Pathophysiology of Status Epilepticus Induced by Pilocarpine

R.M. Freitas[b], A.A. Oliveira[b,*], F.C.F. Sousa[b], S.M.M. Vasconcelos[b], G.S.B. Viana[b] and M.M.F. Fonteles[a,b].

[a]*Department of Pharmacy, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceara, Rua Carpitão Francisco Pedro, 1210, Fortaleza 60000-000, CE, Brazil;* [b]*Department of Physiology and Pharmacology, Laboratory of Neuropharmacology, School of Medicine, Federal University of Ceara, Rua Cel. Nunes de Melo 1127, Fortaleza 60431-970, CE, Brazil*

Abstract: Status epilepticus (SE) is clinically defined as prolonged electrical and clinical seizure activity in which the patient does not regain consciousness to a normal alert state between repeated tonic-clonic attacks. The disorder is a neurological emergency associated with a mortality rate of 10-12% and an even greater morbidity. SE can lead to permanent pathological damage and altered physiological function in certain brain regions and induces major changes in membrane phospholipids, massive increases in arachidonic acid concentrations, diacylglycerol-mediated activation, of protein kinase C, calcium-mediated changes in calmodulin kinase II and possibly generation of free radicals that could play an essential role in the mechanism of oxidative stress involved in neural damage. SE can be characterized by a permanent change in neurotransmitter systems and oxidative stress that it is more facilitated in the brain rather than in other tissues because it contains large quantities of oxidizable lipids and metals. The role of monoamines, amino acid and oxidative stress in pilocarpine-induced SE will be investigated in hippocampus, striatum and frontal cortex of adult rats. The SE studied will be induced by pilocarpine (400mg/kg, s.c.) and the results observed were investigated during acute phase. The data obtained suggests that pilocarpine induced amino acid and oxidative stress changes in brain regions that are similar to the one verified in human temporal lobe epilepsy.

Keywords: Hippocampus, striatum, frontal cortex, oxidative stress, amino acids, seizures, status epilepticus, pilocarpine.

1. INTRODUCTION

Epilepsies are complex neurobehavioral disorders resulting from increased excitability of neurons in several brain regions that involved several neurotransmitters [28]. The cholinergic system plays an important role in generating electroencephalographic (EEG) activity as well as regulating the vigilance states. Pilocarpine is a cholinergic agonist with a moderate affinity for M_1 muscarinic receptors and higher for M_5 ones. Muscarinic cholinergic agonists have effects on rapid eyes movement (REM) and slow wave sleep, playing a role in REM induction [19,26]. On the other hand, pilocarpine high-dose (400 mg/kg, s.c.) administration progresses to a long-lasting status epilepticus (SE) within 1-2h and induces behavioral and EEG alterations in rodents, which are similar to human temporal lobe epilepsy (TLE) [28].

TLE pilocarpine-induced rodent models might provide information regarding neurochemical characteristics associated with seizure activity in young and adult rats [5,11,15,33]. TLE can be characterized by a permanent change in neurotransmitter systems and in oxidative stress (OS) that is more facilitated in the brain rather than in other tissues because for several reasons, its high consumption of oxygen, contains large quantities of oxidizable lipids and pro-oxidative metals, and has comparatively less antioxidant capacity [10,25]. The cells continuously produce free radicals and reactive oxygen species (ROS) as part of their metabolic processes [16,17]. The free radicals are very reactive chemical species and can readily lead to uncontrolled reactions, which may result in oxidative damage DNA, proteins and lipids [27].

ROS can affect the ion transport proteins and channels, via protein oxidation or via peroxidation of membrane phospholipids, resulting in a deleterious on the ionic homeostasis and the neuronal transmission [29,30]. The ROS increased OS induces which is defined as the excessive production of free radicals, such as superoxide (O_2^-), hydroxyl radical ($OH^{\bullet}$), nitric oxide (NO) and their metabolites (nitrate and nitrite) and others, which can dramatically alter the cell function. Besides, an over production of these compounds has been related to seizure-induced neuronal death and SE [9,10,21]. Several compounds can produce free radical such as H_2O_2, that in high concentration can react with O_2^- (Haber-Weiss reaction) or iron (Fenton reaction) producing highly reactive $OH^{\bullet}$. The conversion of H_2O_2 to H_2O and O_2 is made by catalase and glutathione peroxidase [24,32]. The formed $OH^{\bullet}$ radical is likely to react with non-radical molecules, transforming them into secondary free radicals. This reaction occurs during the lipid peroxidation producing hydroperoxides [24,25]. NO can be estimated by their metabolites [37], which are associated with neurodegenerative diseases [38]. Despite the fact that numerous studies clearly indicate the importance of antioxidant enzymatic activities in the epileptic phenomenon the mechanisms by which these enzymes influence seizures and SE are not completely understood [24,32].

*Address correspondence to this author at the Rua Padre Frota, 189, Monte Castelo, Fortaleza, CEP 60325-160, Ceará, Brazil; Tel: (55)-85-288-8337; Fax: (55)-85-288-8333; E-mail: alinealbuquer@hotmail.com

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In the brain, the phenomena of excitotoxicity has been related to an over production of free radicals by the tissue during pilocarpine-induced seizures and SE [32] and in human epilepsy [37]. The increase in levels of ROS can be responsible for this neuropathology and can activate apoptosis processes [29,34]. The free radicals are neutralized by an elaborate antioxidant defense systems consisting of enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase, and numerous non-enzymatic antioxidants such as reduced glutathione (GSH), indicating a cellular response [9]. SE induces ROS production by protein oxidation measured by measuring tyrosine nitration [29] as well as an end-product of lipid peroxidation as indicated by malondialdehyde (MDA) levels [3], and it could also be determined by the effectiveness of the antioxidant enzymes response [8].

The hippocampus might be the principal area affected by pilocarpine-induced seizures and by the SE. Other authors also characterized the neuropathology associated with the SE in striatum, frontal cortex, thalamus and amygdala [12,14, 26].

The principal purpose of the present article to review many studies which have attempted to measure the behavioral and neurochemical alterations in the different brain areas of adult rats after pilocarpine-induced status epilepticus.

2. BEHAVIORAL ALTERATIONS AFTER TREATMENT WITH PILOCARPINE

The pilocarpine model is a useful animal model to investigate the development of acute, silences and chronic phases [5]. Immediately after pilocarpine administration, all animals persistently presented behavioral changes, including initial akinesia, ataxic lurching, peripheral cholinergic signs (miosis, piloerection, chromodacryorrhea, diarrhea and masticatory automatisms), stereotyped movements (continuous sniffing, paw licking, rearing and wet dog shakes that persisted for 10-15 min), clonic movements of forelimbs, head bobbing and tremors.

These behavioral changes progressed to motor limbic seizures as previously described by Tursky *et al.,* (1983a) [35,36]. Limbic seizures lasted for 30-50 min evolving to SE in rats for a period longer than 30 min. In the group observed during 1h, no case of fatality is observed in experiments. During the 24h observation period, 63% of animals died [6,7,22,34].

SE is an emergency situation requiring prompt medical attention if severe permanent brain damage or death is to be prevented. SE often occurs in individuals with a history of seizures, in whom there are neural substrates already predisposed towards supporting seizure activity [1,2]. Our results for the behavioral alterations observed after pilocarpine administration match with previous data described by Marinho et al. (1998) [21].

3. AMINO ACID AFTER PILOCARPINE-INDUCED STATUS EPILEPTICUS

Several neurochemical studies such as neurotransmitter (monoamines, amino acids and peptides), receptor binding (muscarinic, dopaminergic and serotonergic) and enzymatic activities (catalase and superoxide dismutase) determinations have been performed in various brain structures after SE [1,6,15].

Epileptic activity with a wide range of local biochemical changes affects several neurotransmitters (adenosine, norepinephrine, dopamine, serotonin, glutamate (GLU), γ-aminobutyric (GABA), tyrosine (TYR) and glutamine (GLN)) [22,27], and muscarinic [13,34] and dopaminergic [27] receptor densities in hippocampus, frontal cortex and striatum [7,15]. Nevertheless, it is not well established whether other amino acids play a role in the SE process. In addition, little is known about alterations in amino acid content during pilocarpine-induced SE, despite the fact that several studies clearly indicate the importance of amino acids in epileptic phenomenon.

The role for glutamate (GLU), aspartate (ASP), tyrosine (TYR), glutamine (GLN) and other amino acids in seizures process is not clear either and several authors have suggested that the activation of limbic seizures can be induced by acetylcholine and that other systems could be related to epileptogenesis [35]. It is possible that GLU, ASP, TYR and GLN amino acids may also participate in epileptic activity, but when and how it happens has to be determined. SE will observed a significant increase in the GLN level. There was no change in relation to GLU, ASP and TYR concentrations. SE in GLU striatal content is increased. In relation to GLN, ASP and TYR concentrations no alteration is observed. In frontal cortex, ASP content which increase after SE and GLN, GLU and TYR concentrations remained unaltered.

Several amino acids have been associated with the mechanism of pilocarpine-induced SE [6]. Significant differences in amino acid contents were evident in hippocampus, striatum and frontal cortex during development of SE induced by pilocarpine. Increased hippocampal GLN, striatal GLU and frontal cortex ASP levels can be associated with the SE induced by pilocarpine. During the pilocarpine-induced seizures, extracellular GABA, dopamine and GLN levels in hippocampus were increased, suggesting that a neuronal vesicular release may occur and under the same conditions, ASP and GLU decreased [6]. However, more studies including other brain regions should be carried out to identify the importance of amino acids in epileptic phenomenon.

The amino acid (GLN, GLU and ASP) levels increase in hippocampus, striatum and frontal cortex, respectively, suggesting that they have a function in SE. Therefore, it is likely that all three amino acids can be interconnected in epileptic activity. In contrast, loss of control of amino acid oxidation results in growth impairment and epileptic-like seizures [18]. These findings emphasize the importance of control of amino acid catabolism for normal neurological function [18]. The control of catabolism altered can induces SE. The increased levels of GLN observed during SE may suggest that cellular death occurs simultaneously with seizures, as suggested by in intrahippocampal KA-injected rats. The results demonstrate a significant increase in GLN levels in the hippocampus without a significant increase in GLU. However, there is significant glial-dependent uptake of GLU. A safe mechanism for re-supply of GLU to neurons is to convert GLU to GLN and then release this less toxic amino acid

back to the extracellular space for neuronal uptake and reconversion to GLU. Thus, you may be measuring an earlier increase in GLU that has been buffered by glial uptake [18].

It has been observed that during pilocarpine-induced SE, amino acid levels were modified in different ways in hippocampus, striatum and frontal cortex, suggesting that there is a greater involvement of this in the seizures process in comparison to other amino acids which were differently modified during the acute phase of seizures [6].

Other results clearly show that although cholinergic routes were activated by pilocarpine administration, several neurotransmitter systems were involved and that they could be implicated in initiation and/or maintenance of convulsions during establishment of this model [35], but our results can suggest a clear relation between the amino acid systems in the brain structures investigated with the establishment of SE. Studies concerning glutamatergic and GABAergic systems are relevant and can permit the identification of modulators of epileptogenesis. However, this causative relation between amino acid levels and SE has always been easier to propose rather that to demonstrate because there are certain difficulties such as distinct changes in each one of the areas studied.

4. OXIDATIVE STRESS AFTER PILOCARPINE-INDUCED STATUS EPILEPTICUS

The lipid peroxidation (TBARS formed) in the brain homogenates are increased in this model as compared to corresponding values for the control group. During the acute phase of seizures induced by pilocarpine is verified increases in lipid peroxidation level, nitrite concentration and GSH content in striatum, frontal cortex and hippocampus in the same way the TLE [12,13,14]. Our findings show that SE induces different changes in superoxide dismutase activity in brain regions, as such: striatum and hippocampus did not presented any difference, but in frontal cortex is verified with an increase. After the first hour of acute phase of seizures an increase is detected in several regions (striatum, hippocampus and frontal cortex). In catalase activity was verified an increase in striatum, hippocampus and frontal cortex in this epilepsy model [12,13,14].

Lipid peroxidation in a tissue is an index of irreversible biological damage of the cell membrane phospholipid, which in turn leads to inhibition of most of the sulphydryl and some nonsulphydryl enzymes [16]. Lipid peroxidation level increase and reduce, whereas glutathione decrease can be induced by many chemicals (e.g. kainic acid and pilocarpine) and by many tissue injuries, and has been suggested as a possible mechanism for the neurotoxic effects of epileptic activity [13,16,30]. Our findings demonstrated that lipid peroxidation levels increase after 1h and during 24 of the acute phase of seizures induced by pilocarpine in hippocampus, striatum and frontal cortex.

In normal conditions, there is a steady state balance between the production of ROS and their destruction by the cellular antioxidant system. It is demonstrated that the nitrite content in the striatum and frontal cortex is augmented after seizures and SE in adult rats, suggesting a possible increase in the level of ROS which can be involved in the neuronal damage induced SE. Other studies have shown that the level of nitrite and nitrate were not elevated in patients with cryptogenic west syndrome [38], but it is tempting to speculate that the seizure activity per se did not account for the whole of the increase observed in the nitrite and nitrate levels, and other mechanisms may be associated with this parameter in this epilepsy model as well as neuronal degeneration observed in human beings. However, new studies using antioxidants drugs during SE induced by pilocarpine can indicate whether lipid peroxidation, nitrite concentration and GSH are involved in the pathophysiology of SE in this model.

Although there were no selective brain regions particularly vulnerable to oxidative stress, there were some regional variations in the amount of oxidative damage observed. In the regions studied, there were nearly equal elevations in lipid oxidative, nitrite content and GSH markers that persisted during the acute phase of seizures.

All living organisms can suffer oxidative damage, yet the animal brain is often said to be especially sensitive [3,4,16]. The data of our experiments demonstrate that pilocarpine administration and its resulting SE produce significant alterations in hippocampus, striatum and frontal cortex. We recorded alterations in the superoxide dismutase activity in frontal cortex during the seizures, however, no alterations were observed in striatal superoxide dismutase activity of rats under the same conditions. It is likely that the unaltered superoxide dismutase activity in the striatum might not be related to the mechanisms involved in the installation and propagation of seizures and SE induced by pilocarpine, which produces several changes in parameters related to generation and elimination of oxygen free radicals in adult rats [31]. An increase in free radical formation can be accompanied by an immediate compensatory increase of the free radical scavenging enzymatic (superoxide dismutase and catalase) activities and this action was observed during SE in brain regions. Nevertheless, a similar compensatory mechanism of scavenging was observed in catalase activity after SE, suggesting that the enzymatic function of different systems can be modified either during the acute phase of seizures of according to cerebral area investigated.

The present work reports the involvement of catalase activity in hippocampus, striatum and frontal cortex after SE. An increase in the catalase activity in these brain areas can be related to a long-term compensatory mechanism including modulation activity of enzymes from the ROS catabolism. Moreover, the catalase activity might be one of the able mechanisms that avoid the development of neurotoxic effects mediated by SE, indicating that basal-oxygen radical production can damage the cell and that its control is necessary [23,25].

Evidences for the role of free radicals in seizures has been found by using exogenously administered enzymatic and non-enzymatic antioxidants for protection against seizures and SE-induced neuronal damage [14,19]. A steady state level of O_2^- and H_2O_2 is always present within cells as a result of a normal metabolism. SOD and catalase are responsible for degradation of O_2^- and H_2O_2, respectively. The balance between antioxidants enzymes, superoxide dismutase and catalase, can be important during seizures and SE in-

duced by pilocarpine. The present data indicate that pilocarpine treatment and its resulting SE induce neurochemical changes such as an increase in nitrite content and lipid peroxidation level, decrease in GSH content as well as an activation of brain antioxidant mechanisms. The anatomic distribution of alterations observed in the enzymatic activities (superoxide dismutase and catalase) can suggest that the frontal cortex can be extensively involved in the propagation of epileptic activity and further studies should be carried out to ascertain that catabolism of nitrite, ROS and GSH can be involved in the pathogenesis of SE.

The pilocarpine model is essential to investigate the mechanisms for initiation and propagation of seizures and SE. Additionally, it may be assumed that the increased generation nitrite and lipid peroxidation levels after SE is not primary caused by an exhaustion of both the enzymatic and non-enzymatic defense systems measured. Adaptative mechanisms, as the induction of catalase activity, may be taken into consideration to counteract oxidative stress mediated by SE. However, the relation among brain structures, antioxidant systems, lipid peroxidation, nitrite concentration and SE cannot be perfectly established and deserve further studies.

5. PATHOPHYSIOLOGY OF STATUS EPILEPTICUS

Seizures represent one of the most severe *in vivo* stimulatory stresses that the brain is exposed to and generalized SE represents a very severe form of seizures. The international Classification of seizures has defined this condition as a condition characterized by an epileptic seizure that is so frequent or so prolonged as to create a fixed and lasting condition [18]. Major motor SE can lead to permanent pathological damage and altered physiological function in certain brain regions. The pathophysiological changes seen in complex partial, simple partial and absence SE are much less clear [15,18]. SE can cause brain damage, but can also result from it, and it has been difficult to separate the two, particularly in humans [20].

The available experimental data suggests that convulsion generally accelerate brain damage. Limbic SE causes neuronal necrosis in hippocampus, amygdala, pyriform cortex, entorhinal cortex, thalamus, neocortex, striatum and substantia nigra [27]. The neuronal damage depends on synaptic activation [30], probably *via* a glutamatergic, calcium-medited mechanism [20].

SE has been studied very little in animal models. In SE, glutamate, aspartate, serotonin, dopamine and acetylcholine play major roles as excitatory neurotransmitters, and GABA as the dominant inhibitory neurotransmitter [4,7,14]. However, the relation among brain excitatory and inhibitory neurotransmitters and SE yet cannot be perfectly established and deserve further studies with the purpose of clarified the pathophysiology of seizures.

CONCLUSIONS

The pilocarpine model could prove to be useful to delineate and understand the development of behavioral and neurochemical changes associated with temporal lobe epilepsy. Pilocarpine status may provide a model for studying the basic mechanisms responsible for refractory SE, amino acids and oxidative stress in humans and evaluating new drugs. The pilocarpine model may prove useful in the study of SE for number of reasons. First, these seizures accurately model human generalized epilepsy as is seen from the anticonvulsant profile drugs. Secondly, the serve and refractory nature of this model indicates that it should be valuable in the development of new anticonvulsant agents. Finally, the prolonged and uniform degree of SE is useful for metabolic, neurochemical and neuroanatomical studies of the sequelae of prolonged seizure activity.

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