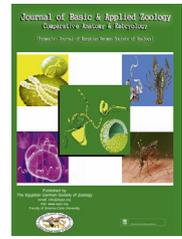




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Locus coeruleus: A brain region exhibiting neuronal alterations in Parkinson's disease rat model



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KEYWORDS

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Abstract Toxic insults lead to increased α -synuclein expression in dopaminergic neurons. However, little information is known about α -synuclein alterations in relation to tyrosine hydroxylase (TH) changes in locus coeruleus (LC) of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) rat model for Parkinson's disease (PD). Four injections (15 mg/kg each) of the neurotoxicant MPTP to rats led to an upregulation of α -synuclein level and increased immunoreactivity with aggregated protein in the MPTP-treated group as revealed by Western blotting and immunohistochemical techniques. Meanwhile, MPTP reduced the level of and caused immunoreactivity toward TH antibody in LC and adjoining noradrenergic neurons. These data indicate that MPTP can induce α -synuclein alterations in other brain regions that have been implicated in the pathogenesis of PD. The findings are also consistent with a pattern that α -synuclein modification influences the TH level.

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Introduction

Parkinson's disease (PD) is a neurodegenerative disease that is accompanied by a group of motor and non-motor symptoms (Fathy and Addelkader, 2015). It was believed that motor manifestations, characterized by bradykinesia, tremor, rigidity, and postural instability, are principally attributable to degeneration of dopaminergic cells and decreased dopamine level in the substantia nigra (SN) region (Masilamoni et al., 2011; Taylor et al., 2014). Dopamine depletion can be assessed by

measuring the level of tyrosine hydroxylase (TH), the rate limiting enzyme for dopamine and noradrenaline (Oaks and Sidhu, 2011). Brain tissue examination revealed reduction of the midbrain dopamine cells and formation of Lewy bodies (LBs); protein aggregates that include various constituents mainly consist of α -synuclein (McCormack et al., 2008). Usually, parkinsonian manifestation is preceded by a preclinical stage that is characterized by a group of symptoms such as sleep disorder (Remy et al., 2005) and cognitive disturbances (Zweig et al., 1993). The previously mentioned non-motor symptoms were reported to be ascribed to degeneration of brain regions other than midbrain and due to the disturbances of neurotransmission in non-dopaminergic systems (Isaia et al., 2011; Taylor et al., 2014).

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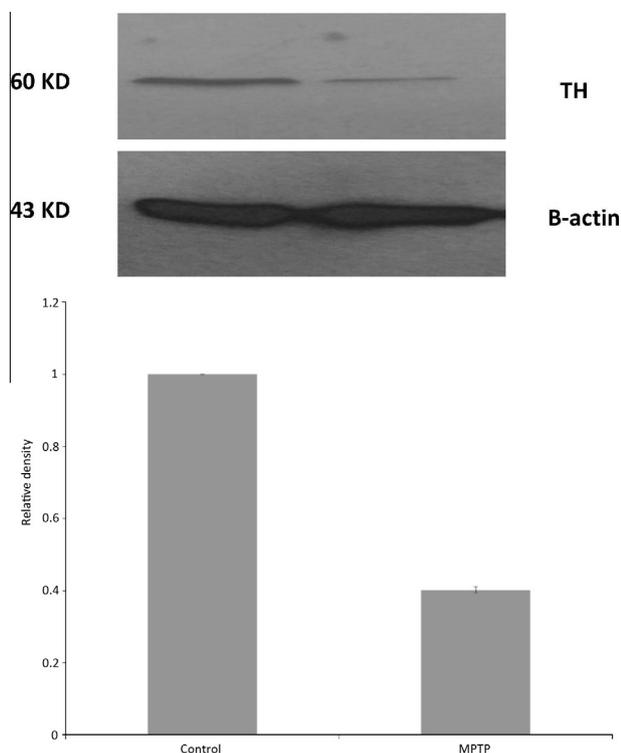


Figure 1 Western blot analysis for TH protein level in pons. The densitometry of TH bands was first normalized to the loading control β -actin. The relative density of the expressions of TH in the MPTP-treated rats relative to that in the saline-treated rats was then calculated. The scanned image of Western blot was analyzed with the software Image J. Data were presented as mean \pm SEM. $P < 0.05$, MPTP-treated group versus saline-treated group.

Locus coeruleus (LC) is a pontine nucleus that was proved to be involved in PD (Isaias et al., 2011). It has been reported that LC is among brain regions that exhibit neuronal degeneration in PD and it encloses projecting neurons controlling the dopaminergic effect of SN. Furthermore, degeneration of LC was postulated to be conjugated with the above mentioned non-motor impairments in PD patients (Isaias et al., 2011).

α -Synuclein is a major protein of the synuclein family of proteins that has been associated with various neurodegenerative diseases such as PD, Alzheimer's disease (AL), and multiple system atrophy (MSA) (McCormack et al., 2008; Oaks and Sidhu, 2011). Lewy bodies and Lewy neuritis are interneuronal inclusions that are recognized in PD and consist of various components, largely including α -synuclein (McCormack et al., 2008). Various evidences supported the link between disturbances of α -synuclein and PD pathology in animal models (McCormack et al., 2008).

Information on the LC in PD is largely dependent on post-mortem examination of histopathological specimens, while its in vivo neuronal changes are largely uncovered. Despite the fact that many studies demonstrated α -synuclein overexpression in the midbrain region of PD, little attention was given to α -synuclein variations in LC region of PD models. Subsequently, the aim of this study is to examine the changes of α -synuclein protein in relation to TH alteration in the LC brain area of a Parkinsonian MPTP rat model.

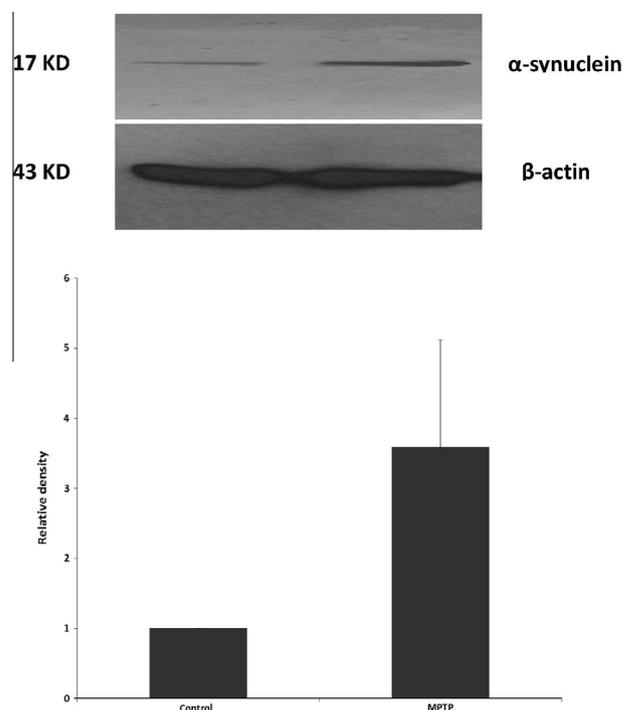


Figure 2 Western blot analysis for α -synuclein protein level in pons. The densitometry of α -synuclein bands was first normalized to the loading control β -actin. The relative density of the expressions of α -synuclein in the MPTP-treated rats relative to that in the saline-treated rats was then calculated. The scanned image of Western blot was analyzed with the software Image J. Data were presented as mean \pm SEM. $P < 0.05$, MPTP-treated group versus saline-treated group.

Materials and methods

Experimental design

The current work was performed according to the Guidelines of Animals Health Research Institute, Egypt. The study protocol was agreed by the Committee on the Ethics of Animals Health Research Institute, Egypt (Permit Number 362). Twenty-four male Wistar rats (200–250 gm) were kept in a temperature controlled room under 12/12 h light/dark cycle with the availability of food pellets and tap water ad libitum. Rats were separated into two groups. The MPTP group was injected intraperitoneally four times, at 2 h apart with 15 mg/kg MPTP (sigma; ST. Louis, MO, USA), while the control rats were injected with physiological saline only. Rats were sacrificed 7 days after administration of MPTP.

Western blot analysis

Quantification of TH and α -synuclein levels was carried out according to (Caudle et al., 2006). For sample analysis, pons regions were mechanically homogenized in the lysis buffer with proteinase inhibitors and cytosolic fractions taken were prepared for reducing and denaturing SDS-PAGE. Proteins were separated on 12% gel and transferred using a Trans-Blot Turbo transfer system (Bio-Rad, UK). Membranes were blocked and probed with polyclonal rabbit anti-TH (Millipore,

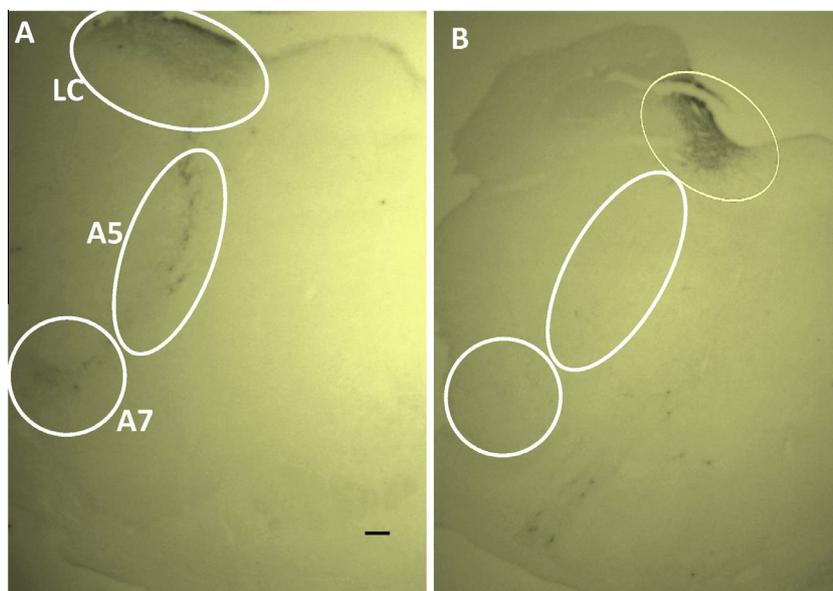


Figure 3 TH immunoreactivity in the LC and adjacent noradrenergic cell groups (A) saline-treated, (B) MPTP-treated rats. Scale bar = 100 μm .

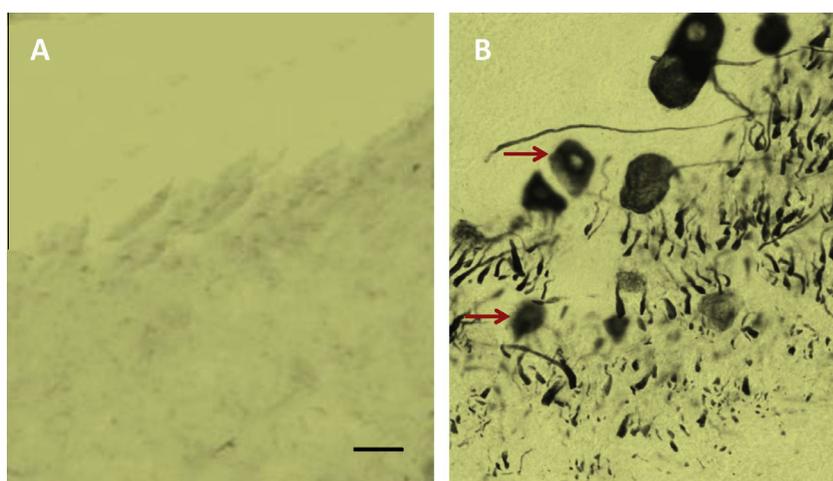


Figure 4 Immunohistochemical analysis of α -synuclein in LC. (A) Saline-treated, (B) MPTP-treated rats, neuronal inclusions immunoreactive for α -synuclein (arrows). Scale bar = 40 μm .

AB152) or monoclonal anti- α -synuclein (Millipore, AB5038) antibodies and visualized with HRP conjugated anti-rabbit IgG (Bio-Rad, UK). Densitometry analysis was performed using Image J software.

Immunohistochemical study

Tissue staining was carried out according to (Caudle et al., 2006; Miller, 1997, 1999). Animals were perfused intracardially with phosphate buffered saline (pH = 7.4) and then with 4% paraformaldehyde and discarded. Afterward, they were placed in 4% paraformaldehyde for 24 h, and finally cryoprotected in 30% sucrose for 48 h. The brains were then cut to a 30 μm thickness using a cryostat microtome (Leica Microsystems). Sections were incubated with either polyclonal anti-TH (1:1000) antibody or monoclonal anti- α -synuclein (1:500)

antibody overnight and then incubated in a biotinylated secondary antibody for 1 h at room temperature. Visualization was performed using 3,3'-diaminobenzidine (DAB) at room temperature. Afterward, all sections were mounted on slides, dehydrated, and coverslipped using DPX Mountant and sections were viewed using a light microscope (Leica). Negative control sections were treated in the same way but in the absence of primary antibodies.

Statistical analysis

Results are presented as mean \pm standard error of the mean. Statistical analysis was performed using an unpaired Student's *t*-test using a statistical package program (Sigma Plot version 11.0). Differences among groups were considered significant at $P < 0.05$.

Results

The results in Fig. 1 displayed the TH levels of both MPTP and saline treated animals. A significant decrease in the protein level in pons of MPTP-treated rats as compared with saline-treated group is shown ($P < 0.05$).

As presented in Fig. 2, immunoblot analysis of the pons homogenate using an antibody for α -synuclein showed a significant variation between MPTP-treated group in relation to normal group. A highly significant increase in the level of the α -synuclein protein in comparison with normal control group is recorded ($P < 0.05$).

Fig. 3 reveals TH immunoreactivity in LC region and adjacent noradrenergic cell groups of both MPTP-treated and saline-treated rats. MPTP treatment induces reduced immunoreactivity toward TH antibody in the LC and related A5 and A7 noradrenaline cell groups in comparison with the saline-treated rats.

Immunohistochemical staining for α -synuclein demonstrated widespread neuronal inclusions of α -synuclein in LC region of the MPTP-treated rats (Fig. 4). The neuronal inclusions' morphology was globular similar to LBs, in addition to the diffuse finely granular pattern of α -synuclein immunoreactivity.

Discussion

Several studies have addressed the relationship between neuronal damage and upregulation of α -synuclein in the midbrain of PD animal models (Vila et al., 2000). In addition, other brain regions have exhibited increased α -synuclein level in response to toxicant induced neuronal destruction (Kiely et al., 2013).

In the current research, MPTP treatment led to depletion of TH and increased level of α -synuclein compared to saline treatment in the LC region. The reduced level and immunoreactivity of TH antibody, confirmed the noradrenergic neuronal degeneration in LC and adjoining noradrenergic areas of pons. In accordance with our findings, Guard et al. (2008) showed degeneration of this region in rats. In addition, it was postulated that degeneration of noradrenergic neurons in LC might start before and accelerate degeneration of dopaminergic neurons in SN as LC region harbors projecting neurons that modulate SN dopaminergic effect (Marien et al., 1993; Fornai et al., 1995, 1997, 2007; Rommelfanger et al., 2007; Rommelfanger and Weinschenker, 2007).

In addition, the current work showed α -synuclein upregulation and accumulation in the LC of MPTP-treated group as compared with saline-treated group. Previous studies have confirmed the overexpression and accumulation of α -synuclein in the dopaminergic system of different PD models (McCormack et al., 2008). It has been reported that α -synuclein upregulation was observed in the midbrain of PD animal models following exposure to neurotoxins; MPTP, Paraquat and Methamphetamine (Fornai et al., 2005; Liao et al., 2005; Manning-Boğ et al., 2002). These previous outcomes were further supported after MPTP injection in non-human primates (McCormack et al., 2008). Furthermore, α -synuclein aggregation was observed in brain regions other than midbrain such as putamen and caudate nuclei as well as hippocampus (Kiely et al., 2013). It was suggested that

augmentation of α -synuclein level is a key factor that might contribute to the PD pathology. The latter finding was ascribed to the interrelation between multiplication of the gene encoding α -synuclein (SNCA), induced expression of the wild-type protein, and the appearance of familial Parkinsonism (Farrer et al., 2004; Singleton et al., 2003).

Moreover, it was reported that α -synuclein is responsible for TH reduction (Giráldez-Pérez et al., 2014). The outcome of the recent work in the LC region can be explained based on the previous postulate. For further support, it was demonstrated that TH enzymatic activity can be controlled via direct relation with α -synuclein (Perez et al., 2002), and that TH expression was elevated in the retina of α -synuclein/ γ -synuclein double knockout (KO) mice as compared with wild type and single KO mice (Surgucheva et al., 2005).

Conclusions

In conclusion, a better understanding of protein changes of α -synuclein as well as TH in the LC of PD rats increases the awareness that PD pathology is not restricted to midbrain only but it is a widespread disease that influences other brain regions.

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