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

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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* cell 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 H_2O_2 (a by-product of dopamine metabolism that increases oxidative stress) to modulate this effect was also evaluated.

METHODS

***Substantia nigra* neuron-glia cocultures**

Substantia nigra cells were isolated from P2-P3 Wistar rat pups. Neurons were plated onto a confluent monolayer of ventral midbrain astrocytes upon conditioning with neuronal culture medium for 1-2 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 electrotransfer 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, 1:20000) for 1 h at RT. Immunoreactive bands were detected using the ECF system (Amersham Life Sciences) and quantified with the Quantity One 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 striatum slices were processed for tyrosine hydroxylase (TH) and GDNF immunoreactivity using a primary antibody to TH (mouse, 1:1000; Transduction Laboratories) and to GDNF (rabbit, 1:500; Santa Cruz), and the biotinylated secondary anti-mouse IgG antibody (1:100; Abcam) and anti-I rabbit (1:20; Sigma) (1h incubation at RT).

RESULTS

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

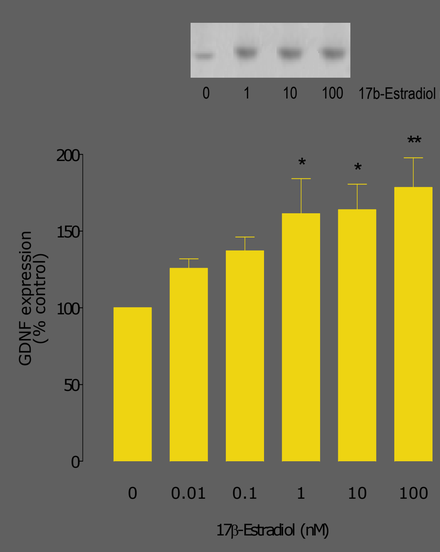

Figure 1 – GDNF expression in *substantia nigra* cultures exposed to different concentrations of 17b-estradiol. The cells were incubated with 17b-estradiol (0.01, 0.1, 1, 10 and 100 nM) for 48h and cell extracts were prepared for Western blot analysis of GDNF levels. Data shown are the mean ± S.E.M.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

L-DOPA and H_2O_2 augmented the effect of 17b-estradiol on GDNF expression in *substantia nigra* cultures

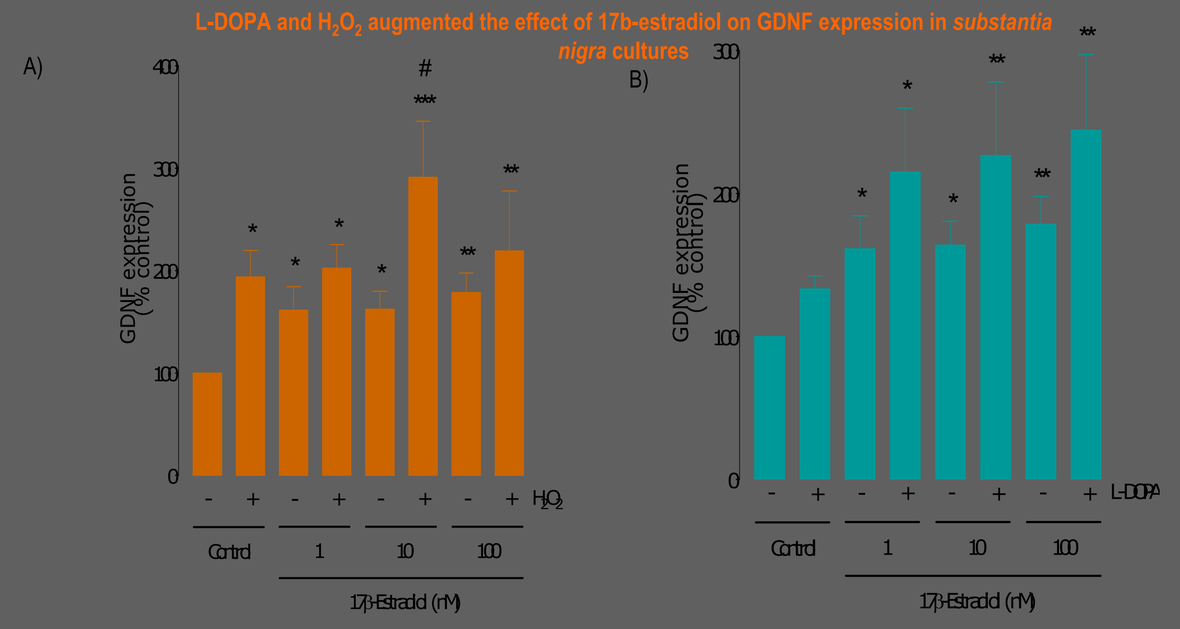

Figure 2 – Modulation of the 17b-estradiol-induced GDNF up-regulation by H_2O_2 (A) and L-DOPA (B) in *substantia nigra* cultures. The cells were incubated with different concentrations of 17b-estradiol (1, 10 and 100 nM) for 48h, in the presence (+) and absence (-) of H_2O_2 50 uM or L-DOPA 200 uM (24 h incubation). GDNF expression was assessed by Western blot analysis of cell extracts. Data shown are the mean ± S.E.M. of up to nine 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* < 0.05 as compared to 17b-estradiol 10 nM.

17b-Estradiol completely prevented the DA cell loss induced by the striatal injection of 6-OHDA

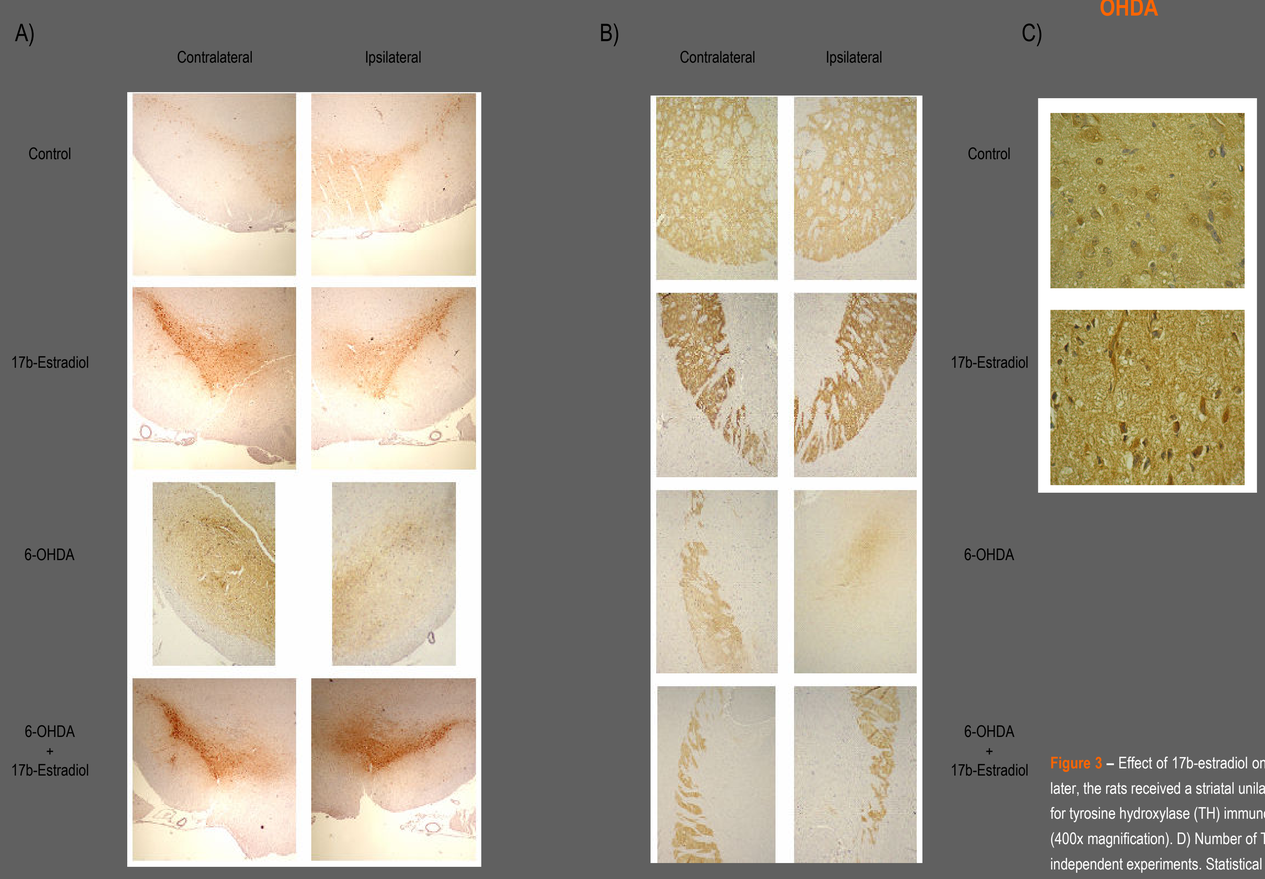

Figure 3 – Effect of 17b-estradiol on the nigrostriatal pathway, *in vivo*. Adult male Wistar rats were implanted s.c. with a 7-day release 17b-estradiol or vehicle (control) microosmotic pump. Ten days later, the rats received a striatal unilateral injection of 6-OHDA or vehicle (control). The *substantia nigra* (A) and striatum (B) coronal slices were obtained five days after 6-OHDA injection and processed for tyrosine hydroxylase (TH) immunoreactivity (40x magnification). C) The *substantia nigra* coronal slices were obtained 15 days after 17b-estradio treatment and processed for GDNF immunoreactivity (400x magnification). D) Number of TH-positive cells in the *substantia nigra*, in the ipsilateral side as compared to the contralateral side (considered 100%). Data shown are the mean ± S.E.M. of three independent experiments. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's test. ****P* < 0.001 as compared to the contralateral side.

CONCLUSIONS

- 17b-Estradiol up-regulates GDNF expression in *substantia nigra* cultures.
- L-DOPA and H_2O_2 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.