Neuroscience 314 (2016) 22–34

ADAPTIVE DOWN-REGULATION OF THE SEROTONIN TRANSPORTER IN THE 6-HYDROXYDOPAMINE-INDUCED RAT MODEL OF PRECLINICAL STAGES OF PARKINSON'S DISEASE AND AFTER CHRONIC PRAMIPEXOLE TREATMENT

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Abstract—Our recent study has indicated that a moderate lesion induced by bilateral 6-hydroxydopamine (6-OHDA) injections into the ventrolateral region of the caudate-putamen (CP) in rats, modeling preclinical stages of Parkinson's disease, induces a ''depressive-like'' behavior which is reversed by chronic treatment with pramipexole (PRA). The aim of the present study was to examine the influence of the above lesion and chronic PRA treatment on binding to the serotonin transporter (SERT) in different brain regions. As before, 6-OHDA (15 μg/2.5 μl) was administered bilaterally into the CP. PRA (1 mg/kg) was injected subcutaneously twice a day for 2 weeks. Serotonergic and dopaminergic neurons of the dorsal raphe (DR) were immunostained for tryptophan hydroxylase and tyrosine hydroxylase, respectively, and were counted stereologically. Binding of [^{3}H]GBR 12,935 to the dopamine transporter (DAT) and [^{3}H]citalopram to SERT was analyzed autoradiographically. Intrastriatal 6-OHDA injections decreased the number of dopaminergic, but not serotonergic neurons in the DR. 6-OHDA reduced the DAT binding in the CP, and SERT binding in the nigrostriatal system (CP, substantia nigra (SN)), limbic system (ventral tegmental area (VTA), nucleus accumbens (NAC), amygdala, prefrontal cortex (PFCX), habenula, hippocampus) and DR. A significant positive correlation was found between DAT and SERT binding in the CP. Chronic PRA did not influence DAT binding but reduced SERT binding in the above structures, and deepened the lesion-induced losses in the core region of the NAC, SN, VTA and PFCX. The present study indicates that both the lesion of dopaminergic neurons and chronic PRA administration induce adaptive down-regulation of SERT binding. Moreover, although involvement of stimulation of dopaminergic transmission by chronic PRA in its ''antidepressant'' effect seems to be prevalent, additional contribution of SERT inhibition cannot be excluded.

Key words: Parkinson's disease, preclinical stages, DAT binding, SERT binding, chronic pramipexole, rat model.

INTRODUCTION

Primary motor symptoms of Parkinson's disease (PD) are generally accepted to result from degeneration of nigrostriatal neurons and subsequent, almost complete loss of striatal dopamine (Ehringer and Hornykiewicz, 1960). However, several non-motor (central and autonomic) disturbances are frequently associated with PD and may appear even in preclinical phases of this disease. Depressive symptoms, including major depression, affect a considerable percentage of PD patients (for review see Ossowska and Lorenc-Koci, 2013), and can occur many years before the onset of the motor signs of PD (Schuurman et al., 2002; Fang et al., 2010).

PD is a multisystem disorder where neuropathological degenerative processes develop in different brain regions. A number of data have suggested that besides other neurons, serotonergic cells are also destroyed in PD. According to Braak et al. (2003), neuropathological alterations (Lewy bodies and Lewy neurites) in neurons of the raphe nuclei antedate the appearance of those in the substantia nigra (SN) in the preclinical period of PD and progress further in its clinical phase. Although not confirmed by all authors (Cheshire et al., 2015), the loss of serotonergic cell bodies in the dorsal (Paulus and Jellinger, 1991) or median raphe (Halliday et al., 1990) has been reported in PD patients. Moreover, decreases in the levels of serotonin, tryptophan hydroxylase (TRPH), as well as in the serotonin transporter (SERT) protein level or binding in the caudate nucleus, putamen, different cortical and limbic regions have been found in the autopsy brains of PD patients (Scatton et al., 1983; Raisman et al.,

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Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 6-OHDA, 6-hydroxydopamine; AMG, amygdala; CP, caudate-putamen; DAB, diaminobenzidine; DAT, dopamine transporter; DG, dentate gyrus; DR, dorsal raphe; HB, habenula; ir, immunoreactive; NAC, nucleus accumbens; NET, noradrenaline transporter; NRS, normal rabbit serum; PBS, phosphate buffer; PD, Parkinson's disease; PF, paraformaldehyde; PFCX, prefrontal cortex; PRA, pramipexole; SAL, saline; SERT, serotonin transporter; SN, substantia nigra; TH, tyrosine hydroxylase; TRPH, tryptophan hydroxylase; VTA, ventral tegmental area.

http://dx.doi.org/10.1016/j.neuroscience.2015.11.049
0306-4522/

1986; D'Amato et al., 1987; Chinaglia et al., 1993; Kish et al., 2008; Rylander et al., 2010; Cheshire et al., 2015).

Binding to SERT has recently been used for positron emission tomography (PET) and single photon emission computed tomography (SPECT) brain imaging in PD patients in order to analyze *in vivo* progress of serotonergic neuron pathology and its relationship to symptoms of this disease. In line with the aforementioned *post mortem* studies, decreases in SERT binding in the caudate and putamen, different cortical regions, limbic structures, hypothalamus and midbrain (including raphe nuclei) have been reported to occur in advanced PD (Kerenyi et al., 2003; Guttman et al., 2007; Politis et al., 2010a,b). In contrast, studies in *de novo* patients or in those with early PD have been less consistent (Albin et al., 2008; Politis et al., 2010b; Beuke et al., 2011; Strecker et al., 2011). The binding data have been suggested to reflect either loss of serotonergic neurons/terminals or down-regulation of SERT in PD (Kerenyi et al., 2003; Guttman et al., 2007; Albin et al., 2008; Politis et al., 2010a,b; Strecker et al., 2011).

Dysfunction of serotonergic transmission is generally accepted to contribute to pathogenesis of major depression (Mann, 1999), and in accordance to this view, the loss of serotonergic neurons in the dorsal raphe (DR) has been suggested to underlie PD-associated depression (Paulus and Jellinger, 1991). On the other hand, however, increases in SERT (Boileau et al., 2008; Politis et al., 2010a), and decreases in 5-HT1A receptor binding (Ballanger et al., 2012) *in vivo,* have been found to correlate with depressive symptoms in PD (Boileau et al., 2008; Politis et al., 2010a). However, since PD patients are chronically treated with different antiparkinsonian drugs, mainly with levodopa which is transformed to dopamine in serotonergic neurons (see Navailles et al., 2011), the aforementioned alterations of serotonin markers in this disease may be a resultant of both pathogenic processes and therapy.

Ex vivo and *in vivo* studies carried out in PD models in rats and primates lesioned with 6-hydroxydopamine (6-OHDA) have also shown decreases in SERT binding in different brain structures (including DR) which was suggested to result from a direct deleterious influence of this toxin on serotonergic neurons (Ma et al., 2008; Rylander et al., 2010; Weng et al., 2013). In fact, 6-OHDA, in spite of being considered selective for dopaminergic neurons, has been reported by some authors to reduce (or tend to reduce) additionally serotonin levels or a number of serotonergic neurons (Annett et al., 1992; Karstaedt et al., 1994; Eskow-Jaunarajs et al., 2012; Chotibut et al., 2012).

Our recent study showed that a medium-sized lesion of dopaminergic neurons (40–50% loss of forebrain dopamine and 30–40% loss of cell bodies), induced by bilateral intrastriatal 6-OHDA injections, modeling preclinical phase of PD, evoked "depressive-like" behavior in rats (Berghauzen-Maciejewska et al., 2014). Like other authors (Bonito-Oliva et al., 2014), we found additionally "antidepressant-like" effect of chronic pramipexole (PRA) but not fluoxetine in lesioned rats. In contrast to dopamine, however, the levels of serotonin in regions infiltrated by 6-OHDA solution (caudate-putamen (CP), nucleus accumbens (NAC), frontal cortex) were not changed, which suggested selective dopaminergic toxicity of this agent in the concentration used by us (Berghauzen-Maciejewska et al., 2014).

The aim of the present study was to examine whether dopaminergic lesion itself may influence binding to SERT in different brain regions and whether its alterations may correlate with the observed by us earlier "depressive-like" behavior and/or "antidepressant-like" drug action (Berghauzen-Maciejewska et al., 2014). In order to make sure that 6-OHDA actually did not destroy serotonergic neurons, the same concentration of this toxin was administered in a separate group of rats and the number of serotonergic neurons was counted stereologically in the DR, the main source of serotonergic innervation of the basal ganglia (Azmitia and Segal, 1978). Moreover, we continued our previous examination of the lesion extent by analyzing the binding to dopamine transporter (DAT) in the CP and the number of dopaminergic neurons of the DR which project to the striatum (Descarries et al., 1986; Stratford and Wirtshafter, 1990).

EXPERIMENTAL PROCEDURES

Animals

The experiments were carried out in compliance with the Animal Experiments Bill of January 21, 2005; (published in Journal of Laws No. 33/2005 item 289, Poland), and according to the EC Directive 86/609/EEC on the protection of animals used for scientific purposes. They received also an approval of the Local Ethics Committee at the Institute of Pharmacology, Polish Academy of Sciences (Permit No. 709 of January 28, 2010). All efforts were made to minimize the number and suffering of animals used.

Male Wistar rats (Charles River, Hannover, Germany) weighing 250–300 g prior to experiments were kept under a 12/12 h light/dark cycle (the light on from 7 am to 7 pm) with free access to food and water. All experiments were carried out during the light period. Behaviors of these rats were analyzed previously in the forced swimming test and in actometers (Berghauzen-Maciejewska et al., 2014).

Operations

Under the pentobarbital anesthesia (Vetbutal, Biowet, Poland; 25 mg/kg, *ip*) the animals were fixed into the stereotaxic instrument (Stoelting, Wood Dale, Illinois, USA) and injected bilaterally with 6-OHDA HBr [Sigma, Aldrich, St. Louis, Missouri, USA, 15 μg (free base)/2.5 μl per side, dissolved in 0.2% ascorbic acid] into the ventrolateral region of the CP (AP: 1.2 mm, L: ±3.1 mm, V: 6.8–7.0 mm from the bregma according to Paxinos and Watson atlas (2007). Sham-operated rats which received 2.5 μl of 0.2% ascorbic acid bilaterally into the above region served as controls in all experiments. The injection cannulae were left in place for 60 s to enable full absorption of the solution. In order to spare noradrenergic terminals, desipramine (Sigma, Aldrich, St. Louis, Missouri, USA) was administered in a dose of