Alteration of enteric monoamines with monoamine receptors and colonic dysmotility in 6-hydroxydopamine–induced Parkinson's disease rats

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Constipation is common in Parkinson's disease (PD), in which monoamines (dopamine (DA), norepinephrine (NE), and 5-hydroxytryptamine (5-HT)) play an important role. Rats microinjected with 6-hydroxydopamine (6-OHDA) into the bilateral substantia nigra (SN) exhibit constipation, but the role of monoamines and their receptors is not clear. In the present study, colonic motility, monoamine content, and the expression of monoamine receptors were examined using strain gauge force transducers, ultraperformance liquid chromatography tandem mass spectrometry, immunofluorescence, and Western blot. The 6-OHDA rats displayed a significant reduction in dopaminergic neurons in the SN and a decreased time on rota-rod test and a marked decrease in daily fecal production and fecal water content. The amplitude of colonic spontaneous contraction was obviously decreased in 6-OHDA rats. Blocking D1-like receptor and β_3-adrenoceptor (β_3-AR) significantly reduced the inhibition of DA and NE on the colonic motility, respectively, whereas the 5-HT and 5-HT_4 receptor agonists promoted the colonic motility. Moreover, DA content was increased in the colonic muscularis externa of 6-OHDA rats. The protein expression of β_3-ARs was notably upregulated, but 5-HT_4 receptors were significantly decreased in the colonic muscularis externa of 6-OHDA rats. We conclude that enhanced DA and β_3-ARs and decreased 5-HT_4 receptors may be contributed to the colonic dysmotility and constipation observed in 6-OHDA rats. (Translational Research 2015;166:152–162)

Abbreviations: β-AR = β-adrenoceptor; DA = dopamine; DAT = dopamine transporter; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; GI = gastrointestinal; 5-HT = 5-hydroxytryptamine; IR = immunoreactive; NE = norepinephrine; 6-OHDA = 6-hydroxydopamine; PD = Parkinson's disease; SN = substantia nigra; TH = tyrosine hydroxylase; UPLC-MS/MS = ultraperformance liquid chromatography tandem mass spectrometry

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AT A GLANCE COMMENTARY

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Background

Constipation is common in Parkinson's disease (PD), in which monoamines and their receptors play an important role. 6-Hydroxydopamine (6-OHDA) rats exhibit colonic dysmotility and constipation, but the role of monoamines and their receptors is not clear.

Translational Significance

The present study demonstrated that enhanced dopamine, upregulated β_3-adrenoceptors, and decreased 5-HT_4 receptors may be responsible for the colonic dysmotility and constipation observed in 6-OHDA rats. This study provides new clues for the diagnosis and treatment of constipation associated with PD.

INTRODUCTION

Dopamine (DA), norepinephrine (NE), and 5-hydroxytryptamine (5-HT) are important monoamines in both the brain and gut. These neurotransmitters have vital functions in regulating gastrointestinal (GI) tract motility, secretion, absorption, and mucosal protection. DA transporter (DAT) knockout (KO) mice display significantly weakened colonic contraction.[1] DA inhibits GI motility via D_1 receptors,[2,3] whereas the total GI and colonic transit time is decreased in D_2 KO mice.[4] The sympathetic noradrenergic system has an inhibitory effect on GI motility. β-Adrenoceptor (β-AR) agonists in a dose-dependent manner relax smooth muscle cells isolated from human colon.[5] According to in vitro and in vivo studies, 5-HT regulates GI motility, mostly via the 5-HT_4 and 5-HT_3 receptors.[6-9] Significant alterations of the monoamine system are observed in the GI tract of the elderly and patients with advanced Parkinson's disease (PD), accompanied with GI dysfunction.[10,11] D_2 receptor antagonists are used as prokinetic drugs in the GI tract.[12,13] Administration of 5-HT_4 receptor agonists and partial 5-HT_3 receptor antagonists is helpful in ameliorating constipation in patients with PD.[14,15]

PD is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra (SN). Recently, degeneration of the NE and 5-HT systems has been observed in patients with PD.[16,17] In addition to the cardinal somatic movement disorders, autonomic dysfunction in PD has attracted strong attention. GI dysfunction, especially constipation, is very frequent in the preclinical stage of PD and has also been identified as a premotor marker of early PD.[18-20] However, constipation is poorly managed in the clinic, as the underlying mechanisms are unclear. Rats with 6-hydroxydopamine (6-OHDA) microinjected into the bilateral SN exhibit delayed gastric emptying and constipation and are widely used to investigate the GI dysfunction in PD.[21-24] The neurons displaying immunoreactivity for vasoactive intestinal polypeptide is degenerated in the enteric nervous system of patients with PD.[25] In addition, we have reported enhanced DA content with upregulation of D_2 receptors and β_1-ARs in the gastric corpus of 6-OHDA rats.[23,24] However, whether enteric DA, NE, 5-HT, and their receptors are altered in the colon of 6-OHDA rats remains unknown. The aim of the present study was to investigate enteric DA, NE, and 5-HT with monoamine receptors' expression and their role in colonic dysmotility in 6-OHDA rats. This study is helpful to find out new biomarker(s) for the early diagnosis and treatment of PD associated with constipation.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (200–250 g) were obtained from the Laboratory Animal Services Center of Capital Medical University, Beijing, China. All animals were housed in groups of 3 on a light-dark (12/12 hours) cycle at 22 ± 1°C. Food and water were provided ad libitum. All experimental procedures were in accordance with the guidelines established by the National Institutes of Health and approved by the Animal Care and Use Committee of the Capital Medical University, Beijing, China.

6-OHDA–treated rat model. The methods used in this study have been described previously.[21-24] Briefly, animals were anesthetized with a mixture of xylazine and ketamine (13 and 87 mg/kg body weight, respectively, intraperitoneal), and placed on a Kopf stereotaxic instrument. Two holes were drilled in the skull at the following coordinates (in millimetres): anteroposterior, −5.6; mediolateral, ±2.0; dorsoventral, −7.5. 6-OHDA (4 μg in 2 μL of 0.9% saline containing 0.05% of ascorbic acid) was bilaterally injected in the experimental group. Injections were performed at 1 μL/min using a 10 μL Hamilton syringe. The needle was left in place for 2 minutes to allow complete diffusion of the medium. Wounds were closed using skin clips. Control rats underwent sham stereotaxic surgery and were injected with saline containing 0.2% ascorbic acid. Subsequent experiments were performed at 6 weeks after 6-OHDA administration.