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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 a electrographic changes that can be divided into 3 distinct periods: (a) an acute period that built up progressive into a limbic *status epilepticus* and that lasts 24 h, (b) a silent period with a progressive normalization of EEG a behavior which varies from 4 to 44 days, and (c) a chronic period with spontaneous recurrent seizures (SRSs). T main features of the SRSs observed during the long-term period resemble those of human complex partial seizures a recurs 2-3 times per week per animal. Therefore, the pilocarpine model of epilepsy is a valuable tool not only to stu the pathogenesis of temporal lobe epilepsy in human condition, but also to evaluate potential antiepileptogenic drug 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 ar brovascular disease, brain tumors, alcohol, tra head injuries, malformations of cortical develo genetic inheritance, and infections of the central n system. In resource-poor countries, endemic infe such as malaria and neurocysticercosis, seem to b risk factors (Duncan et al. 2006).

Epilepsies are characterized by spontaneous rent seizures, caused by focal or generalized pa mal changes in neurological functions triggered normal electrical activity in the cortex (Dichter Because it involves hyperexcitable neurons, a ba sumption links the pathogenesis of epilepsy and t eration of synchronized neuronal activity with an ance between inhibitory [g-aminobutyric acid (G

In commemoration of the 75th anniversary of

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

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

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 5n 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 dif-

and granule cells in the hippocampal complex, terneurons located in the other strata can also b aged. Most notably, hilar mossy cells can be ma damaged by pilocarpine-induced *status epilepti* with large variation in the extent of damage betwe ferent animals. Damage to GABAergic neurons extensive throughout the hippocampus. Howev all GABAergic neurons in the hippocampal comp equally vulnerable, with specific populations in d strata showing different rates of loss. In a recen the density of GAD65 mRNA-positive neuron pro layer III of the entorhinal cortex was similar in and post-*status epilepticus* rats evaluated betwee 7 days after pilocarpine. Similar evaluations have been provided for other brain areas, with the ex of a qualitative assessment of the neocortex (Lei 1990, Turski et al. 1983a, b, 1984).

Glial pathology in the pilocarpine model h received the same level of attention as neuronal ogy. Nevertheless, there have been descriptions ing the proliferation of astrocytes, as shown by creased expression of GFAP and other glial m In addition to glial proliferation, it has been show in the CA1 area of the hippocampal complex of a subjected to pilocarpine-induced *status epilepticu* cells "adapt" to permit rather large increases in cellular potassium accumulation. Microglia an markers of inflammatory tissue reaction have also to be present in the early phases after pilocarp duced *status epilepticus* (Binder and Steinhäuse Garzillo and Mello 2002).

THE PILOCARPINE MODEL OF EPILEPSY: NEUROCHEMICAL ALTERATIONS

Partial or complex seizures, the main character temporal lobe epilepsy (TLE), have been rela important brain impact as well as to the eventu lution of this syndrome. Thus, different autho demonstrated that long-lasting seizures unchain plex chemical cascade, triggering neurochemica ation in neurons and glial cells. These immed long-lasting events can modify the cellular envir

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 endo 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.

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 (RPTPβ), a chondroitim 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 RPTPβ, 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* syn-

modulin or growth associated phosphoprotein (
GAP-43), which is activated by PKC, are mod
the hippocampus of rats in the pilocarpine e
model. GAP-43 has been related to processes
lying cell proliferation in fetal human brain and
related specifically with differentiation and out
of axons. This protein showed its levels incre
the inner molecular layer of the dentate gyrus (
associated with the mossy fiber sprouting), dur
acute, silent and chronic period, in rats submi
pilocarpine-induced epilepsy (Naffah-Mazzaco
al. 1999a). According to several authors, the C
activation may be also induced by glutamate, ac
NMDA receptor, since the blockade of this rece
MK801 prevent the GAP-43 expression as well
mossy fiber sprouting.

During long-lasting seizures, the activation
flammatory processes may also occur. Reactive
such as astrocytes and microglia, appears as a
form. As reported by Garzillo and Mello (200
days (chronic phase) after pilocarpine-induced S
minent astrocytes could still be seen in differen
areas. The activated microglia has been blamed
source of the main inflammatory cytokines. Thu
eral authors described increased expression of
for IL-1β, IL-6, iNOS and TNFα after seizur
Ravizza el al. (2008) showed that specific inflam
pathways are chronically activated during epilept
sis and they persist in chronic epileptic tissue, s
ing that they may contribute to etiopathology of

Another pathway involved in the inflam
processes is linked to prostaglandin (PG) release.
eicosanoids are produced after the action of ph
lipase A2 on phospholipids that releases arach
acid, which could be done by the action of glu
on NMDA receptor. Thus, Naffah-Mazzacor
al. (1995) showed increased release of prostag
PGF2α during the acute phase, PGD_2 during the
silent and chronic period and PGE_2 only duri
chronic phase of the epilepsy model induced b
carpine. During PG formation, free radicals a
duced, increasing the inflammatory process.

H_2O_2, 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-Arg^{9}BK, des-Arg^{10}Kallidin 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 A_2 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 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 (Se-

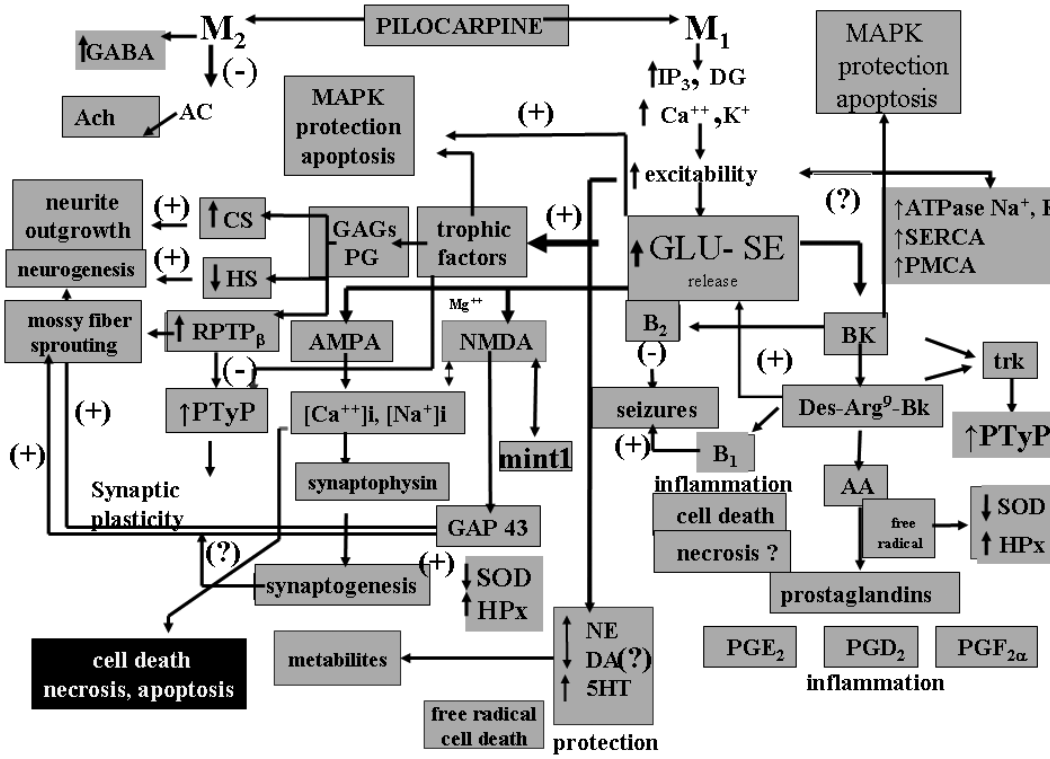

Fig. 1 – Main biochemical pathways and physiological consequences involved in the pilocarpine model. **MAPK** – mitogen activate kinase; **Glu** –glutamate; **ATPase** – ATPase sodium, potassium; **SERCA** – calcium ATPase from sarco-endoplasmatic reticulum; **PMCA** – ATPase from plasma membrane; **BK**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

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

Glutamate on NMDA receptors promotes creased expression of GAP-43, which is linked t sy fiber sprouting and hippocampal plasticity (M Mazzacoratti et al. 1999b).

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 chondroitim sulfate and RPTPβ (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 RPTPβ 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 PGE_2, PGD_2 and PGF2α 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 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

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 medroxiprogesterone reduced the seizure frequency (5 ± 2.8 seizures/week), as well as the treatment with 17β-estradiol + medroxiprogesterone 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 seem in castrated epileptic female and intact epileptic female. In contraposition, in the groups receiving 17β-estradiol + medroxiprogesterone, 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 medroxiprogesterone 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 interneurones (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, and only correlated the density of sp
(in animals and human) with neuronal loss in th
of dentate gyrus. These data were confirmed by
nen et al. (2000), showing that the sprouting dens
not associated with epilepsy severity. Besides th
sprouting could be prevented by cicloheximide,
animals developing epilepsy [revisar] (Longo and
1997, 1998).

In addition to mossy fiber sprouting, the c
in the hippocampus was observed in the chronic
of this model. A visible cellular loss could be
tified in CA1 and CA3, and morphological chan
the hippocampus with a cellular disarrangement a
persion in the hilus of the dentate gyrus could b
alized. Although hippocampal cell loss was pre
the animals submitted to hormonal replacement,
less pronounced (Valente 2005). So, we could ver
the hormonal replacement therapy in castrated a
is important in the epileptogenic process, but i
ciency is dependent of the type of reposition t
animal is submitted.

Another point related to hormones and e
concerns to melatonin, a hormone synthesized
pineal gland with major influence on several cir
physiological activities. It is maximally produc
tween midnight and dawn (Reiter 1986), with lo
els during the light period. Furthermore, melato
been described to act as an anticonvulsant against
ically (Lapin et al. 1998, Yamamoto and Tang 199
electrically (Mevissen and Ebert 1998) induced se
In humans, melatonin has been considered to ac
anticonvulsant following the observation of its ab
reducing the spiking activity and seizures freque
patients with intractable epilepsy (Anton-Tay 19
patients with temporal lobe epilepsy (Bazil et al.
low levels of salivary melatonin were found during
terictal period when compared to controls. On th
hand, high levels of salivary melatonin were ob
during the postictal period (Bazil et al. 2000).

In vitro experiments have shown that me
was able to protect neurons from excitotoxicity m
by kainate-sensitive glutamate receptors and fr
idative stress-induced DNA damage and apoptos

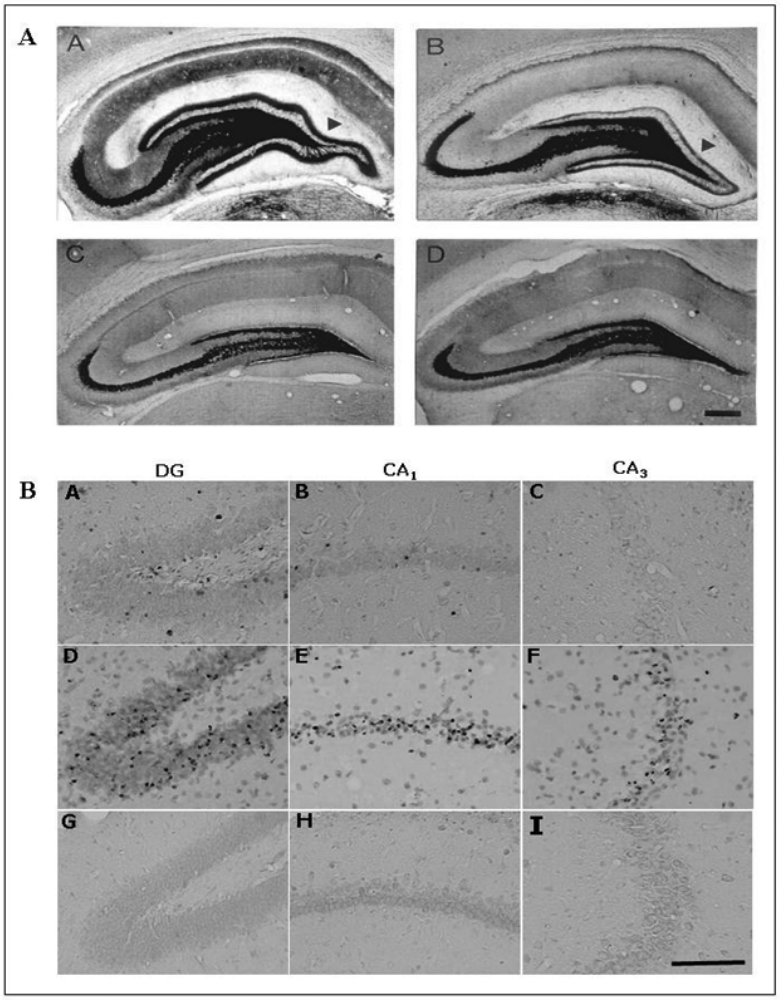

Fig. 2 – **A)** Photomicrographs of neo-Timm stained mossy fibers in the dorsal hippocampus. A – Castrated animal that received pilocarpine and presented SE; B – Non-castrated animal that received pilocarpine and presented SE; C – Castrated animal that received saline; D – Animal that received saline. The arrows indicate the dark band of aberrant mossy fibers in the supragranular layer. Scale bar 300 μm. **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 μm.

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

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 oc-

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 re-

PSE), a community-based study in the United Kin
saw the first case of SUDEP after 11,000 person-y
follow-up (Lhatoo and Sander 2001), and the res
the Medical Research Council Antiepileptic Drug
drawal Study showed that SUDEP is a rare event
patients with epilepsy in remission (1991). Infor
concerning risk factors for SUDEP is conflicti
potential risk factors include: early adulthood, ea
set of epilepsy (Nilsson et al. 1999), long dura
epilepsy (Walczak et al. 2001), uncontrolled s
(mainly in those with TLE) (Walczak et al. 2001
ling et al. 1999), high seizure frequency (Walcza
2001, Langan et al. 2005), certain seizure types
zak et al. 2001, Kloster and Engelskjon 1999),
numbers of AED (Nilsson et al. 1999, 2001, Wal
al. 2001) and winter temperatures (Scorza et al.
Additionally, potential pathomechanisms for S
are unknown, but it is very probable that card
rhythmias during and between seizures, electroly
turbances, arrhythmogenic drugs or transmission
leptic activity to the heart via the autonomic n
system potentially play a role for SUDEP (Stol
and Finsterer 2004).

Cardiac Abnormalities and SUDEP

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

Postmortem examinations in people dy
SUDEP have found hearts that are dilated and
ier than expected (Leestma et al. 1997, Stollberg
Finsterer 2004, Falconer and Rajs 1976, Leestm
1989), and pulmonary edema in approximately 5
of cases (Leestma et al. 1997, 1989, Kloster and
skjon 1999, Stollberger and Finsterer 2004, Thor
2003). Furthermore, others have described pat
cal changes in the hearts of those dying with S
including fibrosis of the walls of small coronary
ies, atrophy of cardiomyocytes, myofibrillar deg
tion, edema of the conductive tissue and morpho
abnormalities of the cardiac conduction system (

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 eletrocardiographic (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 postictal 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, 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

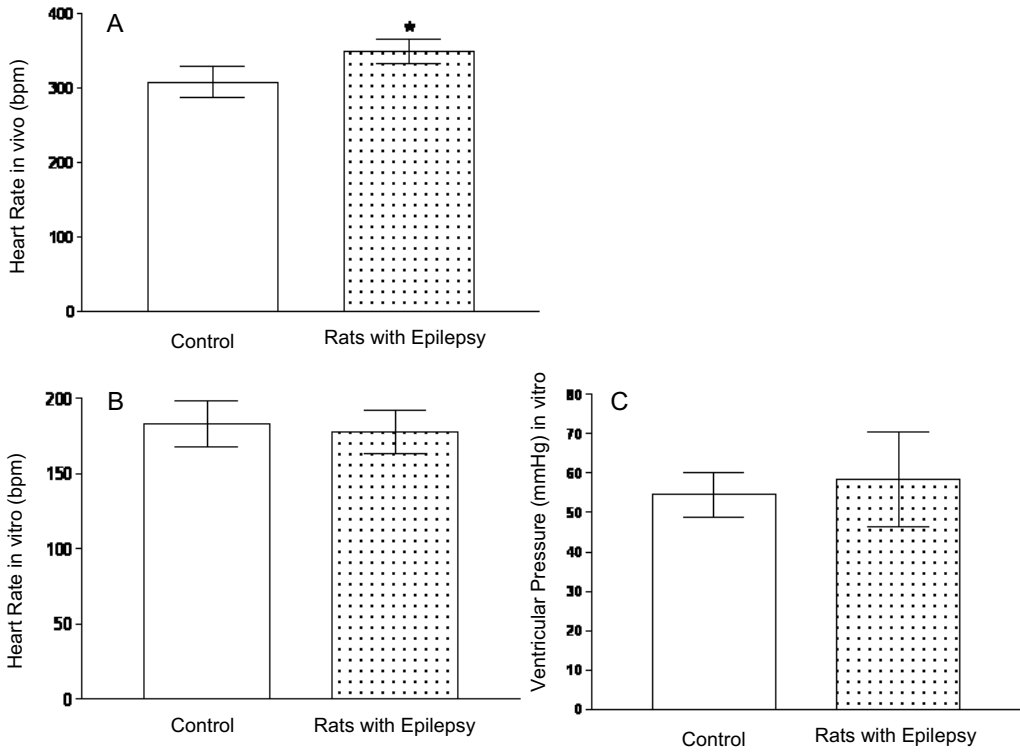

Fig. 3 – Results obtained by Colugnati et al. (2005). Heart rate *in vivo* (A); Heart rate of isolated *ex vivo* preparation (B); Ventricular pr
isolated *ex vivo* situation (C) from control and rats with epilepsy. Note that heart rate *in vivo* is significantly higher in rats with epilepsy. *

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

studies that have been designed to analyze the re
ship between epilepsy and exercise have compare
ical and social activities among patients with e
based on questionnaires and/or clinical studies (
al. 1994, Steinhoff et al. 1996). They have a
sessed physical fitness by using standardized t
physical endurance (Steinhoff et al. 1996, Jala
Sillanpaa 1997) and physical training programs (N
et al. 1990). For instance, a study reported that
ical training program did not change the avera
quency of seizures (Nakken et al. 1990). Anothe
evaluating physical exercise in woman with intr
epilepsy demonstrated that aerobic physical train
creased the number of seizures during the exerc
riod (Eriksen et al. 1994).

Epilepsy and Exercise Physiology

Most experiments on brain electrical activity have

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 (Korczyn 1979). In this study, only

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.

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 epilépticas espontâneas e recorrentes (SRSs). As características das SRSs observadas nos animas durante o período crônico são semelhantes às

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

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

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