



The pilocarpine model of epilepsy: what have we learned?

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ABSTRACT

The systemic administration of a potent muscarinic agonist pilocarpine in rats promotes sequential behavioral and electrographic changes that can be divided into 3 distinct periods: (a) an acute period that built up progressively into a limbic *status epilepticus* and that lasts 24 h, (b) a silent period with a progressive normalization of EEG and behavior which varies from 4 to 44 days, and (c) a chronic period with spontaneous recurrent seizures (SRSs). The main features of the SRSs observed during the long-term period resemble those of human complex partial seizures and recurs 2-3 times per week per animal. Therefore, the pilocarpine model of epilepsy is a valuable tool not only to study the pathogenesis of temporal lobe epilepsy in human condition, but also to evaluate potential antiepileptogenic drugs. This review concentrates on data from pilocarpine model of epilepsy.

Key words: hippocampus, pilocarpine, temporal lobe epilepsy, rat.

EPILEPSY: GENERAL ASPECTS

Epilepsy is the most common serious neurological condition and approximately 50 million people worldwide have it (Sander 2003). In the US, about 100,000 new cases of epilepsy are diagnosed (Begley et al. 1998, Annegers 1997). In the UK, between 1 in 140 and 1 in 200, people (at least 300,000 people) are currently being treated for epilepsy (Yuen and Sander 2004). Epidemiological studies suggest that between 70 and 80% of people developing epilepsy will go into remission, while the remaining patients continue to have seizures and are refractory to treatment with the currently available therapies (Kwan and Sander 2004, Sander 1993).

The most common risk factors for epilepsy are cerebrovascular disease, brain tumors, alcohol, traumatic head injuries, malformations of cortical development, genetic inheritance, and infections of the central nervous system. In resource-poor countries, endemic infections, such as malaria and neurocysticercosis, seem to be major risk factors (Duncan et al. 2006).

Epilepsies are characterized by spontaneous recurrent seizures, caused by focal or generalized paroxysmal changes in neurological functions triggered by abnormal electrical activity in the cortex (Dichter 1994). Because it involves hyperexcitable neurons, a basic assumption links the pathogenesis of epilepsy and the generation of synchronized neuronal activity with an imbalance between inhibitory [g-aminobutyric acid (GABA)-mediated] and excitatory (glutamate-mediated) neurotransmission, in favor of the latter (Dalby and Mody 2001). Seizures and epilepsy are usually divided into 2

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groups: partial and generalized. Partial or focal seizures have clinical or EEG evidence of local onset and may spread to other parts of the brain during a seizure, while generalized seizures begin simultaneously in both cerebral hemispheres (Duncan et al. 2006). Temporal lobe epilepsy (TLE) is the most common form of partial epilepsy, probably affecting at least 20% of all patients with epilepsy (Babb 1999). It is the most common form of drug-refractory epilepsy (Engel 1993). Atrophy of mesial temporal structures is well-known to be associated with TLE and hippocampal sclerosis, which is the most frequent histological abnormality in this form of epilepsy (Cendes 2005).

THE PILOCARPINE MODEL OF EPILEPSY: CHARACTERISTICS and DEFINING FEATURES

The systemic administration of a potent muscarinic agonist pilocarpine in rats promotes sequential behavioral and electrographic changes that can be divided into 3 distinct periods: (a) an acute period that built up progressively into a limbic *status epilepticus* and that lasts 24 h, (b) a silent period with a progressive normalization of EEG and behavior which varies from 4 to 44 days, and (c) a chronic period with spontaneous recurrent seizures (SRSs). The main features of the SRSs observed during the long-term period resemble those of human complex partial seizures and recurs 2-3 times per week per animal (Cavalheiro 1995, Arida et al. 1999a, b).

BEHAVIORAL AND CLINICAL FEATURES

The sequential pattern of electrographic changes during the acute phase, immediately following the injection of pilocarpine, is characterized by a significant theta rhythm that replaces the background activity in the hippocampus and low voltage fast activity in the cortex. This activity progresses to high voltage fast activity with spikes in the hippocampus. The spiking activity spreads to the cortex and evolves into electrographic seizures. Ictal periods recur every 3-5 min and finally lead to sustain discharges 50-60 min after the injection of pilocarpine. This pattern of electrographic activity lasts for several hours and may evolve to a pattern of periodic discharges on a relatively flat background (Cavalheiro 1995, Arida et al. 1999a, b, Leite et al. 1990, Turski et al. 1983a, b, 1984).

Seizure frequency in the chronic period may vary considerably among epileptic rats, and several seizure patterns have been observed. Some pilocarpine-injected rats may present with a low seizure frequency throughout several weeks or months; others may have daily seizures; and some may present clusters of seizures in short periods of time. Such variability in seizure frequency patterns may represent a drawback for behavioral or antiepileptic drug studies. In order to assemble a homogeneous group, it is necessary to identify – through baseline monitoring – a group of rats with regular consistent seizure frequency (Cavalheiro 1995, Arida et al. 1999a, b, Leite et al. 1990, Turski et al. 1983a, b, 1984).

It is important to stress that most behavioral or antiepileptic drug studies rely upon video monitoring to establish seizure frequency. Therefore, class 3-5 limbic seizures are preferentially detected, and less severe (“asymptomatic”) seizure stages (class 1 and 2) are frequently overlooked. This point is particularly relevant because many intractable complex partial seizures in humans rarely generalize, even when antiepileptic drugs are tapered during video-EEG monitoring – and thus are the “equivalent” of class 1 and 2 limbic seizures in rats. High resolution video capturing coupled with EEG is necessary to detect subtle behavioral seizures correlated with, for example, focal ictal activity in the hippocampus or amygdale (Cavalheiro 1995, Arida et al. 1999a, b, Leite et al. 1990, Turski et al. 1983a, b, 1984).

NEUROPATHOLOGY

The induction of *status epilepticus* by pilocarpine leads to severe and widespread cell loss in several brain areas. Dying cells can be assessed via a number of different techniques that characterize a given biochemical or structural aspect of the degenerating cells. Differences in the biochemical and morphological profile of dying cells may indicate whether the cell is suffering from an apoptotic or a necrotic degenerating process. As with a number of different pathological conditions, cell damage in the pilocarpine model has been described as to its necrotic or apoptotic nature. However, classifying cell damage according to these 2 categories may be confusing, and therefore will be avoided here. A more fruitful perspective is to consider that the excitotoxic insult triggered by pilocarpine-induced *status epilepticus*

leads both to immediate cell damage that takes place minutes to a few hours after its onset – and also results in a protracted process of neurodegeneration that may take weeks and months to develop (Cavalheiro 1995, Leite et al. 1990, Turski et al. 1983a, b, 1984).

The initial damage, occurring a few hours after the onset of *status epilepticus*, is most intense in the superficial layers of some neocortical areas, hilus of the hippocampus, endopiriform nucleus, piriform cortex and claustrum. Eight hours after SE onset, damage has further intensified in those areas and, in addition, becomes significant in entorhinal cortex, amygdaloid nuclei, ventromedial nucleus of the hypothalamus, subiculum and the bed nucleus of the stria terminalis. Damage in these distinct brain areas is time-specific (Cavalheiro 1995, Leite et al. 1990, Turski et al. 1983a, b, 1984).

Damage is not restricted to the initial hours and days after SE, and tends to progressively involve other areas in the following months. To this end, damage to the thalamus is often found in animals sacrificed many months after the onset of pilocarpine-induced *status epilepticus*, but is notably less intense (or even absent) in some thalamic nuclei at shorter survival times (Leite et al. 1990, Turski et al. 1983a, b, 1984).

In addition to cell loss, there is also a clear injury resulting in both morphological and functional pathology. Evidence of altered cell morphology in the pilocarpine model has been provided mostly for the hippocampus. Altered distribution of dendritic spines in dentate granule cells and distorted dendritic trees in putative GABAergic hippocampal interneurons are some of these changes. Additional morphological changes are more likely to be reactive rather than a direct consequence of the initial insult. In this sense, the emergence of axonal sprouting – the most notable being the supragranular mossy fiber sprouting, granule cell dispersion, increased rate of neurogenesis, and development of granule cell basal dendrites that are among the morphological changes likely to represent a reactive response (Cavalheiro 1995, Leite et al. 1990, Turski et al. 1983a, b, 1984).

As with any other lesional model, pilocarpine-induced *status epilepticus* does not uniformly damage different cell groups. Here again, cell type-specific vulnerability has been best studied in the hippocampus. There is greater damage to principal cells, that is pyramidal,

and granule cells in the hippocampal complex, but interneurons located in the other strata can also be damaged. Most notably, hilar mossy cells can be markedly damaged by pilocarpine-induced *status epilepticus*, but with large variation in the extent of damage between different animals. Damage to GABAergic neurons is also extensive throughout the hippocampus. However, not all GABAergic neurons in the hippocampal complex are equally vulnerable, with specific populations in different strata showing different rates of loss. In a recent study, the density of GAD65 mRNA-positive neuron profiles in layer III of the entorhinal cortex was similar in control and post-*status epilepticus* rats evaluated between 3 and 7 days after pilocarpine. Similar evaluations have not yet been provided for other brain areas, with the exception of a qualitative assessment of the neocortex (Leite et al. 1990, Turski et al. 1983a, b, 1984).

Glial pathology in the pilocarpine model has not received the same level of attention as neuronal pathology. Nevertheless, there have been descriptions regarding the proliferation of astrocytes, as shown by the increased expression of GFAP and other glial markers. In addition to glial proliferation, it has been shown that, in the CA1 area of the hippocampal complex of animals subjected to pilocarpine-induced *status epilepticus*, glial cells “adapt” to permit rather large increases in extracellular potassium accumulation. Microglia and other markers of inflammatory tissue reaction have also shown to be present in the early phases after pilocarpine-induced *status epilepticus* (Binder and Steinhäuser 2006, Garzillo and Mello 2002).

THE PILOCARPINE MODEL OF EPILEPSY: NEUROCHEMICAL ALTERATIONS

Partial or complex seizures, the main characteristic of temporal lobe epilepsy (TLE), have been related to important brain impact as well as to the eventual evolution of this syndrome. Thus, different authors have demonstrated that long-lasting seizures unchain a complex chemical cascade, triggering neurochemical alteration in neurons and glial cells. These immediate or long-lasting events can modify the cellular environment through changes of ionic gradient across the cell membrane, alteration of gene expression such as receptors, trophic factors, enzymes, proteins from cytoskeleton,

protein from matrix and the phosphorylation of macromolecules. Furthermore, seizures can induce reactive gliosis generated by cell death and induced by these long-lasting convulsions. These modifications promote synaptic remodeling, which can change the excitability of neurons from temporal structures, leading to the appearance of brain damage and a permanent hyperexcitability.

Unfortunately, the temporal lobe epilepsy is not an easily understandable brain dysfunction. The neurochemical alteration found in the brain of experimental animals, as well as in human brain, show high degree of complexity.

Since the hippocampal formation seems to be an important structure in temporal lobe epilepsy, several authors have reported neurochemical alterations in this structure. The hippocampus of rats submitted to the epilepsy model induced by pilocarpine shows increased utilization rate of norepinephrine (NE) and decreased utilization rate of dopamine during the acute, silent and chronic period of this model. As reported, the utilization rate of serotonin was increased only in the acute phase (Cavalheiro et al. 1994). Concerning to aminoacidergic neurotransmission, the acute phase of pilocarpine model was characterized by an increased glutamate release in the hippocampus (Cavalheiro et al. 1994, Costa et al. 2004). Hippocampal synaptosomes from animals presenting long-lasting SE (12 h) still showed increased release of glutamate. However, the uptake of this amino acid is normal in animals presenting 12h of SE (Costa et al. 2004), suggesting an excitatory phenomenon during the acute phase of pilocarpine model. Indeed, when glutamate activates N-methyl-D-aspartate receptors (NMDA) the intracellular Ca^{++} raises inducing activation of lipases, proteases and nucleases, killing the cell by necrosis and/or apoptosis.

Among the mechanisms involved in regulation, the cytosolic calcium is the Ca^{++} ATPases, whose function is to restore the normal level of this ion into the cell. These Ca^{++} ATPases constitute a class of proteins that falls into 2 distinct groups, termed SERCAs and PMCA, depending on whether they are inserted in endoplasmic reticulum or in plasma membrane. SERCAs sequester calcium to sarco/endoplasmic reticulum and SERCA2b is found in several brain structures.

PMCAs promote the extrusion of this ion from neural cell through plasma membrane. According to Funke et al. (2003) in the hippocampus of rats, submitted to pilocarpine model of epilepsy, the expression of SERCA2b, as well as the PMCA enzymes, is increased after 1 h of status epilepticus, showing an attempt to control the tissue excitability during the early stages of the insult. The PMCA remained increased until the silent period, returning to control levels during the chronic phase. In contrast, vulnerable regions to cell death such as CA1, CA3 and hilus presented decreased expression of SERCA2b until the silent period, showing a deficit in the mechanisms related to calcium removal.

The activity of the $Na^{+}K^{+}$ ATPase is also modified in the hippocampus of pilocarpine-treated animals. According to Fernandes et al. (1996) this enzyme has its activity reduced during the acute and silent period and an increased activity during the chronic phase, showing that the hippocampus of these animals also show an ionic imbalance related to its maintained excitability.

The expression of proteins related to NMDA-glutamate receptor is also modified in pilocarpine model of epilepsy. Mint1 or X11 alpha plays an important role in vesicle synaptic transport toward the active zone at presynaptic site, and also participates in the transport of NR2B subunit of NMDA receptor at the postsynaptic site. According to Scorza et al. (2003) this protein, mainly expressed in CA1 regions of control animals, presented its levels decreased 5 h after SE onset and increased levels during the silent and chronic groups, suggesting that this protein is related to plasticity during epileptogenesis.

The silent phase of pilocarpine model is marked by an important unbalance between inhibition and excitation (Cavalheiro et al. 1994). The decreased concentration of GABA in the hippocampus, during the silent period, could suggest an increased release of this amino acid in attempt to control the tissue excitability. In contrast, the increased concentration of glutamate in the hippocampus could suggest a potential excitatory pathway of this structure, probably responsive for the appearance of spontaneous seizures.

Thus, according to several authors, the temporal lobe epilepsy has been related to excessive excitability in limbic structures, low function of inhibitory path-

ways or the association between both events (Meldrum 1991). As a consequence of neurotransmission alteration, the transduction signal through plasma membrane is also modified, changing neuronal metabolism and genes expression.

As a compensatory effect, growth factors can be released and the activation of their receptors induces the auto-phosphorylation of these receptors and activation of different kinase proteins, including the phosphorylation of proteins on tyrosine residues, which are important in cell cycle and intracellular signalling mechanisms. These phosphotyrosine proteins (PTyP), of different molecular weight, have been found to be increased in the hippocampus of rats during the early stages of pilocarpine-induced SE (Funke et al. 1998), showing that several intracellular events could undergo modifications during long-lasting seizures, mainly in CA3 region. The receptor protein tyrosine phosphatase β (RPTP β), a chondroitin sulfate proteoglycan, which is related to plasticity and phosphatase activities, has been associated with the mossy fiber sprouting in the epileptic phenomena (Perosa et al. 2002). The RPTP β , which is expressed only by astrocytes in control tissues, has its synthesis induced in pyramidal neurons from the hippocampus, during the acute and silent phases, of pilocarpine-induced epilepsy, showing that the SE may modify the gene expression in the epileptic rats (Naffah-Mazzacoratti et al. 1999a, b).

The increased expression of growth factors is also related to Mitogen Activated Protein Kinase (MAPK) activation. After binding an agonist, trk receptors phosphorylate themselves on cytoplasmic domains on tyrosine residues, which became docking sites for intracellular signaling proteins. Shc adaptor proteins associate themselves with specific site in trk receptors, activating a signaling pathway involving Ras, Raf, MAPK1, MAPK2, MEK1 and MEK 2. As a consequence, the transcription factors and the regulation of gene expression is modified. As reported by Garrido et al. (1998), several limbic structures showed increased levels as well as increased phosphorylation of MAPKs (ERK1 and ERK2), which are important during the induction of the *de novo* synthesis of several proteins.

Other intracellular signaling pathways may also be modified during epileptogenesis. Levels of the neuro-

modulin or growth associated phosphoprotein (B-50 or GAP-43), which is activated by PKC, are modified in the hippocampus of rats in the pilocarpine epilepsy model. GAP-43 has been related to processes underlying cell proliferation in fetal human brain and is correlated specifically with differentiation and outgrowth of axons. This protein showed its levels increased in the inner molecular layer of the dentate gyrus (regions associated with the mossy fiber sprouting), during the acute, silent and chronic period, in rats submitted to pilocarpine-induced epilepsy (Naffah-Mazzacoratti et al. 1999a). According to several authors, the GAP-43 activation may be also induced by glutamate, acting on NMDA receptor, since the blockade of this receptor by MK801 prevent the GAP-43 expression as well as the mossy fiber sprouting.

During long-lasting seizures, the activation of inflammatory processes may also occur. Reactive gliosis, such as astrocytes and microglia, appears as a tardy form. As reported by Garzillo and Mello (2002), 60 days (chronic phase) after pilocarpine-induced SE prominent astrocytes could still be seen in different brain areas. The activated microglia has been blamed as the source of the main inflammatory cytokines. Thus, several authors described increased expression of mRNA for IL-1 β , IL-6, iNOS and TNF α after seizures, and Ravizza et al. (2008) showed that specific inflammatory pathways are chronically activated during epileptogenesis and they persist in chronic epileptic tissue, suggesting that they may contribute to etiopathology of TLE.

Another pathway involved in the inflammatory processes is linked to prostaglandin (PG) release. These eicosanoids are produced after the action of phospholipase A2 on phospholipids that releases arachidonic acid, which could be done by the action of glutamate on NMDA receptor. Thus, Naffah-Mazzacoratti et al. (1995) showed increased release of prostaglandin PGF2 α during the acute phase, PGD₂ during the acute, silent and chronic period and PGE₂ only during the chronic phase of the epilepsy model induced by pilocarpine. During PG formation, free radicals are produced, increasing the inflammatory process. Against free radicals, the tissues present enzymes such as superoxide dismutase (SOD) and glutathione peroxidase, which are able to remove the superoxide anion (O₂⁻) or

H₂O₂, and considered potent oxidant agents. As reported by Bellissimo et al. (2001), rats presenting SE or spontaneous seizures showed a decreased activity of SOD and increased levels of hydroperoxides (products lipid peroxidation) in the hippocampus of animals submitted to pilocarpine model of epilepsy. As the brain is more vulnerable than others tissues, the decreased activity of SOD could be related to cell death and brain damage that was found in the hippocampus of these animals.

Other compounds related to vessel dilatation, with a consequent rupture of blood brain barrier, edema, pain and inflammatory processes are the kinins. These polypeptides are produced after proteolysis limited action of kallikreins on high and low molecular weight kininogens. These short-living peptides are rapidly degraded by kininases (Bhoola et al. 1992), originating active metabolites such as des-Arg⁹BK, des-Arg¹⁰Kallidin and inactive products. The receptors are denominated B1 and B2, and both are coupled to G protein. Furthermore, stimulation of kinin B1 and B2 receptors induce tissue edema and phospholipase A₂ activation, producing prostaglandins (Bhoola et al. 1992). In addition, kinin B1 and B2 stimulation also activate MAPK (ERK1/ERK2) in cell culture, resulting in AP-1 translocation and modifying the immediate early gene expression.

Usually, kinin B1 receptor is not expressed at a significant level under physiologic conditions in most tissues, but its expression is induced by injury or upon exposure *in vivo* or *in vitro* to pro-inflammatory mediators, such as lipopolysaccharide and cytokines (Marceau 1995). In contrast, kinin B2 receptor is constitutively and widely expressed in all nervous system, and has been found in the nucleus of neurons from hippocampus, hypothalamus and cortex (Chen et al. 2000). Nevertheless, the real function of this receptor in neuronal nucleus is still unknown.

Studying the distribution of kinin B1 and B2 receptors and the expression of mRNA by Real-Time PCR of these receptors during the development of the epilepsy model induced by pilocarpine, Argañaraz et al. (2004a) found increased kinin B1 and B2 mRNA levels during the acute, silent and chronic periods, and changes in kinin B1 receptors distribution. In addition, the immunoreactivity against kinin B1 receptors was increased mainly during the silent period, when clusters of cells could be

visualized suggesting a local inflammation. The kinin B2 receptor immunoreactivity also showed augmentation, but mainly during the acute and silent periods, supporting the hypothesis that both kinin receptors are related to temporal lobe epilepsy.

Trying to understand the role of kinin B1 and B2 receptors in the physiopathology of temporal lobe epilepsy, we developed the epilepsy model induced by pilocarpine in B1 and B2 knockout mice (B1KO and B2KO, respectively), and behavior parameters, cell death and mossy fiber sprouting were analyzed. B1KO mice showed an increased latency for the first seizure, associated to a decreased frequency of spontaneous seizures (chronic phase) when compared with their wild control mice. In addition, B1KO mice showed less cell death in all hippocampal formation associated to a minor grade of mossy fiber sprouting when compared with wild mice. Furthermore, B2KO mice presented minor duration of the silent period and an increased frequency of spontaneous seizures (chronic phase) when compared with wild mice. B2KO and wild mice showed a similar pattern of cell death in the hippocampus, which was very intense when compared with saline-treated animals. The mossy fiber sprouting was also increased in B2KO mice when compared to wild mice and saline-treated animals. Taken together, these data suggest a deleterious effect for B1 receptor and a protective effect for B2 receptor during the development of the temporal lobe epilepsy (Argañaraz et al. 2004b).

Analyzing all these results, we can observe that an insult is able to modify several signaling pathways in central nervous system. Thus, Figure 1 summarizes the main findings since pilocarpine injection until the occurrence of plastics events and cellular death.

Pilocarpine may act on M1 and M2 muscarinic receptors. Activating the M2 one, the adenylate cyclase is inhibited, decreasing the release of acetylcholine and the neuronal excitation. On the other hand, binding to M1, the pilocarpine activates the phospholipase C and therefore produces diacylglycerol (DG) and inositol triphosphate (IP3), which results in alteration in Ca⁺⁺ and K⁺ current and increases the excitability of the brain (Segal 1988). This increased excitability probably occurs due to a decreased activity of ATPases in the hippocampus, which could not repolarize the plasma membrane;

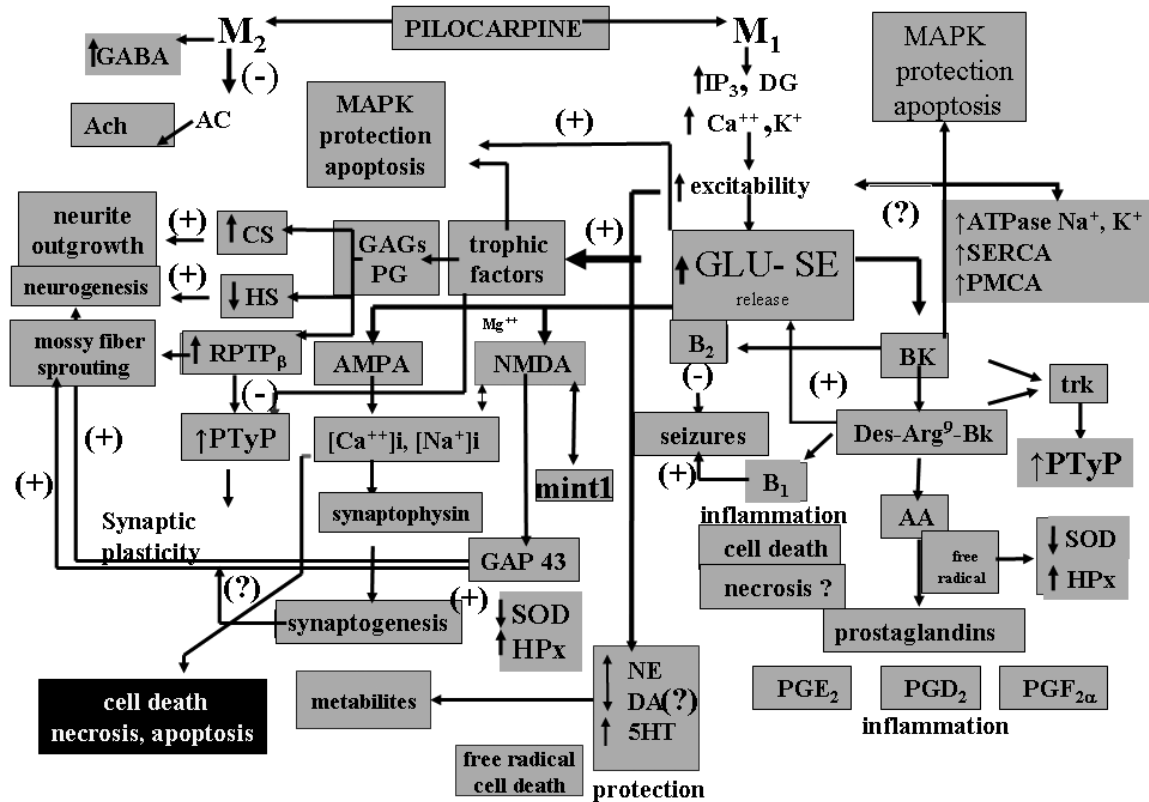


Fig. 1 – Main biochemical pathways and physiological consequences involved in the pilocarpine model. **MAPK** – mitogen activated protein kinase; **Glut** – glutamate; **ATPase** – ATPase sodium, potassium; **SERCA** – calcium ATPase from sarco-endoplasmatic reticulum; **PMCA** – calcium ATPase from plasma membrane; **BK** – bradykinin, **Des-Arg9BK** – des-Arg9 bradykinin; **AA** – arachidonic acid; **PGE2** – prostaglandins E2, **PGD2** – prostaglandin D2, **PGF2α** – prostaglandin F2α; **GAGs** – Glycosaminoglycans; **CS** – chondroitin sulfate; **HS** – heparan sulfate; **RPTPβ** – Receptors Protein Tyrosin Phosphatase β; **PG** – Proteoglycans, **PTyP** – phosphotyrosin protein, **SE** – status epilepticus; **GAP-43** – growth associated phosphoprotein-43, **NMDA** – N-methyl-D-aspartate; **NE** – neradrenaline; **DA** – dopamine, **5HT** – serotonin; **SOD** – superoxide dismutase, **HPx** – hydroperoxide.

neither promotes the calcium extrusion (Fernandes et al. 1996, Funke et al. 2003). The high concentration of Ca^{++} promotes the high release of glutamate, which induces the status epilepticus (SE). The glutamate, acting on AMPA/KA receptors, allows the entrance of Na^{+} and Ca^{++} into the cell and, as a consequence, the Mg^{++} , which blockade the NMDA receptor, is removed inducing the activation of this receptor by glutamate and allowing the entrance of more Ca^{++} into the postsynaptic cell, which will induce excitotoxicity and cell death.

The tissue excitability and/or SE increase the utilization rate of noradrenaline and serotonin with a concomitant decrease in the utilization rate of dopamine (Cavalheiro et al. 1994). After docking to its own receptors, these monoamines are degraded by MAO and

COMT and, during these processes, free radicals can be formed. These free radicals are also freed during glucose metabolism and mitochondrial transport chain, which is over activated during SE. In addition, the superoxide dismutase (SOD) presented a decreased activity during seizures, associated to an increased level of hydroperoxide in the hippocampus of epileptic animals (Bellissimo et al. 2001) showing tissue damage and lipid peroxidation.

Glutamate on NMDA receptors promotes an increased expression of GAP-43, which is linked to mossy fiber sprouting and hippocampal plasticity (Naffah-Mazzacoratti et al. 1999b).

During the SE, the expression of trophic factors such as NGF, BDNF and FGF (Mudo et al. 1996)

increase in the hippocampus, which propitiates MAPK and PTyP activation (Garrido et al. 1998, Funke et al. 1998) and induces modification in genes expression. MAPK also may have protector action or can be related to apoptosis process.

The trophic factor receptors are also associated to proteoglycans (PGs) from extracellular matrix. These proteoglycans (PG) sometimes may function as co-receptors for neurotrophins (Ruoslahti and Yamaguchi 1991). In this context, the increased synthesis of chondroitin sulfate and RPTP β (Naffah-Mazzacoratti et al. 1999b), which is found in the hippocampus of epileptic animals, can be related to neurite outgrowth and/or mossy fiber sprouting. In addition, the RPTP β also presents phosphatase activity, removing the phosphate group from tyrosine residues in PTyP modified during SE (Funke et al. 1998).

The SE or the excess of glutamate in tissue can activate routes that culminate in kinins release, and these polypeptides may act on kinin B1 and B2 receptors, which are over expressed in the hippocampus of epileptic animals (Argañaraz et al. 2004a, b). The kinin B2 receptor has a protector role during epileptogenesis, while B1 is deleterious (Argañaraz et al. 2004a, b). Bradykinin (BK), as well as monoamines, also induces prostaglandins (PG) release (Bazan et al. 1986). As reported by Naffah-Mazzacoratti et al. (1995) the levels of PGE₂, PGD₂ and PGF₂ α is increased in the hippocampus of epileptic rats. During the PG synthesis also occur free radical production, which could be visualized by SOD and HPx analyses (Bellissimo et al. 2001). BK also stimulates the MAPK pathway and binds to neurotrophins receptors, perhaps mediating the phosphorylation of proteins on tyrosine residue (PTyP), changing the gene expression and contributing to plasticity found in the epileptic phenomena.

HORMONAL CHANGES RELATED TO EPILEPSY

Studies have pointed to a great influence of several hormones in the epileptic phenomena (Scharfman and MacLusky 2006, Diamantopoulos and Cunrine 1986, Herzog et al. 1986, Woolley and McEwen 1992, Bazil et al. 2000, Morrell 1991). Gonadal steroids have been shown to exert both excitatory and inhibitory influences on hippocampal excitability and plasticity (Joels 1997,

Herzog 1999). Although both biochemical and physiological evidences exist supporting gonadal hormone modulation of excitability in the hippocampus, the inconsistency of results obtained in past studies makes difficult to draw clear conclusions on how the hormones affects the hippocampal function. Besides that, with the increasing use of hormone and hormone antagonists for contraceptives and the controversies about the use of replacement therapies, it is essential to understand how steroid hormones may alter hippocampal function. Accordingly to the experimental studies, limbic dysfunction might alter hypothalamic tropic hormones release inducing ovulatory failure by affecting the release of pituitary gonadotropins (Herzog et al. 1989, Amado et al. 1993) based on the fact that limbic cortex and the hypothalamus are extensively interconnected (Stuenkel 1991). In addition, female rats submitted to different experimental models of limbic seizures also presented reproductive and endocrine dysfunction (Amado and Cavalheiro 1998, Amado et al. 1993).

It is interesting to observe previous studies showing that sexual hormones protect the brain of female animals against noxious conditions during the reproductive life (Genazzani et al. 1999, Abbasi 1999), but the mechanisms underlying the neuroprotection offered by sexual hormones are not completely known. Some possibilities involve the action of ovarian hormones in brain edema, reduction of free radicals and increase in BDNF mRNA expression.

Amado and Cavalheiro (1998), studying the establishment of this experimental model in female rats, observed that the oestrus cycle was dramatically altered during the acute period of pilocarpine-induced *status epilepticus*. This change was also observed in the other 2 periods of the experimental model, and was accompanied by a decrease in progesterone, LH and FSH levels and by an increase in the estradiol level (Amado and Cavalheiro 1998). When these chronically induced epileptic female rats were mated, it was possible to observe a decrease in the frequency of spontaneous seizures during pregnancy and lactation (Amado and Cavalheiro 1998). As previously described by Valente et al. (2002), the castration in female rats decreased the latency for pilocarpine-induced SE, increased the SE-related mortality and decreased the latent period to spontaneous seizures.

Concerning seizure frequency, Valente (S.G. Valente, unpublished data) showed that only castration do not modify the pattern of seizures in the chronic phase of the model. The animals submitted to 17β -estradiol replacement therapy did not show differences in seizure frequency (7 ± 3.2 seizures/week) either. In contraposition, the treatment with medroxyprogesterone reduced the seizure frequency (5 ± 2.8 seizures/week), as well as the treatment with 17β -estradiol + medroxyprogesterone with a more expressive reduction (3.9 ± 2.1 seizures/week) (Valente 2005).

The mossy fiber sprouting measured by neo-Timm scale (Tauck and Nadler 1985) during the chronic period reached grade 3 for castrated epileptic rats, while the non-castrated epileptic rats showed grade 2. So, as it was seen by Valente et al. (2002), the castrated epileptic female rats present a more intense grade of mossy fiber sprouting in comparison to intact epileptic animals (Fig. 2A). However, animals submitted to 17β -estradiol replacement presented an intermediary grade between that seen in castrated epileptic female and intact epileptic female. In contraposition, in the groups receiving 17β -estradiol + medroxyprogesterone, the sprouting seems to be stabilized in the same level observed in intact epileptic female, showing that the development of sprouting did not progressed. The same fact could be visualized in female treated with medroxyprogesterone replacement (Valente 2005). These results indicate that castration interferes with the epileptogenesis in the pilocarpine model of epilepsy, suggesting that female sexual hormones could have protective effects against pilocarpine-induced SE.

The effect of synaptic sprouting in the hippocampal function in the epilepsy depends, in part, of the balance between the new innervations of granule cells and inhibitory interneurons (Okasaki et al. 1995). However, there are controversies regarding the hippocampal damage, which concerns if it is a cause or consequence of seizures. This point is interesting because, in this study and in that previous study of Valente et al. (2002), we demonstrated that the castrated epileptic animals presented a more intense grade of sprouting compared to that showed in non-castrated epileptic animals, and the frequency seizures in both groups did not show any differences. Mathern et al. (1995, 1996) did not find a correlation between the mossy fiber sprouting and seizure

frequency, and only correlated the density of sprouting (in animals and human) with neuronal loss in the hilus of dentate gyrus. These data were confirmed by Pitkänen et al. (2000), showing that the sprouting density was not associated with epilepsy severity. Besides that, the sprouting could be prevented by cycloheximide, but the animals developing epilepsy [revisar] (Longo and Mello 1997, 1998).

In addition to mossy fiber sprouting, the cell loss in the hippocampus was observed in the chronic phase of this model. A visible cellular loss could be quantified in CA1 and CA3, and morphological changes in the hippocampus with a cellular disarrangement and dispersion in the hilus of the dentate gyrus could be visualized. Although hippocampal cell loss was present in the animals submitted to hormonal replacement, it was less pronounced (Valente 2005). So, we could verify that the hormonal replacement therapy in castrated animals is important in the epileptogenic process, but its efficiency is dependent of the type of reposition that the animal is submitted.

Another point related to hormones and epilepsy concerns to melatonin, a hormone synthesized by the pineal gland with major influence on several circadian physiological activities. It is maximally produced between midnight and dawn (Reiter 1986), with low levels during the light period. Furthermore, melatonin has been described to act as an anticonvulsant against chemically (Lapin et al. 1998, Yamamoto and Tang 1996) and electrically (Mevissen and Ebert 1998) induced seizures. In humans, melatonin has been considered to act as an anticonvulsant following the observation of its ability in reducing the spiking activity and seizures frequency in patients with intractable epilepsy (Anton-Tay 1974). In patients with temporal lobe epilepsy (Bazil et al. 2000), low levels of salivary melatonin were found during the interictal period when compared to controls. On the other hand, high levels of salivary melatonin were observed during the postictal period (Bazil et al. 2000).

In vitro experiments have shown that melatonin was able to protect neurons from excitotoxicity mediated by kainate-sensitive glutamate receptors and from oxidative stress-induced DNA damage and apoptosis (Wu et al. 1999) and, *in vivo*, this hormone has been considered neuroprotective against kainite-induced excitotoxicity (Uz et al. 1996). Taken together, these data

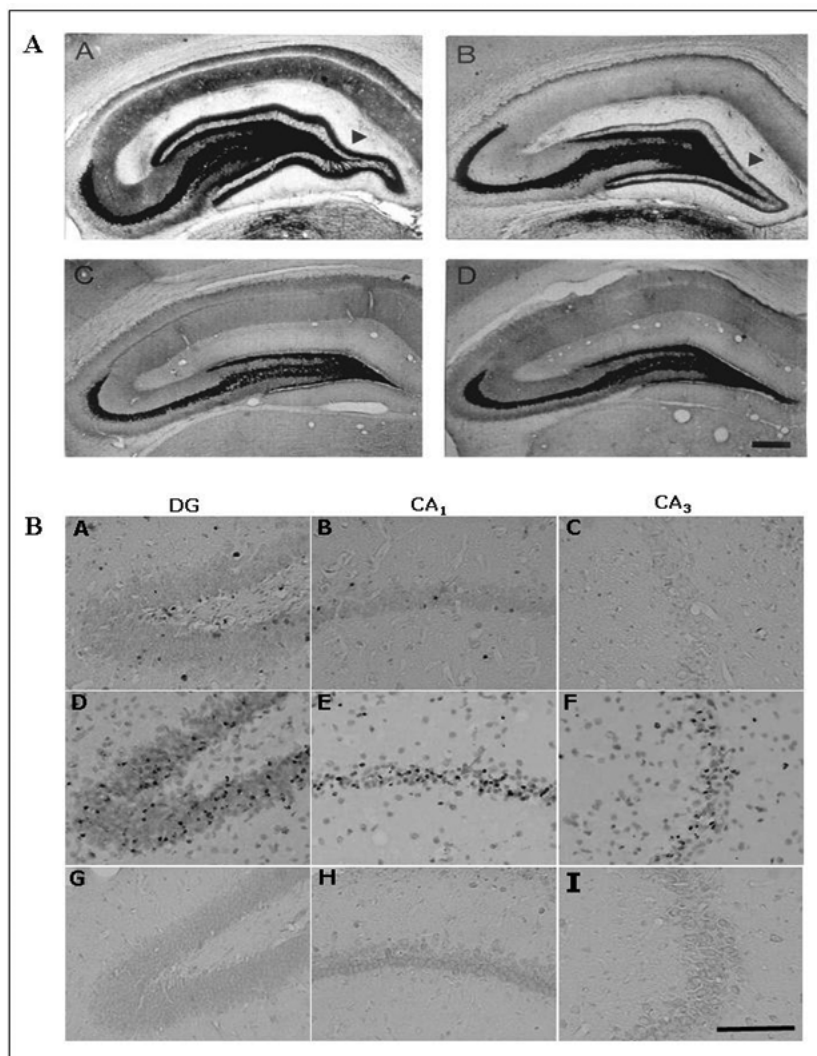


Fig. 2 – **A**) Photomicrographs of neo-Timm stained mossy fibers in the dorsal hippocampus. A – Castrated animal that received pilocarpine and presented SE; B – Non-castrated animal that received pilocarpine and presented SE; C – Castrated animal that received saline; D – Animal that received saline. The arrows indicate the dark band of aberrant mossy fibers in the supragranular layer. Scale bar $300\mu\text{m}$. **B**) Photomicrographs of the coronal section of the hippocampal subfields DG, CA1 and CA3 stained by TUNEL method. A, B, C – Animal that received pilocarpine and presented SE; D, E, F – Pinealectomized rats that received pilocarpine, presented SE and received saline; G, H, I – Pinealectomized rats that received pilocarpine, presented SE and received melatonin treatment. The arrows indicate the DNA damage. Scale bar $160\mu\text{m}$.

are consistent with the hypothesis that melatonin has an inhibitory function on central nervous system activity (Molina-Carballo et al. 1994).

In this context, Chung and Han (2003) suggested that melatonin is a hormone potentially useful in the treatment of acute brain pathologies associated with oxidative stress-induced neuronal damage such as epilepsy, stroke and traumatic brain injury. However, this idea is not widely accepted since several authors

did not find evidences that melatonin deficiency could lead to increased brain vulnerability (Manev et al. 1996).

One of the main characteristics of rats submitted to the pilocarpine model of epilepsy (Cavalheiro et al. 1991) is that the vast majority of spontaneous seizures observed during the chronic period of the model occurred during the day (Cavalheiro et al. 1991, Arida et al. 1999a, b). In this line, Lima et al. (2005) clearly indicate that pinealectomy interferes with the natural course of

the epileptogenesis in the pilocarpine-model of epilepsy in rats by reducing the latency for the first spontaneous seizure (latent period) and increasing the number of spontaneous seizures during the chronic period. Moreover, the reintroduction of melatonin during the *status epilepticus* (acute) period was able to reduce the number of TUNEL-positive cells in several limbic areas (Fig. 2B). In another study, the pre- or post-treatment with melatonin and N-acetylserotonin showed that these hormones have an important neuroprotector effect in the epileptogenesis and in the control of seizures during the chronic period of the pilocarpine model of epilepsy (Lima et al. 2006).

These data are in accordance to other data in the literature which indicate the possibility that, in future therapeutic, attempts might be conducted not only toward the use of pharmacological doses of melatonin, but also to the pharmacological regulation of endogenous melatonin levels in patients with epilepsy.

SUDDEN UNEXPECTED DEATH IN EPILEPSY

GENERAL ASPECTS

Epilepsy is associated with a 2- to 3-fold increase in mortality compared to the general population, and sudden unexpected death in epilepsy (SUDEP) is the most important direct epilepsy-related cause of death (Duncan et al. 2006). SUDEP is defined as a non-traumatic and non-drowning death in patients with epilepsy that is sudden, unexpected, witnessed or unwitnessed, and with or without evidence of a seizure. Also in SUDEP, post mortem examination does not reveal a toxicological or anatomical cause of death (excluding documented *status epilepticus*) (Nashef 1997). Comparisons of incidence estimates for SUDEP are difficult. Since different definitions of SUDEP have been used, not all patients have a post-mortem examination, and case ascertainment methods and source populations have varied (Tomson et al. 2005). The incidence of SUDEP has been estimated to be 3.5/1000 person-years in a lamotrigine clinical trial (Leestma et al. 1997), 0.5-1.4/1000 person-years in people with treated epilepsy (Tennis et al. 1995), 5.9/1000 person-years in outpatients with epilepsy at a tertiary referral centre (Nashef et al. 1995), and 0.35/1000 person-years in a population-based study (Ficker et al. 1998). The National General Practice Study of Epilepsy (NG-

PSE), a community-based study in the United Kingdom, saw the first case of SUDEP after 11,000 person-years of follow-up (Lhatoo and Sander 2001), and the results of the Medical Research Council Antiepileptic Drug Withdrawal Study showed that SUDEP is a rare event among patients with epilepsy in remission (1991). Information concerning risk factors for SUDEP is conflicting, but potential risk factors include: early adulthood, early onset of epilepsy (Nilsson et al. 1999), long duration of epilepsy (Walczak et al. 2001), uncontrolled seizures (mainly in those with TLE) (Walczak et al. 2001, Sperling et al. 1999), high seizure frequency (Walczak et al. 2001, Langan et al. 2005), certain seizure types (Walczak et al. 2001, Kloster and Engelskjøn 1999), higher numbers of AED (Nilsson et al. 1999, 2001, Walczak et al. 2001) and winter temperatures (Scorza et al. 2007). Additionally, potential pathomechanisms for SUDEP are unknown, but it is very probable that cardiac arrhythmias during and between seizures, electrolyte disturbances, arrhythmogenic drugs or transmission of epileptic activity to the heart via the autonomic nervous system potentially play a role for SUDEP (Stollberger and Finsterer 2004).

CARDIAC ABNORMALITIES AND SUDEP

By definition, the cause of death in SUDEP is currently unknown. A number of post-mortem, ictal and interictal cardiac abnormalities do, however, suggest the possibility of seizure-induced cardiogenic SUDEP (Stollberger and Finsterer 2004, Ryvlin et al. 2006).

Postmortem examinations in people dying of SUDEP have found hearts that are dilated and heavier than expected (Leestma et al. 1997, Stollberger and Finsterer 2004, Falconer and Rajs 1976, Leestma et al. 1989), and pulmonary edema in approximately 50-86% of cases (Leestma et al. 1997, 1989, Kloster and Engelskjøn 1999, Stollberger and Finsterer 2004, Thom et al. 2003). Furthermore, others have described pathological changes in the hearts of those dying with SUDEP, including fibrosis of the walls of small coronary arteries, atrophy of cardiomyocytes, myofibrillar degeneration, edema of the conductive tissue and morphological abnormalities of the cardiac conduction system (Kloster and Engelskjøn 1999, Stollberger and Finsterer 2004, Falconer and Rajs 1976, Natelson et al. 1998, Opeskin et al. 2000). These abnormalities may be the conse-

quence of repeated hypoxemia and/or may be associated with the increase of catecholamines during an ictal sympathetic storm (Stollberger and Finsterer 2004, Falconer and Rajs 1976, Natelson et al. 1998).

Several studies have assessed the frequency and character of ictal cardiac rhythm during seizures (Stollberger and Finsterer 2004, Keilson et al. 1987, Opherk et al. 2002), and the most compelling evidence derives from the presence of ictal arrhythmias (Ryvlin et al. 2006). When ictal cardiorespiratory variables were recorded in people with epilepsy, an increase in heart rate in 91% of 41 seizures and a transient bradycardia in 5 seizures (4 patients) were found (Nashef et al. 1996). Another study evaluated the electrocardiographic (ECG) changes during 51 seizures in 43 patients with refractory epilepsy (Nei et al. 2000). This showed that 70% of patients had either ECG abnormalities (16%), tachycardias (30%), or both (23%) during the ictal and/or post-ictal period. These changes may all be relevant to the pathophysiology of SUDEP.

Results of interictal cardiac investigations have also been described. In one study, resting ECGs in 75 patients with epilepsy were compared with normal ECGs recorded in age-matched patients without cardiac or neurological disorders; ventricular rate, PR interval, QRS duration, and QT interval (corrected for heart rate) were compared (Drake et al. 1993). Those with epilepsy had higher heart rates and longer QT durations than the age-matched controls. Heart rate and QT duration were, however, not outside the normal range. Others investigated whether patients with drug refractory epilepsy have cardiovascular abnormalities that might be related to sudden death (Tigaran et al. 2003). Twenty-three subjects underwent comprehensive cardiovascular evaluations (ECG, Holter-monitoring, echocardiography, ergometric exercise test and myocardial scintigraphy; if abnormalities were found, coronary angiography was also performed) before and during video-EEG monitoring. ST-segment depression was found in 40%, and this was associated with a higher maximum heart rate during seizures, suggesting that cardiac ischemia may occur in these patients. Although interictal changes in heart rate variability have been described in patients with epilepsy, their contribution to SUDEP remains to be determined.

Quite interesting, several suggestions have been made concerning the mechanisms behind SUDEP, most

involving speculation about the possible role of autonomic effects disturbances. It has been believed that cardiovascular diseases are often associated with overactivity of the sympathetic nervous system (Schlaich et al. 2004), and that increases in physical activity produce beneficial effects on the cardiovascular system in both normal and diseased individuals via alteration of neural control of the circulation (Billman 2002, Cornelissen and Fagard 2005). These effects include reductions in blood pressure and sympathetic outflow in humans (Pescatello et al. 2004), as well as in animal models of exercise training (De Angelis et al. 2004, Krieger et al. 2001). Since morbidity and mortality in cardiovascular disease are often associated with elevation of sympathetic nervous system activity (Zoccali et al. 2002), the beneficial effects of physical activity are probably related, in part, to the reduction of sympathetic activity. A recent study by our group evaluated the heart rate, *in vivo* (ECG) and isolated *ex vivo* preparation (Langendorf preparation) of rats with epilepsy (Colugnati et al. 2005) (Fig. 3). The results showed differences in the mean heart rate *in vivo* but, surprisingly, no differences in heart rate could be observed in the isolated *ex vivo* situation, suggesting a central nervous system modulation of the heart that could explain SUDEP (Colugnati et al. 2005).

Taking these findings together, it is clear that premature mortality is increased in patients with epilepsy, particularly in those with more severe seizures (Tomson et al. 2005), and it is generally acknowledged that the incidence of cardiac abnormalities between seizures is the very probable cause of SUDEP (Tomson et al. 2005, Stollberger and Finsterer 2004). In conclusion, as reported by others (Bell and Sander 2006), the clarification of risk factors and the establishment of the mechanisms of SUDEP are important for establishing preventative measures for SUDEP and for striving for the best control of seizures. However, it is conceivable that encouraging patients with epilepsy worldwide to receive non-pharmacological treatments will lead to substantial public-health benefits.

EPILEPSY and PHYSICAL EXERCISE

EPILEPSY AND EXERCISE: HUMANS STUDIES

Before we present the data on the role of physical exercise in animal models of epilepsy, brief information

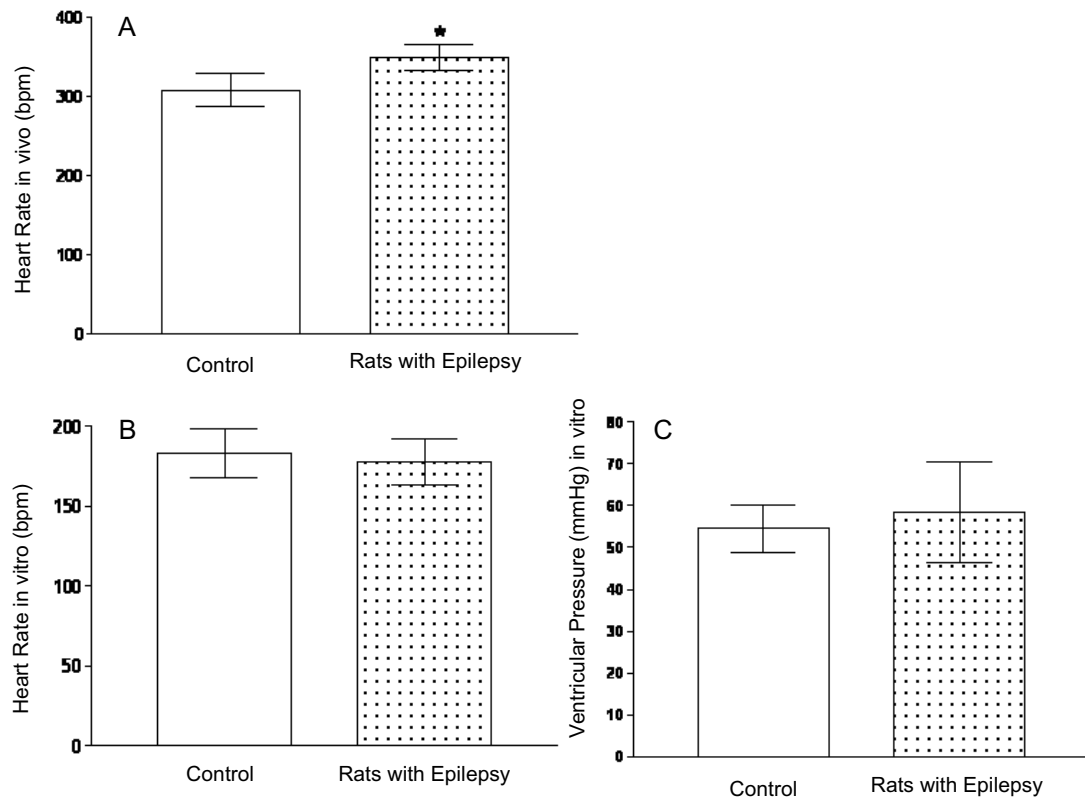


Fig. 3 – Results obtained by Colugnati et al. (2005). Heart rate *in vivo* (A); Heart rate of isolated *ex vivo* preparation (B); Ventricular pressure of isolated *ex vivo* situation (C) from control and rats with epilepsy. Note that heart rate *in vivo* is significantly higher in rats with epilepsy. * $p < 0.05$.

regarding data in humans will be given. Despite this emphasis in today's society on exercise and fitness, people with epilepsy are often excluded from participation in physical activity. This reluctance of both patients and physicians is due in part to fear of injuries and in part to fear that exercise will cause seizures (Bjorholt et al. 1990).

Medical decisions are frequently based on more emotional, anecdotal or personal observations than upon scientific facts. The attitude towards restriction and protection of the epileptic patient has, however, changed dramatically in the last decades, and general recommendations have been recently reviewed. In order to give epileptic patients satisfactory advice about sports, it is essential to understand the factor in sport that could affect the epileptic disorder.

The existing clinical data on the impact of exercise on patient outcomes have limitations. There is a lack of prospective studies, studies using appropriate controls, studies examining behavioral aspects, and studies using a comprehensive approach in an outpatient setting. The

studies that have been designed to analyze the relationship between epilepsy and exercise have compared physical and social activities among patients with epilepsy based on questionnaires and/or clinical studies (Roth et al. 1994, Steinhoff et al. 1996). They have also assessed physical fitness by using standardized tests of physical endurance (Steinhoff et al. 1996, Jalava and Sillanpaa 1997) and physical training programs (Nakken et al. 1990). For instance, a study reported that a physical training program did not change the average frequency of seizures (Nakken et al. 1990). Another study evaluating physical exercise in woman with intractable epilepsy demonstrated that aerobic physical training decreased the number of seizures during the exercise period (Eriksen et al. 1994).

EPILEPSY AND EXERCISE PHYSIOLOGY

Most experiments on brain electrical activity have shown that abnormal discharges disappear in most patients during physical activity, but return at rest (Gotze et al. 1967, Kuijer 1980). It has been also observed that fewer

seizures occur during both mental and physical activity compared with periods of rest (Cordova 1993). The increased vigilance and attention involved in exercise could explain the reduction in the number of seizures (Kuijer 1980).

Although exercise has been shown to reduce epileptic activity on the EEG and the number of seizures, there are numerous factors that could cause seizures during sports and exercise, and any links at this point are largely speculative. It appears that these factors occur as the result of a disturbed balance of physiological parameters such as fatigue, stress, (Temkin and Davis 1984, McLaurin 1973, Cordova 1993), hypoxia (Boucharlat et al. 1973, MacLaurin 1973), hyperhydration (Bennett and Wagner 1983, Noakes et al. 1984), hypoglycaemia (French 1983), hyperthermia (Millington 1985, van Willigen 1988) and hyperventilation. Because hyperventilation in the laboratory may provoke epileptiform discharges on EEG and even seizures, especially absence, some have erroneously believed that increased ventilation during exercise may cause seizures (Esquivel et al. 1991). However, increased ventilation during physical training is a compensatory homeostatic mechanism; the respiratory alkalosis of induced hyperventilation does not occur (Wasserman et al. 1973). In these lines, seizures during exercise may be related to acute metabolic and respiratory changes. How efficient the respiratory control systems are in untrained subjects is not known, but untrained persons lose homeostatic balance more easily than trained persons.

EPILEPSY AND EXERCISE: ANIMALS STUDIES

Experimental studies have also demonstrated a positive effect of physical exercise in animals with epilepsy (Arida et al. 1998, 1999a, b, 2003a, b, 2004, 2007). A study, using the pilocarpine model of epilepsy, evaluated the effect of an aerobic physical program on seizure frequency (Arida et al. 1999a, b). A reduced frequency of seizures in trained animals with epilepsy was observed. The main concern to physical exercise by people with epilepsy has been exercise-induced seizures. Seizures occur during physical exercise, but apparently infrequently (Korczyń 1979). In this study, only 2 animals presented 3 seizures each during 3600 h of exercise and 2 animals presented 1 seizure, 1 min post-exercise.

Further investigations were performed to better clarify the factors that may interfere on this process. A study evaluated by using local cerebral metabolic rates for glucose (LCMRglu) whether physical training modifies the functional activity in rats with epilepsy (Arida et al. 2003a, b). LCMRglu was measured by the quantitative [^{14}C]2-deoxyglucose (2DG) method. In view of the fact that all the animals present seizures at rest and not during exercise (Arida et al. 1999a, b), rats with epilepsy were studied during the interictal phase of the pilocarpine model of epilepsy. The hypothesis that animals with epilepsy submitted to a physical training would exhibit a marked metabolic alteration in the interictal phase was, however, not confirmed. It was observed an increase in interictal LCMRglu in inferior colliculus and auditory cortex in the trained rats with epilepsy when compared to rats with epilepsy. Although no substantial LCMRglu changes were observed after physical training, exercise did reverse the low metabolic rates in several structures of animals with epilepsy. Vissing et al. (1996) reported higher local cerebral glucose utilization in the auditory and visual cortex during exercise, suggesting that these changes are not related directly to the exercise *per se*, but to higher mental alertness in exercise than in resting rats. Since physical activity does need a certain level of alertness, the increased attention and vigilance observed during physical activity could reduce the number of seizures (Kuijer 1980). Although these changes were observed at rest, the increased metabolic rate in these structures could explain a lower seizure number in trained rats with epilepsy in the present and previous work (Arida et al. 1999a, b).

A subsequent study was performed to study the effect of aerobic exercise on *in vitro* hippocampal electrophysiological parameters observed in rats submitted to the pilocarpine model of epilepsy (Arida et al. 2004). Electrophysiological changes were monitored by extracellular field potentials recorded from CA1 area. Trained rats with epilepsy exhibited a reduction in population spikes when compared with nontrained rats. These results indicate that physical training reduces CA1 hyperresponsiveness and can modify synaptic plasticity in rats submitted to the pilocarpine model of limbic epilepsy.

The susceptibility to evoked seizures in the pilocarpine model of epilepsy after a physical training program was tested. The latency of pilocarpine-induced

symptoms and the time when they reached their maximal intensity were much longer in trained animals with epilepsy. Thus, seizures were of lower intensity and frequency, and the SE was considerably shorter than in the non-trained rats (Setkowicz and Mazur 2006).

The analysis of structural changes in hippocampal formation of trained rats with epilepsy by means of an immunohistochemical approach was also performed. Markers of the GABAergic system, such as calcium-binding proteins, parvalbumin and calbindin have been extensively used in different models of epilepsy to visualize morphological changes occurring in the brain (Sloviter 1989, Freund et al. 1991). They can be effective and sensitive markers of hippocampal cells (Célio 1990) and, in particular, of a population of inhibitory interneuron (Freund and Buzsáki 1996). Both voluntary and forced exercise lead to prominent changes in the staining of parvalbumin in the dentate gyrus from control rats and rats with epilepsy. Particularly, the acute physical exercise promoted marked PV-immunoreactivity in number, as well as in fibers staining (hilus) in animals with epilepsy (Setkowicz and Mazur 2006).

On the basis of the available data presented, it seems that physical activity in general cannot be considered a seizure-inducing factor. Furthermore, experimental studies in animal models of epilepsy have confirmed the positive effects of exercise in human's studies. However, the mechanisms by which physical training is able to induce such changes are not completely understood and deserve further investigation.

RESUMO

A administração sistêmica do potente agonista muscarínico pilocarpina em ratos promove alterações comportamentais e eletrográficas que podem ser divididas em três períodos distintos: (a) período agudo o animal evolui progressivamente para o *status epilepticus*, que perdura por até 24h; (b) período silencioso, caracterizado pela normalização progressiva do comportamento e do EEG e pode ter uma duração de 4 a 44 dias; (c) período crônico, aparecimento de crises epiléticas espontâneas e recorrentes (SRSs). As características das SRSs observadas nos animais durante o período crônico são semelhantes às crises parciais complexas dos seres humanos e ocorrem de 2-3 vezes por semana/animal. Além disso, o modelo de epilepsia induzido pela pilocarpina é válido não somente para se estudar a patogênese da epilepsia do lobo temporal em humanos como

também para se testar a viabilidade de drogas antiepilépticas. Esse artigo de revisão aborda diversos aspectos do modelo de epilepsia induzido pela pilocarpina.

Palavras-chave: hipocampo, pilocarpina, epilepsia do lobo temporal, rato.

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