Estrogen protection in Parkinson’s disease – a GDNF role?

C. Batista¹, C. P. Fonseca¹, F. Campos¹, A. Cristóvão¹, L.P. de Almeida³, G. Baltazar¹

¹Health Sciences Research Center, University of Beira Interior, Covilhã,
²Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra,
³Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra, Coimbra,

INTRODUCTION

Parkinson’s disease (PD) is a movement disorder characterized by the progressive degeneration of dopaminergic (DA) neurons projecting to the striatum. Oxidative stress in the nigrostriatal pathway as well as deficient neurotrophic support could be factors triggering neurodegeneration in the substantia nigra. The incidence of PD is greater in men than women, suggesting that estrogens may play a protective role in the progression of this disease. The estradiol is considered a neuroprotective agent for nigral DA neurons and its action has been related to its capacity to reduce the oxidative stress or to regulate the expression of neurotrophic factors. One possible candidate for estradiol regulation is the glial cell line-derived neurotrophic factor (GDNF), a potent factor for the protection of DA neurons which is able to prevent or reverse the neurodegenerative process observed in PD.

This work aimed at studying the effect of 17b-estradiol on GDNF expression in postnatal substantia nigra cultures and the relevance of this effect to the neuroprotective action of this hormone. The ability of levodopa (L-DOPA, the main symptomatic treatment for PD) or H₂O₂ (a by-product of dopaminergic metabolism that increases oxidative stress) to modulate this effect was also evaluated.

METHODS

Substantia nigra-glia co-cultures

Substantia nigra cells were isolated from P0-P2 Wistar rat pups. Neurons were plated onto a collagen-coated petri dish culture conditioning with neuronal culture medium for 7 days. Under these conditions about 7% of total neurons were dopaminergic exhibiting long and branched processes.

Western blot analysis of GDNF levels

The samples (30–40 µg protein) were separated by SDS-PAGE on a 12% acrylamide gel. After electrophoresis onto PVDF membranes and blocking with 5% milk, the membranes were incubated with the primary antibody to GDNF (rabbit, 1:1000; Santa Cruz) overnight at 4ºC, and incubated with a secondary goat anti-rabbit IgG antibody conjugated to alkaline phosphatase (Amersham Life Sciences) and quantified with the QuantiOne software (Bio-Rad).

In vivo experiments

Adult male Wistar rats were implanted s.c. with a 7-day release 17b-estradiol or vehicle (control) microosmotic pump. Ten days later, the rats were unilaterally injected in the striatum with the selective DA toxin 6-hidroxydopamine (6-OHDA) or vehicle (control). Five days later the animals were sacrificed and coronal brain slices were obtained. The midbrain and striatal slices were processed for tyrosine hydroxylase (TH) and GDNF immunoreactivity using a primary antibody to TH (mouse, 1:1000, Transduction Laboratories) and 17b-estradiol (rabbit, 1:500, Santa Cruz), and the biotinylated secondary anti-rabbit IgG antibody (1:100, Vector) and an ABC reagent (Vector), followed by 3,3′-diaminobenzidine tetrahydrochloride (DAB-CH). and the brain sections were counterstained with neutral red.

RESULTS

17b-Estradiol up-regulated GDNF levels in substantia nigra cultures

Figure 1 - GDNF expression in substantia nigra cultures treated with different concentrations of 17b-estradiol. The cells were incubated with 17b-estradiol (0.01, 0.1, 1, 10 and 100 nM) for 48h and cell extracts were prepared for Western blot analysis of GDNF levels. Data shown are the mean ± S.E.M. of at least three independent experiments performed in triplicate. Statistical analysis was performed using one-way ANOVA followed by Dunnett’s test. *P < 0.05 as compared to control.

L-DOPA and H₂O₂ augmented the effect of 17b-estradiol on GDNF expression in substantia nigra cultures

Figure 2 - Modulation of the 17b-estradiol-induced GDNF up-regulation by H₂O₂ and L-DOPA in substantia nigra cultures. The cells were incubated with different concentrations of 17b-estradiol (1, 10, 100 nM) in the presence or absence of H₂O₂ (50 uM or L-DOPA 200 uM (24 h incubation). GDNF expression was assessed by Western blot analysis of cell extracts. Data shown are the mean ± S.E.M. of at least three independent experiments performed in triplicate. Statistical analysis was performed using one-way ANOVA followed by Dunnett’s test. *P < 0.05; **P < 0.01 as compared to control. ***P < 0.001 as compared to 17b-estradiol 10 nM.

CONCLUSIONS

- 17b-Estradiol up-regulates GDNF expression in substantia nigra cultures.
- L-DOPA and H₂O₂ augmented the effect of 17b-estradiol on GDNF expression, in vitro.
- 17b-Estradiol exerts a neuroprotective effect in vivo on selectively injured substantia nigra DA neurons. This effect may be mediated by an up-regulation of GDNF expression.