RESEARCH

Oxidative stress indicators in brains of cognitive-deficient elderly rats

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ABSTRACT

The role of reactive oxygen species (ROS) in the pathogenesis of Alzheimer´s disease derives from a prolonged pro-oxidant state that induces the synthesis by immunocompetent mononuclear cells of soluble mediators in the brain of patients. The present paper focuses on determining the levels of oxidative metabolism indicators and pro-inflammatory cytokines in brain tissues of cognitive-deficient elderly rats, to evaluate the probable interaction among these variables and their implication in the neuropathology of this disease. Behavioral studies were carried out in animals to demonstrate cognitive deterioration, the biochemical and immunological indicators quantified by spectrophotometric and immunoenzymatic methods. Changes were observed in aging-associated oxidative metabolism indicators. The oxidative stress showed a changing pattern of antioxidant enzymes in the brain, where the superoxide dismutase was over-activated in all the regions studied and catalase were only over-activated in those regions where the neurodegenerative process was prominent (hipocampus and striata). The concentrations of malondialdehyde, phospholipase A_2 and Tumor necrosis factor α changed with aging, confirming ROS as cellular messengers and not only as deleterious agents. This study evidences a strong relationship between oxidative metabolism and aging-associated cognitive processes.

Keywords: Reactive oxygen species, aging, brain tissue, cognitive disorders

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RESUMEN

Indicadores de estrés oxidativo en el cerebro de ratas viejas con déficit cognitivo. Las especies reactivas de oxígeno (ERO) inducen la síntesis de mediadores solubles por las células mononucleares inmunocompetentes en el cerebro de pacientes con enfermedad de Alzheimer. Se determinaron los niveles de indicadores del metabolismo oxidativo y las citocinas proinflamatorias en el tejido cerebral de ratas viejas con déficit cognitivo, con el objetivo de evaluar la interacción probable de estas variables y su implicación en la neuropatología de la enfermedad. Se hicieron estudios conductuales en los animales para demostrar el deterioro cognitivo. La cuantificación de los indicadores bioquímicos e inmunológicos se realizaron por métodos espectrofotométricos e inmunoenzimáticos. Se observaron cambios en los indicadores del metabolismo oxidativo asociados con la edad. El estrés oxidativo mostró un patrón de cambios en la actividad de las enzimas antioxidantes en el cerebro. La superóxido dismutasa se sobreactivó en todas las regiones estudiadas, y la catalasa solo en aquellas donde el proceso neurodegenerativo es más prominente, como el hipocampo y el estriado. Existen cambios en las concentraciones de malondialdehído, fosfolipasa A2 y en el factor de necrosis tumoral α asociados con la edad, que confirman que las ERO son mensajeros celulares y no simples agentes deletéreos. Este estudio evidencia un vínculo estrecho entre el metabolismo oxidativo y los procesos cognitivos asociados con la edad.

Palabras clave: Especies reactivas de oxigeno, envejecimiento, tejido cerebral, trastornos cognitivos

*I*ntroduction

The increase in life expectancy has brought about population aging, leading to a relative rise in their related diseases, including neurodegenerative disorders such as Alzheimer´s disease (AD).

Clinical observations related to dementia show a heterogeneity of symptoms, reflecting not a single but a heterogeneous syndrome in contrast to most aging-associated neurodegenerative diseases. Additionally, they include homogeneous etiopathogenic subsets that are still hypothetical and seemingly multifactorial and polygenic [1, 3], difficulting the development of an efficacious therapy [4].

AD is neuropathologically characterized by neuronal loss in several cortical areas, mainly in the temporal lobe and the entorrinal cortex, and also in the cholinergic complex of the anterior basal brain. It has been suggested that the anatomical isolation of the hippocampus results in cognitive disorders (dementia) associated to AD [5, 6]. From the clinical point of view, it is characterized by neuropsychiatric symptoms, such as dementia, confusion, irritability, and language and spatial memory deterioration.

The brain is a highly oxygenated structure. Several factors specifically predispose this tissue to oxidative damage compared to other body tissues. It has a very poor activity of antioxidant enzymes, while showing increased concentrations of iron and other substrates that are sensitive to oxidation like poly-unsaturated fatty acids and catecholamines [7].

The reactivity of oxygen and the derived species, due to their electrophilic nature, form the basis of some toxic events in aerobic systems. In spite of this, much experimental evidence suggest that reactive oxygen species (ROS) act not only as noxious agents, but are also part of physiological mechanisms of intracellular signaling. Therefore, maintaining the balance between

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oxidant generation and consumption is essential to maintain cellular viability [8].

On the other hand, many years ago it was considered that the immune system was involved in AD pathogenesis, based on studies evidencing a chronic pro-inflammatory process in the brain of AD patients [9]. The immune system activation process induces the synthesis of soluble mediators by immunocompetent mononuclear cells, a process that takes place during the development of an autoimmune response [10, 11].

Finally, is important to notice that the ROS are part of physiological mechanisms of intracellular signaling, as in the case of the Nuclear Factor kappa B (NF-κB) and the transcription activator protein 1 (AP-1) [12-15]. These factors modify their own expression and alter their capacity to bind to the genomic elements controlled by the ROS, depending on the concentration of ROS, kinases and phosphatases subjected to oxidative modulation and probably involved in neuronal death during AD.

The present paper focuses on determining the levels of oxidative metabolism indicators and pro-inflammatory cytokines in brain tissues of cognitive-deficient elderly rats, to evaluate the probable interaction among these variables and their implication in the neuropathology of aging-associated diseases.

Materials and methods

Animals

The thirty-five male Sprague-Dawley rats that were used for the study were from the National Center for the Production of Laboratory Animals (CENPALAB, Cuba), and formed by two age groups: young, (n = 15; 2 months old) with a lifeweight of 272 ± 36 g (mean ± standard deviation, SD); and old (n = 20; 22 months old), showing cognitive deficiency, and weighing 520 ± 46 g.

General housing requirements

The animals were housed at a rate of 4 rats per cage; the cage was changed twice a week. Temperature and humidity were kept at 22-24 ºC and 60 ± 5%, respectively, with dark/light cycles every 12 hrs and water and food were supplied *ad libitum*.

Criteria

Inclusion criteria: Cognitive-deficient elderly rats (See the Behavioral studies section).

Exclusion criteria: Presence of tumoral conditions; walking disorders; body mutilations; weighing less than 520 g (old animals) or 190 g (young animals); changes in fur color. "Unsuitable" clinical criteria are expressed by veterinary inspection using routine sanitary controls, according to Clark and coworkers [16] and specifications for aged animals of Hubel and Brener [17] and the Canadian Council for Animal Care (CCAC) [18,19].

Behavioral studies

I. Passive avoidance test

A two-compartment light-dark step-through box (Electromedicina CIREN) of plastic and steel was used. The walls and ceiling of the compartments were dark and transparent, respectively, while placing a source of light (100 W) in front of the last dark compartment.

Procedure

It was carried out as described by Graham and Buccafusco [20]. The experiment included an adaptation period, followed by the test period consisting of two consecutive phases of learning and retention, with a 24 hrs interval between them.

- Adaptation: The rat was placed in the transparent compartment, behind the dark compartment (initial position), that allowed it to move freely in the cage. After 6 minutes, it was returned to the initial cage.

- Learning (phase 1): The previously described procedure was repeated 24 hrs later, also registering latency 1 (L1, time taken for it to enter the dark compartment) and the number of times it goes from one to the other compartment (C1). After 6 minutes, the communicating door was closed, leaving the rat enclosed in the dark compartment, while receiving a 75-80 Hz and 1.5 mA electrical shock for 2 s. Immediately afterwards, the rat was returned to its original cage.

- Retention (phase 2): Twenty four hours later, the phase 1 procedure was repeated, also registering L1 and C1 parameters, now called L2 and C2, respectively, for 6 minutes and without applying the electrical shock.

II. Learning and spatial memory in a Morris' Aquatic Maze (MAM)

Equipment

Stainless-steel circular pool, with a white bottom and walls, and a circular translucent escape platform submerged 1 cm under water that is invisible for the rat swimming at the surface.

Experimental conditions

No noise or other signals, or signals outside the maze were generated that could distract or confuse the animals. The researcher stood far away from the MAM and came nearer through a constant movement to avoid being used as a false reference point. Hypothermia was avoided in the animals after the assay.

Procedure

It was carried out as described by Morris *et al.* [21] and Terry [22]. Number of days of the assay: 5, with up to 37 assays, performing 8 assays per day during the first 4 days, and 5 on the last day, respectively. The animal was released in the water facing the wall, at any of the 8 cardinal points randomly selected, letting it swim for 60 s. If the rat was unable to find the submerged plat-form during that time, it was placed on the platform for 30 s, starting the next assay immediately afterwards. The following parameters were recorded:

- Trajectory

- Latency of escape per assay (LE): the time it took the animal to find the submerged platform. The longest time was 60 s.

- The number of analogous crossings at the time of assay 37. In assay 37, the platform was eliminated, and the number of times the rat crossed the area where the platform was placed was recorded.

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Cognitive deterioration criterion

The criterion followed for old animals corresponds to that described by Gage and coworkers [23]. The rat with an average total latency of more than two-fold the SD of the average total latency of the reference group of young animals during the first 36 assays in the MAM, was considered to be suffering cognitive deterioration.

Biological sampling

Following profound anesthesia and decapitation, the brains were extracted, washed with cold 0.9 % NaCl and the brain areas of interest were desiccated: septum, hippocampus, striata and the frontal cortex. All the tissue samples were frozen in liquid nitrogen, weighted and stored at -70 ºC until they were assayed.

Immunological studies

The levels of circulating Tumor Necrosis Factor α (TNF-α) were quantitatively determined in samples by a commercial ELISA kit (Kit Biosource Int, Bender Medsystem, NIBSC), which is based on a double-site immunoenzymatic method.

Biochemical analyses

Total glutathione (GSH) was determined by Tietze's enzymatic recycling assay, as described by Azbill *et al*. (1997) [24]. The chloramphenicol acetyltransferase (CAT) and superoxide dismutase (SOD) activities were determined by Aebi's [25] and Marklund's [26] methods, respectively. Lipoperoxide concen-trations were quantified as malondialdehyde (MDA) by Ohkawah's method [27], and the phospholipase A_2 enzyme activity was determined by the method described by Hotter and Radvani (1987) [28].

GSH and MDA assays and enzyme activity determinations were carried out by a continuous method, employing a spectrophotometer (Shimadzu, Kyoto, Japan) that automatically reports the slope of the group of optical density (O.D.) *vs* time.

Bioethics

International regulations for these species in chronic disease investigations [17, 29, 30] were implemented, also following standards for the use of animal models in psychological and neuroscience studies [18, 19].

Statistical analyses

Data were statistically analyzed with the Statistica software (version 4.5), Windows 98. The normal distribution and homogeneity of variance were first verified by Kolmogorov-Smirnov's and Levene's tests, respectively. A one-tail ANOVA variance analysis was applied.

Duncan's Multiple Range test was used to evaluate significant differences between groups in oxidative stress and immunological indicators. The MAM and passive avoidance behavioral studies in rats were compared by Mann-Whitney's U non-parametric test. A significance value of $p \leq 0.05$ was used in all tests.

Results

Data of MAM ($p = 0.036$) and Passive Avoidance ($p = 0.028$) behavioral studies are shown in tables 1 and 2, respectively. The behavior of the old rats was significantly different to young animals, and was used to select the working sample group.

Table 1. Average values of the mean ± standar error of the mean (SE) of total latency and the number of crossings for the MAM assay 37 in groups of young and old rats

Rats	Total latency (seconds)	Number of crossings
Young	10.15 ± 4.38	7.50 ± 2.30
Elderly	35.55 ± 1.12*	0.90 ± 1.60*

*$p < 0.05$. Statistical significance for Mann-Whitney's U test.

Table 2. Average values of the mean ± SE of the behavior of old and young rats during the Passive Avoidance test

Rats	Lat 1 (s)	# Crossings 1	Lat 2 (s)	# Crossings 2
Young	23.26 ± 8.16	11.4 ± 0.16	347.50 ± 2.13*	1.04 ± 0.06*
Elderly	34.84 ± 3.41	8.14 ± 0.36	187.35 ± 12.39*	6.62 ± 1.75*

*$p < 0.05$ *vs* Condition 1 (Lat1 and Crossings 1, respectively) for Mann Whitney's U test.

Table 3. Average values of the mean ± SE for GSH concentrations (mg/mg of protein)

Rats	Frontal cortex	Hippocampus	Striata	Septum
Young, n = 15	7.670 ± 0.028^a	5.800 ± 0.032^a	5.670 ± 0.045^a	6.750 ± 0.049^a
Elderly, n = 20	1.200 ± 0.071^b	0.211 ± 0.028^b	1.410 ± 0.028^b	0.560 ± 0.014^b

[a,b] Statistical significance for Duncan's multiple range test ($p < 0.001$).

Table 3 shows the values of GSH concentrations in the four brain areas studied, either for cognitively deficient old or young rats, respectively. A marked decrease of tripeptide content ($p < 0.001$) was observed in all the regions, especially at the hippocampus where it decreased in 96% in respect to the rest of brain areas studied.

GSH concentration at the hippocampus and septum showed a similar behavior, which was more evident in old animals.

In young rats, the highest SOD activity was observed at the septum, while having similar values in old animals, with the cortex showing the lowest values (Table 4).

The CAT activity showed a different behavior among the brain areas studied, with a heterogeneous pattern according to age. In old animals, the CAT activity decreased at the cortex and septum, augmenting at similar levels at the striata and the hippocampus, with the

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Table 4. Average values of the mean ± SE for the SOD activity (U/mg of protein).

Rats	Frontal cortex	Hippocampus	Striata	Septum
Young, n = 15	1.190 ± 0.021^a	1.290 ± 0.052^a	1.850 ± 0.023^a	2.660 ± 0.014^a
Elderly, n = 20	2.870 ± 0.007^b	3.680 ± 0.077^b	4.030 ± 0.016^b	3.840 ± 0.034^b

[a,b] Statistical significance for Duncan's multiple range test ($p < 0.01$).

Table 5. Average values of the mean ± SE for the CAT enzyme activity (KU/mg of protein)

Rats	Frontal cortex	Hippocampus	Striata	Septum
Young, n = 15	57.200 ± 0.004^a	35.000 ± 0.000^a	37.400 ± 0.002^a	80.200 ± 0.007^a
Elderly, n = 20	45.500 ± 0.007^b	66.700 ± 0.009^b	71.200 ± 0.014^b	26.600 ± 0.002^b

[a,b] Statistical significance for Duncan's multiple range test ($p < 0.01$).

lowest CAT activity detected at the septum in cognitive deficient elderly rats (Table 5).

Significant increases ($p = 0.0044$) in MDA concentrations were detected for all regions studied, while behavior varied according to the area. The highest MDA concentrations were found at the hippocampus and striata areas; the levels at the striata were 5-fold in old animals compared to young ones (Table 6). These results reflect the susceptibility of these two regions to ROS.

A relationship was found between PLA_2 activity and age, while differences between brain areas were also observed. The highest levels of activity for this enzyme were detected at the striata in both young and elderly rats (Table 7).

The specific activity of TNF-α significantly varied ($p = 0.026$) with age, increasing in all brain areas for old animals, compared to young rats where the TNF-α levels were similar in all the areas studied. The striata was where the highest activity was found (Table 8).

Table 6. Average values of the mean ± SE for MDA concentrations (nmol/mg of wet tissue)

Rats	Frontal cortex	Hippocampus	Striata	Septum
Young, n = 15	36.600 ± 0.679^a	15.160 ± 0.293^a	18.070 ± 0.587^a	12.400 ± 0.312^a
Elderly, n = 20	46.030 ± 0.007^b	75.900 ± 0.382^b	64.550 ± 0.191^b	51.380 ± 0.014^b

[a,b] Statistical significance for Duncan's multiple range test ($p < 0.01$).

Tabla 7. Average values of the mean ± SE for PLA_2 activity (U/mg of protein)

Rats	Frontal cortex	Hippocampus	Striata	Septum
Young, n = 15	0.140 ± 0.001^a	0.120 ± 0.032^a	0.180 ± 0.002^a	0.190 ± 0.005^a
Elderly, n = 20	0.260 ± 0.063^b	0.300 ± 0.004^b	0.410 ± 0.012^b	0.310 ± 0.003^b

[a,b] Statistical significance for Duncan's multiple range test ($p < 0.01$).

Table 8. Average values of the mean ± SE for TNF-α specific activity (pg/mg of protein)

Rats	Frontal cortex	Hippocampus	Striata	Septum
Young, n = 15	1.230 ± 0.039^a	0.980 ± 0.062^a	1.430 ± 0.054^a	0.760 ± 0.002^a
Elderly, n = 20	2.400 ± 0.022^b	2.060 ± 0.079^b	2.620 ± 0.007^b	1.980 ± 0.053^b

[a,b] Statistical significance for Duncan's multiple range test ($p < 0.05$).

Discussion

GSH acts as a proton donor while neutralizing H_2O_2 and organic peroxides, while directly reacting with radical species and intervening in the regeneration of other antioxidants such as tocopherol and ascorbic acid. This compound, however, not only maintains the cellular reducing potential, it is also involved in intra- and extracellular signaling in the brain, *e.g.* capture, synthesis and release of glutamic acid and Gamma-aminobutyric acid [31], substrate and allosteric modulator of eicosianoid synthesis [32, 33], regulator of NMDA and non-NMDA glutamatergic receptors [34, 35], as an excitatory neurotransmitter through its own recep-tors and as an activator of transcription factors [35].

The acute decrease in GSH concentrations, in the brain regions of all the cognitively-defective elderly rats, causes an oxidative stress in the cell that promotes incomplete oxidation of mitochondrial substrates. The subsequent escape of electrons from the normal sequence of the mitochondrial electron transport chain increases a great variety of ROS that diffuse through the mitochondrial membrane and damage other cellular structures. It is plausible to assume that they generate compensatory responses in the anti-oxidant pathways, not only in the ROS metabolic pathways enzymes but also in other antioxidant enzymes such as SOD and CAT.

It is well known that the ROS-related cellular damage does not appear until GSH reaches 50% of the tissue [37]. Even when GSH concentrations were different in the brain areas studied, there was a difference between age groups, mainly in septal and hippocampal areas in up to 96%. These results agree with previous reports [36-39]. The topographic specificity of this effect could be related to the special sensitivity of the hippocampus to low GSH concentrations, and its early damage with aging. These results agree with our behavioral studies evaluating an inhibitory modality of learning and memory (Passive Avoidance), and the MAM spatial memory assay. The behavior of old animals, with low GSH content, showed a lower profile in task acquisition or consolidation during spatial learning. Significant differences were found between the experimental groups in the capacity of animals to maintain an inhibitory behavior. Our results agree with those of other groups [40] that showed that the GSH content did not significantly affect the acquisition during the Passive Avoidance test while it does produce a specific effect on spatial learning.

In recent years, the molecular events responsible for learning and memory are being classified by identifying brain areas and neuronal circuits related to information acquisition and storage, and the recognition of plastic synaptic connections while fixing cognitive processes. The mechanisms involved in memory formation and preservation comprise the NMDA type glutaminergic receptors' activity, the stimulation of enzymes like the calcium-dependent protein kinase II and calmodulin, the activation of transcription factors and the induction of synthesis of neurotrophic factors [41].

On the other hand, most biomolecules involved in these processes are sensitive to changes in the intracellular redox state [42]. Nevertheless, there is still limited knowledge on the influence of the oxidative imbalance on cognitive processes. Nonetheless, the use of a broad spectrum of antioxidants has been proposed to achieve a balanced oxidative metabolism, expecting to obtain an improvement in brain functioning that could lead to a significant decrease in alterations in understanding and memory during aging [42].

The decreased intracellular GSH could also be a part of the excitotoxic mechanism resulting from energy failure, because the transitory insufficiency of GSH increases the oxidant potential in the medium and diminishes NMDA receptor conductivity. This leads to a cascade of neurodegenerative unleashing events that ultimately affect synapse efficiency and cognitive function, resulting in neuronal atrophy and death. It is also partially derived from the generation of ROS beyond the homeostatic capacity of the tissues.

A significant increase in antioxidant enzymes levels were found for SOD in cognitively-deficient elderly animals, demonstrating a similar behavior for all areas studied in the brains of young and old rats. The CAT

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activity showed a similar behavior in all areas studied, with a heterogeneous pattern according to age, diminishing in the cortex and septum, increasing in the hippocampus and remarkably in the striata.

As mentioned above, studies on the effects of brain aging on antioxidant enzymes have shown no consistent results. Experimental differences in species, varieties, sex, age and brain areas among other factors, have contributed to these results. Our results agree with those reported by Cirolo and coworkers [43], in the age-associated increase in the hippocampal SOD activity. Increases of this enzyme activity with age, as well as of other antioxidant enzymes such as glutathione transferase, have been found in the liver of rodents. The oxidative damage on proteins and lipids rise with age, with the subsequent spikes in SOD and CAT activities as a compensating intracellular mechanism to counteract ROS, leading to a reduction on H_2O_2, lipoperoxides and other electrophylic compounds such as aldehides and quinones contents, which corresponds to their induction by ROS [44].

It is noteworthy that, the SOD became highly activated in all brain areas studied, with the most intense activity in the hippocampus and striata, while CAT activity only increased in these two regions. CAT is involved in recycling the cellular H_2O_2; the enzyme being characterized by a high reaction capacity and a low substrate affinity, requiring high concentrations for an optimal activity. The joint effect of both enzymes is required to maintain cellular oxidative balance. The increase of SOD without the concurrent CAT activity would accumulate H_2O_2 and its inefficient recycling would generate other reactive species that would degenerate the cell by oxidative stress.

During aging, an increased monoamine oxidase B (MAO-B) activity has been observed at the hippocampus [45], with H_2O_2 being generated as a by product of this reaction. Also, dopamine metabolism itself makes striata dopaminergic neurons more sensitive to oxidative stress, since dopamine is enzymatically metabolized by MAO, generating H_2O_2, with the simultaneous self-oxidation of dopamine generating semiquinones that are highly toxic to dopaminergic neurons. Floyd [46] and Pellmar [47] reported the formation at high levels of the hydroxyl radical related to the high concentration of iron in these two areas.

The increased CAT activity at the hippocampus and striata suggests that H_2O_2 levels reach the threshold required for activation, which did not occur in the septum and frontal cortex, where the action of the glutathione oxidase was able to eliminate H_2O_2 generated by the SOD. An alternative explanation is the progressive accumulation of ROS and electrophilic compounds in these regions, which is required to trigger CAT by ROS, while knowing that the striatal CAT is induced in glutamatergic hyper-stimulating conditions associated to excitotoxicity.

Finally, it is important to stress that the diminished CAT enzyme activity in the cortex and septum areas could be due to the regulatory inhibition of enzyme expression and/or activity. A large amount of experimental evidence supports the concept that oxidative damage resides in self-perpetuating mechanisms, lasting more than the trigger event; it is probable that the compensating over-expression of this enzyme would not be accompanied by its increased enzyme activity, due to the oxidative post-translational damage of the protein. Based on the role of CAT in oxidative metabolism, its inhibition exacerbates intracellular oxidative damage.

Altogether, these findings denote increased enzyme activity with specificity for each brain area, in which the SOD becomes over-activated in all the areas examined, while the CAT is only activated in areas where the neurodegenerative processes is more prominent. This pattern suggests levels of sensitivity for the activation induced by the oxidative damage for each enzyme, the lowest threshold being that of SOD.

MDA concentration is a direct indicator of ROS cellular damage, and more precisely the hydroxyl radical. We found statistically significant increases in MDA concentration with age in all brain areas studied and a different behavior according to the specific area, where high MDA concentrations were observed in the hippocampus and striata. These results agree with the increase in antioxidant enzymes activity and decrease in GSH concentration.

The PLA_2 has a key role in membrane turnover, exocytosis and oxidative damage repair [48]. Its reaction products (free fatty acids and lisophosphoglycerides) can act as intracellular signaling molecules or they can be modified into biologically active compounds as the platelet activating factor, prostaglandins and leukotrienes [49]. The central nervous system expresses a significant PLA_2 activity, and it is also rich in arachidonic acid-enriched phospholipids. There are reports on the high concentration of arachidonic acid and its metabolites in processes such as brain ischemia, hypoglycemia and inflammation [50]. However, a markedly decreased PLA_2 activity has been found in brain areas of autoimmune encephalitis patients [51].

The increased activity found for this enzyme in our study during aging could be related to its membrane turnover and oxidative damage repairing activity, eliminating peroxidated fatty acids, although its role in signal transduction through the synthesis of signaling molecules like arachidonic acid and diacylglycerol would not be underestimated. This activity is distinctive of acute events like schemia-reperfusion, hypoxia and inflammation.

Lipid peroxidation products are involved in inflammation, producing changes in vascular permeability, edema formation, changes in ionic channels and alterations in the function of membrane receptors. Therefore, membrane potential is altered and membrane permeability to certain ions such as calcium increases. At the same time, it activates PLA_2 with the subsequent release of arachidonic acid from membrane phospholipids.

The capacity of these species to oxidize GSH, the increase in SOD activity without changing CAT activity and the high concentration of MDA, and high PLA_2 in all areas studied could be involved in decreasing GSH content in brains of elderly animals.

Cytokines produce an oxidative stress response, and therefore the signals induced by them, in the role of first messengers, involve the generation of ROS. Cytokines activate transcription factors, such as NF-κB, also inducing apoptosis [52].

TNF-α seems to play an essential role during the development of several pathological processes in the

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CNS, including neuronal degeneration, demyelination and gliosis, also acting as a direct destroyer of myelin or oligodendrocytes, contributing to demyelinating lesions. The activity of TNF-α and interleukin 1β also generates large amounts of the superoxide anion. Here we observed high levels of TNF-α, related to aging in all brain areas, its activation increasing concentrations of H_2O_2 and radical hydroxyl, partially in agreement with the induction of CAT activity in the hyppocampal and striata areas studied in elderly rats with cognitive deficiencies.

Conclusions

1. There are changes in oxidative metabolism indicators that are associated to aging in response to oxidative stress in rodents with cognitive deficiencies, in which oxidative stress induces a pattern of changes in the activity of antioxidant enzymes in the brain. The SOD became over-activated in all the areas studied, while the CAT was activated in areas where the neurodegenerative process is more prominent, such as the hippocampus and striata.

2. There are aging-associated changes in MDA, PLA_2 and TNF-α concentrations, confirming the role of ROS as cellular messengers and not only as simple deleterious agents.

3. The present study evidences a narrow relationship between the oxidative stress and the aging-associated cognitive process, in which the mechanisms to maintain the oxidative homoeostasis of the brain could simultaneously act as modulators of cognitive functions and as targets of neurodegenerative events.

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