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Beneficial effects of sodium butyrate in 6-OHDA induced neurotoxicity and behavioral abnormalities: Modulation of histone deacetylase activity

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HIGHLIGHTS

- 6-OHDA administration leads to neurotoxicity in rats.
- Sodium butyrate attenuated 6-OHDA-induced motor deficit in rats.
- Sodium butyrate reduced striatal neuro-inflammation and oxidative stress markers.
- Sodium butyrate elevated the striatal dopamine, BDNF and histone acetylation level.

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ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disorder. Recent studies have investigated the involvement of epigenetic modifications in PD. Histone deacetylase (HDAC) inhibitors have been reported to be beneficial in cognitive and motor deficit states. The present study was designed to investigate the effect of sodium butyrate, a HDAC inhibitor in 6-hydroxydopamine (6-OHDA) – induced experimental PD like symptoms in rats. To produce motor deficit, 6-OHDA was administered unilaterally in the right medial forebrain bundle. Three weeks after 6-OHDA administration, the rats were challenged with apomorphine. Following this, the animals were treated with sodium butyrate (150 and 300 mg/kg i.p.) once daily for 14 days. Movement abnormalities were assessed by battery of behavioral tests. Biochemically, oxidative stress markers, neuroinflammation and dopamine were measured in striatal brain homogenate. Further, to explore the molecular mechanism(s), we measured the level of global H3 histone acetylation and brain derived neurotrophic factor (BDNF). 6-OHDA administration results in significant motor deficit along with reduction in striatal dopamine level. 6-OHDA treated rats showed elevated oxidative stress and neuroinflammatory markers. Treatment with sodium butyrate results in significant attenuation of motor deficits and increased striatal dopamine level. Moreover, sodium butyrate treatment attenuated the oxidative stress and neuroinflammatory markers. These effects occur concurrently with increased global H3 histone acetylation and BDNF levels. Thus, the observed results of the present study are indicative for the therapeutic potential of HDAC inhibitors in PD.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting approximately 2% of the population over the age of 65 [1]. Selective dopaminergic neuronal degeneration in substantia nigra pars compacta (SNpc) and depletion of dopamine in striatal projections at relatively early stages are the prominent features of PD pathology [1]. Although, the exact molecular mechanism (s) which results in neuronal cell death still remains unclear. However, increasing evidence suggests the involvement of transcriptional dysregulation in the disease pathology [2]. Recent investigations suggest that gene expression modulated by histone acetylation has been found to be altered in animal models as well as in PD patients (for review [2]). Rouaux et al. were the first to identify alterations of histone acetylation levels during neuronal damage. They demonstrated that histone

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acetylation levels were decreased globally in neurons under neurodegenerative conditions [3]. Since then, the linkage between histone hypoacetylation and neurodegeneration has been well established in numerous cognitive and movement disorders. Histone deacetylases (HDACs) along with histone acetyltransferases (HATs) are the enzymes that regulate the homeostasis of histone acetylation. Inhibitors of HDACs, which were initially characterized as anticancer drugs, are recently suggested to act as neuroprotective agents in a wide range of neurodegenerative disorders, including Alzheimer's disease (AD), PD and Huntington's disease (HD) [2,4–6]. Moreover, we have previously demonstrated the beneficial effects of HDAC inhibitors in various animal models of cognitive deficits and AD [7,8]. The most widely used HDAC inhibitors are sodium butyrate, phenylbutyrate, valproate, trichostatin A, and suberoylanilide hydroxamic acid (SAHA). Among these, the butyrates have been the best clinically studied compounds and are known to readily reach the brain [9]. Sodium butyrate has been previously reported to exert neuroprotective actions in animal and cell culture models of various neurological diseases [10–12]. Moreover, sodium butyrate has also been reported to protect dopamine neurons against various insults in cell culture and α-synuclein transgenic fly models of PD [13–15]. Thus, these results suggest that HDAC inhibitors may be potentially neuroprotective against dopaminergic cell death and should be explored in animal models of PD. However, the exact molecular mechanisms by which sodium butyrate possess neuroprotection still remains elusive. The administration of 6-OHDA directly into the rat brain produces a well established model of PD [16–17]. Thus, in the present study, we have used 6-OHDA to induce PD like symptoms in rats.

Based on recent evidence regarding the role of HDAC inhibitors in neurodegenerative disease, we hypothesize that pharmacological inhibition of HDAC activity would alleviate 6-OHDA-induced neurotoxicity and PD like symptoms in rats. Therefore, the present study was designed to investigate the therapeutic potential of sodium butyrate, a HDAC inhibitor, against 6-OHDA induced experimental PD like symptoms in rats.

2. Materials and methods

2.1. Animals

The experiments were carried out in male Wistar rats (280–350 g) obtained from Central Animal House of Birla Institute of Technology & Science, Pilani, India. The rats were kept in polyacrylic cages and maintained under standard husbandry conditions (room temperature 22 ± 1 °C and relative humidity of 60%) with 12 h light/dark cycle. The animals were fed with normal pellet diet and filtered water ad libitum. All the behavioral assessments were carried between 09:00 and 17:00 hrs. The experimental procedures on animals were in compliance with the Institutional Animal Ethics Committee of BITS, Pilani, Rajasthan (India).

2.2. Materials

6-OHDA, desipramine, apomorphine and TNF-α ELISA kit were purchased from Sigma–Aldrich, USA. Sodium butyrate was purchased from Cayman Chemicals. BDNF ELISA kit was purchased from Boster Biological Tech. Co., Ltd., CA, USA. EpiQuik global Histone H3 acetylation assay Kit was purchased from Epigentek, NY. All other chemicals used in the study were of analytical grade. Solutions of the drugs and chemicals were always prepared afresh before use. Sodium butyrate was dissolved in saline and always prepared afresh before administration.

2.3. Induction of experimental Parkinson's disease by 6-OHDA administration

Rats were anesthetized using ketamine (100 mg/kg, i.p.) and xylazine (5 mg/kg i.p.) and placed into a stereotaxic frame (Inco, Ambala). Since 6-OHDA damages both dopaminergic and noradrenergic axons in the MFB, we administered desipramine (25 mg/kg i.p.) to rats in order to prevent the damage to noradrenergic neurons. A midline saggital incision was made in the scalp and bregma was determined. A dental drill was used to make a hole through the skull. All the rats except vehicle control were infused with 6-OHDA unilaterally into the right MFB using following coordinates: −4.4 mm posterior to bregma; 1.2 mm lateral to sagittal suture and −7.8 mm ventral from the surface of the brain [18]. Immediately after surgery, the rats were injected with gentamicin (5 mg/kg i.p.) and housed individually in polypropylene cages for a week, and then they were re-grouped in their home cages.

2.4. Experimental groups

The selection of experimental animals was based on their performance in behavioral tasks during training phase. The rats which fail to cross the narrow beam in 60 s were excluded from study. Similarly, the rats which were not able to hold the rotating rod in rotarod task for a minimum of 30 s were also excluded from study. All rats were given training for a period of one week on rotarod and narrow beam walk test. One day after training session, brain surgery was performed. Group 1 received 4 μl ascorbic acid: saline solution into the right MFB. Group 2 received 6-OHDA in right MFB and later the animals received saline i.p. as a vehicle for sodium butyrate for 14 days. 6-OHDA was used at a concentration of 4 μg/μl dissolved in a solution of 0.02% w/v ascorbic acid in 0.9% w/v sterile saline. A total volume of 4 μl was injected using the stereotaxic coordinates. Groups 3 and 4 received 6-OHDA followed by sodium butyrate (150 and 300 mg/kg i.p., respectively) for 14 days (Fig. 1). Behaviorally effective doses of sodium butyrate were selected based upon pilot study conducted in our lab and some previous reports [19–20].

2.5. Apomorphine challenge

Three weeks following 6-OHDA infusion rats were treated with apomorphine (3 mg/kg i.p.) to check rotational behavior response. Apomorphine shows a characteristic contralateral turning behavior when the supersensitive receptors in the lesioned side of the brain are activated and the rats starts showing turning behavior to the contralateral side. Out of 36 rats only 30 showed significant rotations following apomorphine challenge and were randomly divided into 3 groups. The number of contralateral rotations was counted for 30 min. Dopamine deafferentation was considered successful in those animals that made at least 50 contralateral rotations within 20 min of the apomorphine injection.

2.6. Behavioral parameters

2.6.1. Narrow beam walk test

Gait abnormalities and foot slip count were measured by narrow beam walk apparatus as per method described by Sharma and Deshmukh [1]. Briefly, the apparatus consists of a horizontal narrow beam (1 cm × 100 cm) suspended 1 m above a foam-padded cushion. A black box was placed at the end of the beam as the finish point. A lamp (with 60 W light bulb) was used to shine light above the start point and serves as an aversive stimulus. Time taken to cross the beam was measured by stopwatch manually. During testing, the rats were given 1 min to traverse the beam. The latency to cross the beam along with their number of foot slips was recorded. If the rats did not complete the task or if they fall off the beam or