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Lipoic acid increases glutathione peroxidase, Na^+, K^+-ATPase and acetylcholinesterase activities in rat hippocampus after pilocarpine-induced seizures?

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ABSTRACT

In the present study we investigated the effects of lipoic acid (LA) on acetylcholinesterase (AChE), glutathione peroxidase (GPx) and Na^+, K^+-ATPase activities in rat hippocampus during seizures. Wistar rats were treated with 0.9% saline (i.p., control group), lipoic acid (20 mg/kg, i.p., LA group), pilocarpine (400 mg/kg, i.p., P400 group), and the association of pilocarpine (400 mg/kg, i.p.) plus LA (20 mg/kg, i.p.), 30 min before of administration of P400 (LA plus P400 group). After the treatments all groups were observed for 1 h. In P400 group, there was a significant increase in GPx activity as well as a decrease in AChE and Na^+, K^+-ATPase activities after seizures. In turn, LA plus P400 abolished the appearance of seizures and reversed the decreased in AChE and Na^+, K^+-ATPase activities produced by seizures, when compared to the P400 seizing group. The results from the present study demonstrate that preadministration of LA abolished seizure episodes induced by pilocarpine in rat, probably by increasing AChE and Na^+, K^+-ATPase activities in rat hippocampus.

Key words: glutathione peroxidase, Na^+, K^+-ATPase, acetylcholinesterase, hippocampus, seizures.

O ácido lipóico aumenta as atividades da glutationa peroxidase, da Na^+, K^+-ATPase e da acetilcolinesterase no hipocampo de ratos após convulsões induzidas por pilocarpina?

RESUMO

No presente estudo nós investigamos os efeitos do ácido lipóico (AL) sobre as atividades da acetilcolinesterase (AChE), da glutationa peroxidase (GPx) e da Na^+, K^+-ATPase no hipocampo de ratos durante crises convulsivas. Ratos Wistar foram tratados com solução salina a 0,9% (i.p., grupo controle), ácido lipóico (20 mg/kg, i.p., grupo AL), pilocarpina (400 mg/kg, i.p., grupo P400), e a associação de AL (20 mg/kg, i.p.) com a pilocarpina (400 mg/kg, i.p.), 30 min antes da administração de pilocarpina (grupo AL + P400). Após os tratamentos todos os grupos foram observados durante 1 h. No grupo P400, houve um aumento significativo na atividade da GPx, assim como uma diminuição das atividades da AChE e Na^+, K^+-ATPase. Por sua vez, o pré-tratamento com AL aboliu o aparecimento de convulsões e reverteu a diminuição das atividades da AChE e da Na^+, K^+-ATPase causadas pelas convulsões, quando comparada com o grupo P400 sozinho. Os resultados do estudo demonstram que o pré-tratamento com AL aboliu os episódios de convulsão induzido pela pilocarpina em ratos, provavelmente por meio do aumento das atividades das enzimas AChE e Na^+, K^+-ATPase no hipocampo de ratos.

Palavras-chave: glutationa peroxidase, Na^+, K^+-ATPase, acetilcolinesterase, convulsões, hipocampo.

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Oxidative stress is attractive as a possible mechanism for the pilocarpine-induced seizures for many reasons. The brain processes large amounts of O_2 in relatively small mass, and has a high content of substrates available for oxidation in conjunction with low antioxidant activities, making it extremely susceptible to oxidative damage[1,2]. In addition, certain regions of central nervous system (CNS), such as the hippocampus, may be particularly sensitive to oxidative stress because of their low endogenous levels of antioxidants[3]. Such a depressed defense system may be adequate under normal circumstances. However, in pro-oxidative conditions, such as seizures, these low antioxidant defenses can predispose the brain to oxidative stress.

The mechanism behind seizures-induced oxidative stress is not well understood, but several explanations have been proposed. These include excitotoxicity associated with excessive neurotransmitter release and oxidative stress leading to free radical damage[2,4]. Recently, several studies have examined the role of oxidative stress on pilocarpine-induced seizures whose underlying mechanisms are not yet fully established[3].

Na^+, K^+-ATPase is a crucial enzyme responsible for maintaining the ionic gradient necessary for neuronal excitability. It is present at high concentrations in brain cellular membranes, consuming about 40-50% of the ATP generated in this tissue[5]. It has been demonstrated that this enzyme is susceptible to free radical attack[6]. Besides, there are some reports showing that Na^+, K^+-ATPase activity is decreased in various chronic neurodegenerative disorders[6-8].

On the other hand, there is considerable evidence showing that oxidative stress is an important event occurring in various common acute and chronic neurodegenerative pathologies[9]. This is understandable since the CNS is potentially sensitive to oxidative damage due to its great oxygen consumption, high lipid content and poor antioxidant defenses[10]. We have recently shown that pretreatment with lipoic acid (LA) induces alterations in antioxidant enzymatic activities in rat hippocampus, suggesting a direct effect of this antioxidant on this enzymatic activity[11].

In addition, cholinergic transmission is mainly terminated by ACh hydrolysis by enzyme acetylcholinesterase (AChE)[12,13]. This enzyme substantially contributes to synaptic transmission during seizures, thus, it would be important to describe the effects of LA on this enzymatic activity. In the present study we investigated the LA effects on AChE, glutathione peroxidase and Na^+, K^+-ATPase activities in rat hippocampus after pilocarpine-induced seizures.

METHOD

Adult male Wistar rats (250-280 g) maintained in a temperature controlled room (26±1°C) with a 12-h light/dark cycle with food and water ad libitum were used. All experiments were performed according to the Guide for the care and use of laboratory the US Department of Health and Human Services, Washington, DC[14]. The research project was approved by the Ethics Committee of the Federal University of Piaui, Brazil (Protocol Number 038/09). The following substances were used: pilocarpine hydrochloride and alpha-lipoic acid (Sigma, Chemical USA). All doses are expressed in milligrams per kilogram and were administered in a volume of 10 ml/kg injected intraperitoneally (i.p.). In a set of experiments, the animals were divided in four groups and treated with LA (20 mg/kg, i.p., n=36) or 0.9% saline (i.p., n=36) and 30 min later, they received pilocarpine hydrochloride (400 mg/kg, i.p.), and in this 30-min interval rats were observed for the occurrence of any change in behavior. The treatments previously described represent the LA plus P400 and P400 groups, respectively. Other two groups received 0.9% saline (i.p., n=36, control group) or lipoic acid alone (20 mg/kg, i.p., n=36, LA group). After the treatments, the animals were placed in 30 cm x 30 cm chambers to record: latency to first seizure (any one of the behavioral indices typically observed after pilocarpine administration: wild running, clonus, tonus, clonic-tonic seizures)[15], number of animals that died after pilocarpine administration. Previous work have shown that the numbers of convulsions and deaths occurring within 1 h post-injection always follow the same pattern, so we decided to observe the animals for 1 h as pilocarpine-induced convulsions occur in 1 h and deaths within 1 h after pilocarpine injection. The survivors were killed by decapitation and their brains dissected on ice to remove hippocampus for determinations AChE, glutathione peroxidase and Na^+, K^+-ATPase activities. The pilocarpine group was constituted by those rats that presented seizures for over 30 min and that did not died within 1 h.

The drug dosages were determined from both dose-response studies, including pilocarpine (data not shown), and observations of the doses currently used in animals studies in the literature[16,17]. The doses used are not equivalent to those used by humans because rats have different metabolic rates.

GPx was measured by method described by Sinet et al.[18] using t-butyl-HPx as substrate. The protein concentration was measured according to the method described by Lowry et al.[19]. The results expressed as mU per mg of protein (mU/mg of protein).

Na^+, K^+-ATPase activity was determined by method described by Wyse et al.[20]. Released inorganic phosphate (Pi) was measured by method of Chan et al.[21]. Specific activity of the enzyme was expressed as nmol Pi released per min per mg of protein (nmol Pi/min/mg of protein).

AChE activity was determined according to Ellman et

Table. Effect of pretreatment with lipoic acid on pilocarpine-induced seizures and lethality in adult rats.

Groups	Latency to first seizures (min)	Percentage seizures	Percentage survival	Number of animals / group
Pilocarpine	35±0.70	60	60	36
LA plus pilocarpine	79.15±1.05[b]	25[a]	100[a]	36
LA	0	0	100[a]	36

[a]p<0.0001 as compared with pilocarpine group (χ^2-test); [b]p<0.0001 as compared with pilocarpine group (ANOVA and Student-Newman-Keuls test).

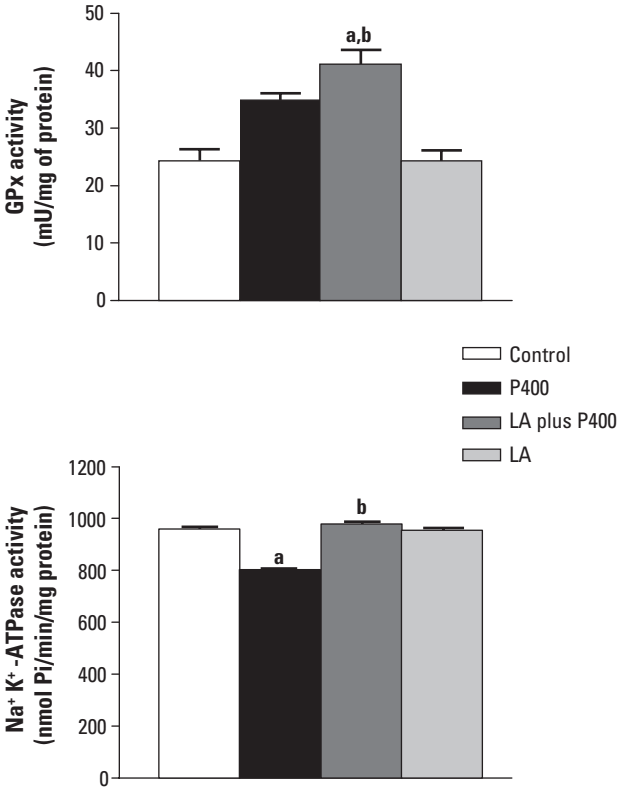


Fig 1. Effect of lipoic acid in adult rats prior to pilocarpine-induced seizures on glutathione peroxidase (GPx) and Na^+, K^+-ATPase activities in hippocampus of adult rats.

al.[22] with some modifications. The protein was measured by the method of Lowry et al.[19] using bovine serum albumin as standard. The results expressed as nmol acetylthiocholine hydrolyzed per min per mg protein (nmol/min/mg of protein).

Results of latency to first seizure and neurochemical alterations were compared using ANOVA and the Student-Newman-Keuls test as post hoc test, because these results show a parametric distribution. The number of animals that seized and the number that survived were calculated as percentages (percentage seizures and percentage survival, respectively), and compared with a nonparametric test (χ^2). In all situations statistical significance was reached at p less-than-or-equals, slant 0.05. The statistical analyses were performed with the software GraphPad Prism, Version 3.00 for Windows, GraphPad Software (San Diego, CA, USA).

RESULTS

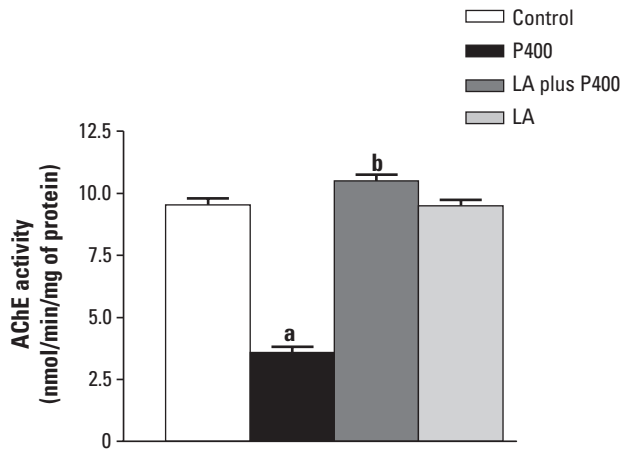
Animals studied showed generalized tonic-clonic convulsions (60%) with status epilepticus (SE), and 60% survived the seizures. Pilocarpine induced the first seizure at 35±0.70 min. Animals pretreated with the LA selected for this study were observed for 1 h before pilocarpine injection and its manifested alterations in behavior, such as peripheral cholinergic signs (100%), tremors (50%), staring spells, facial automatisms, wet dog shakes, rearing and motor seizures (25%), which develop progressively within 1-2 h into a long-lasting SE (25%) (Table). Results showed that when administered at the dose (20 mg/kg) before pilocarpine, LA reduced by 35% the percentage of animals that seized (p<0.0001), increased (126%) latency to the first seizure (79.15±1.05 min) (p<0.0001) and increased (40%) the survival percentage (p<0.0001) as compared with the pilocarpine-treated group (Table). No animal that received injections of isotonic saline (control) or LA alone showed seizure activity (Table).

Fig 1 shows the LA effects on glutathione peroxidase (GPx) and Na^+, K^+-ATPase activities in the hippocampus during seizures induced by pilocarpine. Post hoc comparison of means indicated a significant (52%) increase in hippocampal GPx activity in the hippocampus during seizures (p<0.0003), when compared with the control group. The pretreatment with LA also produced a significant increases in hippocampal GPx activities (20%; p<0.0001), when compared with the P400 group. In addition, the pretreatment with LA, 30 min before administration of pilocarpine also produced a significant increased of 81% in GPx (p<0.0228) activities, when compared with corresponding values for the control group (Fig 1).

Na^+, K^+-ATPase activity in the hippocampus during seizures showed a significant (17%) decrease in P400 group, when compared with corresponding values for the control group (p<0.0001). However, post hoc comparison of means indicated that hippocampal Na^+, K^+-AT-

Pase activity in the rat hippocampus pretreated with LA was not markedly altered during acute phase of seizures (p=0.1334), when compared with the control group (Fig 1). Post hoc comparison of means indicated a significant (23%) increase in hippocampal Na^+, K^+-ATPase activity of rats pretreated with LA (p<0.0001) when compared with the P400 group (Fig 1). However, no adult rats that received LA alone showed alterations in GPx (p=0.8913) and Na^+, K^+-ATPase activities (p=0.7039), when compared with the control group (Fig 1).

Fig 2 shows the LA effects in AChE activity in hippocampus during seizures induced by pilocarpine. Hippocampal AChE activity of rats in pilocarpine group was markedly decreased (63%) (p<0.0001), when compared with corresponding values for the control group. However, post hoc comparison of means indicated a significant (197%) increase in hippocampal AChE activity of rats pretreated with LA (p<0.0001), 30 min before administration of pilocarpine (LA plus P400 group), when compared with the P400 group. In addition, in LA plus P400 group it was observed no changes in AChE activity (p=0.0534), when compared with corresponding values for the control group (Fig 2). Moreover, AChE activity in the hippocampus adult rats that received lipoic acid alone (LA group) was not markedly altered (p=0.9823), when compared with corresponding values for the control group, but showed a significantly increased (169%) (p<0.0001), when compared with corresponding values for the P400 group (Fig 2).



a: p<0.05 as compared to control animals (t-Student-Neuman-Keuls test); b: p<0.05 as compared to P400 group (t-Student-Neuman-Keuls test).

Fig 2. Effect of lipoic acid in adult rats prior to pilocarpine-induced seizures on acetylcholinesterase (AChE) activity in hippocampus of adult rats.

DISCUSSION

The CNS contains some antioxidant enzymes, including superoxide dismutase (SOD) and GPx that are expressed in higher quantities than catalase[23]. This spectrum of enzymatic defense suggests that the brain may efficiently metabolize superoxide but may have difficulties in eliminating the hydrogen peroxide produced by this reaction[24]. In the present study we have examined whether the pretreatment with LA can reverse the alterations in the AChE, Na^+, K^+-ATPase and GPx activities in rat hippocampus caused by seizures. Generation of reactive oxygen species (ROS) is currently viewed as one of the process through which epileptic activity exert their deleterious effects on brain[22]. These ROS in the absence of an efficient defence mechanism cause peroxidation of membrane poly unsaturated fatty acids[25]. Brain is particularly susceptible to peroxidation due to simultaneous presence of high levels of poly unsaturated fatty acids and iron[24], which is the target of free radical damage.

Previous studies conducted in our laboratory have shown that during seizures there are no alterations in hippocampal superoxide dismutase and catalase activities[11]. Furthermore, other antioxidant systems such as glutathione peroxidase can be responsible by inhibition of neurotoxicity induced by acute phase of seizures activity. It has been demonstrated that pretreatment with LA during acute phase of seizures induced by pilocarpine produces increase in SOD, catalase activities[11] and GPx in rat hippocampus. The increase in antioxidant enzymes activities, after pretreatment with LA, is most readily explained as a necessary consequence of inhibiting formation of free radicals during convulsive process[26-28].

LA plus P400 and P400 groups showed an increase in the GPx activities. These data suggests that H_2O_2, which is generated during superoxide dismutation, could be sufficiently removed by GPx during seizures and after the pretreatment with lipoic acid. Previous studies showed an increased in hippocampal GPx activity after seizures[26]. In addition, during the convulsive process, neuronal activities changes are accompanied by alterations in the cerebral metabolic rate[29]. Considering that an increased metabolic demand can be observed during the epileptic activity, we can suggest that GPx activity is modified by seizures. This finding might suggest that pretreatment with LA produces an increase in this enzymatic activity. Its compensatory mechanisms against oxidative stress observed during seizures can explain the anticonvulsant actions of LA. The seizures induced by pilocarpine are prevented by LA, suggesting a role of free radical in controlling seizures installation and propagation. In fact, we found that pretreatment with LA is able to inhibit pilocarpine-induced seizures. In addition, the present data suggest evidence that free radical formation have a relevant role in the propagation and/or maintenance of convulsive activity. Meanwhile free radical formation reduc-

es, an increase in antioxidant enzymes activities produced by LA produces a significant decrease in the susceptibility to seizures induced by pilocarpine.

LA administration to convulsive animals has been shown to protect hippocampus against oxidative stress. LA has been observed to act as antioxidants towards hydroxyl radicals and to inhibit the oxidation of lipids and protein[4,9]. Results of animal studies have demonstrated that LA can reduce damage to neurons caused by free radicals that are produced in neurodegenerative diseases.

The underlying mechanisms of brain dysfunction in seizures are poorly understood. Regarding this, it has been demonstrated that elevated free radical concentrations can be highly toxic, and that nitric oxide metabolites produced by the oxidative stress pathway such as nitrite and nitrate might contribute to this toxicity[30]. It is also known that hydroxyl radical has a synergistic effect on seizures elicited by pilocarpine.

Considering that Na^+, K^+-ATPase is decreased by free radical formation[6], lipid peroxidation[31] and that –SH groups of cell proteins are highly susceptible to oxidative stress[32], we also investigated the LA effects on inhibitory action of seizures on this enzyme activity. We verified that seizures significantly inhibited this enzymatic activity. On the other hand, we have shown in present work that LA increases this enzymatic activity during seizures in rat hippocampus[11]. These observations may explain, at least in part, the neuroprotective effects of LA against oxidative stress caused by seizures. Although the exact mechanism through which seizures inhibits Na^+, K^+-ATPase activity is yet unknown, the present findings suggest the involvement of ROS probably by oxidizing SH groups of the enzyme and/or by peroxidation of membrane lipids, in which the enzyme is embedded. In this context, it should be noted that LA acts directly as a thiol-reducing agent, as well as a scavenger of free radicals and lipid peroxidation products[33]. In turn, LA can be able to interact with cell membranes, trapping ROS and interrupting the chain of oxidative reactions that damage cells. Furthermore, there are studies in the literature showing that antioxidant compounds can effectively slow down the progression of neurodegenerative diseases[34-36].

Finally, we also evaluated the effect of LA on AChE activity in rat hippocampus. Our results show that this enzyme activity was decreased in seized rats. In order to confirm these findings, we verified the effect of a single injection of LA on AChE activity. Results show that LA alone administration did not alter this enzyme activity in rat hippocampus killed 1 h after pilocarpine administration. Moreover, a single injection of LA, 30 min before administration of pilocarpine produces increased on AChE activity. The increases in AChE may be due to the compensatory mechanism of long-term administration with LA may be due to the up-regulation of AChE activity. The results obtained by AChE activities measurements could be further supported by Western blot analysis, which did not show higher protein contents of AChE (data not show).

Although it is difficult to extrapolate our animal model data to the human condition[37-39], it is tempting to speculate that neurological symptoms observed in seizures may be related to high tissue concentrations of free radicals having an adverse effect on brain function through oxidative stress and inhibition of Na^+, K^+-ATPase and AChE activities. However, whether these or other abnormalities are the main factors responsible for the brain damage in seizures remains to be elucidated. Furthermore, future studies should be carried out to provide additional information so as to clarify the action mechanisms of lipoic acid during the establishment of seizures.

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