

Nitric Oxide (no) and Alpha-Tubulin Nitrotyrosination: A Possible Role in the Pathogenesis of Depression? Proposal for Concomitant Voltammetric and Molecular Biology Study

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Abbreviations: NO: Nitric Oxide; TTCP: Tubulin Tyrosine Carboxypeptidase; TTL: Tubulin Tyrosine Ligase

ABSTRACT

Nitric Oxide (NO) is a free radical involved in diverse cellular functions, it also mediates cellular damage through direct reaction with cell proteins or through generation of a number of chemical products such as peroxynitrite ONOO- A number of works suggest a role of NO in psychiatric illness such as schizophrenia and anxiety disorders and that SSRI such as paroxetine act as NOS inhibitor. Our hypothesis is that the increase in nNOS observed after stressful conditions may be followed by intracellular NO increase. This increase could lead to a major production of ONOO- and then to the α -tubulin nitrotyrosination which is irreversible and result in the block of the tyrosination/detyrosination cycle and to the microtubular dysfunction that may be responsible for the observed neuronal plasticity failure (atrophy, cell death and neurogenesis decrease) in response to stress and depression. The present Research Proposal aims to verify such hypothesis that could lead to potential new therapeutic indication for SSRI i.e. the modulation of NOS activities and therefore modulation of NO levels within abnormal conditions in which the increase of NO activity would influence negatively cell functions in particular at the level of cytoskeleton.

Mini Review

Nitric Oxide (NO)

Nitric Oxide (NO) is a free radical produced from L-arginine in a reaction catalyzed by a set of related enzymes known as NO synthetases (NOS). NO is involved in diverse cellular functions, such as long-term potentiation, macrophage cytotoxicity, vasodilatation, and neurotransmission. This free radical also mediates cellular damage through direct reaction with cell proteins or through generation of several chemical products such as peroxynitrite (ONOO-), which mediates much of NO's cytotoxicity. One of the products of NO-derived reactions is nitro tyrosine, which is elevated in a number of pathological conditions [1,2].

Tubulin and Nitrotyrosination

Microtubules are a candidate for nitro tyrosine-mediate protein dysfunction. It is well known that microtubules are spatially

and functionally associated with a wide range of eukaryotic cell activities such as cell division, motility and transport, maintenance of cell shape and signal trasduction. Tubulin, the building block of microtubules, is a heterodimer composed of α - and β - Tubulin. α -Tubulin is subject to the post-traslational C-terminal removal and subsequent addition of tyrosine in reactions catalyzed by two enzymes: tubulin tyrosine carboxypeptidase (TTCP) and Tubulin tyrosine ligase (TTL). Recently it has been demonstrated that the modified amino acid Nitro tyrosine is post-translationally incorporated onto α -Tubulin, in several cell types, leading to cell dysfunction and apoptosis [3,4].

NO, Stress and Depression

Up-regulation of the neuronal NOS (nNOS) expression has been shown in response to stressful conditions in various brain areas following stressful conditions such as restraint [5-7]. In addition, a

number of works suggest a role of NO in psychiatric illness such as schizophrenia and anxiety disorders [8-10]. [11] have reported for the first time that an SSRI (Paroxetine) is a NOS inhibitor *in vivo* and *in vitro*. Accordingly, we have observed that the SSRI fluoxetine could reduce NO levels when recorded *in vivo* in the striatum of anaesthetised rats [12].

Tubulin and Depression

We have previously demonstrated that in rat submitted to an animal depression model (Forced Swimming Test [FST]) the brain tyrosinated α -Tubulin expression and 5-HT levels significantly decrease ($p < 0.01$) about 20% and 40% respectively [13]. We have also demonstrated using acute and sub-chronic restraint stress as well as various pharmacological treatments that parallel variation of the activity of these two systems is directly correlated [13-15]. Indeed, our results suggest that the 5-HT system may exert a role of modulator on the microtubular network dynamics that in the case of depression may lead to an observed consequent reduction of the neuronal plasticity events [16,17]. Additionally, the observation that NOS inhibitors such as SSRI (i.e. fluoxetine) influence either cerebral serotonergic as well as NO levels [12] may lead to consider the presence of a complex interaction between these three systems and therefore to consider that inadequate production NO may also be implicated in stress and depression.

Hypothesis

In particular our hypothesis is that the increase in nNOS observed after stressful conditions [5-7] may be followed by intracellular NO increase. This increase could lead to a major production of ONOO- and consequently to the α -tubulin nitro tyrosination. The nitro tyrosination of α -tubulin is irreversible and result in the block of the tyrosination/detyrosination cycle [3,18]. This microtubular dysfunction may be responsible for the observed neuronal plasticity failure (atrophy, cell death and neurogenesis decrease) in response to stress and depression [16,17]. Among the various stress-related brain areas the hippocampus seems to be the most vulnerable to stressful conditions. In particular a chronic restraint stress cause atrophy of apical dendrites in CA3 pyramidal neurons of the hippocampus [19,20]. It is important to underline that the antibody specific for the tyrosinated α -tubulin used in our works (clone TUB-1A2) is incapable to bind the nitrotyrosinated α -tubulin [3]. These findings can suggest that the observed decrease of tyrosinated α -tubulin expression subsequent to FST may be more related to the nitrotyrosination of the protein than to a reduced amount of tyrosinated α -tubulin. In order to scrutiny our hypothesis 2 experiments could be done:

Experiment 1: α -Tubulin Tyrosination and Nitrotyrosination expression in rat brain areas after acute and chronic restraint stress

Rats (200-250g) will be submitted to acute (one day) or chronic (seven days) daily restraint periods of 2h (group 1) or

6h (group 2) repetitively in a cylindrical chamber. Control rats will remain undisturbed in their cages. According to de Oliveira et al. [5], the animals will be sacrificed twenty-four hours after the last immobilization period. The brain will be rapidly removed, hippocampus, cortex and striatum dissected and then processed for a Western Blot method [13-15] allowing to analyze the expression of: I) total α -tubulin II) tyrosinated α -tubulin III) nitrotyrosinated α -tubulin.

Experiment 2: *In vivo* NO level in freely moving rat submitted to restraint stress and *in vivo* α -Tubulin Tyrosination and Nitrotyrosination expression

Rats (200-250g) will be prepared as previously described for *in vivo* amperometric measurement of NO levels in specific brain areas, by means of specifically treated carbon fiber microelectrodes (CFE) [21,22]. The electrochemical recordings will be taken before (control period), during and after the restraint stress. The restraint stress will be made as described above in a cylindrical chamber modified to permit the electrochemical recordings. Twenty-four hours after the last immobilization period the animals will be sacrificed the brain removed and processed for Western Blot Analysis.

Experimental Procedure

The objectives of this work will be challenged via various approaches. In particular:

- Forced swimming test (FST) or other animal stress models [14,15,23] to obtain the stress-induced cytoskeletal changes observed in our previous studies [13,15].
- In vivo* voltammetry associated with carbon fiber micro biosensors [24,25] to monitor NO levels in animals submitted to the behavioral models mentioned above.
- Electrophysiological analysis of cell firing in various brain areas [26] this will allow the monitoring of 5-HT cell activity, the parallel detection via concomitant voltammetry measurements of serotonergic release [27] will indicate the putative correlation between the NO and the 5-HT systems in the experimental conditions applied. In particular the data gathered will add value to the hypothesis of an interrelationship between these two cerebral systems within the induced pathological conditions.
- Molecular biology investigation to analyse the tyrosinated and nitrotyrosinated
- α -tubulin expression by means of the Western Blot method [15]
- Pharmacological studies to analyse the effect of the SSRI upon NO production and nitrotyrosinated α -tubulin expression [24,28,29]

Conclusion

Data gathered will be of help in supporting the hypothesis that the microtubular network is a potential new pathway that may increase our understanding of stress-related events. In addition, while confirming the involvement of the serotonergic system in such pathologic conditions, they will propose a potential new therapeutic application for SSRIs i.e. the modulation of NOS activities and therefore that of NO levels within abnormal states in which the increase of NO activity would influence negatively cell functions in particular at the level of cytoskeleton.

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