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**PHD. THESIS ABSTRACT**

**Researches regarding the biochemical bases of the relationships between oxidative and nitrosative stress**

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In the recent days, particular attention is given to the relations between cellular and molecular alterations, caused by oxygen free radicals and normal or pathological implications of the nitric oxide and nitrosative stress. Thus, given the lack of studies in this research area, both nationally and internationally, in this paper we try to describe the latest knowledge on the reactive species of oxygen and nitric acid, as well as their main effects upon body level, organizing the most recent data of the specialty literature upon stress as a biomedical and biochemical notion, but also the original research on the oxidative, nitrosative stress, and, in particular, the interactions that appear between these processes (the so called stress radicalar cascade).

Thus, in this work, we focused on studying some changes of the specific enzymatic systems, as well as of some reactive species of oxygen and nitric oxide, in conditions of oxidative and nitrosative stress, opting for experimental situations that included determining the level of oxidative and nitrosative stress (and more important, the interactions that appear between these) in case of specific behavioral tests (cognitive tests – particularly for determining the memorization processes, the motor activity, the affective-emotional and pain activity in rats), after administering angiotensin II (Ang II) and some of its specific agonists / antagonists (taking also into consideration the tradition of our laboratory in studying angiotensinic peptides, initiated nationally and even internationally by Haulic Academy, since the beginning of 1960), as well as the effect that the rat induced-contention stress has got upon the main markers of oxidative and nitrosative stress, and the interactions that appear between them.

Regarding the angiotensinic peptides, taking into consideration the fact that, lately the specialty literature mentions more often some antagonistic effects of the peptide called Ang (1-7) upon Ang II, as well as the lack of studies on this aspect, we have decided to study the effects that Ang (1-7) administration has got upon the above mentioned behavioral tests and the way in which these are expressed within the interactions between the oxidative and nitrosative stress.

Thus, the obtained results showed that Ang II exerts important behavioral influences – expressed by short term memory diminishment, motor activity decrease (Y maze), long term memory diminishment (passive avoidance test), anxiety and affective-emotional states increase (plus elevated maze) and antinociceptive effects (hot plaque test). Thus, the Y maze results showed a significant decrease of the spontaneous alternation percentage in rats treated with Ang II, compared to the control group, which suggests a short term spatial memory diminishment. On the other hand, administering different Ang II antagonists (specific blockers of receptors AT 1 – losartan and AT 2 - PD-123177 or captopril – blocker of the angiotensin converting enzyme) generated a significant increase of the spontaneous alternation percentage compared to the control group.

We also report here a decrease of the motor activity when Ang II is administered, as it can be seen after the significant decrease of the number of entries in the arms of the Y maze.

Furthermore, the data obtained in the passive avoidance test confirmed an amnesic effect of Ang II administration, considering the significant decrease of the latency time in the test retention, compared to the control group. This aspect was also confirmed by the fact that the central blocking of the AT 1 receptors, by administering losartan, resulted in a significant increase of the latency time.

It was observed that Ang II administration generated an increase of the anxiety states, as it is observed from the significant decrease of the time spent in the open arms of the elevated plus maze, compared to the control group. We also report here a decrease of the anxiety in rats treated with losartan and captopril, considering the significant increase of the amount of time they spent in the open arms of the elevated plus maze.

Ang II administration determined antinociceptive effects, considering the increase of the latency time in the hot plaque test, compared to the control group. On the other hand, its agonist administration generated reverse effects, manifested by significant decrease of the latency time for captopril, losartan and PD-123177.

Regarding the oxidative stress status, about the first determined anti-oxidant enzyme, superoxide dismutase (SOD), we could notice that Ang II administration resulted in a significant decrease of the SOD specific activity in the temporal lobe, compared to the control group, suggesting pro-oxidant effects. On the other hand, administering specific antagonists of Ang II determined a SOD significant decrease, especially for captopril.

Regarding the second antioxidant enzyme we managed to analyze in this study, glutathione peroxidase (GPX), we could notice a significant decrease of the specific activity in the temporal lobe in case of Ang II administration, compared to the control rats. In this case, the captopril administration statistically generated a significant increase of GPX specific activity, also.

Furthermore, a significant increase of the malondialdehyde (MDA) levels was noticed in the temporal lobe in rats treated with Ang II, compared to the control group. These results were complemented by a decrease of the lipid peroxidation level in the case of the animals to which captopril and PD-123177 were administered.

Regarding the nitrosative stress, there was a significant increase of the nitrite anion quantity in animals treated with Ang II, compared to the control group, which suggests the apparition of the nitrosative stress at the level of the temporal lobe of these animals. A significant decrease of the NO2 quantities was as also noticed in groups of rats that received captopril and losartan.

Important to be mentioned is the fact that the effects of Ang II upon the mentioned behavioral indices also effect the level of the oxidative stress markers (SOD, GPX and MDA), being a strong correlation between the majority of the mentioned behavioral indices and the oxidative stress at the level of the temporal lobe. There is a strong connection between the behavioral indices and the level of the nistrosative stress markers, represented here by the nitrite anion, after administering Ang II.

A special relevance for the main research theme of this paper has got the correlation between how the dynamics of the oxidative and nitrosative stress markers correlates statistically, after administering Ang II.

Statistically significant correlations were identified between the indices of the oxidative and nitrosative stress when the rats were subjected to contention stress.

Furthermore, Ang (1-7) has got important behavioral influences, most of them with an antagonistic expression against the Ang II effects and expressed at short term memory level, motor activity (Y maze), long term memory (maze with 8 radial arms), affective-emotional states (plus elevated maze) and nociceptive sensitivity (hot plaque test). Thus, about the effects that Ang (1-7) administration had upon the short term spatial memory, studied in Y maze, a significant increase of the spontaneous alternation percentage was noticed, compared to the control group. Thus, these results suggest a facilitating effect of Ang (1-7) in case of short term spatial memory, studied within Y maze.

Regarding the motor activity in rats treated with Ang (1-7), studied in the Y maze by the number of entries of the rats in the arms of the maze, there were no significant differences between the Ang 1-7) injected group and the control one. Regarding the number of the work memory within the 8 radial arm maze, during the 7 day experiment, their significant decrease can be seen, starting from the 2-nd experimental day. This aspect suggests a positive effect of Ang (1-7) administration upon the short term memory, studied in the radial maze. A significant decrease of the numbers of errors of the reference memory after administering Ang (1-7), compared to the control group, suggested facilitating effects upon the long term period, too. These positive effects upon the reference memory were obvious during the whole experiment, except for the 4-th testing day.

About the anxiety states and the results of the elevated plus maze, a significant increase of the time the rats spent in the open arms was noticed, expressed in percentages, after administering Ang (1-7), compared to the operated control group. These results suggested a possible anxiolitic effect of the Ang (1-7) administration. Moreover, regarding the nociceptive sensitivity, a significant decrease of the latency time was noticed, expressed in seconds, during the hot plaque test, in rats treated with Ang (1-7), when compared to the operated control group.

Regarding the oxidative status, there were no significant differences between the groups treated with Ang (1-7) and the operated control group, about the SOD specific activity. On the other hand, Ang (1-7) administration determined a significant increase of the specific activity in case of GPX, the second determined antioxidant enzyme, compared to the control group, suggesting the damages of the antioxidant effects.

The possible antioxidant effect of Ang (1-7) was also demonstrated, when the level of lipid peroxidation was determined, expressed through MDA concentration at the level of the temporal lobe. Thus, a significant decrease of temporal MDA concentration when administering Ang (1-7) was observed, compared to the operated control group.

Regarding the nitrosative stress, a significant decrease of the anion nitrite concentration in the temporal lobe was noticed, as main marker of the nitrosative stress, in the rat group to which Ang (1-7) was administered, compared to the operated control group. Moreover, the behavioral influences of Ang (1-7) affect the oxidative stress markers level (SOD,GPX and MDA), a significant correlation existing between the majority of the above mentioned indices and the oxidative stress at the level of the temporal lobe. There is also a strong relation between the behavioral indices and the level of the nitrosative stress markers, represented here by the nitrite anion, after administering Ang (1-7).

A very important relevance for the main research theme of this paper is also the correlation between the way in which the dynamics of the oxidative and nitrosative stress markers correlate statistically, after administering Ang (1-7). Thus, our results sustain the theory about the existence of a real radicalar cascade that would be made between the oxygen and nitrogen reactive species as well as the interactions between them.

Regarding the future research prospective as a result of the studies presented in this paper, our group aims particularly to the more advanced study of the relevance the central renin-angiotensin system has got and the influences they exert upon the oxidative and/or nitrosative stress, during some different neuropsychiatric deficiencies, by using some specific test-animals for each disease, like Alzheimer type dementia, Parkinson’s disease or deficiencies like anxiety and depression.

As noted throughout this paper, besides the originality of the main study subject (interactions between the oxidative and nitrosative stress) which was the subject of only a couple of international studies, other essential original aspects were represented by demonstrating some anxiolitic effects of Ang (1-7) for the first time in the specialty literature, as well as clarifying some amnesic effects of Ang II, the main peptide of the renin-angiotensin system.