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XLVII CONGRESSO DELLA SOCIETA' ITALIANA DI NEUROLOGIA
Venezia, 22 - 25 Ottobre 2016

Neuronal toxicity induced by mitochondrial complex IV inhibition is enhanced during experimental multiple sclerosis: possible neuroprotection through the nitric oxide pathway

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INTRODUCTION

A close link between inflammation and neurodegeneration has been demonstrated during multiple sclerosis (MS), leading to the hypothesis that immune mechanisms may control and even promote neuronal degeneration and irreversible disease progression. In this scenario, it has been proposed a potential pathogenic role for mitochondrial dysfunction[1]. The main aim of our project was to investigate, with electrophysiological recordings, if the neuroinflammatory process associated with experimental autoimmune encephalomyelitis (EAE) may increase neuronal vulnerability to mitochondrial impairment induced by mitochondrial complexes inhibitors, and the possible molecular pathways of this effect. Indeed, inflammation and mitochondrial dysfunction could be intertwined in a vicious cycle, ultimately converging into a common synergistic process responsible of neuro-axonal degeneration during the course of MS.

MATERIALS AND METHODS

Chronic-relapsing EAE induced in Biozzi ABH mice was chosen as experimental model of MS. Animals were monitored and weighed daily, to assess the development of relapsing-remitting paralysis. The initial (acute) phase of EAE usually occurred around 15-18 days post injection (Fig. 1). At the time of this first episode of neurological deficit, animals with a significant clinical disability score were selected for the experiments in the EAE group.

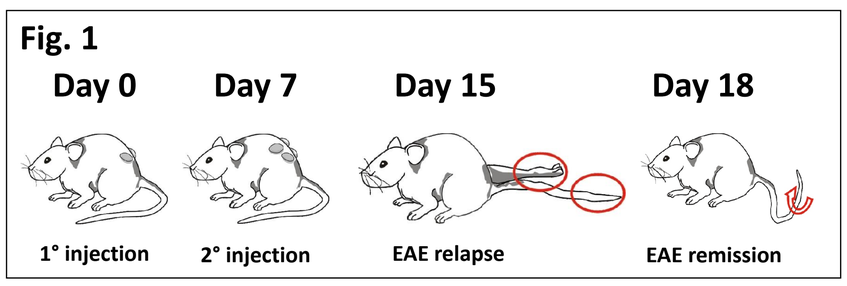

Fig. 1: Clinical course for EAE mice.

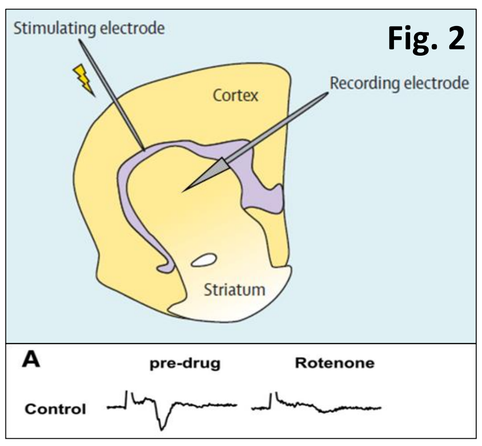

Fig. 2: Electrophysiological recordings in cortico-striatal brain slices. (A) Example of an evoked excitatory field potential recording, before and after rotenone exposure.

Corticostriatal coronal slices (thickness, 240-270 µm) were cut using a vibratome. A single slice was then transferred to a recording chamber and submerged in a continuously flowing Kreb's solution (34°C; 2.5–3 ml/min) bubbled with a 95% O2–5% CO2 gas mixture. Extracellular recordings were obtained by using microelectrodes pulled from borosilicate glass pipettes backfilled with 2 M NaCl (15–20 MΩ), connected to an Axoclamp 2B amplifier. The stimulating electrode was located in the white matter between the cortex and the striatum to activate corticostriatal fibers (Fig. 2).

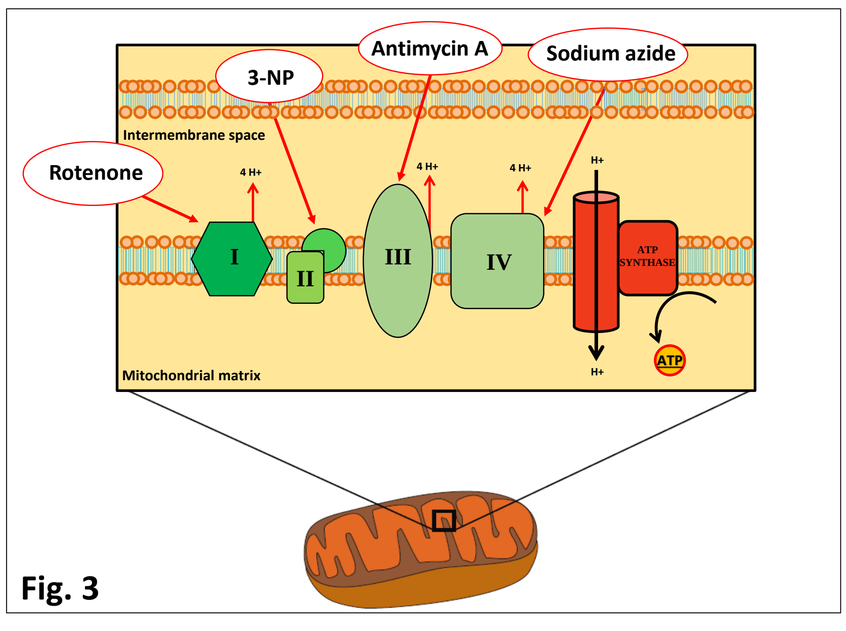

Fig. 3: Schematic representation of mitochondrial respiratory chain and mitochondrial complexes inhibitors.

In order to induce mitochondrial complexes dysfunction, we utilized rotenone (complex I), 3-nitropropionic-acid (3-NP, complex II), antimycin A (complex III) and sodium azide (NaN3, complex IV) as specific mitochondrial inhibitors (Fig. 3).

RESULTS

We found that the pathogenic process associated to acute demyelination/neuroinflammation enhanced neuronal toxicity caused by exposure to 1 µM rotenone (worsening the field potential amplitude loss by 14.21 % at 35' post exposure) and particularly by 1 mM NaN3 (26.64 % worsening). Conversely, no significant difference between control and EAE group during the exposure to 10 mM 3-NP (3.85 % worsening) and 25 µM antimycin A (1.97 % worsening) (Fig. 4a,b).

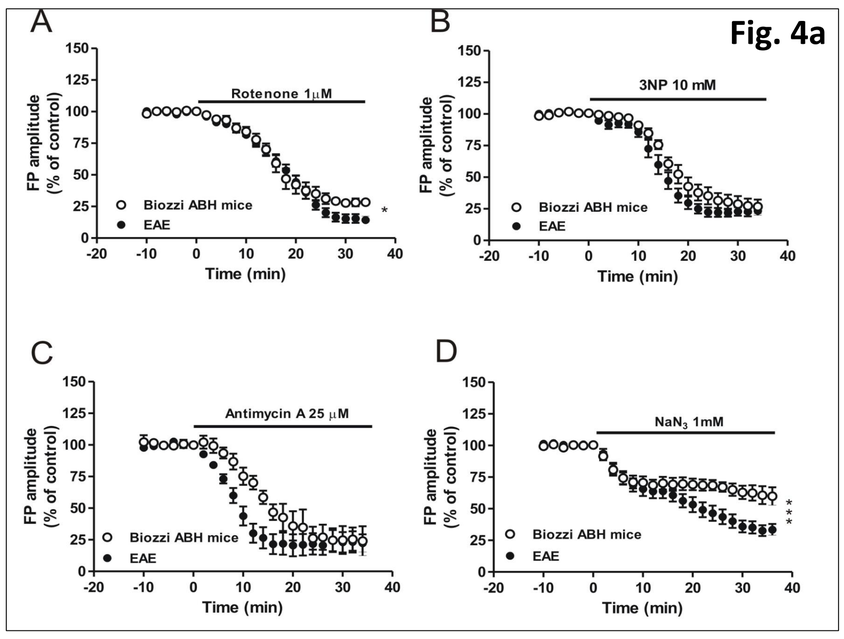

Fig. 4a: Time courses of field potential amplitude loss during exposure to 1 µM Rotenone (A), 10m M 3-NP (B), 25 µM Antimycin A (C) and 1mM NaN3 (D); p < 0.001: *** p<0.05: *

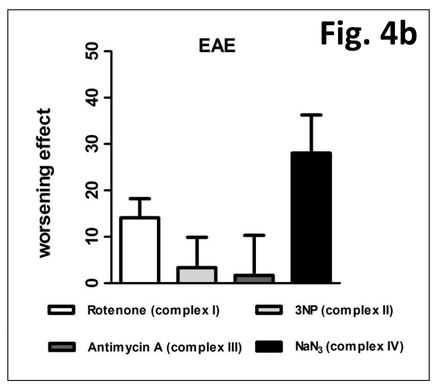

Fig. 4b: Worsening effect of EAE on neuronal dysfunction/toxicity induced by mitochondrial complexes inhibition.

Since NO seems to exert a crucial role in mitochondrial dysfunction during acute demyelination[2], in particular directly inhibiting mitochondrial complex IV, we investigated if the modulation of NO-activated intracellular pathway was able to influence the progressive loss of field potential amplitude induced by NaN3 during EAE. In detail, we utilized 7-NINA (7-nitroindazole), a specific nitrix oxide synthase (NOS) inhibitor, ODQ (1H-1,2,4 oxadiazolo-4,3-a quinoxalin-1-one), a selective inhibitor of soluble guanylyl cyclase (sGC), and Rp-8Br-PET-cGMP, a specific inhibitor of protein kinase G (PKG) (Fig. 5).

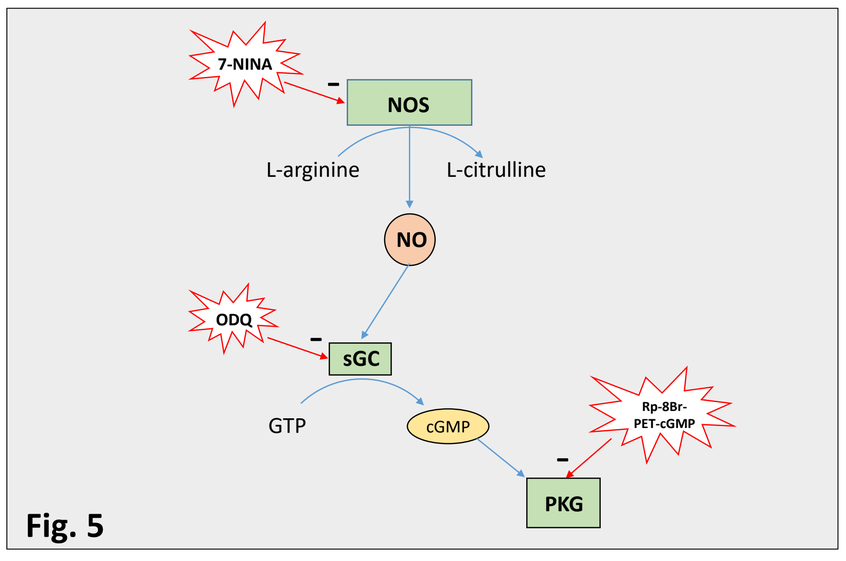

Fig. 5: NO and its intracellular pathway. Once synthesized by NOS, NO activates sGC that catalyses the transformation of guanosine triphosphate (GTP) into 3',5'-cyclic guanosine monophosphate (cGMP). cGMP has several downstream effectors, the most important being PKG.

Interestingly, we found that the inhibition of NO synthesis and of its intracellular pathway markedly counteracted the enhancing effect of inflammation on NaN3 neuronal toxicity, suggesting a crucial role of this pathway in the observed effect. In particular the application of 7-NINA 10 µM, ODQ 10 µM and Rp-8Br-PET-cGMP 1 µM markedly reduced the progressive loss of field potential amplitude induced by NaN3 in EAE mice (respectively a reduction of 34.53 %, 52.96 %, 32.08%) (Fig. 6). Moreover, there was no protective effect of these compounds against NaN3-induced neuronal toxicity in control conditions, suggesting that only during neuroinflammation the modulation of NO-activated pathway would exert a protective role.

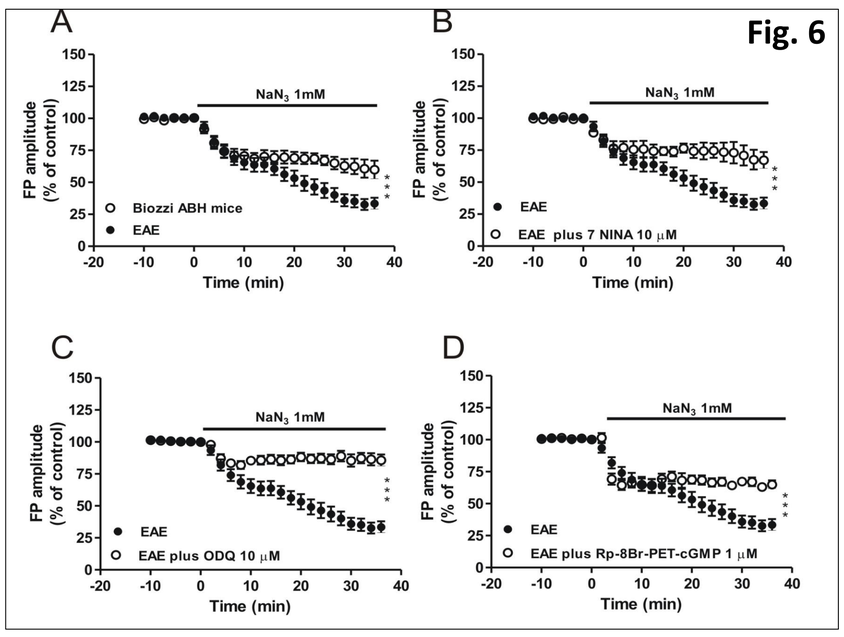

FIG 6: Time course of field potential amplitude loss during exposure to 1mM NaN3 (A) and co-exposure to 1mM NaN3 and 10 µM 7-NINA (B), 10 µM ODQ (C) and 1 µM Rp-88Br-PET-cGMP (D); p < 0.001: *** p<0.05: *

Since we found that inflammation worsened NaN3-induced neuronal toxicity, we investigated if soluble immune products, such as pro-inflammatory cytokines (interleukin-1β, tumour necrosis factor α, interferon γ) were able to influence the progressive loss of field potential amplitude during exposure to NaN3 in control conditions. Interestingly none of this inflammatory molecules, alone or in variable association, influenced NaN3-induced toxicity. Finally, we tested if the exposure to pharmacological agonists of the NO-activated pathway in control conditions was an element sufficient to mimic the worsening effect of inflammation on complex IV toxicity. In particular we applied SNAP 100 µM (S-nitroso-N-acetylpenicillamine), an NO donor, YC-1 1µM (3-5-hydroxymethyl-2-furyl-1 -benzyl-indazole), an activator of sGC, and 8-Br-cGMP 1µM , an activator of PKG. Interestingly, we found that none of these pharmacological compounds, if applied alone and acutely, was able to mimic the enhancing effect of EAE on NaN3 toxicity.

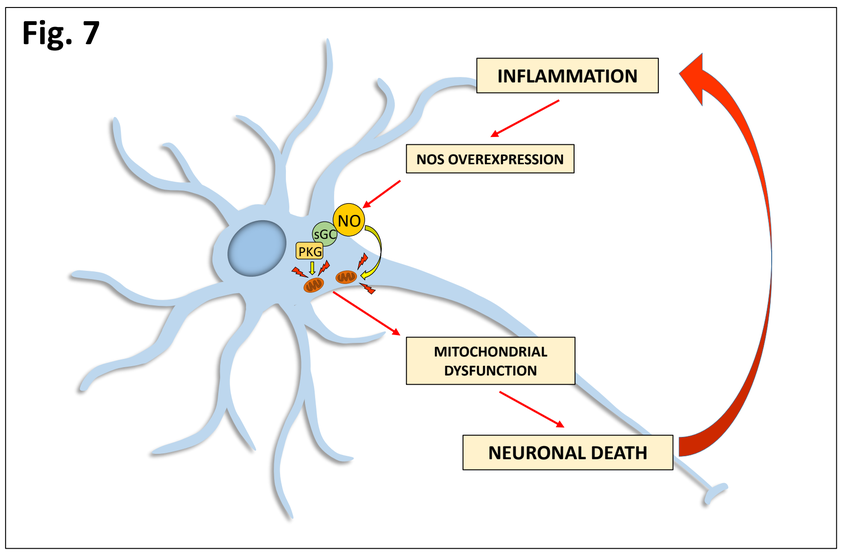

Fig. 7: Schematic representation of the possible links between inflammation, mitochondrial dysfunction and neuro-axonal degeneration. NO could be harmful for mitochondrial homeostasis directly and indirectly, through the chronic activation of its intracellular pathway (sGC and PKG).

CONCLUSIONS

- **During experimental MS, neuroinflammation enhances neuronal susceptibility to mitochondrial dysfunction, ultimately leading to neuronal death.**
- **Neuro-axonal degeneration and inflammation could be intertwined in a vicious cycle, linked by mitochondrial dysfunction (Fig. 7).**
- **Mitochondrial complex IV appears to be particularly susceptible to the detrimental effect of neuroinflammation.**
- **Inflammation-related NO synthesis and the chronic activation of its intracellular pathway, involving sGC and PKG, seem to play a crucial role in this process, representing a promising pharmacological target for neuro-protective strategies aimed at counteracting neuronal death in MS.**

References:

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