INTERACTIONS BETWEEN THE OXIDATIVE AND NITROSATIVE STRESS IN NOCICEPTIVE PROCESSING IN RAT

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THE INTERACTION BETWEEN OXIDATIVE AND NITROSATIVE STRESS IN NOCICEPTIVE PROCESSING IN RAT (Abstract): During the last years, special emphasis was directed towards the relation between the molecular and cellular alterations produced by free oxygen radicals and the normal and pathologic implications of nitric oxide. Moreover, lately it is believed that a true radical cascade might exist between the reactive species of oxygen and nitrogen during stress of various causes. In this way, their succession would produce a temporal prolongation of the complex cellular alterations produced by stressing aggressions.

In the present paper, we were interested in presenting some selective aspects regarding the interactions between oxidative and nitrosative stress in the modulation of the nociceptive behavior in rats. We report here a significant correlation between the results of the hot-plate latency time and the values of some oxidative and nitrosative stress markers. **Key words: OXIDATIVE STRESS, NITROSATIVE STRESS, NOCICEPTION**

Researches carried on in the last two decades with more and more advanced technical means, starting from the level of the entire organism continuing to a cellular and molecular level, have established that during the exaggerated oxido-reductive energogenic reactions there are made up variable quantities of free radicals of oxygen, generating multiple biochemical alterations, generically called oxidative stress (1).

In normal conditions of rest, the molecular oxygen, as a strongly oxidant element which is indispensable to the cellular energetic metabolism, suffers processes or bivalent reduction by the capture of two electrons with the participations of the mitochondrial respiratory chain, represented by the system cytochromes - cytochrome oxidase. Only small quantities (of 2-3%) of the molecular O₂ reaching the cellular level “escapes” from the bivalent mitochondrial oxidative-reductive reactions and take part to the process of univalent partial reduction by a single electron for making up the superoxide anion (O₂●⁻), as a precursor of the radical reactions producing hydrogen peroxide (H₂O₂ – oxygenated water), hydroxyl radicals (HO●) and singlet oxygen (1O₂).
The intensity of the univalent reduction making new reactive species of O₂ depends both on the activation of pro-oxidant enzymes of the type of NADPH-oxidase, xanthine oxidase and myeloperoxidase, and on the participation of the means of endogenous anti-radical counteraction and defense, represented by the antioxidant enzymes – superoxide dismutase (SOD), catalase and glutathione-peroxidase (GPX) (2, 3).

Being much more reactive than the molecular O₂ reduced on bivalent way, the radical species of the oxygen determine the holding or the dislocation of some molecular components of lipids, proteins and cellular nucleoproteins (DNA, RNA), producing multiple tissue injuries, mainly cardiovascular and cerebral ones (4-6).

Disequilibria created by the oxidative stress by the predominance of the radical species of O₂ on the activity of antioxidant enzymes contribute on functional plan both to the modulation of some physiological reactions and to the genesis and evolution of different pathologic conditions.

One of these is represented by pain. Pain as complex psycho-sensorial cerebral-spinal phenomenon, having as a basis the integration and modulation of nociceptive information existent both at the level of the posterior cusp of the spinal marrow and at the level of the spinal-thalamic-cortical supraiacent structures, is one of the most common cause of human suffering generating stress. Concomitantly with the specific sensorial, perceptual, affective and behavioral manifestations, pain releases non-specific neuro-endocrino-metabolic disequilibria, similar to the other types of stressing aggressions inducing the oxidative and nitrosative stress (7).

Generally, the variable reactivity to pain and stress is based both on the multiple individual features of the cerebral chemical mediators, of the type of opioid neuropetides (endorphins, enkephalins, dinorphins), and on the genic substratum at the level of the cortical-subcortical polyneural matrix, recently described under the denomination of „Body-self neuro-matrix” (8).

Depending on the genetic substratum and on the previous experience, the central cortical-subcortical structures evaluate and release individual reactions of different intensities and durations, depending on the constitutional type, on the features of stressing aggression and on the capacity of adaptation of the subject.

Furthermore, recently, more and more studies remind of the implications of the oxidative and nitrosative stress in the nociceptive processes. As a matter of fact, in the last years, a special attention is given to the relations between the cellular and molecular alterations produced by the free radicals of O₂ and the normal or pathological implications of the nitric oxide (NO). This results from the oxidation of the terminal azoth of the amino-acid L-arginin on the shunted course of the cycle of urea, under the influence of one of the three isoenzymes of nitrooxide sintetase (constitutive NOS calcium dependent), which is present in almost all the tissues and organs (9).

On a functional plan, it was proved that NO, as the free radicals of O₂, possesses dual biological properties, depending on concentration and on the individual reactivity. In small physiological concentrations, NO contributes to fix the local vasodilatation, the cardiovascular and cerebral circulation, to platelet aggregation and to anti-proliferative and protective action against bacterial and viral infections. The multiple biological properties of NO are achieved both by the mechanism of activating the
guanilatcyclase forming the cyclical GMP, as an intracellular main secondary messenger, and by nitrosation of proteins forming oxidant nitrosotiolis of some amino-acids (cysteine, methionine, glutathione, tyrosine) (10).

In this context, in the present study we have intended to describe the possible relations that establish between the oxidative stress and the nitrosative one in the modulation of the nociceptive processes. These aspects shall be proved by the use of a model of induced pain and by the administration of angiotensin II, of which was initially proved to detain a hyperalgesic role, as well as the use of an experimental model of stress by contention in rats, followed by the determination of some markers of the nitrosative/oxidative stress and the determination of correlations that occur between these markers and the indices of the painful perception.

MATERIAL AND METHODS

Experiments were carried on lots of male Wistar rats, with an average weight of 230-250 g, kept in normal laboratory conditions (constant temperature of 21°C ± 2°C, food and water ad libitum, alternating lighting regime – 12 hours light – 12 ore dark). The experimental techniques that were used have been in compliance with the international regulations regarding the work on laboratory animals.

Techniques of Neurosurgery

For the purpose of the stereotaxic intervention, Wistar rats were anesthetized, by intraperitoneal injection (i.p.) with a solution of Nembutal (45 mg/ kg b.w.) (Sigma). The heads of animals were fixed in the stereotaxic device so that the plan that passes through the auditory channels and the back of incisors to form an angle of 11°. Angiotensin II has been administered (3 consecutive days, 0.1μg/kg b.w.) by a plastic cannula (Portex, with the diameter of 0.44), stereotaxically implanted in the left ventricle, with the following coordinates: 0.5 mm posterior to the bregma; 1.3 mm laterally to the median line; 4.3 mm ventrally of the surface of the cortex.

The false-operated animals have been injected with physiological serum, the behavioral testing starting 7 days after the treatment.

Hot-plate test

The investigation of painful sensitivity was achieved by means of the hot-plate test. For this test, rats were placed in a closed cylindrical space, provided with an electrically hot plate and maintained constant at the temperature of 55ºC with the aid of an ultrathermostat. The hot plate produces two behaviors and their times of reaction can be measured: paw licking and the bounce.

Stress of contention

Stress of contention consists in the immobilization of the animal for 30 minutes. In this manner, it was considered that the stress of contention is one of the most repeatable experimental protocols, with the possibility of minimizing the implied variables and which in the same time presents enough similarities with the other types of nociceptive stress.

Determination of the markers of the oxidative and nitrosative stress was carried on as described above (6, 7). As the statistical analysis of data is concerned, it was used ANOVA one-way (the values of p ≤ 0.05 have been considered significant) for the results of the behavioral tests and the Pearson correlations in order to establish the relations between the markers of the oxidative/nitrosative stress and the
behavioral indices.

RESULTS
As mentioned above, the administration of angiotensin II has determined a significant decrease (F (1.8)=13, p=0.006) of the time of latency for the hot-plate test, related to the witness lot (fig. 1), fact that suggests the nociceptive effects of angiotensin II.

![Fig. 1. The effects of administrating Ang. II on the time of latency of hot-plate. The results are expressed by average ± E.S.M. (n=5). *p=0.006 compared to the control.](image)

Furthermore, we have noticed a significant correlation between these effects and the levels of some different markers of the oxidative stress that were previously determined (11). In this way, as the relation between the time of latency of hot-plate vs. the specific activity of superoxide dismutase (SOD) are concerned, the Pearson correlations have shown an r = - 0.798 and a p of 0.006, for n= 10 (fig. 2). In the same manner, in the case of the second determined antioxidant enzyme have been noticed significant correlations with the time of latency in the hot-plate (n=10, r= -0.541, p=0.045) (fig. 3). Moreover, we have obtained significant correlations between the level of lipidic peroxidation (shown by the concentrations of MDA) vs. the time of latency (n=10, r=0.857, p=0.002) (fig. 4).

As the level of markers of the nitrosative stress is concerned, it was opted for the determination of the nitrite anion (NO$_2^-$), considering that this is the main mediator in the reaction of nitrosylation of the tyrosine amino-acids in the structure of different proteins, this reaction representing the foundation of the phenomenon of nitrosative stress. In this case was also noticed an important correlation with the behavioral processes, considering the decreased level of NO$_2^-$ after those 30 minutes of stress of contention (F(1,8)=60, p<0.0001), compared to the witnesses (fig. 5).
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Fig. 2. The degree of correlation between the time of latency in the hot-plate and SOD.

Fig. 3. The degree of correlation between the time of latency in the hot-plate and GPX.

Fig. 4. The degree of correlation between the time of latency in the hot-plate and MDA.
DISCUSSION

In the present study we have confirmed the existence of some relations between the oxidative stress and the nitrosative one in the modulation of the nociceptive processes, by the use of a model of pain induced by the administration of angiotensin II, as well as by the use of an experimental model of stress by contention in rats, followed by the determination of some markers of the nitrosative/oxidative stress and proving the correlations that occur between these markers and the indices of the painful perception.

In this manner, previous studies have proved the involvement of angiotensin II in the modulation of the nociceptive perception. Therefore, Kaneko and collaborators have proved that the intracerebroventricular administration of angiotensin II has led to the diminishment of the analgesia induced by the initial administration of morphine (12). Also, it was proved that the administration of some inhibitors of the conversion enzyme, as well as of some antagonists specific for angiotensin II lead to the diminishment of nociceptive responses in specific tests such as the hot-plate test (13). Similarly, in the case of the present paper we have proved that the administration of angiotensin II determines a significant decrease of the time of latency for the hot-plate test, compared to the witness lot, fact that suggests its nociceptive effects.

Moreover, in the present article we have reported a direct correlation between the above-mentioned effects in the behavioral tests of pain (hot-plate) and the markers of the oxidative stress, represented by two enzymes with antioxidant effect (SOD and GPX), as well as of a marker of the processes of lipidic peroxidation (MDA).

Also, there was presented a possible relation between an important marker of the stress (NO$_2^-$) and the stress of contention (it was considered that the stress of contention is one of the most repeatable experimental protocols, with the possibility of minimizing the implied variables and which in the same time presents enough similarities with the other types of nociceptive stress). Further-
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more, nitrite anions are the main mediators in the reaction of nytrosilation of the tyrosine amino-acids in the structure of different proteins. This reaction represents the basics of the phenomenon of nitrosative stress and as a result, the variations of concentration of these radicals are direct witnesses of the nitrosative stress. Therefore, from a statistical point of view, there was a significant increase of the quantity of nitrite anions, fact that is an incontestable proof in the favor of the occurrence of the nitrosative stress at the level of the nervous system of animals subjected to the stress of contention.

Therefore, the nociceptive stress produces modifications at the level of the central nervous system, of oxidative and nitrosative type, modifications that can variably affect the capacity of transmission and perception of nociceptive signals by the animal of experience. Moreover, these data seem to suggest the fact that the intraneural and/or extraneural effects of modification of proteins involved in the phenomena of transduction of the painful signal are truly due to the effects of the presence of mediators of the nitrosative stress, which are in increased quantities.

As a matter of fact, it is considered that a real radical cascade would produce between the reactive species of the oxygen, azoth and newly of the carbon during the stress having different causes (14, 15). Their succession would realize amplification and implicitly the prolongation in time of the complex cellular disorders produced by the stressing aggressions, such as those related to nociception. Also, on a pharmacotherapeutic plan there occur differences between the three categories of radical species involved in stress: while the oxidative component of the stress benefits of antioxidant therapy based on the high consumption of vitamins C, A and E, betacarotene, flavonoids, selenium, polyphenols, anthocians and procyanidins, fighting the stress caused by pain situates to the fore the adequate analgesic, anxiolitic and hormonal medication with glucocorticoids or non-steroidal inhibitors of the cyclooxygenases of the type of indomethacin, diclophenac, ketoprophen or celebrex in the case of the inflammatory pain (16-18). Moreover, new therapeutic opportunities open the clinical-experimental researches referring to the neurosedative and anti-stressing properties of melatonin or inhibitors of receptors of the hypothalamic CRF (Corticotropin Releasing Factor) and the rennin-angiotensin cerebral system (19, 20), discussed in the present paper.

Although these studies show the involvement of the free radicals in different nociceptive mechanisms, much more detailed studies are necessary for the independent replication of these results and the use of the proper antioxidants as a treatment, having in regard the complexity of the biochemical systems and numerous interactions between the antioxidant and the prooxidant molecules from the biological processes.

CONCLUSIONS

In this study we have confirmed the existence of some relations between the oxidative stress and the nitrosative one in the modulation of the nociceptive processes, by the use of a model of pain induced by the administration of angiotensin II, as well as by the use of an experimental model of stress by contention in rats, followed by the determination of some markers of the nitrosative/oxidative stress and proving the correlations that occur between these markers and the indices of the painful per-
ception. These aspects could have a special importance in the therapy of different mechanisms of nociception.

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REFERENCES