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# Molsidomine, a nitric oxide donor, modulates rotational behavior and monoamine metabolism in 6-OHDA lesioned rats treated chronically with L-DOPA

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ABSTRACT

Some biochemical and histological studies of Parkinson's disease patients' brains and 6-OHDA-lesioned rats suggest that dopaminergic dennervation of the striatum leads to the nitrergic system hypofunction in this structure. Hence, recently the modulation of nitric oxide (NO)– soluble guanylyl cyclase–cyclic GMP signaling is considered to be a new target for the treatment of Parkinson's disease. The aim of our study was to examine the impact of chronic combined treatment with low doses of the NO donor molsidomine (2 and 4 mg/kg) and L-DOPA (12.5 and 25 mg/kg) on rotational behavior and monoamine metabolism in the striatum (STR) and substantia nigra (SN) of unilaterally 6-OHDA-lesioned rats.

Chronic administration of molsidomine at a dose of 2 mg/kg jointly with 25 mg/kg of L-DOPA significantly decreased the number of contralateral rotations when compared to L-DOPA alone. Other combinations of the examined drug doses were less effective. The tissue DA levels in the ipsilateral STR and SN after the last chronic doses of molsidomine (2 mg/kg) and L-DOPA (12.5 or 25 mg/kg), were significantly higher than after L-DOPA alone. Chronic L-DOPA treatment alone or jointly with a lower dose of molsidomine decreased 5-HT levels and accelerated its catabolism in the examined structures. However, combination of a higher dose of molsidomine with L-DOPA (25 mg/kg) did not reduce 5-HT content while its catabolism was less intensive. The obtained results show that low doses of molsidomine can modulate rotational behavior and tissue DA and 5-HT concentrations in the STR and SN of 6-OHDA-lesioned rats treated chronically with L-DOPA.



## 1. Introduction

A growing body of evidence derived from animal models reveals a compelling role of nitric oxide (NO), a unique gaseous neurotransmitter and neuromodulator with a broad spectrum of activities in the mammalian brain (Guix et al., 2005), in the regulation of motor function. Behavioral studies have demonstrated that acute administration of selective or non-selective neuronal nitric oxide synthase (nNOS) inhibitors to rodents, reduced spontaneous locomotor activity (Stewart et al., 1994; Dzoljic et al., 1997; Sandi et al., 1995) and hyperlocomotion induced by cocaine (Pudiak and Bozarth, 1993; Przegaliński and Filip, 1997), morphine (Calignano et al., 1993), substance P (Mancuso et al., 1994) and amphetamine or methamphetamine (Przegaliński and Filip, 1997; Ohno and Watanabe, 1995; Abekawa et al., 1994). Locomotor activity enhanced by selective dopamine $D_1$ and $D_2$ receptor agonists (Starr and Starr, 1995; Przegaliński and Filip, 1997) and the NMDA receptor antagonist MK-801 (Deutsch et al., 1996) was also decreased by these inhibitors.

In addition to reduction of the spontaneous and stimulated locomotor activities, injections of nNOS inhibitors evoked a distinct catalepsy (Marras et al., 1995; Krząścik and Kostowski, 1997; Del Bel et al., 1998). Cataleptic activity of nNOS inhibitors was aggravated by selective dopamine $D_2$ receptor antagonists (Marras et al., 1995; Krząścik and Kostowski, 1997; Cavas and Navarro, 2002; Del Bel et al., 2005). In line with these data, an additive long-lasting, cataleptic effect of the non-selective nNOS inhibitor $N^G$-nitro-L-arginine (L-NOARG) and the dopamine $D_2$ receptor antagonist haloperidol, both administered at non-cataleptic doses, was described in rats (Krząścik and Kostowski, 1997). These behavioral data clearly indicate that disturbances in dopaminergic and nitrergic transmissions can cause deficits in motor function. On

*Abbreviations:* ANOVA, analysis of variance; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HPLC, high-pressure liquid chromatography; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; 6-OHDA, 6-hydroxydopamine hydrochloride; HVA, homovanillic acid; MAO, monoamine oxidase; PD, Parkinson's disease; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulate; STR, striatum.

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the other hand, the attenuation of catalepsy induced by nNOS inhibitors or neuroleptics, by the NO precursor, L-arginine or the NO donor molsidomine (Marras et al., 1995; Krząścik and Kostowski, 1997; Cavas and Navarro, 2002; Del Bel et al., 2005) suggests that compounds which increase NO levels in the brain could have a beneficial therapeutic effect in the treatment of motor disorders.

In Parkinson's disease (PD), the principal neuropathology that gives rise to the motor deficit, is the loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNc) which results in a severe depletion of DA in the caudate-putamen (in the rat corresponding to the corpus striatum). Apart from this well-documented pathological alteration in the nigrostriatal dopaminergic system, it has been demonstrated that the number of nitric oxide (NO) synthesizing neurons (Böckelmann et al., 1994) and the expression of nNOS mRNA were markedly decreased in the caudate-putamen of Parkinson's disease patients' brains (Eve et al., 1998). The biosynthesis of NO by nNOS is strictly dependent on the cofactor (6R)-tetrahydrobiopterin ($BH_4$), concentration of which is greatly reduced in the caudate nucleus of parkinsonian patients (Kuiper et al., 1994). Hence, in the cerebrospinal fluid of these patients, the level of nitrate which is considered to be a measure of NO biosynthesis in the brain was found to be significantly reduced when compared to controls (Kuiper et al., 1994).

Consistently with these clinical data, Sancesario et al. (2004) have reported that the level of nNOS protein was decreased by 42% and the number of nNOS-immunopositive intrastriatal fibers but not nNOS-immunopositive cell bodies was markedly reduced in the DA-deafferented rat striatum. Moreover, using an enzymatic method based on conversion of $^3$H-L-arginine to $^3$H-citruline for assessment of nNOS activity, it has been demonstrated that lesion of the nigrostriatal dopaminergic innervation resulted in a 50% decrease in the activity of this enzyme in the ipsilateral striatum (de Vente et al., 2000). In addition, recent biochemical and histological studies performed in animal models provide convincing evidence clearly indicating that DA by stimulation of dopamine $D_1$ receptors localized on striatal nNOS-positive interneurons increases NO biosynthesis in the striatum while acting indirectly via activation of dopamine $D_2$ heteroreceptors, produces opposite effects (Sammut et al., 2006, 2007; Park and West, 2009; Hoque et al., 2010). Hence, it is reasonable to think that the loss of striatal DA due to degeneration of the nigrostriatal dopaminergic neurons would be expected to induce a down-regulation of the striatal NO production.

In contrast to the above-described hypofunction of nitrergic system in the caudate-putamen of parkinsonian patients and in the striatum of 6-OHDA-lesioned rats, an excessive production of NO by microglia in the SN is also suggested as an important factor associated with the pathogenesis of PD. In fact, accumulation of reactive microglia surrounding the remaining DA neurons has been found in the SN of parkinsonian patients (Hirsch et al., 1998) and MPTP-treated monkeys (McGeer et al., 2003). NO is a key molecule produced by reactive microglia that can affect neuronal viability (Block et al., 2007). Consistently, in *in vitro* experiments on the rat midbrain slice cultures treated with interferon-γ (IFN-γ) followed by lipopolysacharide (LPS) as well as in *in vivo* experiments in rats receiving supranigral infusion of these compounds, pharmacological inhibition of inducible isoform of nitric oxide synthase (iNOS) rescued dopaminergic neurons from degeneration induced by activated microglia (Iravani et al., 2002; Shibata et al., 2003; Singh et al., 2005). Also, nNOS inhibition protected the rodent SN against MPTP- and 6-OHDA-induced depletion of DA (Schulz et al., 1995; Di Matteo et al., 2009) and degeneration of DA neurons (Haik et al., 2008; Watanabe et al., 2008; Yuste et al., 2012). Thus, this experimental evidence clearly indicates that NOS inhibitors may exert a beneficial effect on survival of dopaminergic neurons in animal models of PD, in contrast to their deleterious effects upon locomotor activity and catalepsy in non-lesioned animals. Therefore, the use of NOS inhibitors in the treatment of PD is widely discussed.

On the other hand, although IFN-γ/LPS caused death of dopaminergic neurons in the rat midbrain slice cultures in an NO-dependent manner, the inhibition of soluble guanylyl cyclase (sGC) and cyclic GMP-dependent protein kinase exacerbated IFN-γ/LPS-induced loss of DA cells (Kurauchi et al., 2009). The latter effects suggest that the NO–sGC–cGMP signaling paradoxically increases survival of dopaminergic neurons. Kurauchi et al. (2009) have demonstrated that cytoprotective actions of the NO–sGC–cGMP signaling was associated with the induction of heme oxidase-1 (HO-1) whose robust expression was visible in the surviving dopaminergic neurons challenged with IFN-γ/LPS. What is more, HO-1 activity in microglia has been reported to inhibit iNOS-mediated NO production (Min et al., 2006). Interestingly, pretreatment of the rat midbrain slice cultures with low concentrations (0.1–3 μM) of 3-(4-morpholinyl)sydnonimine hydrochloride (SIN-1), an active metabolite of NO donor molsidomine, prevented dopaminergic neuron loss induced by subsequent high SIN-1 concentration (1 mM). SIN-1 at low concentrations also significantly increased the number of HO-1-positive dopaminergic neurons in the examined cultures (Kurauchi et al., 2009). The above-cited study and many others have shown that physiological amounts of NO produced from NOS or released by NO donors are neuroprotective whereas higher concentrations are clearly neurotoxic (Calabrese et al., 2007). Moreover, it is well-known that NO at physiological concentrations acts as an important regulator of synaptic plasticity, neurosecretion and neurotransmission (Calabrese et al., 2007).

Taking into account a broad spectrum of NO actions in the striatum and disturbances in the nitrergic transmission under conditions of DA depletion, it seems that apart from supplementation of deficient DA by L-DOPA, the enhancement of NO level by addition of a low dose of an NO donor could be beneficial for improvement of motor function in PD therapy. Therefore, the aim of the present study was to examine mainly chronic effects of the NO donor, molsidomine (2 and 4 mg/kg) and L-DOPA (12.5 and 25 mg/kg), administered alone or in combination, on asymmetric behavior and DA and serotonin (5-HT) metabolism in the striatum and substantia nigra (SN) of unilaterally 6-OHDA-lesioned rats. Two doses of L-DOPA, low and high, were used to check, how the effect of each of them on the studied parameters will be modulated by low doses of molsidomine administered chronically. The number of contralateral rotations increased with the prolonged L-DOPA treatment. Thus, for comparison we analyzed the effects of acute administration of the used doses of L-DOPA and molsidomine, alone and in combination, on rotational behavioral. As to acute effects on DA and 5-HT metabolism, only combined treatment of mol(2) and L-DOPA (25) was studied. We hope that this set of experiments sheds a new light on a potential role of NO donors in the therapy of PD.

## 2. Materials and methods

The studies were conducted on 3 months old male Wistar rats of an initial body weight between 280 and 320 g kept under standard laboratory conditions; 5 animals per a large cage, at room temperature (22 °C) under an artificial light/dark cycle (12/12 h; lights on from 7 am, lights off from 7 pm), with free access to standard laboratory food and tape water. All procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and received a prior approval from the Bioethics Commission of the Polish Academy of Sciences, as compliant with the Polish Law (of January 21, 2005).