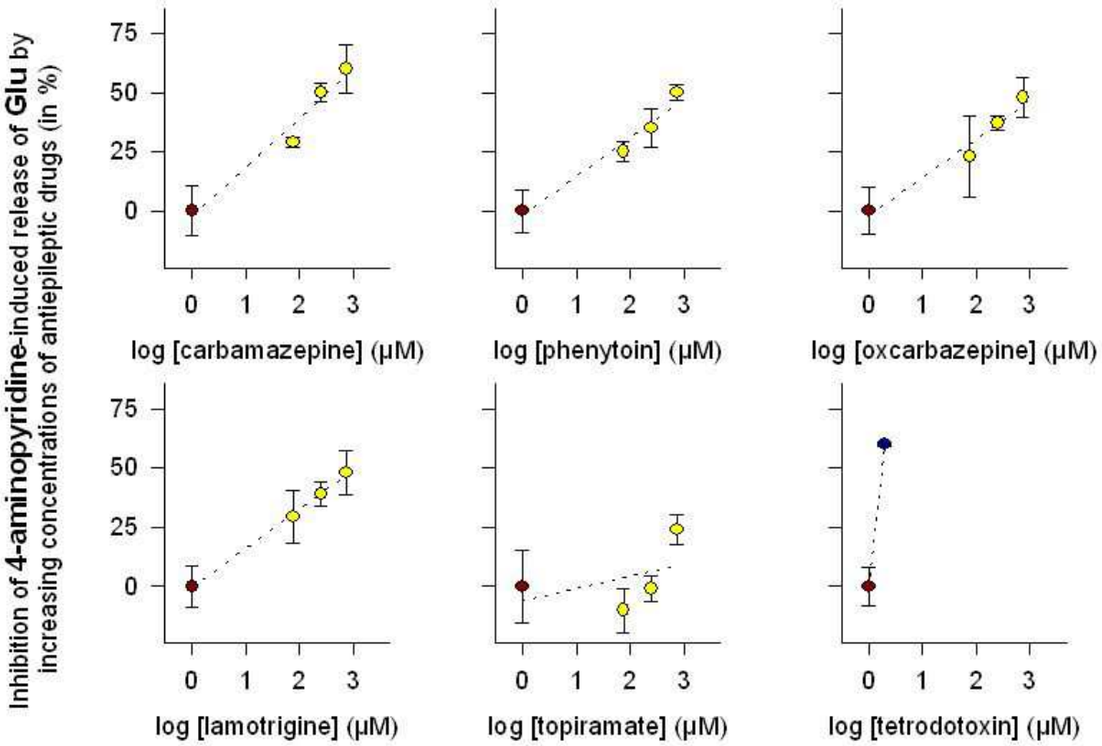

Fig. 7. Inhibition (in percentage of control) exerted by the indicated compounds at increasing concentrations on glutamate (Glu) release induced by 4-aminopyridine in hippocampus synaptosomes.

Figure 8 shows that similarly to glutamate release induced by 4-aminopyridine, the rise in Ca^{2+} induced by 4-aminopyridine also was partially sensitive to the blockade of Na^+ channels with 1 µM tetrodotoxin or with 250µM carbamazepine, phenytoin, lamotrigine and oxcarbazepine, and insensitive to topiramate at that concentration.

The antiepileptic drugs valproate and levetiracetam, even at a very high (1000 µM) concentration were unable to inhibit the rise in Ca^{2+} induced by 4-aminopyridine in hippocampus isolated nerve endings. In line, levetiracetam, like valproate, also was unable to inhibit glutamate release induced by the Na^+ channel opener, veratridine or by high K^+ (data not shown), suggesting that levetiracetam mechanism of action does not involve inhibition of cerebral presynaptic Na^+ or Ca^{2+} channels permeability as well.

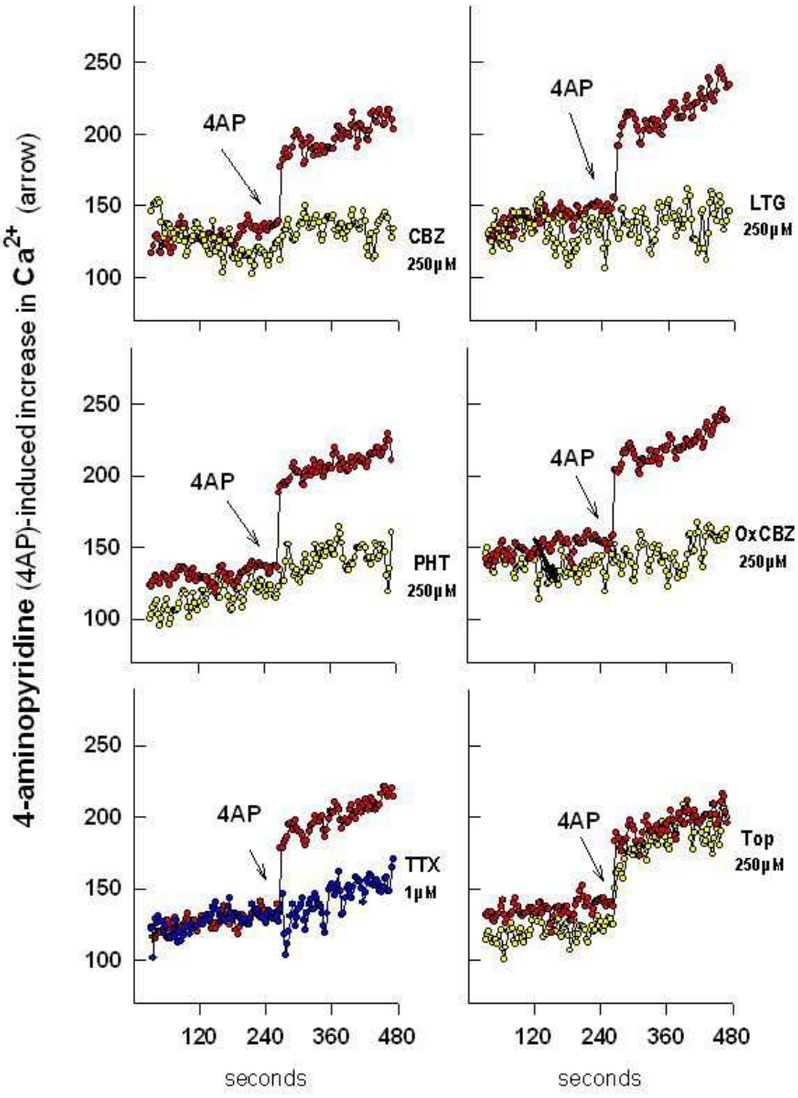

Fig. 8. The elevation of Ca^{2+} (in mM) induced by 4-aminopyridine in hippocampus isolated nerve endings is sensitive to carbamazepine (CBZ), lamotrigine (LTG), phenytoin (PHT), oxcarbazepine (OxCBZ) and tetrodotoxin (TTX), and insensitive to topiramate (Top).

5. Presynaptic Na^+ channels are better targets of antiepileptic drugs than presynaptic Ca^{2+} channels

Comparison of the inhibition exerted by the antiepileptic drugs on glutamate release triggered by the selective activation of Ca^{2+} channels with high K^+ with the inhibition exerted by the antiepileptic drugs on glutamate release evoked by the activation of Na^+ channels induced by veratridine, clearly shows that antiepileptic drugs targeting cerebral presynaptic channels are in general more effective blockers of presynaptic Na^+ than of presynaptic Ca^{2+} channel mediated responses.

Moreover, it is likely that all the compounds that inhibited the increase in Ca^{2+} and the release of glutamate induced by 4-aminopyridine were reducing presynaptic Na^+ channels

permeability, and by this mean the entrance of Ca^{2+}. In agreement with this conclusion, lamotrigine that barely reduced the Ca^{2+} dependent release of glutamate induced by high K^+ (Fig. 6), markedly inhibited glutamate release induced by veratridine (Fig. 3). Thus, the inhibition exerted by lamotrigine on the rise in Ca^{2+} induced by 4-aminopyridine may also result from a blockade of tetrodotoxin sensitive Na^+ channels. In line with an indirect effect of lamotrigine, as well as of carbamazepine, on the rise in Ca^{2+} induced by 4-aminopyridine, a detailed model of the binding sites for carbamazepine, lamotrigine and phenytoin in the inner pore of voltage-gated Na^+ channels was recently provided by Lipkind and Fozzard (2010).

6. Effect of the new potential antiepileptic drug vinpocetine on presynaptic ion channels

Although there is an uncovered medical need for the treatment of epilepsies, neurologists are with reasons reluctant to believe in new antiepileptic drugs. Because also new antiepileptic drugs produce several secondary effects that in some cases are severe. In addition to the fact that as antiepileptic drugs control seizures but do not cure the illness, they have to be taken for all the life span.

Vinpocetine (ethyl apovincamine-22-oate) is a nootropic drug with neuroprotective capabilities discovered during the late 1960s that in animal models of hypoxia and ischemia exerts beneficial effects against neuronal damage and has been used in the treatment of central nervous system disorders of cerebral-vascular origin for decades. In brain isolated nerve endings vinpocetine inhibited the rise in the internal concentration of Na^+ and neurotransmitter release induced by veratridine (Tretter & Adam-Vizi, 1998; Sitges & Nekrassov, 1999; Trejo et al., 2001; Sitges et al., 2006), as well as the tetrodotoxin sensitive fraction of the rise in Na^+ and Ca^{2+} induced by 4-aminopyridine (Sitges et al., 2005). In hippocampus isolated nerve endings, vinpocetine inhibited glutamate release induced by increasing presynaptic Na^+ channels permeability with veratridine and by increasing presynaptic Ca^{2+} channels permeability with high K^+ in a much lower range of concentrations than carbamazepine, phenytoin, lamotrigine and oxcarbazepine (Sitges et al., 2007a; 2007b).

Moreover, in contrast to carbamazepine, phenytoin, lamotrigine and oxcarbazepine, which that at the highest dose tested (750 μM) only inhibited glutamate release to 4-aminopyridine between 50-60%, vinpocetine completely abolished glutamate release to 4-aminopyridine at a concentration of 25 μM, which is a much lower concentration. Since in molluscan neurons, 30 μM vinpocetine, but not other antiepileptic drugs, increases the fast inactivating 4-aminopyridine-sensitive K^+ current (IA) (Bukanova et al., 2002), one possible explanation of the higher efficacy of vinpocetine to inhibit glutamate release induced by 4-aminopyridine in the hippocampus nerve endings could be that, in addition to its action on Na^+ and Ca^{2+} channels, vinpocetine is capable to overcome the blockade of the IA current produced by 4-aminopyridine. Also at a tenfold lower concentration than of carbamazepine, phenytoin, lamotrigine and oxcarbazepine, vinpocetine reduced the Ca^{2+} response to 4-aminopyridine in hippocampus isolated nerve endings. Figure 9 summarizes some of the above findings.

Combination of antiepileptic drugs is a common practice in refractory epileptics not responding to mono-therapy. Interestingly, in striatum isolated nerve endings vinpocetine facilitated the inhibition exerted by carbamazepine on the rise in Na^+ and glutamate release induced by veratridine activation of Na^+ channels (Sitges et al., 2006).

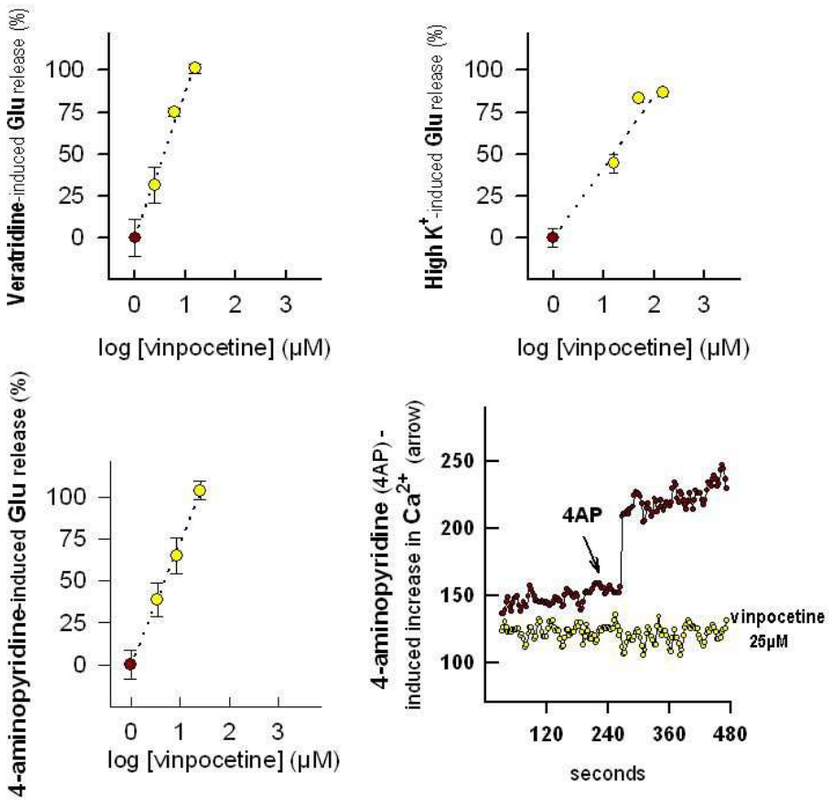

Fig. 9. Inhibition (in percentage of control) exerted by vinpocetine at increasing concentrations on the release of glutamate (Glu) induced by: veratridine, high K^+ and 4-aminopyridine, and on the elevation of Ca^{2+} induced by 4-aminopyridine in hippocampus synaptosomes.

7. Comparison of vinpocetine and some antiepileptic drugs effects on seizures and hearing in the animal *in vivo*

The high antiepileptic potential of vinpocetine also was evidenced in the guinea pig *in vivo;* where vinpocetine completely prevented seizures and the epileptic-like cortical activity induced by 4-aminopyridine at a convulsive dose (Sitges & Nekrassov, 2004).

The top traces in figures 10a and 10b show that the EEG recordings under control conditions (i.e. before the injection of the convulsive agent, 4-aminopyridine) in an animal administered with vehicle and an animal administered with vinpocetine are similar. In contrast, the abnormal EEG changes accompanying seizures observed 20, 30, 60 and 80 min after 4-aminopyridine administration in the control animal administered with vehicle are not observed in the animal pre-administered with 2 mg/kg vinpocetine.

Also seizures and the epileptiform activity induced by the convulsive agent pentylenetetrazole were vinpocetine sensitive (Nekrassov & Sitges, 2004). Representative EEG recordings before and 10, 20, 30 and 50 min after pentylenetetrazole administration are shown in figure 11a, and representative EEG recordings before and at the same periods of time after pentylenetetrazole administration in an animal pre-administered with vinpocetine are in figure 11b.

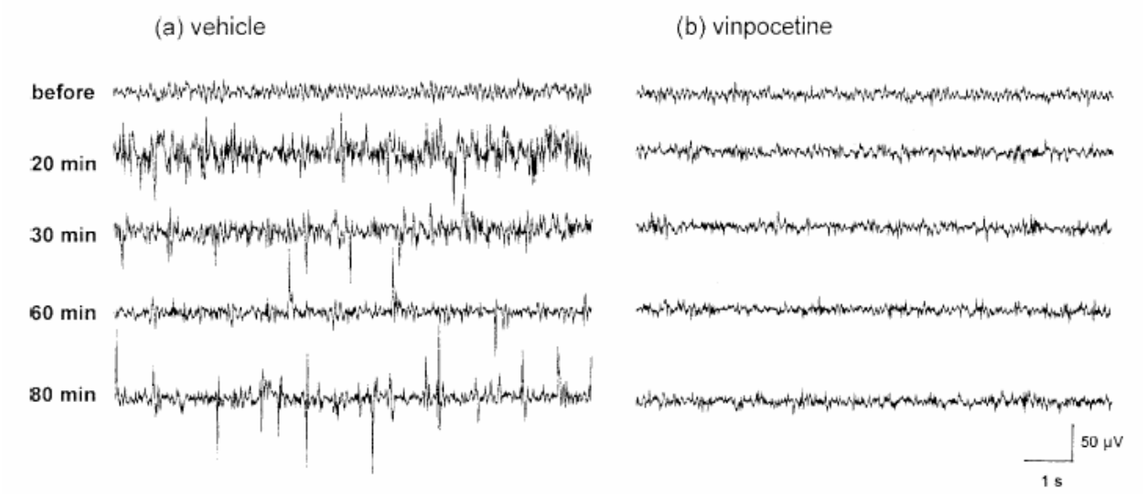

Fig. 10. Representative EEG recordings of the cortical activity before and at the indicated times after 4-aminopyridine in: (a) a control animal and (b) an animal pre-administered with vinpocetine.

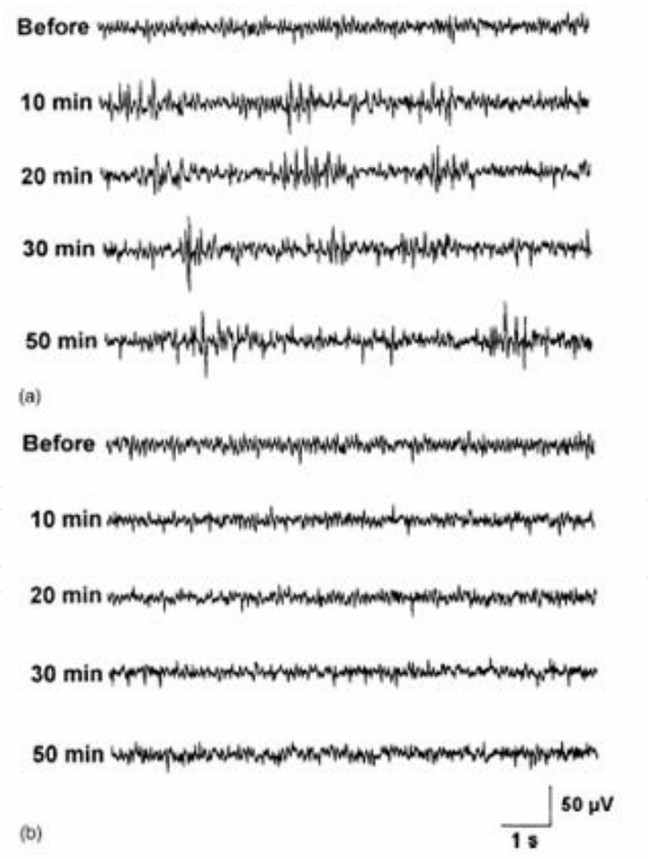

Fig. 11. Vinpocetine prevents pentylenetetrazole-induced epileptiform activity accompanying seizures.

Moreover, the epileptiform activity accompanying seizures induced by pentilenetetrazole was inhibited by vinpocetine at a lower dose than the classical antiepileptic drugs: carbamazepine, phenytoin and valproate (Nekrassov & Sitges, 2006), and a higher potency of vinpocetine than carbamazepine to inhibit seizures induced by 4-aminopyridine was observed too (Nekrassov & Sitges, 2008).

In a previous study, in which the acute, chronic and post-treatment effects of carbamazepine and vinpocetine were investigated on seizures induced by 4-aminopyridine in the guinea pig *in vivo* (Nekrassov & Sitges 2008) we found that: all the control animals developed seizures upon 4-aminopyridine exposure regardless on the time of vehicle administration; namely vehicle injection one hour before 4-aminopyridine (acute), 13 days of vehicle injections before 4-aminopyridine (chronic) or 4-aminopyridine one month after the end of the vehicle injections (post-treatment), as illustrated in figure 12a adapted from Nekrassov & Sitges, 2008. We also found that in the carbamazepine animal group, the acute carbamazepine treatment failed to prevent 4-aminopyridine-induced seizures in all the animals, whereas the chronic carbamazepine treatment, protected about half of the animals from developing seizures after 4-aminopyridine. However, one month after the end of treatment, all the animals of the carbamazepine group developed seizures again after 4-aminopyridine (Fig. 12b). In the vinpocetine animal group, the acute vinpocetine treatment already protected 43% of the animals from developing seizures and the chronic vinpocetine treatment 70% of the animals. Interestingly, 40% of the animals in the vinpocetine group did not developed seizures upon 4-aminopyridine administration one month after the end of treatment (Fig. 12c).

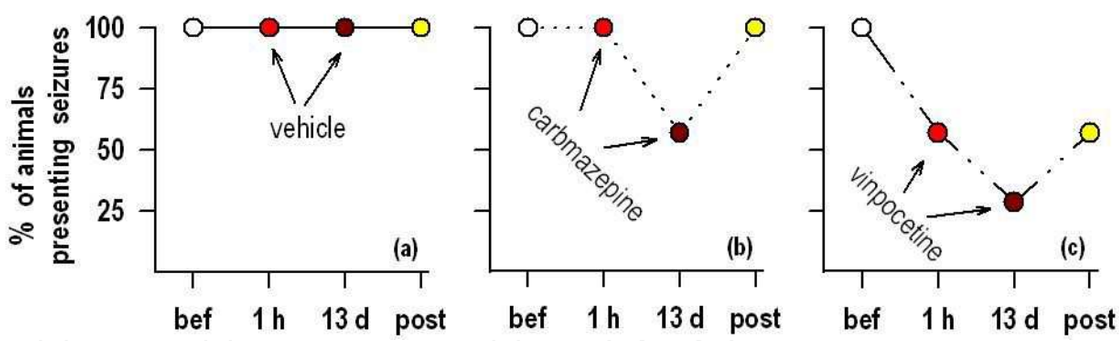

Fig. 12. Acute, chronic and post-treatment effects of carbamazepine and vinpocetine on seizures induced by 4-aminopyridine at a highly convulsive dose. Guinea pigs presenting seizures (in percentage) after the injection of 4-aminopyridine before any treatment was started (bef.), one hour after the first (1h) or of the last (13 d) injection of: (a) vehicle, (b) 17 mg/kg carbamazepine or (c) 3 mg/kg vinpocetine, and one month after the end of the above treatments (post).

Since the available antiepileptic drugs control seizures but do not cure the illness, the finding that vinpocetine even after post-treatment was able to prevent 4-aminopyridine-induced seizures is very hopefulness.

In another study, (Nekrassov & Sitges, 2006) we also investigated the auditory sensitivity, as indicated by brainstem auditory evoked potential thresholds at two tone frequencies (4 and 8 kHz) in guinea pigs daily injected with vehicle (control), 20 mg/kg carbamazepine, 6 mg/kg phenytoin, 30 mg/kg valproate or 2 mg/kg vinpocetine for 28 days before and after

the administration of pentylenetetrazole at a convulsing dose (100 mg/kg). In that study we found that the long term treatment with carbamazepine, phenytoin or valproate increased the auditory threshold to a similar extent as the convulsing agent, pentylenetetrazole. In contrast, the 28 days treatment with vinpocetine even decreased the auditory threshold. Moreover, the increases exerted by the antiepileptic drugs and by pentylenetetrazole on the auditory thresholds were additive, indicating that the hearing loss produced by the long term treatment with the most commonly used antiepileptic drugs could be aggravated by the illness. On the contrary, vinpocetine at the anticonvulsive dose prevented the hearing decline accompanying seizures. In other words, oppositely to the classical antiepileptic drugs carbamazepine, phenytoin and valproate, vinpocetine was able to improve hearing loss by itself and to prevent hearing loss accompanying seizures (Nekrassov & Sitges, 2006).
Figure 13 adapted from data reported in : (a) Nekrassov & Sitges, 2004 and (b) Sitges & Nekrassov, 2004 shows that vinpocetine pre-administered at a dose of 2 mg/kg (i.p.) prevents the hearing loss induced by pentylenetetrazole and 4-aminopyridine at convulsive doses in the guinea pig *in vivo*. Hearing loss was assessed by recording the auditory threshold at 8 kHz before, 30 and 60 min after administration of the convulsive agents.

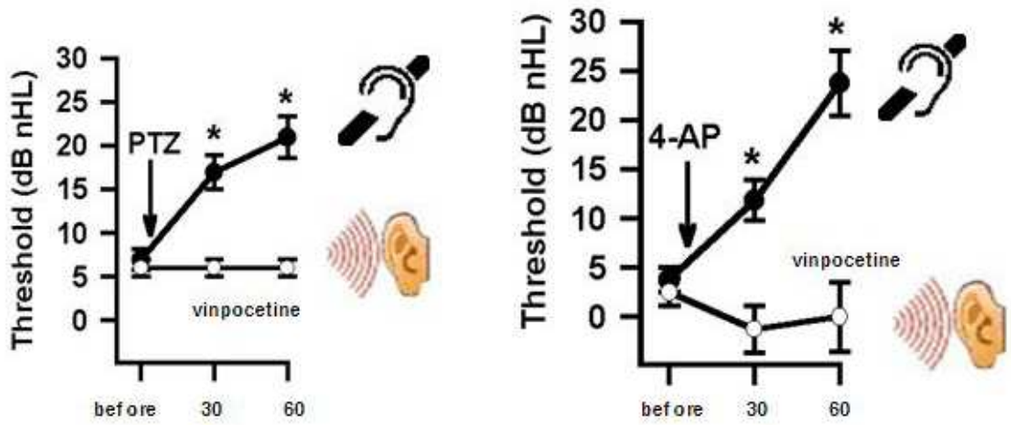

Fig. 13. Vinpocetine inhibits the rise in the auditory threshold induced by pentylenetetrazole (PTZ) and 4-aminopyridine (4-AP) at convulsive doses.

The high doses of antiepileptic drugs required to control seizures are frequently accompanied by adverse secondary effects. A great number of epileptic patients suffer from memory disturbances which are consequence of both, the disease (Prevey et al., 1998; Theodore et al., 1999; Meador, 2001; Elger et al., 2004) and the antiepileptic medication; as several studies show that antiepileptic drugs of either the "old and new generations" are also a causal factor (Vermeulen & Aldenkamp, 1995; Gates, 2000; Kwan & Brodie, 2001; Brunbech & Sabers, 2002; Schmidt, 2002). For instance, the classic antiepileptic drug carbamazepine deteriorates cognitive function particularly when administered at high doses or after a long term treatment (O´Dougherty et al., 1987; Gallassi et al., 1988; Forsythe et al., 1991; van der Meyden et al., 1992; Seidel & Mitchell, 1999). Fascinatingly previous studies in animals and humans show that vinpocetine is also a memory enhancer (Subhan & Hindmarch, 1985; Bhatti & Hindmarch, 1987; DeNoble, 1987: Lendvai et al., 2003).
The higher potency of vinpocetine not necessarily has to indicate a best side-effect profile than the conventional antiepileptic drugs. Nevertheless, vinpocetine has shown to be well tolerated and without contraindications (Hindmarch et al., 1991). Therefore, the higher

potency and efficacy of vinpocetine to reduce the permeability of presynaptic ionic channels controlling the release of the most important excitatory neurotransmitter in the brain must be advantageous in seizures control and epilepsy treatment. In line with this assumption it is worthy to mention that an unpublished investigation in course in epileptic children resistant to classic antiepileptic drugs the add-on-therapy of vinpocetine effectively controlled seizures at a dose more than tenfold lower than the dose of the classical antiepileptic drugs.

8. Conclusion

The findings summarized in the present chapter show that cerebral presynaptic ion channels, and particularly presynaptic Na^+ channels controlling glutamate release, are among the most important targets of various anticonvulsant drugs. Therefore, the pharmacological down-modulation of those channels in situations in which all neurons are firing is likely to be particularly beneficial in the control of epileptic seizures. In addition, since there is an uncovered medical need for the treatment of epilepsies and cerebral presynaptic channels are targets of the most effective antiepileptic drugs, the *in vitro* techniques presented in this chapter may represent powerful tools for the future screening and discover of anticonvulsive drugs controlling excitation by targeting brain presynaptic channels. The findings presented also show that the higher potency and efficacy of vinpocetine than the most effective antiepileptic drugs to inhibit presynaptic Na^+ and Ca^{2+} channels permeability is extensive to the control of seizures in experimental animal models of epilepsy. Current unpublished studies carried out in epileptic inpatients refractory to the classic antiepileptic drugs also show the high efficacy of this third generation antiepileptic drug in seizures control.

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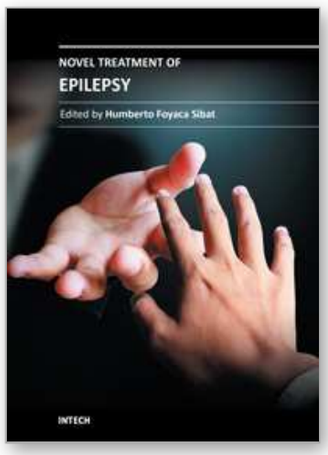

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Epilepsy continues to be a major health problem throughout the planet, affecting millions of people, mainly in developing countries where parasitic zoonoses are more common and cysticercosis, as a leading cause, is endemic. There is epidemiological evidence for an increasing prevalence of epilepsy throughout the world, and evidence of increasing morbidity and mortality in many countries as a consequence of higher incidence of infectious diseases, head injury and stroke. We decided to edit this book because we identified another way to approach this problem, covering aspects of the treatment of epilepsy based on the most recent technological results â€œin vitroâ€ from developed countries, and the basic treatment of epilepsy at the primary care level in rural areas of South Africa. Therefore, apart from the classic issues that cannot be missing in any book about epilepsy, we introduced novel aspects related with epilepsy and neurocysticercosis, as a leading cause of epilepsy in developing countries. Many experts from the field of epilepsy worked hard on this publication to provide valuable updated information about the treatment of epilepsy and other related problems.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

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