

Neuroprotective effect of Vinpocetine against 3- NP Induced reduction of body weight and oxidative stress in Rats

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Abstract

Huntington's disease is a progressive, degenerative disease characterized by abnormal body movements symptoms like chorea and a reduction of body weight. Recently, it has been reported that oxidative stress, which is one of the pathological hallmarks of various neurodegenerative disorders, also plays an important role in the pathogenesis of Huntington's disease. 3- Nitropropionic acid, a neurotoxin treatment significantly reduction in body weight. Intraperitoneal administration of 3-nitropropionic acid (10 mg/kg for 14 days) caused significant loss of body weight and poor retention of memory. Biochemical analysis revealed that 3-NP administration significantly increase in lipid peroxidation in the brains of rats. The present study demonstrated that inhibition of type 1 phosphodiesterase (PDE1) by vinpocetine (5, 10 & 20mg/kg) significantly reversed behavioral and biochemical dysfunction in 3-NP treated group. The result of the present study suggests facilitatory role of PDE1 enzyme in loss in body weight and oxidative stress following 3-NP injection.

Keywords: 3- Nitropropionic acid, Vinpocetine, Huntington Disease.

Introduction

Huntington's disease is a dominantly inherited neurodegenerative disorder characterized by progressive worsening chorea, motor impairment and psychiatric disturbances leading to inexorable decay and death¹. The degenerative process involves medium spiny striatal neurons and lesser extent cortical neurons². To investigate the mechanism of neurodegeneration in HD, animal models of HD have been generated using

genetic manipulations, excitotoxins and neurotoxins. Injections of NMDA receptor agonists, such as quinolinic acid, into the striatum, induce HD like pathology, with a loss of projecting MSN and sparing of cholinergic and NADPH diaphorase neurons³. 3-NP, a mycotoxin, is a suicide inhibitor of succinate dehydrogenase (SDH), enzyme located in mitochondrial inner member. Inhibition of SDH interferes with electron cascade and interrupts oxidative phosphorylation. This phenomenon leads to reduced ATP synthesis and oxidative

stress⁴. Growing body of evidences suggests the involvement of impaired energy metabolism, excitotoxicity and production of inflammatory cytokines leading to neuronal death, by both necrosis and apoptosis. These events of neurodegeneration are relevant to the striatal cell loss seen in HD⁵. Elevated levels of oxidative damage products, such as malondialdehyde (MDA) and 3-nitrotyrosine has been reported in areas of degeneration in the HD brain⁶. The levels of cAMP and cGMP are reported to be decreased in neuropathological conditions⁷. cAMP system is closely involved in the regulation of important BDNF expression too⁸ which play important role neuronal survival⁹, memory & body weight¹⁰. Among different iso forms of PDEs, PDE1 is found to localize in striatum and cortex, which play important role in body weight and motor functioning¹¹. Despite substantial research into neuroprotection, treatment options for HD are still limited to supportive care and the management of complications. Currently available drugs provide symptomatic relief but do not stop progression of disease¹². Thus, the development of new therapeutic strategies remains unmet medical need. Vinpocetine is a specific inhibitor of basal and calmodulin-activated¹³ PDE-1. Based on important and versatile role of cAMP and cGMP signaling in regulation of neuronal functions, the present study has been designed to investigate the role of PDE1 inhibition in 3-NP induced loss in body weight & oxidative stress. Vinpocetine is well known for its antioxidant and anti-cancer disease effects^{15,16}. Recently, neuroprotective effects of Vinpocetine have been well reported in an animal model of neurodegenerative disease¹⁷. Thus the present study was carried out to investigate the effect of vinpocetine on loss of body weight and oxidative stress induced by 3-NP in a rat model of HD.

Material and Methods

Animals

The experiments were carried out in adult (5-6 months old) male wistar rats (220-250 g) obtained from Central Animal House of I.S.F. College of Pharmacy, Moga, Punjab (India). They were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 22±20C and relative humidity of 60-65%) with 12h light/dark reverse cycle. The food in the form of dry pallets and water were made available *ad libitum*. All behavioural experiments were carried out between 10 AM and 4 PM. The protocol was reviewed and approved by the Institutional Animal Ethics Committee and the animal experiments were carried out in accordance with the Indian National Science Academy Guidelines for use and care of animals.

Drugs and Chemicals

3-Nitropropionic acid was purchased from Sigma-Aldrich Corp. Vinpocetine was provided as *ex-gratia* sample by M/S Covex Pharma Ltd., Germany. All other chemicals used in the study were of analytical grade. Solutions of the drug and chemicals were freshly prepared before use.

Drugs Administration

3-NP was diluted with saline (pH 7.4) and administered intraperitoneally at a dose of 10 mg/kg for 14 days. Vinpocetine was dissolved in normal saline containing 1% ascorbic acid and administered intraperitoneally at different doses viz 5, 10 and 20 mg/kg for 14 days.

Grouping of Animals

Animals were divided in five groups and each group comprised of five animals.

Group 1: Vehicle (normal saline containing 1% ascorbic acid, 0.5ml/rat, i.p)

Group 2: 3-NP control (10mg/kg, i.p. for 14days)

Group 3: 3-NP+Vinpocetine (5mg/kg, i.p.)

Group 4: 3-NP+Vinpocetine (10mg/kg, i.p.)

Group 5: 3-NP+Vinpocetine (20mg/kg, i.p.)

3-NP = 3nitropropionic acid and VIN = Vinpocetine.

Parameters Evaluated

Measurement of Body Weight

Body weight was noted on the first and last days of the experiment. Percentage change in the body weight was calculated in comparison with the initial body weight on the first day of the experimentation.

$$\frac{\text{Body Weight (Ist day- 15}^{\text{th}} \text{ day)}}{\text{Ist day body weight}} \times 100$$

Ist day body weight

Estimation of biochemical parameters

All the biochemical parameters were measured in the brain homogenate on day 15th following 3-NP i.p injection.

Brain homogenate preparation

Animals were sacrificed and decapitation and brains were removed and rinsed with ice -cold isotonic saline . Brain tissue samples were then homogenized with ice cold 0.1 M phosphate buffer (pH 7.4) in a volume 10 times the weight of the tissue. The homogenate was centrifuged at 10,000×g for 15 min and aliquots of supernatant separated and used for biochemical estimation.

Estimation of Malondialdehyde (MDA)

The quantitative measurement of malonialdehyde – the end product of lipid peroxidation –in brain homogenate was performed according to the method¹⁸. The amount MDA was measured after its reaction with thiobarbituric acid at 532 nm using spectrophotometer (Shimadzu, UV 1700). The concentration of MDA was determined from a standard curve expressed as nmol per mg protein.

Result

Effect of Vinpocetine on body weight in 3-NP injected rats

There was no significant difference in the initial and final body weight of the vehicle treated animals. However, 3-NP treatment caused a significant decrease in body weight on the day 15th as compared to vehicle treated group.

Vinpocetine , dose dependently attenuated the decrease in body weight. However, there was no significant difference in Vin 10 and 20mg/kg dose i.p. (Table 1, Fig 1)

Evaluation of biochemical parameters

Malondialdehyde (MDA) Level

The MDA levels significantly increased on day 15th ($377.8 \pm 16.44 \mu\text{M}/\text{mg}$ protein) following Ist 3- NP injection as compared to vehicle ($144.3 \pm 7.950 \mu\text{M}/\text{mg}$ protein , $P < 0.001$). Vinpocetine shows dose dependent significant decrease in MDA levels (5mg/kg , i.p $323.5 \pm 11.55 \mu$ mole /mg protein , $p < 0.05$; 10 mg /kg, i.p $218.4 \pm 11.71 \mu$ mole /mg protein, $P < 0.001$; 20mg /kg , i.p $208.2 \pm 12.58 \mu$ mole /mg protein, $P < 0.001$) compared to 3-NP treated rats (Fig.2, Table 2) compared with those of 3-NP treated animals [Total F (4.49) = 58.29, $P < 0.001$] . Between groups viz vehicle and Vin there was no significant difference in the levels of MDA [Total F(4.49) = 58.29, $p > 0.05$] (Fig.2, Table 2).

Statistical analysis

The results are expressed as means±SD. The behavioral values were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. $p < 0.05$ was considered statistically significant.

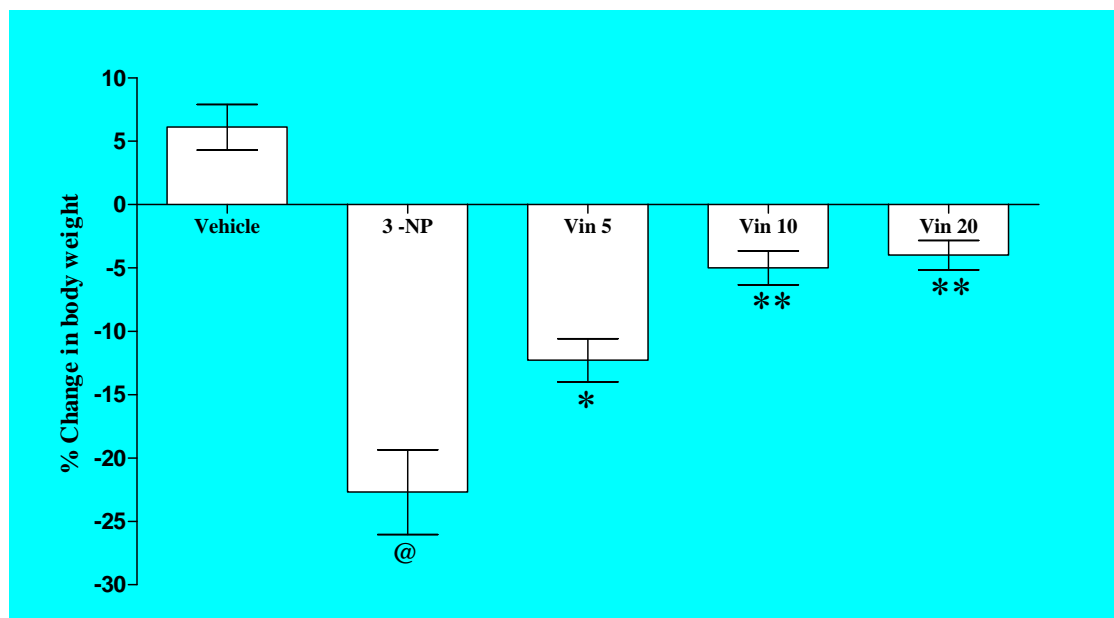
Discussion

Vinpocetine- (14-ethoxycarbonyl-(3a,16a-ethyl)-14,15-eburnamine; Cavinton) – a PDE1 inhibitor is a synthetic derivative of lesser periwinkle plant (Vinca minor) alkaloid. Vincamine is widely used as a neuroprotective agent for the prevention and treatment of central nervous system disorder of cerebrovascular origin^{19,20}. 3-NP has been observed to cause significant reduction in body weight and behavioral abnormalities including muscle weakness and rigidity in animals. Huntington's disease patients often show degeneration of hypothalamic neurons and loss of body weight²¹. Reduced body weight can be considered as an indicator of 3-NP neurotoxicity.

Table.1 Effect of Vinpocetine on % change in body weight in 3-NP treated rats

Treatment	% Change in Body weight
Vehicle	6.100 ± 0.5667
3-NP	-22.70 ± 1.055 @
Vin (5 mg/kg) + 3-NP	-12.30 ± 0.5385*
Vin (10 mg/kg) + 3-NP	-5 ± 0.4216**
Vin (20 mg/kg) + 3-NP	-4 ± 0.3651**

Values are expressed as mean ± SD, @ signifies P<0.05 as compared to vehicle, *P< 0.05 versus 3-NP control, ** P<0.05 versus Vin 5 and 3-NP control.

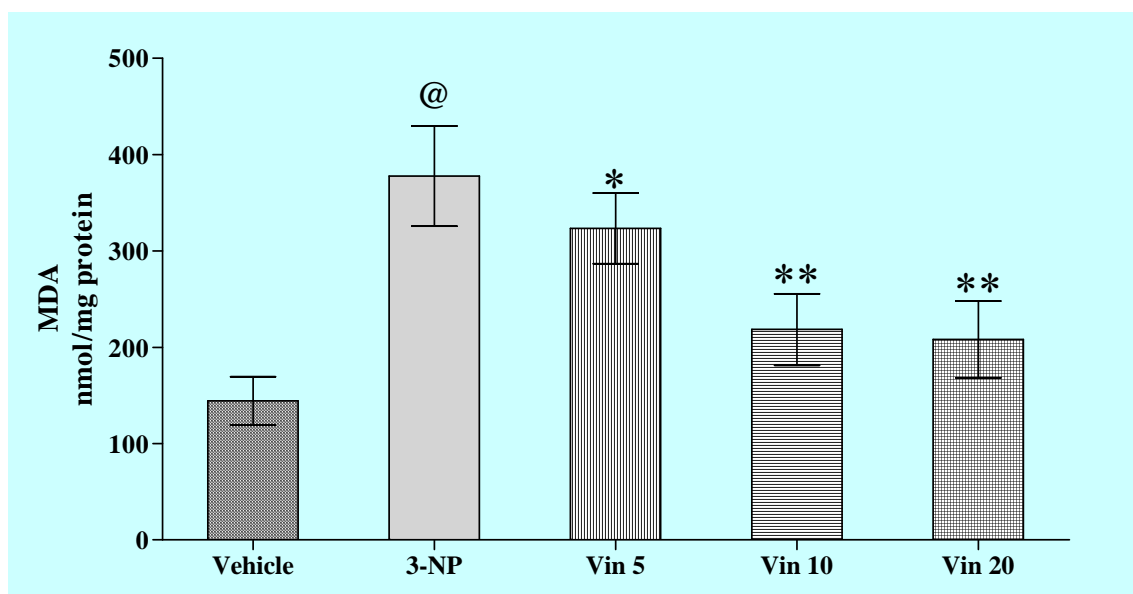
**Fig.1 Effect of vinpocetine on body weight in 3-NP treated rats**

Values are expressed as mean ± SD, @ signifies P<0.05 as compared to vehicle, *P< 0.05 versus 3-NP control, ** P<0.05 versus Vin 5 and 3-NP control rat.

Table.2 Effect of Vinpocetine on Lipid peroxidation (MDA levels) in brain homogenate of rat

Treatment	Malondialdehyde (nm/mg protein)
Vehicle	144.3± 7.950
3-NP	377.8± 16.44@
Vin (5 mg/kg) + 3-NP	323.5 ± 11.55*
Vin (10 mg/kg) + 3-NP	218.4 ± 11.71**
Vin (20mg/kg) + 3-NP	208.2 ± 12.58**

Values are expressed as mean ± SD, @ signifies P<0.05 as compared to vehicle, *P< 0.05 versus 3-NP control, ** P<0.05 versus Vin 5 and 3-NP control.

**Fig.2 Effect of Vinpocetine on MDA levels**

Values are expressed as mean ± SD, @ signifies P<0.05 as compared to vehicle, *P< 0.05 versus 3-NP control, ** P<0.05 versus Vin 5 and 3-NP control.

Whereas Vinpocetine treatment has been shown to significantly improve body weight and attenuate 3-NP induced hypoactivity in animals. The hypoactivity is a major late stage symptom in HD patients²². Loss in body weight and hypoactivity could be simply because of decreased energy metabolism after 3-NP treatment. 3-NP induces energy deficit leads to depolarization of membrane potential, followed by release of substrate for radical species production and consequently oxidative stress²³. The lesions occur by a mechanism involving secondary excitotoxicity. It has shown that excitotoxicity may be linked to free radical generation²⁴. Previous evidence for the involvement of the oxidative stress in 3-NP induced neurotoxicity includes the production of hydroxyl free radicals, changes in endogenous antioxidants, and increased in 3-nitrotyrosine, a marker of peroxynitrite-mediated damage²⁵. Recently it was reported that systemic administration of 3-NP leads to oxidized proteins in the striatum and cortex as well as massive loss of striatal neurons²⁶. Moreover, mitochondrial dysfunction and oxidative stress has also been implicated in Pathophysiology of HD²⁷. Malondialdehyde (MDA) is an end product of lipid peroxidation and it was suggested that plasma MDA may be used as potential biomarker to test treatment efficacy of drugs used in HD²⁸. Supporting to earlier reports, in the present study of 3-NP significantly increased lipid peroxidation in rats brain^{5,23}. Whereas, Vinpocetine treatment in these animals has shown to significantly attenuate an increase in the levels of MDA (indicator of lipid peroxidation due to free radicals) following 3-NP administration, suggesting antioxidant action of Vinpocetine. In addition of Vinpocetine has been demonstrated to have antioxidant potential and reported to scavenge hydroxyl radicals^{29,30}. In conclusion, using a rodent model system that shows impairment in body weight and increase in oxidative stress, we obtained results suggesting that Vinpocetine has neuroprotective effects against 3-NP induced neurotoxicity. In the current study, for the first time, we have tried to

further explore the role of PDE1 inhibition by using Vinpocetine as a pharmacological tool in 3-NP induced neurotoxicity.

Conclusion

The present data demonstrate that vinpocetine can improve body weight and oxidative stress in 3-NP-induced neurotoxicity, an animal model of HD. The results suggest that vinpocetine may have therapeutic benefit in the treatment of HD.

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References

- [1]. Vonsattel JP, DiFiglia M. Huntington disease, *J. Neuropathol Exp Neurol* 1998;57:369-384.
- [2]. Martin JB. and Gusella JF. Huntington's disease: pathogenesis and management. *N Engl J. Med* 1986; 315: 1267-1276.
- [3]. Cruz, V and Santamaria, A. Integrative Hypothesis for Huntington's disease: A Brief Review of Experimental Evidence. *Physiol. Res* 2007; 56: 513-526.
- [4]. La Fontaine MA, Geddes JW, Banks A and Butterfield DA. Effect of exogenous and endogenous antioxidants on 3-nitropropionic acid – induced in vivo oxidative stress and striatal lesions: insights into Huntington's disease. *J Neurochem* 2000; 75: 1709- 1715.
- [5]. Kumar P, Padi SSV, Naidu PS and Kumar A. Possible Neuroprotective of Curcumin in Attenuating 3-Nitropropionic Acid –

- Induced Neurotoxicity. *Methods Find. Exp. Clin. Pharmacol* 2006; 17: 485- 92.
- [6]. Kim YJ, Yi Y, Sapp, Wang Y, Cuiffo B, Kegel, Qin ZH, Aronin, DiFiglia M. Caspase3- cleaved N- terminal fragments of wild type and mutant huntingtin are present in normal and Huntington's disease brains, associate with membranes, undergo calpain – dependent proteolysis. *Proc. Natl. Acad Sci . U.S.A.* 2001; 98: 12784- 12789.
- [7]. Vis JC, Van Huizen RT, Veerbeek MM, de Waal RM, ten Donkelaar HJ and Kremer B. Creatine protects against 3-nitropropionic acid-induced cell death in murine corticostriatal slice cultures. *Brain Res* 2004; 1024: 16-24.
- [8]. Beal MF. Mitochondrial dysfunction in neurodegenerative disease. *Biochem Biophys Acta* 1998; 1366: 211-223.
- [9]. Nagakura M, Oosumi K, Hirano H, Onishi Y. Pharmacogenomics and therapeutics strategies for Dementia . *Science direct* 2002; 34:357- 379.
- [10]. Puzzo D, Vitolo O , Trinchese F, Joel P, Jacob AP, Arancio . Neurobiology of Disease Amyloid β Peptide Inhibits Activation of the Nitric Oxide/cGMP/cAMP-Responsive Element-Binding Protein Pathway during Hippocampal Synaptic Plasticity. *J Neuroscience* 2005; 25: 6887– 6897.
- [11]. Fujita SS, Zghb J, Hong . Species differences in Brain phosphodiesterase levels . *J Neural Transm* 2008; 41:185.
- [12]. Nibuya M , Nestler EJ, Duman RS. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *J Neurosci* 1996; 16: 2365–2372.
- [13]. Chun YJ, Kim MY, Guengerich FP. Resveratrol is a selective human cytochrome P450 1A1 inhibitor. *Biochem Biophys Res Commun* 1999; 19 :20-24.
- [14]. Song, Perides, Liu and YF .Expression of full length polyglutamine expanded Huntington disrupt growth factor receptor signaling in rat. *J Biolchem* 1997; 277:6703-6707.
- [15]. Bishop VS, DM Farrell. The role of cGMP and cAMP in active thermoregulatory vasodilation. *Neurobiol Dis* 1997; 272: R975- R981.
- [16]. O'Donnell JM and Zhang HT. Antidepressant effects of inhibitors of cAMP phosphodiesterase. *Trends Pharmacol. Sci* 2004; 25: 158–163.
- [17]. K Tarnok, E Kiss, PGM Luiten, C Nyakas, K Tihanyi, K Schlett ,and ULM Eisel. Effects of Vinpocetine on mitochondrial function and neuroprotection in primary cortical neurons. *Curr Pharm Des* 2008; 66: 63-65.
- [18]. Lugnier C: Cyclic nucleotide phosphodiesterase superfamily. *Pharmacol Ther* 2006; 109:366-398.
- [19]. Wills ED. Mechanism of lipid peroxide formation in animal. *Biochem J.* 1966; 99: 667-676.
- [20]. Kumar P, Padi SSV, Naidu PS and Kumar A. Effect of resveratrol on 3-nitropropionic acid- induced biochemical and behavioural changes: possible neuroprotective mechanisms. *Behav. Pharmacol*, 2006 ; 17: 485- 492.
- [21]. Rose GM, Hopper A, De Vivo M and Tehim A. Phosphodiesterase inhibitors for cognitive enhancement. *Curr Pharm Des* 2005; 11(26): 3329-3334.
- [22]. Vas A and Gulyas D. Eburnamine derivatives and the brain. *Med Res Ev* 2005; 25: 737-57.
- [23]. Kaudac D and Mittlemon A , Serpico R, Sinha AA, Natale C, Pani P, Simone S

and Farber E. Cell death: apoptosis versus necrosis. *Int J on col* 2002; 21: 165- 70.

- [24]. Koutouzis TK , Borlongan, Scorcia and Creese PR , Systemic 3-NP : long term effects on locomotor behavior. *Brain Res* 1994; 646:242- 46.
- [25]. Tunes I, Montilla P, Munoz and Tariq MC. Treatment with dehydroepiandrosterone prevents oxidative stress induced by 3-nitropropionic acid in synaptosomes. *Pharmacology* 2005; 74:113-118.
- [26]. Borlongan CV , Freeman TK, DW Cahill. Behavioral pathology induced by repeated injection of 3-NP mimic the symptoms of HD . *Brain Res* 1995; 697: 254-257.
- [27]. Borlongan CV, Kanning K ,Freeman. Free radical damage and oxidative stress in Huntington's disease. *JFLA Med Assoc* 1996; 83: 335-41.
- [28]. Haik KL, Haik, DA,Shear Sabel, Dunbar GL, Quinolinic acid released from polymeric brain implants causes behavioral and neuroanatomical alterations in a rodent model of HD. *Exp Neurol* 2000; 163: 430- 439.
- [29]. Kodsi, Swerdlow. Mitochondrial 3-NP produce abnormalities in rats that model of HD. *Depart Psych* 1997; 231 : 103-07.
- [30]. Sun Y. Free radicals and antioxidant enzymes. *Free Radic Biol Med* 1990; 8: 583-99.
- [31]. Stolc S. Indole derivatives as neuroprotectants .*Life Sci* 1950; 65: 1943-50.
- [32]. Santos MS, Durate AL, Oliveria. Synaptosomal response to oxidative stress: effects of Vinpocetine. *Free Radic Res* 2000; 32: 57-66.