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 in exorable decay and death. The degenerative process involves medium spiny striatal neurons and lesser extent cortical neurons. 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 neurons. 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 stress.
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 HD. Elevated levels of oxidative damage products, such as malondialdehyde (MDA) and 3-nitrotyrosine has been reported in areas of degeneration in the HD brain. The levels of cAMP and cGMP are reported to be decreased in neuropathological conditions. cAMP system is closely involved in the regulation of important BDNF expression which play important role neuronal survival, memory & body weight. Among different iso forms of PDEs, PDE1 is found to localize in striatum and cortex, which play important role in body weight and motor functioning. 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 disease. Thus, the development of new therapeutic strategies remains unmet medical need. Vinpocetine is a specific inhibitor of basal and calmodulin-activated 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. Recently, neuroprotective effects of Vinpocetine have been well reported in an animal model of neurodegenerative disease. 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±20°C 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 administrated intraperitonely at a dose of 10 mg/kg for 14 days. Vinpocetine was dissolved in normal saline containing 1% ascorbic acid and administered intraperitonely 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.

Body Weight (1st day- 15th day) X100

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 malonaldehyde – the end product of lipid peroxidation – in brain homogenate was performed according to the method. 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 ± 16.44 µM/mg protein) following 1st 3- NP injection as compared to vehicle (144.3 ± 7.950 µM/mg protein, P< 0.001). Vinpocetine shows dose dependent significant decrease in MDA levels (5mg/kg, i.p 323.5 ±11.55µ mole /mg protein, p< 0.05 ; 10 mg /kg, i.p 218.4 ± 11.71 µ mole /mg protein, P< 0.001; 20mg /kg, i.p 208.2± 12.58 µ 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. 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 weight. 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

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

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Malondialdehyde (nm/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>144.3 ± 7.950</td>
</tr>
<tr>
<td>3-NP</td>
<td>377.8 ± 16.44@</td>
</tr>
<tr>
<td>Vin (5 mg/kg) + 3-NP</td>
<td>323.5 ± 11.55*</td>
</tr>
<tr>
<td>Vin (10 mg/kg) + 3-NP</td>
<td>218.4 ± 11.71**</td>
</tr>
<tr>
<td>Vin (20 mg/kg) + 3-NP</td>
<td>208.2 ± 12.58**</td>
</tr>
</tbody>
</table>

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 patients\(^{22}\). 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 consequentially oxidative stress\(^{23}\). The lesions occur by a mechanism involving secondary excitotoxicity. It has shown that excitotoxicity may be linked to free radical generation\(^{24}\). 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 damage\(^{25}\). 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 neurons\(^{26}\). Moreover, mitochondrial dysfunction and oxidative stress has also been implicated in Pathophysiology of HD\(^{27}\). 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 HD\(^{28}\). 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.

**Acknowledgement**

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