available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Adrenalectomy counteracts the local modulation of astroglial fibroblast growth factor system without interfering with the pattern of 6-OHDA-induced dopamine degeneration in regions of the ventral midbrain

Gerson Chadi[a,*], *Camila Silva*[a], *Jessica Ruivo Maximino*[a], *Kjell Fuxe*[b], *Gilberto Ochman da Silva*[a]

[a]*Neuroregeneration Center, Department of Neurology, University of São Paulo School of Medicine, University of São Paulo, 01246-903, São Paulo, Brazil*
[b]*Department of Neuroscience, Karolinska Institute, S-171 77, Stockholm, Sweden*

ARTICLE INFO

Article history:
Accepted 7 November 2007
Available online 22 November 2007

Keywords:
Adrenalectomy
Glucocorticoid
6-OHDA
FGF-2
Receptor
Basal ganglia
Paracrine action

ABSTRACT

The present study investigated the effects of bilateral adrenalectomy (ADX) on the synthesis of basic fibroblast growth factor (bFGF, FGF-2) mRNA and on the expression of its FGF receptor subtype-2 (FGFR2) mRNA after a 6-hydroxydopamine (6-OHDA)-induced lesion of nigrostriatal dopamine system. In previous papers we have demonstrated that corticosterone increases FGF-2 immunoreactivity mainly in the astrocytes of the substantia nigra [Chadi, G., Rosen, L., Cintra, A., Tinner, B., Zoli, M., Pettersson, R.F., Fuxe, K., 1993b. Corticosterone increases FGF-2 (bFGF) immunoreactivity in the substantia nigra of the rat. Neuroreport 4, 783–786.] and that 6-OHDA injected in the ventral midbrain upregulates FGF-2 synthesis in reactive astrocytes in the ascending dopamine pathways [Chadi, G., Cao, Y., Pettersson, R.F., Fuxe, K., 1994. Temporal and spatial increase of astroglial basic fibroblast growth factor synthesis after 6-hydroxydopamine-induced degeneration of the nigrostriatal dopamine neurons. Neuroscience 61, 891–910.]. Rats were adrenalectomized and received a 6-OHDA stereotaxical injection in the ventral midbrain 2 days later. Seven days after the dopamine lesion, Western blot analysis showed a decreased level of tyrosine hydroxylase in the lesioned side of the midbrain, an event that was not altered by ADX or corticosterone replacement. Moreover, the degeneration of nigral dopamine neurons, which was confirmed by the disappearance of acidic FGF (FGF-1) mRNA and the decrement of tyrosine hydroxylase mRNA labeled nigral neurons, was not altered by ADX. The FGF-2 protein (23 kDa isoform but not 21 kDa fraction) levels increased in the lesioned side of the ventral midbrain. This elevation was counteracted by ADX, an effect that was fully reversed by corticosterone replacement. *In situ*

* *Corresponding author*. Department of Neurology, University of São Paulo, Av. Dr. Arnaldo, 455 2nd floor, Room 2119, 01246-903, São Paulo, Brazil.
E-mail address: gerchadi@usp.br (G. Chadi).

Abbreviations: ADX, adrenalectomy; aFGF, FGF-1, acidic fibroblast growth factor; BDNF, brain derived neurotrophic factor; bFGF, FGF-2, basic fibroblast growth factor; DAB, 3,3′; diaminobenzidine tetrahydrochloride, ; FGFR2, fibroblast growth factor receptor subtype-2; GFAP, glial fibrillary acidic protein; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; NGF, nerve growth factor; NT-3, neurotrophin-3; PBS, phosphate-buffered saline; SNc, pars compacta of the substantia nigra; SNr, pars reticulata of the substantia nigra; SSC, standard saline citrate; 6-OHDA, 6-hydroxydopamine; TGF-beta, transforming growth factor beta; TH, tyrosine hydroxylase; VTA, ventral tegmental area

0006-8993/$ – see front matter
doi:10.1016/j.brainres.2007.11.024

hybridization revealed that ADX counteracted the elevated FGF-2 mRNA levels in putative glial cells of the ipsilateral pars compacta of the substantia nigra and in the ventral tegmental area. The ADX also counteracted the increased density and intensity of the astroglial FGF-2 immunoreactive profiles within the lesioned pars compacta of the substantia nigra and the ventral tegmental area as determined by stereology. The stereotaxical mechanical needle insertion triggered the expression of FGFR2 mRNA in putative glial cells, spreading to the entire ipsilateral ventral midbrain from the region of needle track, an occurrence that was partially reversed by ADX. In conclusion, bilateral ADX counteracted the increased astroglial FGF-2 synthesis in the dopamine regions of the ventral midbrain following a 6-OHDA-induced local lesion and interfered with FGF receptor regulation around injury. These findings give further evidence that adrenocortical hormones may regulate the astroglial FGF-2-mediated trophic mechanisms and wound repair events in the lesioned central nervous system.

1. Introduction

Basic fibroblast growth factor (bFGF, FGF-2) is a member of the heparin-binding growth factors. The presence and action of FGF-2 on the nigrostriatal dopamine system have been described (Ferrari et al., 1989; Engele and Bohn, 1991; Park and Mytilineou, 1992; Chadi et al., 1993a; Otto and Unsicker, 1990, 1993; Cintra et al., 1991; Bean et al., 1991). Increasing evidence has indicated that the FGF-2-induced protection of dopamine neurons of the substantia nigra is mediated by paracrine trophic actions of FGF-2 synthesized by activated astrocytes (Gaul and Lubbert, 1992; Chadi et al., 1993a). Furthermore, glial FGF-2 has been suggested to act as a potent regulator of the repair events following brain damage (Finklestein et al., 1988; Frautschy et al., 1991; Takami et al., 1992; Humpel et al., 1994; Logan et al., 1992). In a previous study we have observed that a 6-hydroxydopamine (6-OHDA)-induced lesion in the nigrostriatal dopamine system upregulates astroglial FGF-2 synthesis in the ascending dopamine pathways (Chadi et al., 1994), emphasizing the importance of astroglial paracrine mechanisms in the lesioned basal ganglia (Chadi and Gomide, 2004). Moreover, it is likely that FGF-2 action on nigral dopamine neurons is mediated by its high affinity FGF receptor subtypes 1, 2 and 3, which are called FGFR1, FGFR2, and FGFR3, respectively. While FGFR1 and FGFR2 may act in an autocrine fashion, the FGFR2 seems to be particularly involved in paracrine astroglial modulation within the substantia nigra, especially after dopamine lesion (Belluardo et al., 1997; Grothe and Timmer, 2007; Timmer et al., 2007). Furthermore, FGFR1 mRNA level in PC12 cells was not affected by steroid hormones (Meisinger et al., 1996).

It has been postulated that the actions of steroid hormones on brain plasticity/trophism of adult animals (Pollock et al., 1990; Laping et al., 1991; Chao et al., 1998) may be related to their ability to regulate the expression of neurotrophic factors (Barbany and Persson, 1993; Chao and McEwen, 1994; Riva et al., 1995a, 1995b; Smith et al., 1995). Moreover, corticosterone was shown to increase FGF-2 immunoreactivity in the substantia nigra of the rat (Chadi et al., 1993b). Thus, it becomes important in the analysis of the hormonal influences on neurodegenerative/neuroprotection events in the basal ganglia, with particular interest to pathophysiology of Parkinson's disease. The present study evaluated whether a bilateral removal of the adrenal gland can alter the synthesis of FGF-2 and the expression of its high affinity receptor FGFR2 as well as the levels of tyrosine hydroxylase (TH) in the dopamine regions of the ventral midbrain after a 6-OHDA-induced degeneration of dopamine neurons. The study was performed by means of Western blot as well as *in situ* hybridization and immunohistochemistry in combination with image analysis and stereology. The disappearance of dopamine neurons containing acidic

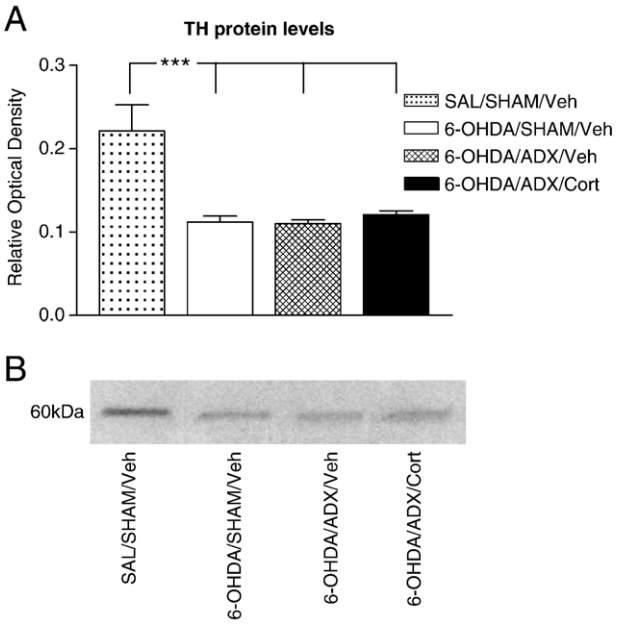

Fig. 1 – The figure shows the tyrosine hydroxylase (TH) protein levels (relative optical density in A and illustrated in B) by means of Western blot in the biochemical experiments in the ipsilateral rat ventral midbrain following an unilateral nigral injection of saline or 6-hydroxydopamine (6-OHDA). Nigral saline injected rats were sham operated for bilateral adrenalectomy (ADX) and received daily injections of vehicle (SAL/SHAM/Veh groups, $n=6$). Nigral 6-OHDA injected rats were sham operated for ADX and received daily injection of vehicle (6-OHDA/SHAM/Veh, $n=6$). Moreover, an additional number of 6-OHDA injected rats received ADX and were treated with daily injection of vehicle (6-OHDA/ADX/Veh, $n=6$) or corticosterone (6-OHDA/ADX/Veh, $n=6$). One-way analysis of variance (ANOVA) was applied for comparisons at the ipsilateral lesioned side, using the protected Fisher test. Mean ± s.e.m. $^{***}p<0.001$.