Study of mitochondrial death pathways in different cellular models of Parkinson's disease: effect of 17β-Estradiol.

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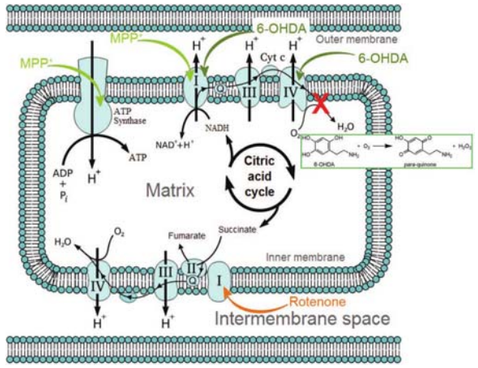
NEUROSCIENCE 2014

1 Introduction

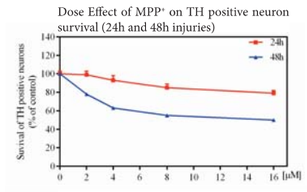
Parkinson's disease (PD) is the second most common neurodegenerative disorder in the United States. The predominant motor symptoms of PD including slow movement, resting tremor, rigidity and gait disturbance are caused by the loss of dopaminergic neurons in the substantia nigra (SN). Epidemiological studies suggest that the use of pesticides increases the risk of PD, possibly via reduced activity of complex I in the mitochondrial respiratory chain in the substantia nigra and result in the pathogenesis of PD. 6-hydroxydopamine (6-OHDA), a H2O2 pro-oxidant, a natural dopaminergic catabolite, 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP), a mitochondrial complex I inhibitor, and Rotenone, a nature-derived pesticide, are used to mimic in vitro model of PD (see figure for possible mode of action).
In this study, the neuronal death of primary mesencephalic neurons induced by these 3 toxins was studied and the neuroprotective effect of 17 β-Estradiol (β-Estr) was assessed.

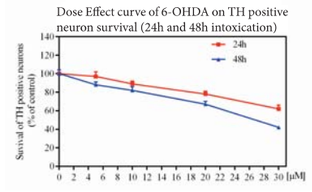


2 Methods

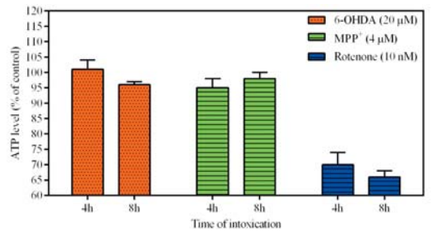
Culture of rat mesencephalic dopaminergic neurons: Neurons were cultured as described by Visanji *et al.*, 2008. Briefly, the midbrains obtained from 15-day old rat embryos were dissected. Cells were seeded at a density of 40 000 cells/well in 96 well-plates pre-coated with poly-L-lysine maintained in a humidified incubator at 37°C. Six wells were used per conditions.
Pharmacological treatments: On day 6, fresh medium was added, without or with 6-OHDA, MPP⁺ or Rotenone. 1h before addition of neurotoxins, the cells were incubated with β-Estr at 100 nM.
Survival of TH positive neurons: After 24 and 48 hours of intoxication, cells were fixed by a solution of 4% paraformaldehyde. The cells were incubated with Monoclonal Anti-Tyrosine Hydroxylase (TH) antibody that was revealed with Alexa Fluor 488 goat anti-mouse IgG. The immuno-labelled cultures were examined with MetaXpress (Molecular Devices, USA) at X 10 magnification.
Apoptosis and necrosis evaluation: 4, 8, 24 and 48h after injuries apoptosis or necrosis were assessed using Glomax system and Apotoxglo triplex AC Kit (Promega, USA).
ATP evaluation: 4 and 8h after injuries, ATP level in culture wells was measured using Glomax system and Celltiter-Glo luminescent assay kit (Promega, USA).

3 Results



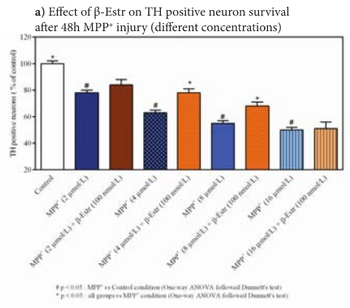


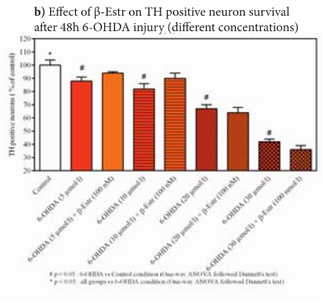
Time/dose effect of MPP⁺, 6-OHDA and Rotenone on TH positive neuron survival: The 3 toxins induced TH neuron cell death. This effect was depending on the time of application and dose.

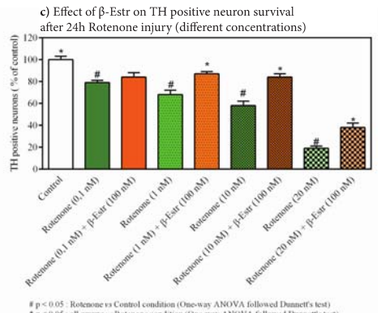


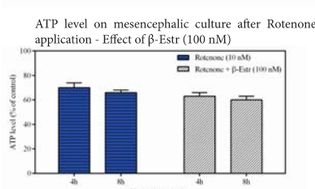
Effect of MPP⁺, 6-OHDA and Rotenone on the ATP pools in mesencephalic cells : Only rotenone (10 nM) induced a large decrease of ATP pools. This major depletion started 4h after application.

Neuroprotective effect of β-Estr on a) MPP⁺, b) 6-OHDA and c) Rotenone on TH positive neuron survival: β-Estr was able to protect TH neurons from injuries induced by Rotenone (all doses), MPP⁺ (except for the highest toxic concentration 16 µM) and has no effect on the 6-OHDA (all doses) toxicity.





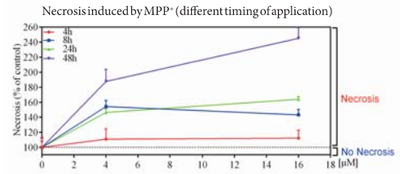


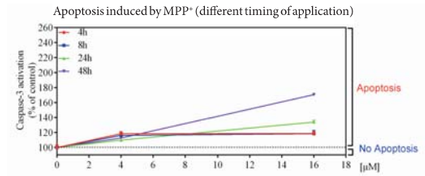


β-Estr did not protect cell from the ATP depletion induced by Rotenone application.

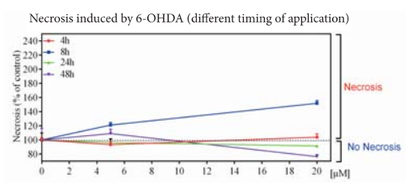
Apoptosis (caspase-3 activation) or necrosis induced by MPP⁺, 6-OHDA and Rotenone application in mesencephalic neuronal culture:

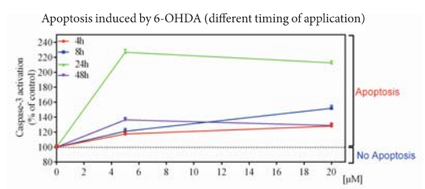
1. The cell death induced by MPP⁺ is mainly caused by a necrosis process. The number of cells entering in necrosis increases with the dose and the time of contact with the toxin. The necrosis process started 8h after contact.



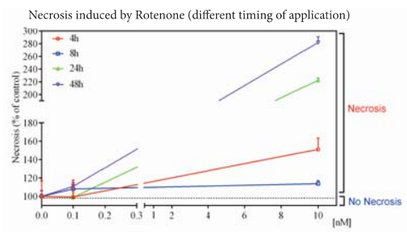


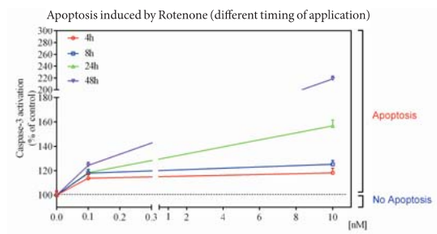
2. 6-OHDA induced a large and significant apoptosis, the caspase-3 activation started 4h after application and increased with doses, the peak of caspase-3 activation was recorded after 24h of contact. No major cellular necrosis was observed.





3. By contrast, Rotenone induced cell death by apoptosis process at low doses (0,1 nM), whereas at the highest doses (10 nM) necrosis mecanisms appeared.





4 Conclusions

We showed that despite the fact that all the 3 toxins are able to block the respiratory chain complexes (see above figure); they seem to have well distinct pathways leading to dopaminergic neuron death. β-Estr (100 nM) was able to protect injuries induced by Rotenone (all doses) as well as MPP⁺ (all doses accept the highest). But interestingly, β-Estr was not able (at the dose used) to protect from 6-OHDA injuries (for all doses of toxin). In regards of these results, a deep knowledge of the cytopathologic effects of each toxin is definitely fundamental in the process of drug discovery of neuroprotective compounds in PD indication.