MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Effect of 6-OHDA growth hormone on the calcium binding proteins located in the bulbus olfactorius in Parkinson model rats

Ozlem KIRAZLI[1], Hatice EFEKAN[2], Mehmet ACINIKLI[3], Mesut OZTURK[4], Serhat DEMIR[5], Zeynep BUYUKYILMAZ[3], Umit S. SEHIRLI[1]

[1] Department of Anatomy, School of Medicine, Marmara University, Istanbul, Türkiye.
[2] Department of Anatomy, School of Medicine, Eastern Mediterranean University, Mersin, Türkiye.
[3] Taksim Training and Research Hospital, Istanbul, Türkiye.
[4] Department of Otolaryngology, Fatih Sultan Mehmet Training and Research Hospital, Health Sciences University, Istanbul, Türkiye.
[5] Plastic, Reconstructive and Aesthetic Surgery Clinic, Kartal Dr. Lütfi Kırdar City Hospital, Istanbul, Türkiye.
Corresponding Author: Ozlem Kirazli
E-mail: ozlemsarioz@msn.com

Submitted: 15.03.2024 **Accepted:** 14.05.2024

ABSTRACT

Objective: Parkinson's disease is the second most common neurodegenerative disease. One of the earliest signs is hyposmia which may appear in the premotor period resulting from the alpha-synucleinopathy that affects the neurons along the olfactory pathway. Growth hormone (GH) stimulates cell growth and regeneration. The recovery of neuronal functions may be correlated with the GHs. Calretinin can buffer calcium when intracellular calcium levels increase. This study aims to investigate the effect of GH on calretinin-positive neurons in the olfactory bulb of Parkinsonian rats.

Materials and Methods: 6-hydroxydopamine (6-OHDA) was injected intracranially. GH (0.15 mg/kg/day) and saline were administered subcutaneously for treatment and control groups respectively. Calretinin staining was performed on the sections and calretinin-positive neurons in the lateral olfactory tract were counted.

Results: Thickness of the lateral olfactory tract was lesser in the control group. The decrease in the thickness of the tract may be an important sign of hyposmia. The calretinin-positive neurons in the olfactory bulb of the treatment group are more in number when compared with the control group.

Conclusion: The decrease in expression of calcium-binding proteins is closely related to neurodegenerative diseases. As a result, the slight increase compared to the control group may be due to the neuroprotective effect of GH.

Keywords: Parkinson's disease, Growth hormone, Calretinin, Lateral olfactory tract

1. INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease characterized by the degeneration of dopaminergic neurons mostly located in the substantia nigra pars compacta (SNc). The cardinal signs of PD are bradykinesia, rigidity, tremor, and postural instability which are attributable to the loss of dopaminergic (DA) neurons in the SNc that innervate the basal ganglia. The results showed that the prevalence of PD in industrialized countries may be seen as 1% in people over 60 years of age and this percentage may increase to 4% in people over 80 years of age [1].

No cure has been found for this disease. Various treatment modalities like drug therapy, and deep brain stimulation are available to improve the quality of life. The golden standard way of therapy is L-dopa which can replace the deficiency of dopamine. Unfortunately, the long-term use of L-dopa may cause L-dopa-induced dyskinesia, and dystonia in the clinical aspect **[2].**

To investigate the different treatment modalities and neuroprotective agents for PD, numerous experimental studies are being carried out also. In the experimental procedures, injection of 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), into the medial forebrain bundle or striatum may lead to the degeneration of dopaminergic neurons and affect the motor and cognitive functions of the animals.

How to cite this article: Kirazli O, Efekan H, Acinikli M et al. Effect of 6-OHDA growth hormone on the calcium binding proteins located in the bulbus olfactorius in Parkinson model rats. Marmara Med J 2025;38(1): 36-42. doi: 10.5472/marumj.1627842

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1627842
Marmara Med J 2025;38(1): 36-42

Due to the loss of the dopaminergic neurons, 95% of the striatal dopamine level significantly decreases and for this reason, the decrease in motor performance and cognitive functions may rise. The unilateral injection of the 6-OHDA can cause movement disorder [3]. The intraperitoneal injection of amphetamine into Parkinsonian rats induces a discharge of dopamine release from the intact dopaminergic neurons located on the ipsilateral side. Therefore, the rats are prone to rotate towards the contralateral side of the lesion. The number of these rotations is related to the severity of the lesion [4].

To understand the mechanism of the pathology of neurodegenerative diseases, the effect of several neuroprotective agents is being searched. In this study, we focused on the effect of growth hormone (GH). The GH is a 22 kDa protein that has crucial functions in lipid, carbohydrate, and protein metabolism [5]. The GH is synthesized and secreted by the somatotropic cells located in the anterior lobe of the pituitary gland. The secretion of the GH is regulated by many physiological processes. The GH-releasing hormone (GHRH), GH secretagogue (GHS), and somatostatin have a fundamental role in regulating the secretion of the GH [6]. The GH is a pleiotropic hormone that is a vital component for the development of brain functions. The modulator effect on the neurotransmitters is related to neuronal differentiation and regulation of the functions [7]. The capillary blood of the blood-brain barrier can absorb the GH as was also shown in the experimental models [8].

The studies showed that the neuronal regeneration followed by a head trauma may be related to the GH injection. For the first time, Gustafson et al., revealed the recovery effect of the GH after brain injuries [9]. The efficacy of some hormones like the GH-insulin-like growth factor-1 (GH–IGF-I) is based on the mechanisms of neurogenesis and neuroplasticity [10].

Heredia et al., put forward that the rats with frontal lobe lesions were injected with GHs and these rats had better performance in motor activity tests. Moreover, the injection of the GH enhanced the neurogenesis in the lesioned side and increased the brain plasticity in the motor area of the contralateral side. The expression of the GH increases after brain damage [11].

Many studies claim that the GH is responsible for the neuroprotective mechanisms in neurodegenerative diseases. The mechanism of the neuroprotective effect should be studied in clinical and experimental studies. Hyposmia may be a clinical sign of neurodegenerative diseases like PD, Alzheimer's disease, dementia with Lewy bodies, and synucleinopathies. In PD, olfactory dysfunction is an early symptom before the characteristic motor and cognitive signs [12].

This may be an indicator of the early diagnosis of the patients. The protein accumulations like amyloid (A), hyperphosphorylated Tau (p-Tau), and alpha-synuclein (a-Syn) aggregates, appear in the olfactory bulb. The increase in the density of microglia in the anterior olfactory nucleus is also seen in humans [13]. In the adult mammalian brain, the newly formed neurons move to the olfactory bulb from the sub-ventricular zone. These neurons are classified as two neurogenic niches which are glomerular (synapses between mitral, periglomerular, and tufted cells, juxtaglomerular neurons) and the granular cell layer (GABA-ergic inhibitory neurons) [14].

Ansari and Johnson put forward that PD patients most probably have anosmia when compared to stroke, epilepsy, and head trauma [15]. Furthermore, another study explained that the patients had some problems while detecting and discriminating the smell [16].

Ninety-five percent of the patients have an olfactory disease and 12% of the patients have problems with the function of smelling which may be seen as an early symptom. This is a result of the deficiency of dopamine innervation in the sub-ventricular region and olfactory region [17]. The neurons of the olfactory system are affected by the Lewy bodies. In this way, the modifications of the olfactory system can be used as a marker for early diagnosis [18]. The synthesis of neurotransmitters has a vital role in physiological processes like enzyme activities on the permeability of the membranes, axonal transport, and neuronal sensitivity. The calcium-binding proteins like parvalbumin, and calretinin consist of 240 proteins. The expression of calretinin (D28k and D9k) is dependent on vitamin D.

The calretinin D-28k is expressed in the neurons with long axons, thalamic projection neurons, nigro-striatal neurons, basal ganglia, Meynert neurons, Purkinje cells, retinal, cochlear, and vestibular ganglion cells. Moreover, calretinin is also expressed in the cells with short axons, spinal interneurons, and interneurons of the cortex. The calretinin D-28k is the mostly found one, through the calcium-binding proteins in the nervous system. Calretinin can buffer the calcium and protect the neurons when the intracellular calcium level increases. The ability to buffer calcium provides calcium entry through the mitochondrial membrane and depolarisation, thus preventing the apoptosis of the cell [19]. In addition, the knockout of calretinin in the brains of rats and mice produces disruption in motor coordination [20].

In the preclinical, experimental studies on the animals, some neuroprotective drugs showed neuroprotective effects. However, there is still no cure for preventing cell death or recovering the damaged cells in the clinical aspect. The cardinal motor symptoms may appear following the degeneration of 60-70% dopaminergic neurons in the substantia nigra pars compacta and an 80% decrease in the striatal dopamine level. Therefore, clinical studies related to neuroprotective agents have difficulties in being effective in the early phase of the disease. In Parkinson's disease, cholinergic, adrenergic, and serotonergic neurons may be damaged. Due to the loss of these neurons, several disturbances like sleep disorders, amnesia, anxiety, depression, and gastrointestinal diseases may arise before the motor disorders. Unfortunately, the symptoms of PD, except the motor symptoms, do not respond to the treatment of dopaminergic agents [20].

Various studies focused on the symptoms of anosmia in many diseases, particularly PD [21,22]. PD is known as a 'resting tremor' disease but the symptoms of anosmia should not be also ignorable for early diagnosis [23].

Although, many promising studies searched for the precursor or fatal cell transplant for experimental animal models, any procedure has not been approved ethically in the clinical aspect. The GH may support the treatment of neurodegenerative diseases. In the literature, no study shows the direct influence of the GH on Parkinson's disease.

In this study, we focused on observing the effect of the GH injection on the calretinin-positive neurons in the olfactory bulb of the Parkinsonian rat brain.

2. MATERIALS and METHODS

Male Sprague-Dawley albino rats (300–350 g, 10 weeks) were used in the experiments. The animals were supplied by the Marmara University Animal Center and caged individually at 21 °C on a 12-h light/dark cycle (lights out from 08:00 to 20:00) with unlimited food and water access. All experiments were carried out with the approval of the Marmara University Ethical Committee for Experimental Animals (06.2018.mar). 6-OHDA (Sigma H116, St Louis, MO, USA; dissolved in saline 0.9% NaCl) was used to PD rat model. To achieve extensive degeneration in dopaminergic neurons, animals had two unilateral injections of 6-OHDA (8 µg/4 µl per site) into the medial forebrain bundle along the nigrostriatal pathway using the stereotaxic method.

The bregma was used as the reference point and the coordinates of the lesions were anteroposterior: – 2.1 mm, lateral: 2.0 mm, ventral: – 7.8 mm for the first injection, and anteroposterior: – 4.3, lateral: 1.5, ventral: – 7.8 for the second injection [24].

Following the stereotaxic surgery, the rats were divided into two groups:

1. The rats with 6-OHDA injection (n=5)
2. The rats with 6-OHDA and the GH injections (n=5)

Growth hormone injection

Following the 6-OHDA injection, the GH was delivered by subcutaneous injection with a concentration of 0.15 mg/kg/day for the treatment group. The dosage was determined and calculated by considering their weight. The GH was dissolved in the saline. The control group was injected with physiologic saline with a concentration of 0.15 mg/kg/day.

Rotation test

The rotation test was performed to measure the impairment. The test was held on the 21st day and following 6-OHDA injections. The rotational behaviour was induced by subcutaneous apomorphine injection (0. 05 mg/kg) and the number of contralateral and ipsilateral (injection side) rotations were counted for 30 min. The number of rotations was noted and statistically analysed.

Following the rotational behaviour test, the animals were deeply anesthetized with ketamine (100 mg/kg, intraperitoneal), transcranial perfused with 50 mM phosphate-buffered saline (PBS), and fixed with 4% paraformaldehyde (PFA) in 100 mM phosphate buffer at pH 7.4. The brain was removed, post-fixed in the fixative overnight, and transferred into a 30% sucrose solution for cryoprotection.

For immunohistochemistry investigations, serial coronal brain sections (40 µm) were taken in the anteroposterior direction (striatum and substantia nigra) using a sliding microtome. One of the three sections was taken for tyrosine hydroxylase (TH) and calretinin immunohistochemistry procedures, respectively.

TH immunohistochemistry procedure

The sections were rinsed in PBS three times for 5 min and incubated in 80 ml methanol and 3% H2O2 for 20 min (at room temperature) to block endogenous peroxidase activity.

After rinsing in PBS, the sections were incubated in blocking serum solution (10 ml PBS, 1 ml normal goat serum, 0.1 g bovine serum albumin (BSA), and 30 µl Triton™ X-100 (Merck, Darmstadt, Germany) for 1 h, followed by incubation in primary antibody (anti-tyrosine hydroxylase antibody (Pel Freez/cat no: P40101) in 1:500 10 ml carrier solution) for overnight at room temperature. After the rinsing period, sections were incubated in 150 µl biotinylated anti-rabbit IgG secondary antibody for 2 h (Vector labs, CA, USA), then rinsed in PBS and subsequently incubated in the avidin-biotinylated complex for 1 h at room temperature (Vector lab, ABC kit, CA, USA). Immunoreactivity was visualized by incubating the sections in a solution of 3, 3′-diaminobenzidine (DAB) (10 ml PBS, 5 mg DAB, 100 µl 3% H2O2). The sections were mounted on gelatin-coated slides and examined under a light microscope.

Calretinin immunohistochemistry procedure

Calretinin immunohistochemistry was performed by rinsing the mounted sections three times in PBS and incubating overnight at room temperature in PBS containing 1:500 anti-calretinin antibody (Merck, Istanbul, Turkey), 0.3 Triton™ X-100 (Merck, Istanbul, Turkey), and 1% serum (Sigma-Aldrich, Istanbul, Turkey). After PBS rinsing, the sections were incubated for 90 min in a secondary antibody solution of Alexa Fluor 488 donkey anti-mouse IgG (Invitrogen, Carlsbad, CA, USA).

The sections were examined at×40 magnification, under a fluorescence microscope. The stereotaxic coordinates of the lateral olfactory tract (LOT) (5.64mm anterior to bregma) were determined from a rat brain atlas [24]. The five sections from each brain were obtained and the calretinin-positive interneurons were counted (Figure 1 and Figure 2).

Statistical Analysis

The GraphPad Prism 6 software was used for the analysis of the data. The non-parametric t test was performed. Data in the text and figures were shown as mean. A $p < 0.05$ value was considered statistically significant.

3. RESULTS

The mean number of the calretinin-positive neurons in the olfactory bulb of the treatment group (Figure 1) was counted as approximately 534.18 whereas the mean number of the

http://doi.org/10.5472/marumj.1627842
Marmara Med J 2025;38(1): 36-42

calretinin-positive neurons in the control group (Figure 2) was counted as 473.90. The number of calretinin-positive neurons located in the olfactory bulb of the treatment group displayed a slight increase compared to the control group but this increase was statistically not significant (Figure 5).

On the other hand, the density of TH-positive neurons in the substantia nigra pars compacta did not show any difference between the treatment and Parkinsonian groups (Figure 3 and Figure 4). The microscopic observations also exhibited that the thickness of the LOT of the control group decreased when compared to the treatment group.

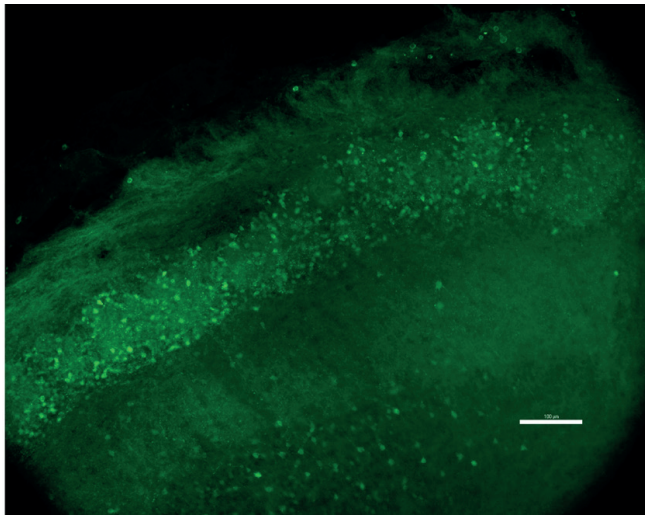

Figure 1. *Calretinin-positive neurons located in the olfactory bulb of the treatment group*

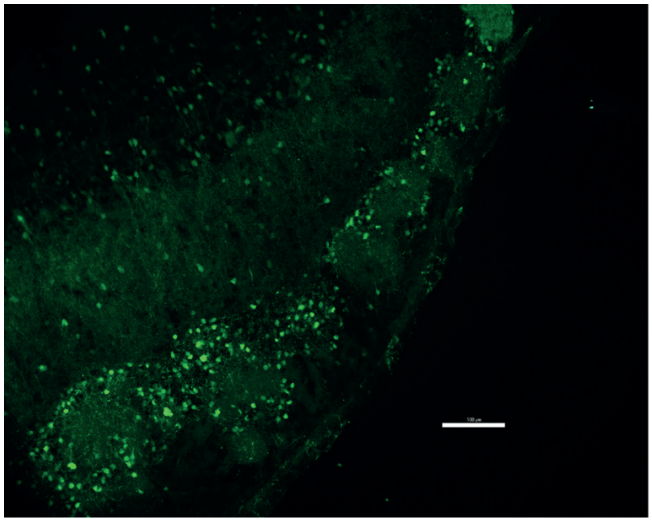

Figure 2. *Calretinin-positive neurons located in the olfactory bulb of the control group*

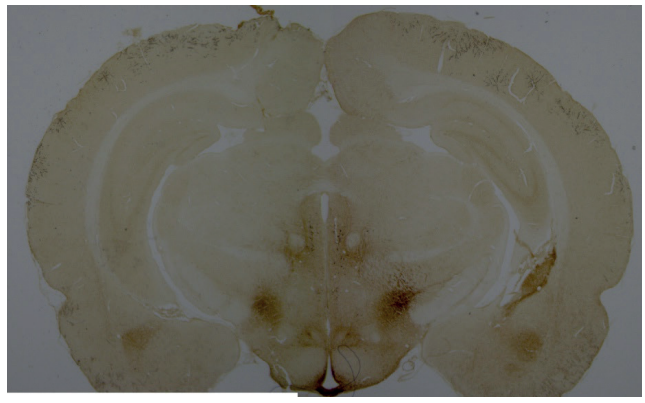

Figure 3. *TH-positive neurons in substantia nigra pars compacta of the control group*

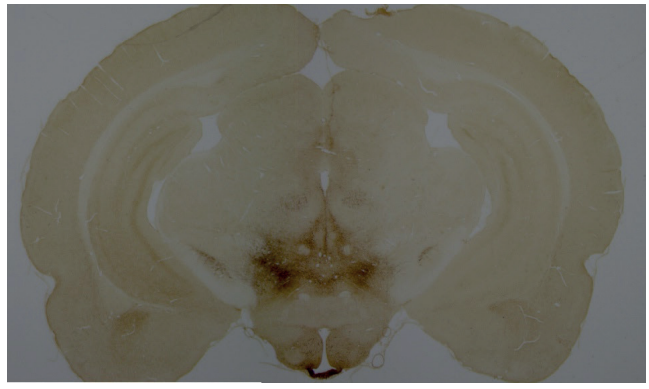

Figure 4. *TH-positive neurons in substantia nigra pars compacta of treatment group*

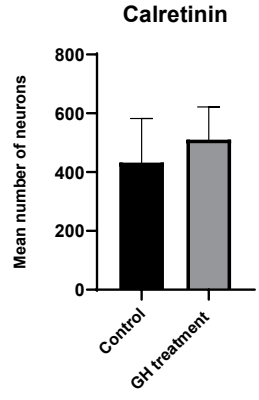

Figure 5. *The mean number of the calretinin-positive neurons in the treatment group is counted as approximately 534.18 whereas the mean number of the calretinin-positive neurons in the control group is counted as 473.90.*

4. DISCUSSION

In this study, the number of calretinin-positive neurons located in the olfactory bulb of Parkinsonian rats was investigated following a GH injection. The number of calretinin-positive neurons in the olfactory bulb of the treatment group showed a slight increase compared to the control group (Figure 5). This result put forth that the increase in calretinin-positive neurons may be related to the neuroprotection mechanism because the literature supposes that the increase in calcium-binding proteins like calretinin, calbindin, and calmodulin may be neuroprotective for degenerative diseases. Based on this interaction between the effect of GH and calcium-binding proteins, new approaches to treatment modalities for PD may appear in further studies.

The GH is a mainstay in the growth and development of the brain, as well as in processes like neurogenesis and plasticity [25]. Both GH and IGF-1 may exhibit similar functions in the physiological processes and they can cross the blood-brain barrier [26].

A wide range of pathways of the neurotransmitters regulates GH secretion in different ways. The neurotransmitters may either act directly on the anterior pituitary gland or modulate GH-releasing hormone (GHRH) or somatostatin release [27]. The anterior pituitary gland secretes GH. The liver and brain can affect the activation of GH receptors and the secretion of IGF-1 [28]. The GH receptors on the membrane of the neurons in the hypothalamus, thalamus, cortex, cerebellum, brainstem, and retinal ganglion cells are activated by the GH. Furthermore, the glial cells (microglia, astrocytes, and oligodendrocytes) are also affected by the GH [29]. The growth factors have essential roles in the signalling mechanisms related to the astrocytes and neurons [30]. The GH may inhibit the caspase 3 expression and the hormone can provide a neuroprotective effect by deactivating the apoptosis-promoting gene [12].

The role of calcium-binding proteins is very debatable in the literature. Several studies suggested that calretinin acts as a calcium modulator due to its limited calcium binding capacity. Calretinin may trigger the binding capacity of the EF-hand domain when intracellular calcium levels increase [31].

The decrease in the level of calretinin and calbindin in the striatum may be responsible for the motor disturbance [32]. Many factors like age, the severity of an injury, and the type of degeneration may be effective in the expression of calcium-binding proteins [19]. In a study, the upregulation of brain-derived neurotrophic factor (BDNF) induced a reduction in calretinin expression [33]. On the other side, in our study, the GH caused an increase in the number of calretinin-positive neurons in the olfactory bulb.

The calcium-binding proteins like calretinin and calbindin are very closely associated with resistance [19]. These proteins also have a sensor function when there is a dysregulation related to the intracellular calcium concentration. In this way, calretinin and calbindin can act as neuroprotective agents in neuroprotective diseases like Alzheimer's and Parkinson's disease [34].

Tsuboi et al., revealed that the calretinin-positive dopaminergic neurons located in the substantia nigra pars compacta were intact following the intra-striatal injection in a rat brain of the Parkinsonian group [35].

This result showed that calretinin expression is essential in the neuroprotective mechanism for the dopaminergic neurons. Another study found that the number of calretinin-positive interneurons located in the striatum decreased after a 6-OHDA nigro-striatal injection. This decrease might be due to the hyperactivity of the glutamatergic receptors and a reduction in the number of dopaminergic axons. In this study, the number of parvalbumin-positive interneurons in the striatum remained the same [36]. A study searched for the relationship between estrogen, calretinin, and dopaminergic neurons. Short-term estrogen treatment increased the calretinin expression on the dopaminergic neurons and presented neuroprotection on the dopaminergic neurons [37].

It is still difficult to identify possible biomarkers and early molecular pathways in PD. According to recent studies, the early stages of the disease may involve new functions for post-translational citrullination/deimination brought on by the calcium-activated enzyme family known as peptidylarginine deiminases (PADs). The current study evaluated PAD isozymes in the 6-OHDA driven rat model of pre-motor PD. The pre-motor PD model and controls were examined in six different brain regions: the cortex, hippocampus, striatum, midbrain, cerebellum, and the olfactory bulb. The citrullinated protein IDs of the PD model and the controls differed significantly for every area of the brain [38]. As it is known that striatal activity is related to the motor performance of rats, an experimental study focused on the effect of GH injection on dendrite morphology in the dorsal striatum of the 6-OHDA-induced rats. The number of mushroom and stubby dendritic spine types increased in the GH-injected group brains compared to the control group brains on the 21st day, 2nd month, and 3rd month following the procedure [39].

Our study also indicated that the injection of GH following the 6-OHDA injection did not show any difference in the dopaminergic neurons. The period of 1 month may be an insufficient time to elicit a neuroprotective effect on the dopaminergic neurons in a Parkinsonian rat brain.

As a result, this study put forward that the slight increase in the number of calretinin-positive neurons in the treatment group was seen due to a neuroprotective effect of GH for PD. Moreover, the change in morphology of the olfactory tract may be related to hyposmia. Further and long-term studies may enlighten the relationship between the mechanism of GH and calcium-binding proteins in the pathology of PD.

Compliance with Ethical Standards

Ethical approval: All experiments were carried out with the approval of the Marmara University Ethical Committee for Experimental Animals (approval number: 06.2018.mar).

Conflict of interest: The authors declare that there is no conflict of interest.

http://doi.org/10.5472/marumj.1627842
Marmara Med J 2025;38(1): 36-42

Financial support: This study received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Authors contributions: OK: Responsibility for all of the work, HE: Writing the manuscript and literature search, MA and SD: Literature search, MO and ZB: Data collection, USS: Responsibility for all of the work. All authors approved the final version to be published.

REFERENCES

[1] de Lau LM, Koudstaal PJ, Hofman A, Breteler MM. Subjective complaints precede Parkinson disease: the Rotterdam study. Arch Neurol 2006;63:362-5. doi: 10.1001/archneur.63.3.noc50312.

[2] P Soares-da-Silva 1, M P Serrão, M A Vieira-Coelho, M Pestana. Evidence for the involvement of P-glycoprotein on the extrusion of taken up L-DOPA in cyclosporine A treated LLC-PK1 cells. Br J Pharmacol 1998;123:13-22. doi: 10.1038/sj.bjp.0701572

[3] Ungerstedt U. Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol Scand Suppl 1971:367:95-122. doi: 10.1111/j.1365-201x.1971.tb11001.

[4] Emborg ME. Evaluation of animal models of Parkinson's disease for neuroprotective strategies. J Neurosci Methods 2004;139:121-43. doi: 10.1016/j.jneumeth.2004.08.004.

[5] Davidson MB. Effect of growth hormone on carbohydrate and lipid metabolism. Endocr Rev 1987 ;8:115-31. doi: 10.1210/edrv-8-2-115

[6] Tannenbaum GS. Growth hormone-releasing factor: direct effects on growth hormone, glucose, and behavior via the brain. Science 1984;226(4673):464-6. doi: 10.1126/science.6436973.

[7] Arce VM, Devesa P, Devesa J. Role of growth hormone (GH) in the treatment on neural diseases: from neuroprotection to neural repair. Neurosci Res 2013;76:179-86. doi: 10.1016/j.neures.2013.03.014

[8] Pan, W, Yu Y, Cain CM, Nyberg F, Couraud PO, Kastin AJ. Permeation of growth hormone across the blood-brain barrier. Endocrinology 2005, 146, 4898-904. doi: 10.1210/en.2005-0587.

[9] Gustafson K, Hagberg H, B A Bengtsson BA, Brantsing C, Isgaard J. Possible protective role of growth hormone in hypoxia-ischemia in neonatal rats. Pediatr Res 1999 ;45:318-23. doi: 10.1203/00006.450.199903000-00005

[10] Jan Frystyk. Exercise and the growth hormone-insulin-like growth factor axis. Med Sci Sports Exerc 2010 ;42:58-66. doi: 10.1249/MSS.0b013e3181b07d2d

[11] Heredia M, Fuente A, Criado J, Yajeya J, Devesa J, Riolobos A S. Early growth hormone (GH) treatment promotes relevant motor functional improvement after severe frontal cortex lesion in adult rats. Behav Brain Res 2013;247:48-58. doi: 10.1016/j.bbr.2013.03.012.

[12] Li R C, Guo S Z, Raccurt M, et al. Exogenous growth hormone attenuates cognitive deficits induced by intermittent hypoxia in rats. Neuroscience 2011, 196, 237-50.

[13] Kohl Z, Schlachetzki JC, Feldewerth J, et al. Distinct pattern of microgliosis in the olfactory bulb of neurodegenerative proteinopathies. Neural Plast 2017:2017:3851262. doi: 10.1155/2017/3851262.

[14] Lois C, García-Verdugo JM, Alvarez-Buylla A. Chain migration of neuronal precursors. Science 1996 ;271:978-81. doi: 10.1126/science.271.5251.978

[15] Ansari KA, Johnson A. Olfactory function in patients with Parkinson's disease. J Chronic Dis 1975 ;28:493-7. doi: 10.1016/0021-9681(75)90058-2.

[16] Hang W, Liu G, Han T, Zhang P, Zhang J. Olfactory dysfunction and neurotransmitter disturbance in olfactory bulb of transgenic mice expressing human A53T mutant α-synuclein. PLoS One 2015 ;10:e0119928. doi: 10.1371/journal.pone.0119928

[17] Schwerk A, Altschüler J, Roch M, et al. Human adipose-derived mesenchymal stromal cells increase endogenous neurogenesis in the rat subventricular zone acutely after 6-hydroxydopamine lesioning. Cytotherapy 2015;17:199-214. doi: 10.1016/j.jcyt.2014.09.005. Epub 2014 Oct 24.

[18] Mucignat C, Caretta A. Drug-induced Parkinson's disease modulates protein kinase A and olfactory marker protein in the mouse olfactory bulb. Behav Brain Funct 2017;13:1. doi: 10.1186/s12993.017.0119-

[19] Fairless R, Williams SK, Diem R. Calcium-binding proteins as determinants of central nervous system neuronal vulnerability to disease. Int J Mol Sci 2019; 20:2146. doi: 10.3390/ijms20092146

[20] Castro ES, Santos-García D, de Deus Fonticoba T, et al. Causes, and factors related to dopamine agonist withdrawal in Parkinson's disease. Brain Behav 2016 ;6: e00453. doi: 10.1002/brb3.453

[21] Leboucq N, Menjot de Champfleur N, Menjot de Champfleur S, Bonafé A. The olfactory system. Diagn Interv Imaging 2013;94:985-91. doi: 10.1016/j.diii.2013.06.006.

[22] Welge-Lüssen A. Ageing, neurodegeneration, and olfactory and gustatory loss. B-ENT 2009;5 Suppl 13:129-32.

[23] Hawkes CH. Is Parkinson's disease inherited? Br J Hosp Med 1997;4:130-3.

[24] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th edition. Massachusetts: Academic Press, 2006.

[25] Aberg ND, Brywe K G, Isgaard J. Aspects of growth hormone and insulin-like growth factor-i related to neuroprotection, regeneration, and functional plasticity in the adult brain. Sci World J 2006; 6:53-80. doi: 10.1100/tsw.2006.22

[26] Nagaraja TN, Patel P, Gorski M, et al. In normal rat, intraventricularly administered insulin-like growth factor-1 is rapidly cleared from CSF with limited distribution into brain. Cerebrospinal Fluid Res 2005;2:5. doi: 10.1186/1743-8454-2-5

[27] Giustina A, Veldhuis JD. Pathophysiology of the neuroregulation of growth hormone secretion in experimental

animals and the human. Endocr Rev 1998;19:717-97. doi: 10.1210/edrv.19.6.0353

[28] Frago LM, Pañeda C, Dickson SL, et al. Growth hormone (GH) and GH-releasing peptide-6 increase brain insulin-like growth factor-i expression and activate intracellular signaling pathways involved in neuroprotection. Endocrinology 2002;143:4113-22. doi: 10.1210/en.2002-220261

[29] Grasso S, Bramanti V, Tomassoni D, et al. Effect of lipoic acid and α-glyceryl-phosphoryl-choline on astroglial cell proliferation and differentiation in primary culture. J Neurosci Res 2014;92:86-94. doi: 10.1002/jnr.23289

[30] Lobie PE, García-Aragón J, Lincoln DT, et al. Localization and ontogeny of growth hormone receptor gene expression in the central nervous system. Dev Brain Res 1993;74:225-33. doi: 10.1016/0165-3806(93)90008-X

[31] Baimbridge KG, Celio MR, Rogers JH. Calcium-binding proteins in the nervous system. Trends Neurosci 1992;15:303-8. doi: 10.1016/0166-2236(92)90081-I

[32] Grillner S, Hellgren J, Menard A. Mechanisms for selection of basic motor programs – roles for the striatum and pallidum. Trends Neurosci 2005;28:364-70. doi: 10.1016/j.tins.2005.05.004

[33] Fiumelli H, Kiraly M, Ambrus A, et al. Opposite regulation of calbindin and calretinin expression by brain-derived neurotrophic factor in cortical neurons. J Neurochem 2000;74:1870-7. doi: 10.1046/j.1471-4159.2000.074.1870.x

[34] Schwaller B. Calretinin: from a "simple" Ca2+ buffer to a multifunctional protein implicated in many biological processes. Front Neuroanat 2014;8. doi: 10.3389/fnana.2014.00003

[35] Tsuboi K, Kimber TA, Shults CW. Calretinin-containing axons and neurons are resistant to an intrastriatal 6-hydroxydopamine lesion. Brain Res 2000;866:55-64. doi: 10.1016/S0006-8993(00)02219-8

[36] Ma Y, Zhan M, OuYang L, et al. The effects of unilateral 6-OHDA lesion in medial forebrain bundle on the motor, cognitive dysfunctions and vulnerability of different striatal interneuron types in rats. Behav Brain Res 2014;266:37-45. doi: 10.1016/j.bbr.2014.02.039

[37] Yi H, Bao X, Tang X. Estrogen modulation of calretinin and BDNF expression in midbrain dopaminergic neurons of ovariectomised mice. J Chem Neuroanat 2016;77:60-7. doi: 10.1016/j.jchemneu.2016.05.005

[38] Mercer A, Sancandi M, Maclatchy A, Lange, S. Brain-Region-specific differences in protein citrullination/deimination in a pre-motor Parkinson's disease rat model. Int J Mol Sci 2024; 25: 11168. doi: 10.3390/ijms252011168

[39] Kirazli O, Ozkan M, Verimli U, Gulhan R, Arman A, Sehirli U S. The effect of growth hormone on motor findings and dendrite morphology in an experimental Parkinson's disease model. Anat Sci Int 2024 10.1007/s12565.024.00790-6. doi.org/10.1007/s12565.024.00790-6 Epub ahead of print.