Alterations on monoamines concentration in rat hippocampus produced by lipoic acid

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ABSTRACT
The purposes of the present study were to verify monoamines (dopamine (DA), norepinephrine (NE), serotonin (5-HT)), and their metabolites (3,4-hydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA)) contents in rat hippocampus after lipoic acid (LA) administration. Wistar rats were treated with 0.9% saline (i.p., control group) and LA (10, 20 or 30 mg/kg, i.p., LA10, LA20 and LA30 groups, respectively). After the treatments all groups were observed for 24 h. The NE and DA levels were increased only in 20 mg/kg dose of LA in rat hippocampus. Serotonin content and in their metabolite 5-HIAA levels was decreased in same dose of LA. On the other hand, in DOPAC and HVA levels did not show any significant change. The alterations in hippocampal monoamines can be suggested as a possible of brain mechanism of action from this antioxidant. The outcome of the study may have therapeutic implications in the treatment of neurodegenerative diseases.

Key words: lipoic acid, hippocampus, dopamine, norepinephrine, serotonin.

Alterações na concentração de monoaminas no hipocampo de ratos produzidas pelo ácido lipóico

RESUMO
O objetivo do presente estudo foi verificar a concentração das monoaminas (dopamina (DA), norepinefrina (NA), serotonina (5-HT)), e seus metabólitos (ácido 3,4-hidroxifenil (DOPAC), ácido homovanilico (HVA) e 5 ácido hydroxiindolacético (5-HIAA)) no hipocampo de ratos após administração do ácido lipóico (AL). Ratos Wistar foram tratados com solução salina 0,9% (i.p., grupo controle) e AL (10, 20 ou 30 mg/kg, i.p., AL10, AL20 e AL30 grupos, respectivamente). Após os tratamentos todos os grupos foram observados durante 24 h. O conteúdo de DA no hipocampo de ratos foi aumentado apenas com AL na dose de 20 mg/ kg dose. A concentração de serotonina e do seu metabólito 5-HIAA também foi diminuída com esta dose de AL. Por outro lado, os níveis de DOPAC e de HVA não mostram nenhuma mudança significativa. As alterações na concentração das monoaminas hipocampais podem ser sugeridas como um possível mecanismo de ação cerebral deste antioxidante. O resultado do estudo pode ter implicações terapêuticas no tratamento de doenças neurodegenerativas.

Palavras-chave: ácido lipóico, hipocampo, dopamina, norepinefrina, serotonina.

Alpha-lipoic acid (LA) also known as thiocitic acid contains two thiol groups, which may be oxidized or reduced (Fig 1). As the thiol antioxidant glutathione, LA is part of a redox pair, being the oxidized partner of dihydroalpha acid (DHLA), the reduced form. Unlike glutathione reduced which only the reduced form is an antioxi-
LA possesses a modulatory role in the pathogenesis of neurodegenerative diseases. Thus, LA may possess a modulatory role in the treatment of neurodegenerative diseases, since this compound interrupts cellular oxidative processes in both its oxidized and reduced forms. The effects of LA in the central nervous system (CNS) have not yet been determined, therefore, would be important to conduct these studies to clarify its brain mechanism of action.

Dopamine (DA), serotonin (5-HT), norepinephrine (NE) and their metabolites 3,4-hydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), represent the most important monoamines and metabolites-derived neurotransmitters. DA and NE are the most abundant excitatory monoamines neurotransmitter and are widely distributed in the mammalian brain, including the hippocampus. There are some diseases associated with changes in these neurotransmitters levels. A role for the disrupted dopaminergic transmission in the etiology of chronic neurodegenerative diseases was postulated. Pharmacological studies with LA can reveal their effects in monoamines concentrations, since when established may justify the use of this antioxidant in the clinic treatment of neurodegenerative diseases.

A naturally occurring compound that is synthesized in small amounts by plants and animals, including humans. Endogenously synthesized LA is covalently bound to specific proteins, which function as cofactors for mitochondrial dehydrogenase enzyme complexes. In addition, to the physiological functions of protein-bound LA, there is an increasing scientific and medical interest in potential therapeutic uses.

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According to Packer and colleagues, an ideal therapeutic antioxidant should fulfill several criteria. These include absorption from the diet, conversion in cells and tissues into usable form, the presence of variety antioxidant actions in both membrane and aqueous phases, and low toxicity. LA is unique among natural antioxidants in its ability to fulfill all of these requirements, making it a potentially highly effective therapeutic agent for a number of conditions in which oxidative damage has been implicated. LA’s antioxidant properties consist of the following: 1) its capacity to directly scavenge reactive oxygen species (ROS); 2) its ability to regenerate endogenous antioxidants, such as glutathione reduced, vitamins E and C; and 3) its metal chelating activity, resulting in reduced ROS production.

LA has a long-established efficacy in slow-onset and long-term reduction of oxidative processes involved in the pathogenesis of neurodegenerative diseases. Although the effect was slow, drug’s cheapness, low toxicity and the ability to ameliorate other unpleasant side effects of these distressing conditions make it a practical choice. Protecting the central nervous system (CNS) against oxidative damage may be a useful therapeutic approach. Thus, LA may possess a modulatory role in the treatment of neurodegenerative diseases, since this compound interrupts cellular oxidative processes in both its oxidized and reduced forms. The effects of LA in the central nervous system (CNS) have not yet been determined, therefore, would be important to conduct these studies to clarify its brain mechanism of action.

METHOD

In each experiment, male Wistar rats weighing (250-280 g) provided by the Animal House of the Federal University of Piaui (Brazil) were used. The animals were housed in groups of 30, into plastic cages with sawdust as beddings, and kept in a room with controlled temperature (26±1°C) and a 12-h light/dark cycle, with food and water ad libitum, except during the experiments. The animals were treated in accordance to the current law and the NIH Guide for Care and Use of Laboratory Animals. The project was previously submitted to Animal’s Ethics Committee of the Federal University of Piaui.

Animals were treated with 0.9% saline (controls) and lipoic acid at the doses of 10, 20 or 30 mg/kg, i.p. (LA10, LA20 or LA30 groups, respectively). Twenty-four hours after the drug administration, the animals were sacrificed by decapitation, and the hippocampus was dissected. After dissection, the area was used for monoamines and metabolites determinations.

Analyses of monoamines and metabolites were car-
ried out with a high-performance liquid chromatography (HPLC, Shimadzu, Japan) apparatus. For all the experimental procedures, 10% (w/v) homogenates of hippocampus of the LA10 (n=7), LA20 (n=7), LA30 (n=7) and control (n=9) groups were prepared. The endogenous levels of DA, NE, 5-HT and their non conjugated metabolites DOPAC, HVA, and 5-HIAA were determined by reverse-phase high-performance liquid chromatography (HPLC) with electrochemical detection. Briefly, a C18 reverse phase column (Shim-pack, CLC-ODS 150 mm × 4.6 mm; Shimadzu, Kyoto, Japan), an amperometric detector (Shimadzu, LECD-6A) and a liquid chromatography work station were used. The hippocampus was immediately homogenized in 0.2 M perchloric acid. The homogenates 10% (w/v) were centrifuged (20,000 × g per min for 30 min). After centrifugation 20 µl of the supernatant was injected into the chromatograph. The mobile phase (pH 3.0) used at a flow rate of 0.6 ml/min was of the following composition: 15.7 g of citric acid, 471.5 ml of twice-distilled water, NaOH sufficient to bring pH to 7.0, 3.78 mg of octyl sodium sulfate, 20 ml of acetonitrile and 10 ml of tetrahydrofuran. Oxidation potential was fixed at 0.85 V using a glass carbon working versus an Ag/AgCl reference electrode. The peak areas of the external standards were used to quantify the sample peaks. The values obtained were expressed as ng/g wet tissue.

All results are presented as mean ± S.E.M of number of animals used in experiments. The results were compared using ANOVA followed by Student-Newman-Keuls test. The significance level was set at p<0.05.

RESULTS

DA levels in rat hippocampus increased by 9% after LA administration (20 mg/kg), when compared to the controls (p<0.0001). No significant changes were observed in DA levels after LA treatment (10 or 30 mg/kg, i.p.), when compared to control group (Fig 2). When dopamine level at LA20 group was compared to the LA10 group a significant increase (11%) was also found. In addition, when dopamine level at LA30 group was compared to the LA20 group a significant decrease (10%) was also found (p=0.0489). No significant changes were observed in DOPAC and HVA levels in rat hippocampus, after the administration of LA (10, 20 or 30 mg/kg i.p.) when compared to the control (Fig 2).

NE level in rat hippocampus increased by 11% after LA administration (20 mg/kg) as compared to controls (p<0.0001). At LA20 group, 5-HT level decreased by 9% (p=0.0345) and 5-HIAA level decreased by 21% (p<0.0001) after LA treatment, compared to the controls. No significant changes were observed in NE, 5-HT and 5-HIAA levels in rat hippocampus, after the administration of LA (10 or 30 mg/kg, i.p.) when compared to the control (Fig 3). When NE level at LA20 group was compared to the LA10 group a significant increase (13%) (p<0.0121) was found, instead 5-HT and 5HIAA levels decreased by 12% (p<0.0006) and 20% (p<0.0004) compared to LA10 group, respectively. In addition, when NE level at LA30 group was compared to the LA20 group a significant decrease (11%) (p<0.0049) was found, instead 5HIAA levels increased by 13% (p<0.0014) compared to LA20 group (Fig 3).

DISCUSSION

Most of the studies with LA were carried out in organs and systems other than the CNS. In the present
work, DA, NE, 5-HT and their non-conjugated metabolites DOPAC, HVA, and 5-HIAA levels were determined in rat hippocampus, after the LA administration. In the present study after the LA acute administration (20 mg/kg), DA and NE levels were increased in rat hippocampus. In a previous study, we observed that LA in same dose increased the locomotor activity, in the open field test, confirming that LA do not posses inhibitory effect and the behavior presented may be related to changes in these monoamines concentrations in the hippocampus (data not shown). Recently, Meurs and colleagues, described an increase in hippocampal DA content in the establishment of neurodegenerative diseases. DA has a modulatory effect on the dopaminergic system, in which increase of DA in the hippocampus is correlated with decreased glutamate levels. Dopamine modulates fast excitatory synaptic transmission in several brain regions. Early studies in the hippocampus revealed that DA, in particular via D2 receptors, indirectly stimulates Y2 receptor causing inhibition of glutamatergic system. These results suggest that LA probably exert their effects in CNS through of indirect stimulation of Y2 receptor, since, that produces an increase in DA levels.

The result suggests that the observed increase in dopamine level was not produced by the activation of the sparse dopaminergic projection from ventral tegmental area to the hippocampus, this increase was probably produced by inhibition of glutamatergic system by LA. Furthermore, we suggest that the increased dopamine and norepinephrine levels can be produced by three mechanisms: [1] by increasing their synthesis and/or release; [2] by elevation in their metabolization rate; and/or [3] by decreasing their activity via serotonergic receptors, as well as serotonergic receptors, is followed by the sequential intervention of several metabolic pathways mediated by pre- and post-synaptic protein kinases that also activate proteins necessary to the formation of memory. In this case, we suggest that the LA can change the concentration of these, as well as can produce effects indirect on amino acids contents in the hippocampus. Therefore, further studies should be performed to confirm the hypothesis of that LA exert effects in serotonin concentration.

During chronic mild stress leads to altered synaptic function and cellular survival in the ventral hippocampus. These impairments of the hippocampus may be compatible with altered emotionality, cognitive impairment, social isolation and disturbed memory. Acute antioxidant treatment can modified hippocampal serotonergic transmission. Serotonin is suggested to regulate aspects of the development and the plasticity of brain circuits. Serotonin and GABA show a reciprocal modulatory function in the hippocampus. This region is rich in both 5-HT and GABA terminals and receptors. Projections from serotonergic neurons of the raphe nuclei terminate on GABAergic hippocampal interneurons increasing or decreasing their activity via serotonergic receptors. On the other hand, inhibition of the GABA receptor decreases hippocampal 5-HT level, while application of GABA agonists to hippocampus decreases 5-HT release. This reciprocal modulation of 5-HT and GABA in the hippocampus appears incompatible with the simplistic hypotheses of decreased 5-HT and GABA levels in neurodegenerative diseases. Hence, the study of LA effects in hippocampus may be useful to study the respective contribution direct of serotonin and/or indirect of GABA in the treatment of CNS diseases. We suggest that the decreased in serotonin levels can be produced by three mechanisms: [1] by decreasing of their synthesis and/or release; [2] by elevation in their metabolization rate; and/or [3] by increasing its reuptake brain. However, further studies should be performed to confirm the hypothesis of that LA exert effects in serotonin concentration.

In addition, the hippocampus is responsible for some aspects of the neurobiology of memory, as the consolidation of memory. The selective activation of dopaminergic receptors, as well as serotonergic receptors, is followed by the sequential intervention of several metabolic pathways mediated by pre- and post-synaptic protein kinases that also activate proteins necessary to the formation of memory. In this case, we suggest that the LA can change the concentration of these, as well as can produce effects indirect on amino acids contents in the hippocampus. Therefore, further studies should be performed with LA, since these amino acids may interfere with various brain mechanisms, altering several neurotransmission systems and producing neurodegenerative diseases. Thus, more specific studies must be conducted in order to further clarify these questions.

This study showed, for the first time, the effects of LA on monoamines levels, in the CNS. In conclusion, our results are consistent with the hypothesis that LA stimulates the release and/or synthesis or reduces the metabolism rate of endogenous monoamines. This substance increased the dopamine and norepinephrine levels in rat hippocampus. Moreover, serotonin levels were decreased.
Together, these results are of interest, considering that some neurodegenerative diseases are related to the imbalance of these monoamines levels in the CNS.

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