

## Vasoactive intestinal peptide and Parkinson's disease

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Using an experimental model which represents the end-stage of Parkinson's disease (PD), we aimed to measure the levels of glutathione reductase activity, lipid peroxidation in different brain regions (cortex, hippocampus) in the presence or absence of vasoactive intestinal peptide (VIP), which is a 28-amino acid "brain-gut" neuropeptide. A total of 20 male Wistar rats, weighing 150-200 g at the time of surgery, were randomly divided in groups and housed in cages with free access to rat chow and water. The rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), had their heads shaved, and placed in a stereotaxic apparatus. The target coordinates were: AP = +0.2; LR = -3.0; H = -5.6 according to the stereotaxic atlas. The experimental group received an injection of 20 µg/2 µl of 6-hydroxydopamine (6-OHDA), while the control group received an injection of 2 µl saline. All injections were made into the right striatum area by a Hamilton microsyringe at a rate of 1 µl/min. The wound was closed with stainless steel clips and the rat was allowed to recover before being returned to its cage. VIP (13µg/2 µl) was injected into the right striatum 15 min before 6-OHDA lesion and at the 21<sup>st</sup> day after surgery. Our experiments showed that the neuropeptide decreased the activity of enzyme glutathione reductase and inhibited lipid peroxidation in the experimental model of Parkinson's disease counteracting in such way against membrane damage and ameliorating the cell viability.

**Keywords:** Vasoactive intestinal peptide; Parkinson's disease; Lipid peroxidation; Glutathione reductase

### INTRODUCTION

Parkinson's disease (PD) is currently regarded as the most common neurodegenerative disorder of the aging brain after the Alzheimer's dementia. Clinically, PD is characterized by the tremor at rest, slowness of voluntary movements, rigidity, and postural instability [1]. The cardinal biochemical abnormality in PD is the profound deficit in brain dopamine level, primarily, but not exclusively, attributed to the loss of neurons of the nigrostriatal dopaminergic pathway [2]. Parkinson's disease is among the causes of death in people over 65 years of age. For example the boxing legend Muhammad Ali, who recently died, suffered from PD. Although the pathogenesis of PD are still unknown there is increasing evidence that impairment of mitochondrial function, oxidative damage and inflammation are certainly involved [3]. Some new knowledge about this neurodegenerative disorder has been achieved by in vitro and in vivo experimental models of PD [2]. Most popular of them is 6-hydroxydopamine-induced Parkinson's disease rat model. Injected stereotaxically, unilateral in striatum this dopamine analog

produces a more protracted retrograde degeneration of nigrostriatal system which can last from 1-3 weeks after lesion [4, 5]. The toxic effects of 6-OHDA are due to enhanced oxidative stress, inflammatory processes and apoptosis [6].

We aimed to measure the levels of glutathione reductase activity and lipid peroxidation in different brain regions (cortex, hippocampus) in the presence or absence of vasoactive intestinal peptide (VIP) by means of 6-hydroxydopamine-induced PD rat model, which represents the end-stage of this disease.

### EXPERIMENTAL

All experiments have been performed according to the "Principles of laboratory animal care" (NIH publication No. 85-23), and the rules of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences (registration FWA 00003059 by the US Department of Health and Human Services).

#### *Surgical procedures*

A total of 20 male Wistar rats, weighing 150-200 g at the time of surgery, were randomly divided in groups and housed in cages with free access to rat chow and water. The rats were anesthetized with

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chloral hydrate (400 mg/kg, i.p.), had their heads shaved, and placed in a stereotaxic apparatus. The scalp was cleaned with a jodine solution, incised on the midline and a burr hole was drilled through the skull at the appropriate location. The target coordinates were: AP = +0.2; LR = -3.0; H = -5.6 according to the stereotaxic atlas [7]. The experimental group received an injection of 20 µg/2 µl of 6-OHDA (Sigma-Aldrich, St. Louis, MO, USA; calculated as free base, dissolved in ice-cold saline with 0.02 % ascorbic acid) while the control group received an injection of 2 µl saline. All injections were made into the right striatum area by a Hamilton microsyringe at a rate of 1 µl/min. The needle was left in place an additional 2 min before being slowly withdrawn. The wound was closed with stainless steel clips and the rat was allowed to recover before being returned to its cage. VIP (13µg/2µl) was injected in the striatum twice: 15 min before 6-OHDA lesion and at the 21<sup>st</sup> day after surgery.

#### Biochemical procedures

Protein content was measured by the method of Lowry et al. [8]. Lipid peroxidation in the absence and in the presence of an inducer (5.10–5 M Fe<sup>2+</sup>) was determined by the amount of the thiobarbituric acid-reactive substances, formed in fresh preparations for 60 min at 37°C [9]. The absorbance was read at 532 nm against appropriate blanks; the absorbance at 600 nm was considered to be a non-specific baseline and was, therefore, subtracted from A532. Glutathione reductase activity was measured by the method of Pinto & Bartley [10].

#### Statistical analysis

Results were expressed as mean ± S.E.M. Statistical analysis of the data was performed by Student's *t*-test for unpaired data or by one-way analysis of variance (ANOVA) followed by Newman-Keuls post-test. *P*-values < 0.05 were considered significant.

### RESULTS AND DISCUSSION

It is considered that the reduction of apomorphine-induced rotational behavior in 6-OHDA-lesioned rats is the most utilized method for assessing functional efficacy in this model of PD [11]. The rotations were measured according to a method as described previously [12]. Briefly, the animals were allowed to habituated for 10 min and then 1 min after the injection (apomorphine 2mg/kg, s.c), the rotations were counted. Number of rotations was monitored in a cylindrical container

(a diameter of 33 cm and a height of 35 cm) for 1 hour in a dimly-lighted room. The ipsilateral rotation was not significant (Fig. 1). All animals that made more than 30 turns/30 min opposite to the lesion were selected for the experiments (Fig.2).

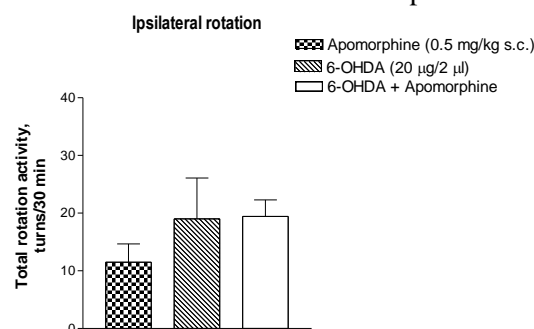


Fig. 1. Rotational behavior of rats to the same side of the lesion.

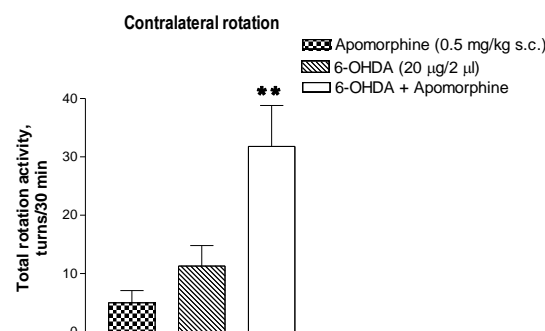


Fig. 2. Rotational behavior of rats opposite to the side of the lesion; \*\**P* ≤ 0.01.

Vasoactive intestinal peptide (13µg/2 µl), injected into the right striatum 15 min before 6-OHDA lesion and at the 21<sup>st</sup> day after surgery decreased the levels of enzyme glutathione reductase significantly in the cortex (Fig. 3) and lowered it in the hippocampus of Parkinsonian rats (Fig. 4).

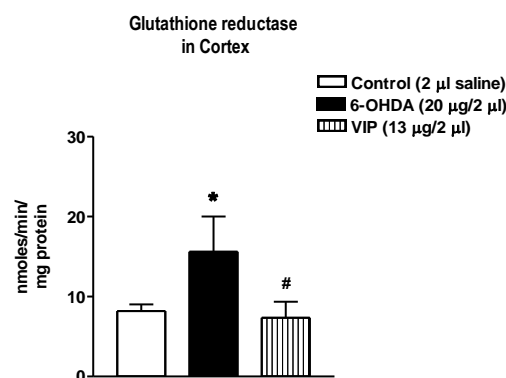
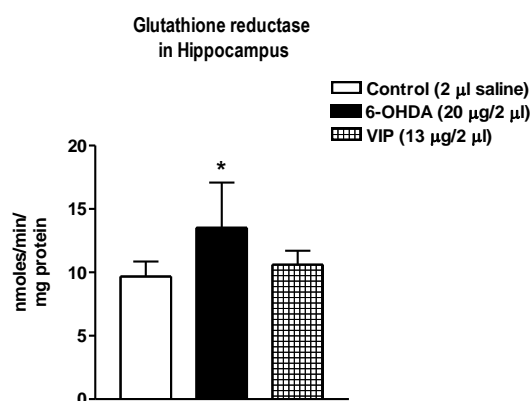


Fig. 3. Levels of glutathione reductase in the cortex of control and Parkinsonian rats. *n* = 5; \**P* ≤ 0.05 vs Control; #*P* ≤ 0.05 vs 6-OHDA-lesioned rats.

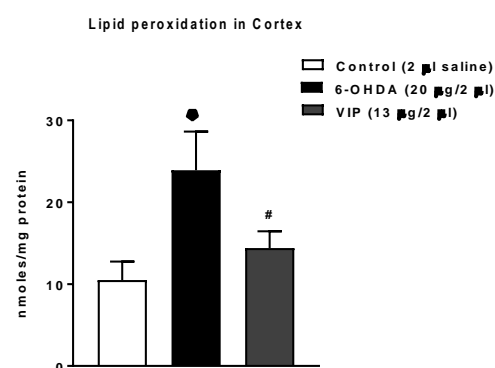


**Fig. 4.** Levels of glutathione reductase in the hippocampus of control and Parkinsonian rats.  $n = 5$ ; \* $P \leq 0.05$  vs Control.

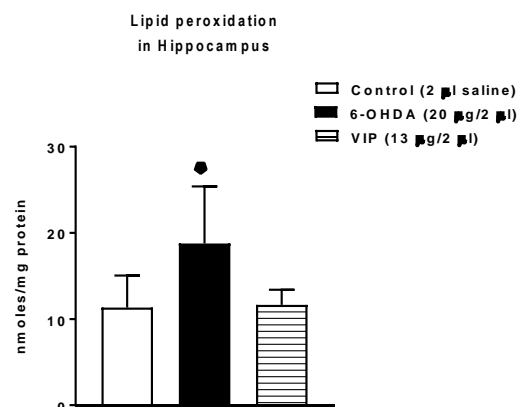
Vasoactive intestinal peptide is now recognized as a major neuropeptide in the brain, with function ranging from neurotransmission to neuromodulation with neurotrophic properties. This neuropeptide is found in high concentration in the cerebral cortex, amygdala, striatum, hippocampus, midbrain [13]. VIP is a peptide with potent anti-inflammatory, anti-oxidant and anti-apoptotic effect [14, 15, 16]. Neuroprotective effect of vasoactive intestinal peptide in a mouse model of Parkinson's disease by blocking microglial activation was shown [17]. Moreover, the team of professor IllanaGozes reported neuroprotection by stearyl-Nle17-VIP, vasoactive intestinal peptide, and NAP (8aa) against the buthioninesulfoximine, a selective inhibitor of glutathione synthesis, suggesting that the mechanism may involve the glutathione antioxidant system [18]. Our results are in accordance with the above-mentioned hypothesis, showing that vasoactive intestinal peptide decreased the activity of the enzyme glutathione reductase in a Parkinson's disease model.

We also demonstrated that VIP (13µg/2 µl) decreased lipid peroxidation both in cortex (Fig. 5) and hippocampus (Fig. 6) in the 6-hydroxydopamine-induced rat model of Parkinson's disease. Lipid peroxidation is a crucial step in the pathogenesis of several disease states in adult and infant patients. The reactive oxygen species (hydroxyl radical, hydrogen peroxide etc.) readily attack the polyunsaturated fatty acids of the fatty acid membrane, initiating a self-propagating chain reaction. The destruction of membrane lipids and the end-products of such lipid peroxidation reactions are especially dangerous for the viability of cells, even tissues. Since lipid peroxidation is a self-propagating chain-reaction, the initial oxidation of only a few lipid molecules can result in significant tissue damage. Lipid peroxidation has

been implicated in Parkinson's disease. It was also reported that both VIP and PACAP have neuroprotective effects in PD models by inhibiting the production of inflammatory mediators [19]. Vasoactive intestinal peptide family was proved to be a therapeutic target for Parkinson's disease [20].



**Fig. 5.** Levels of Fe-ascorbat induced lipid peroxidation in the cortex of control and Parkinsonian rats.  $n = 4$ ; \* $P \leq 0.05$  vs Control; # $P \leq 0.05$  vs 6-OHDA-lesioned rats.



**Fig. 6.** Levels of Fe-ascorbat induced lipid peroxidation in the hippocampus of control and Parkinsonian rats.  $n = 4$ ; \* $P \leq 0.05$  vs Control.

## CONCLUSION

In the present study we demonstrated that vasoactive intestinal peptide decreased the activity of enzyme glutathione reductase and inhibited lipid peroxidation in the experimental model of Parkinson's disease counteracting in such way against membrane damage and ameliorating the cell viability.

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## ВАЗОАКТИВЕН ИНТЕСТИНАЛЕН ПЕПТИД И БОЛЕСТ НА ПАРКИНСОН

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(Резюме)

Цел на нашите експерименти беше, използвайки експериментален модел на болестта на Паркинсон, да се проследят промените в активността на ензима глутатион редуктаза и нивата на липидна пероксидация в две различни мозъчни структури (мозъчна кора и хипокам) в присъствие или отсъствие на вазоактивен интестинален пептид (ВИП), който е 28 аминокиселинен пептид, принадлежащ към групата на „мозъчно-чревните“ пептиди. Използвани бяха 20 мъжки, половозрели плъха от породата Wistar с тегло 150-200 г. Животните бяха разделени на групи на случаен принцип със свободен достъп до храна и вода. Плъховете бяха анестезирани с хлоралхидрат (400 mg/kg, интраперитонеално), главата им се обръсваше и се поставяха на стереотаксичен апарат. Таргетните координати за стриатум бяха: AP = +0.2; LR = -3.0; H = -5.6 в съответствие със стереотаксичен атлас. На експерименталната група животни се инжектираше 20 µg/2 µl of 6-хидроксидопамин (6-OHDA), а на контролната 2 µl физиологичен разтвор. При всички групи животни инжектиранията се осъществяваха в десен стриатум посредством микроспринцовка Хамилтон при скорост на вливането 1 µl/min. Раната се затваряше чрез неръждаеми клипсове след което плъховете се оставяха да се възстановят. ВИП (13 µg/2 µl) се инжектираше в десен стриатум 15 минути преди лезията с 6-OHDA и на 21<sup>ия</sup> ден след операцията. Получените от нас резултати показват, че при използвания експериментален модел на болестта на Паркинсон е налице понижение в активността на ензима глутатион редуктазата и потискане на липидната пероксидация, което означава, че увреждането на клетъчните мембрани е намалено, а жизнеспособността на клетките е повишена.