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Research report

Positive association between striatal serotonin level and abnormal involuntary movements in chronic L-DOPA-treated hemiparkinsonian rats

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ABSTRACT

Although L-DOPA represents the standard of care in Parkinson's disease, long-term treatment may be compromised by L-DOPA-induced dyskinesia (LID), with adverse fluctuations in motor responsiveness and progressive loss of control. Here we show that in rats with 6-hydroxydopamine-induced lesions of the median forebrain bundle, LID correlates with 5-HT levels. Rats were treated with L-DOPA (6 mg/kg) and benserazide (15 mg/kg) daily for 3 weeks to induce the development of abnormal involuntary movements (AIMs).

After this chronic L-DOPA treatment, the lesion side of the rats displayed significant changes in striatal dopamine (DA) and 5-HT levels. Striatal DA and 5-hydroxytryptamine (5-HT) levels were inversely correlated, and AIMs were strongly positively correlated with DA levels and negatively correlated with 5-HT levels. Axial AIMs were more strongly correlated with DA and 5-HT levels than were the other AIMs subtypes, while locomotive AIMs showed no significant correlation at all. In addition, striatal 5-HT was more strongly (negatively) correlated with the AIMs than striatal DA levels. These results demonstrate that 5-HT contributes to LID and that both striatal DA (positively) and 5-HT (negatively) affect the severity of LID. We suggest that by strategic modification of the serotonin system it may be possible to attenuate the adverse effects of chronic L-DOPA therapy.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta, and a reduction in striatal dopamine (DA) [23]. L-DOPA continues to be the most effective agent for the symptomatic treatment of PD. However, the initial benefits decline over the time due to the development of motor complications, some of which, e.g., abnormal involuntary movements (AIMs) are directly related to the L-DOPA therapy [1,28]. Although the exact mechanism of L-DOPA-induced dyskinesia (LID) remains unknown, evidence suggests that it results in part from the supersensitization of striatal DA D_1 and D_2 receptors following DA depletion [8,24]. A search for new ways to suppress the dyskinesia is ongoing. One potential non-dopaminergic target of this search may be the serotonin system [22,30]. Following DA denervation, neuroadaptive changes in striatal serotonergic fibers and up-regulation of 5-hydroxytryptamine (5-HT) receptors allow this system to more readily influence basal ganglia activity [5,6,10].

Serotonergic neurons are interesting in this context, because they can convert L-DOPA to DA, store the newly formed DA in vesicles, and release it in an activity-dependent manner [10]. The DA released from dopaminergic terminals is controlled via DA reuptake by DA transporter (DAT) and DA D_2 autoreceptor feedback control [4]. In the absence of such autoregulatory mechanisms, DA released from serotonergic terminals might perturb motor control and induce dyskinesia [16]. In support of this hypothesis, some reports suggested recently that in rats with complete lesions of the ascending DA pathway, L-DOPA administration reduced striatal 5-HT levels by about 50% and that LID could be almost completely blocked in these animals by either lesioning the serotonin system or activating serotonergic autoreceptors with 5-HT_{1A} or 5-HT_{1B} receptor agonists [9,11]. These findings suggest that L-DOPA-derived DA, released as a "false transmitter" from serotonergic terminals, is the principal trigger of dyskinesia in the rat PD model. To extend these finding, which concern only presynaptic serotonergic functions, we investigated the effects of chronic L-DOPA on DA and 5-HT levels, and evaluated the correlation between these monoamines and AIMs.

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2. Materials and methods

2.1. Animals

Adult female Sprague–Dawley rats, 225–250 g, were housed in a room at constant temperature (18 ± 2 °C) and humidity (50 ± 10%) with an automatic 12/12 light/dark cycle. Food and water were available *ad libitum*. All surgical procedures were performed in accordance with regulations for the use of laboratory animals established by the Ethics Committee of Hanyang University.

2.2. 6-OHDA lesioning

All 6-OHDA injections were performed under anesthesia induced by injection of lumpun plus ketamine (5 mg/kg), 7:3 (Apoteksbolaget, Sweden), using a stereotaxic frame (Stoelting, Wood Dale, IL) with an attached Hamilton syringe. The animals received 3 μl 6-OHDA (Sigma–Aldrich AB, Sweden) into the median forebrain bundle (MFB) (8 mg/ml free base in 0.02% ascorbic acid in saline) to achieve a complete lesion of the nigrostriatal pathway. The following coordinates relative to the bregma were used: AP, −4.4 mm; ML, −1.2 mm; DV, −7.8 mm with the incisor bar positioned at 2.4 mm below the interaural line [26]. Injection speed was 1.0 μl/min and the syringe was kept in place for an additional 5 min before it was slowly retracted. To minimize mechanical damage, all injections were performed with a 26-gauge needle on a Hamilton syringe.

2.3. Experimental design

At 4 weeks post-lesion, forty rats were tested for amphetamine-induced rotation (2.5 mg/kg D-amphetamine i.p.; 60 min testing), and twenty-four rats showing individual means >5 full turns per min in a direction ipsilateral to the lesion were selected for study. Animals were injected with L-DOPA (6 mg/kg) and normal saline (control) daily for 3 weeks and the cylinder test was evaluated off (baseline) and on L-DOPA (6 mg/kg). Finally, 2 weeks after the last experiment, twelve rats were killed and performed the high performance liquid chromatography (HPLC) for DA and 5-HT. A subset of these animals was injected with either L-DOPA at 6 mg/kg or vehicle 60 min before being killed in order to investigate the impact of L-DOPA treatment on striatal DA and 5-HT levels.

2.4. L-DOPA-induced dyskinesia

To induce stable AIMs, L-DOPA methyl ester (6 mg/kg; Sigma–Aldrich, Sweden) combined with the peripheral DOPA–decarboxylase inhibitor, benserazide (15 mg/kg, Sigma–Aldrich, Sweden), was dissolved in physiological saline and injected i.p. daily into each rat for 3 weeks. AIMs were evaluated on the rat dyskinesia scale as described [13,20,21]. Briefly, the animals were placed individually in transparent plastic cages without bedding material and observed for 5 min every 20 min for 3 h following injection of L-DOPA and their behavior recorded on video. The experienced observer was kept blinded to the animal grouping and treatment regimen throughout the entire experiment. The AIMs were further classified into four subtypes according to their topographic distribution as Forelimb (Li), Orolingual (Ol), and Axial (Ax) behaviors. Forelimb and orolingual dyskinesias manifest predominantly as hyperkinesia, and axial dyskinesia as dystonia. The locomotive dyskinesia is expressed as circling movements away from the lesioned side. These rating system do not include exaggerated expressions of normal behaviors, such as grooming, gnawing, rearing, and sniffing. Scores from 0 to 4 indicate the severity of each AIM subtype (0: absent, 1: occasional, i.e. present less than 50% of the time; 2: frequent, i.e. present more than 50% of the time; 3: continuous, but interrupted by strong sensory stimuli and 4: continuous, not interrupted by strong sensory stimuli). After chronic L-DOPA, the animals were divided into L-DOPA-treated non-dyskinesia (LND) and LID groups according to their AIMs.

2.5. Quantification of DA and 5-HT levels

For measuring DA and 5-HT ($n = 12$), after removing whole brains (decapitation), the striata were rapidly isolated using a rodent brain matrix and a standard rat brain atlas [26], and sectioned coronally from the rostro-caudal levels through the striatum (range; +1.18 to −0.46 mm relative to bregma). The striata were collected as fragments of approximately 1 mm × 1 mm × 1 mm. The tissues were immediately stored at −80 °C. At the time of assay, tissues were homogenized in 0.2 N perchloric acid in 0.1 M EDTA, centrifuged at 13 000 rpm for 10 min, and filtered though minispin filters for an additional 5 min at 13 000 rpm. Alumina extraction for DA and 5-HT analysis was performed by the method of Anton and Sayre [3]. Samples (20 μl) were injected using a Rheodyne injector and separated with a reverse phase μ-Bondapak C18 column (150 mm × 3.0 mm, Eicom, Japan) maintained at 30 °C with a column heater (Waters, Cotland, NY). The mobile phase consisted of 0.05 M citric acid, 0.05 M disodium phosphate (pH 3.1), 3.2 mM 1-octanesulphonic acid (sodium salts), 0.3 mM EDTA and 12% methanol pumped at a flow rate of 0.5 ml/min using a Waters solvent delivery system. Electroactive compounds were analyzed at +750 mV using an analytical cell with an amperometric detector (Eicom, Model ECD-300, Japan). Elution peaks were processed using the DS Chromatographic Software (Donam, Korea), and DA and 5-HT levels were calculated using internal DA and 5-HT standard injected immediately before and after each experiment, and corrected for sample protein content. Protein concentrations were determined by the method of Bradford [7].

2.6. Statistical analysis

For statistical analyses, we used SPSS/PC software. Data were expressed as mean ± standard errors of the mean (S.E.M.), and the significance of differences between mean values was assessed by one-way ANOVA coupled with Duncan's multiple range tests at $p < 0.05$. Correlations were analyzed using Pearson's correlation (bivariate two-tailed).

3. Results

3.1. Changes in DA and 5-HT levels after 6-OHDA lesioning

The effect of unilateral 6-OHDA injection into the MFB was evaluated by TH immunohistochemistry. As expected, 6-OHDA lesioning severely reduced TH-immunoreactivity in the striatum. Using HPLC, we also measured striatal DA and 5-HT levels, and found significantly reduced striatal DA levels (92.6%) compared to intact striata ($n = 12$; $P < 0.01$; Fig. 1A). However, 5-HT levels did not decrease ($n = 12$; $P = 0.497$; Fig. 1B).

3.2. Effects of chronic L-DOPA on striatal DA and 5-HT levels in lesioned striata

After chronic L-DOPA, animals were divided into LND or LID groups according to their axial, limb, and orolingual (ALO) AIMs (Fig. 2A and B), and striatal DA levels were measured in the lesioned striata in the LND ($n = 8$; 0.83 ± 0.14 ng/mg protein) and LID ($n = 12$; 1.78 ± 0.32 ng/mg protein) rats after chronic L-DOPA (Fig. 2C). The LID rats had higher striatal DA levels than the LND rats ($P < 0.05$; Fig. 2B). Striatal 5-HT levels were also compared in the LND ($n = 8$; 0.042 ± 0.021 ng/mg protein) and LID ($n = 12$; 0.142 ± 0.048 ng/mg protein) rats (Fig. 2D). The LID rats had higher 5-HT levels than the LND rats ($P < 0.05$; Fig. 2C).

3.3. Correlation of DA levels with AIMs in L-DOPA-treated rats

Striatal DA levels were significantly correlated with both ALO, and with axial AIMs (adjusted $R = 0.550$, $P = 0.046$ for DA levels and ALO; adjusted $R = 0.672$, $P = 0.018$ for DA levels and axial AIMs, $n = 12$, Fig. 3A and B, respectively), but were not correlated with either locomotive or limb + orolingual AIMs (adjusted $R = 0.465$; $P = 0.121$ for DA levels and limb + orolingual; adjusted $R = 0.362$, $P = 0.134$ for DA levels and locomotive, $n = 12$, Fig. 3C and D, respectively). For analyzing the correlation between DA and AIMs, we used the same LID rats as in Fig. 2.

3.4. Correlation of 5-HT levels with AIMs in L-DOPA-treated rats

Striatal 5-HT levels were negatively correlated with ALO, axial, and limb + orolingual AIMs (adjusted $R = -0.655$, $P = 0.021$ for 5-HT levels and ALO; adjusted $R = -0.789$, $P = 0.006$ for 5-HT levels and axial; adjusted $R = -0.559$, $P = 0.038$ for 5-HT and limb + orolingual, $n = 12$, Fig. 4A, B and C, respectively), but were not correlated with locomotive AIMs (adjusted $R = -0.405$, $P = 0.072$ for 5-HT level and locomotive, $n = 12$, Fig. 4D). These results suggest that DA released from serotonergic fibers contributes to LID and that both striatal DA (positively) and 5-HT (negatively) influence the severity of LID. For analyzing the correlation between DA and AIMs, we used the same LID rats as in Fig. 2.