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Original article

Contribution of the nitric oxide donor molsidomine and the antiparkinsonian drug L-DOPA to the modulation of the blood pressure in unilaterally 6-OHDA-lesioned rats

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ABSTRACT

Background: Interaction between dopaminergic and nitrergic neurotransmission in the brain plays a crucial role in the control of motor function and in the regulation of blood pressure (BP). In Parkinson's disease (PD), dopaminergic denervation of the striatum leads to disturbances in the nitrergic system in the basal ganglia. Recently, it has been demonstrated that addition of a low dose of the nitric oxide donor molsidomine to L-DOPA therapy improves dopaminergic neurotransmission in the denervated nigrostriatal system and weakens dyskinesias in rodent models of the disease.
Methods: The aim of the present study was to examine the impact of chronic administration of molsidomine (2 mg/kg) and L-DOPA (25 mg/kg), alone and in combination, on systolic (SBP) and diastolic (DBP) blood pressure in the anesthetized, unilaterally 6-OHDA-lesioned rats. The measurement of SBP and DBP was performed 24 h after the penultimate and immediately after the last drug doses.
Results: In 6-OHDA-lesioned rats receiving saline, spontaneous, small decreases in SBP and DBP were observed during the measurements lasting 60 min. Administration of molsidomine alone or in combination with L-DOPA distinctly decreased the BP in 6-OHDA-lesioned rats already after 10 min compared to those treated with saline or L-DOPA alone, respectively. In both groups, the molsidomine-mediated declines in BP persisted till the end of measurement but they disappeared after 24 h.
Conclusions: Our results indicate that in this PD model molsidomine evokes a short-lasting decrease in BP in contrast to conventional antihypertensive drugs that maintain long-term effect and worsen orthostatic hypotension in parkinsonian patients.

Introduction

It is commonly accepted that dopamine (DA) and nitric oxide (NO) play a significant role in the central regulation of blood pressure (BP) and sympathetic tone [1–6]. Some experiments performed in the anesthetized rats showed that NO produced endogenously or released from NO donors, in such brain structures as the nucleus tractus solitaris (NTS), rosral ventrolateral medulla (RVLM), paraventricular nucleus of hypothalamus (PVN) and medial prefrontal cortex (mPFC) decreased BP [1–4], while other studies carried out in the unanesthetized rats demonstrated that NO acting locally in the PVN or mPFC increased BP [4–6]. NO-mediated BP decreases in anesthetized rats as well as BP increases in unanesthetized rats were abolished by NO synthase inhibitors [1–6]. As to the role of the central dopaminergic system in BP regulation, its activation increased BP [7–9]. Hence, in spontaneously hypertensive rats, intravenous injection of the DA D_2 agonist quinpirole induced a rapid increase in BP which was blocked by the centrally acting DA antagonist haloperidol or sulpiride [7]. Other evidence strongly suggests that substantia nigra pars compacta (SNc) is involved to the modulation of BP [8]. Consistently, it was demonstrated in anesthetized rats that electrical stimulation of DA neurons in the SNc, increased DA release in the striatum (STR), and

Abbreviations: BP, blood pressure; DA, dopamine; MFB, medial forebrain bundle; NO, nitric oxide; nNOS, neuronal nitric oxide synthase; 6-OHDA, 6-hydroxydopamine; OH, orthostatic hypotension; PD, Parkinson's disease; SNc, substantia nigra pars compacta; STR, striatum.

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induced hypertension that was attenuated by prior blockade of postsynaptic DA receptors by intrastriatal administration of DA antagonists (haloperidol or pimozide) or by a spinal transection [8]. Furthermore, the physical or chemical stimulation of DA neurons in the ventral tegmental area has also been shown to increase BP in rats [9]. Both the SNc stimulation-induced hypertension and the increased striatal DA release were attenuated by prior destruction of the nigrostriatal DA pathway by an injection of 6-OHDA into the medial forebrain bundle (MFB) [8]. The above data clearly indicate that stimulation of the dopaminergic neurotransmission in the brain increases BP in rats while damage of central DA system can decrease it. On the other hand, stimulation of peripheral DA receptors by DA agonists decreases BP in rats [7].

The most characteristic neuropathological feature of Parkinson's disease (PD), *i.e.* the loss of DA neurons in the SNc, results in a severe depletion of DA in the caudate-putamen that gives rise to the appearance of motor symptoms. However, besides motor deficits, inherent consequences of the dopaminergic deafferentation of the STR include disturbances in the nitrergic transmission in the basal ganglia [10–14]. Consistently with the above, significant decreases in the number of nNOS containing neurons [10], in the level of nNOS mRNA expression [11] and in the concentration of (6R)-tetrahydrobiopterin (BH_4), which is an important cofactor in NO biosynthesis in the caudate-putamen of parkinsonian brains, as well as in the content of nitrate in the cerebrospinal fluid [15] strongly suggest hypofunction of the nitrergic system in this disease. Similar changes in the striatal nitrergic system as that observed in PD were also found in the 6-OHDA rat model of this disease [12–14,16]. Furthermore, it has been demonstrated that activation of DA D_1 and D_2 receptor in the STR contributes to the regulation of nNOS activity [17–20]. Thus, DA *via* a direct stimulation of DA $D_{1/5}$ receptors co-expressed on striatal nNOS interneurons facilitates NO production while acting indirectly *via* DA D_2 heteroreceptors expressed on other striatal neurons attenuates it [17,18]. Hence in PD, due to deficiency of both DA and BH_4, the endogenous NO biosynthesis in the STR and possibly in other dopaminergic brain structures may be reduced.

In PD therapy, the metabolic precursor of DA, L-3,4-dihydroxyphenylalanine (L-DOPA) is still the most effective and frequently used drug for reliving motor disturbances. However, a long-term administration of this drug leads to the reduction of its therapeutic efficacy and to the appearance of motor complications, such as dyskinesias [21]. Assuming the hypofunction of the nitrergic transmission in 6-OHDA-lesioned rats, in our recently published study we have demonstrated that chronic administration of a low dose of the NO donor molsidomine (2 mg/kg) in combination with L-DOPA (25 mg/kg) increased the tissue concentration of L-DOPA-derived DA in the STR and SN on the lesioned side more distinctly than L-DOPA alone [22]. Furthermore, a slight decrease in the number of L-DOPA-induced contralateral rotations observed in the latter study under the influence of molsidomine, was interpreted as a potential antidyskinetic effect [22] and in fact antidyskinetic activity of a low dose of molsidomine has been recently confirmed in the genetic mouse model of PD [23]. The above-presented beneficial effects of molsidomine and L-DOPA in 6-OHDA-lesioned rats implicate potential usefulness of this drug combination in PD therapy.

Since both DA and NO exert potent modulatory effects on BP, it was of great importance to check how the combination of molsidomine with L-DOPA affects this parameter in the rat model of PD, especially because in this disease, cardiovascular autonomic dysfunctions are commonly observed [24–26]. They include orthostatic hypotension (OH), supine hypertension (HT), nocturnal hypertension (NT), labile blood pressure and heart rate (HT) variability [24–27]. Cardiovascular autonomic dysfunctions are associated with sympathetic noradrenergic neurons, resulting in cardiac and extra-cardiac noradrenergic denervation [25,27]. It is also supposed that these dysfunctions are related to the use of L-DOPA and/or DA agonists. However, these results are contradictory; some studies suggested that OH was associated with L-DOPA treatment while others did not confirm it [26,27]. Therefore, in the present study, an analysis of systolic and diastolic blood pressure (SBP and DBP) was carried out in unilaterally 6-OHDA-lesioned rats treated chronically with the same doses of molsidomine (2 mg/kg) and L-DOPA (25 mg/kg), alone or in combination, as in our previous study [22]. Experiments were performed in the anesthetized rats because L-DOPA induces intensive contralateral rotation directly after *ip* injection. BP was measured 24 h after administration of the penultimate doses of molsidomine and L-DOPA ("OFF phase") to check their long-lasting effects and immediately after the last doses to assess their direct action ("ON phase"). Additionally, effects of single doses of these drugs on BP were studied in the intact anesthetized rats. We hope that these experiments shed new light on the disorders of BP in PD and on application of NO donors in the therapy.

Materials and methods

Animals

The experiments were carried out in compliance with the Animal Experiments Bill of January 21, 2005 reapproved of January 15, 2015 (published in Journal of Laws no 33/2005 item 289 and no 23/2015 item 266, Poland), and according to the Directive of the European Parliament and of the Council of Europe 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes. They were also approved by the Local Ethics Committee at the Institute of Pharmacology Polish Academy of Sciences. All efforts were made to minimize the number and suffering of animals used.

The experiments were conducted on normotensive male Wistar rats (Charles River, Germany) 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 chow and tape water. All experiments were carried out during the light period.

Stereotaxic operation

Thirty minutes prior to surgery, animals received *ip* 15 mg/kg of desipramine hydrochloride in order to inhibit 6-OHDA-induced degeneration of noradrenergic pathways [28]. Then rats were lightly anesthetized with a mixture (1:1 v/v) of ketamine (50 mg/kg, Biowet, Poland) with diazepam (2.5 mg/kg, Polfa, Warsaw, Poland) administered in a volume of 1 ml/kg body weight and were placed in a stereotaxic apparatus. A stainless steel needle (0.28 mm o.d.) was inserted unilaterally through a small hole in the skull and the needle tip was placed in the left medial forebrain bundle (MFB). The stereotaxic coordinates according to the atlas of Paxinos and Watson [29] were as follow: A/P = −2.8 mm, L = +1.8 mm, D/V = −8.6 mm. The solution of 6-hydroxydopamine (6-OHDA) was freshly prepared before surgery. 6-OHDA hydrochloride, at a dose of 8 μg (calculated as the free base) dissolved in a volume of 4 μl of sterile 0.9% NaCl supplemented with 0.05% ascorbic acid, was slowly infused into the left MFB at a flow rate of 0.5 μl/min using a 10-μl Hamilton syringe. After stopping the infusion of 6-OHDA, the cannula was left in place for a further 5 min for complete diffusion of the toxin and then was slowly retracted. Sham-operated rats were treated in the same manner but received equivalent volumes of vehicle instead of 6-OHDA.

Assessment of the extent of the lesion

As established in our previous study, in rats injected with 6-OHDA (8 μg/4 μl) unilaterally into the MFB, which exhibited more than 100 contralateral turns per 1 h in the apomorphine test (APO; 0.25 mg/kg *sc*) two weeks after lesion, the levels of DA in the ipsilateral STR and SN were drastically reduced by 95 and 80% of control, respectively [22]. Therefore, in the present study, 6-OHDA-lesioned rats were selected based on the rotation behavior in response to APO two weeks after surgery. To this aim, individual rats were placed for 1 h in automated rotameters (Panlab, Barcelona, Spain) [30] equipped with a computer software for recording rotations in ipsiversive and contraversive directions. All 6-OHDA-lesioned rats used, exhibited 241.2 ± 20.3 (n = 26) contralateral and 1.6 ± 0.5 (n = 26) ipsilateral turns per 1 h, fulfilling behavioral criterion of an extensive unilateral lesion of nigrostriatal dopaminergic system.

Drugs and treatment

Desipramine hydrochloride, 6-hydroxydopamine hydrochloride (6-OHDA), L-ascorbic acid, apomorphine hydrochloride, benserazide hydrochloride, N-(ethoxycarbonyl)-3-(4-morpholino)sydnone imine, SIN-10 (molsidomine), 3,4-dihydroxy-L-phenylalanine (L-DOPA) were provided by the Sigma-Aldrich Chemical Company (Steinheim, Germany).

The experiment was performed in two series. In the first series of experiments, a group of unilaterally sham-operated (n = 6) and 4 groups of 6-OHDA-lesioned male Wistar rats (n = 26), 12 weeks of age, weighing 280–320 g were treated chronically *ip*, with molsidomine (2 mg/kg) or benserazide (6.25 mg/kg) plus L-DOPA (25 mg/kg), alone or in combination, once daily for successive 14 days. Control groups were treated chronically *ip* with 0.9% NaCl solution (2 ml/kg). Administration of drugs or saline started on the following day after the APO test. Molsidomine and benserazide were dissolved, respectively, in 2 ml of 0.9% NaCl or the redistilled water while L-DOPA was suspended in 2 ml of propylene glycol. Benserazide was injected 30 min before L-DOPA, while molsidomine was given 20 min after benserazide *i.e.* 10 min before L-DOPA. Administration of benserazide + L-DOPA was defined in the whole text as L-DOPA treatment.

In the second series of experiments, 4 groups of naive male Wistar rats (n = 24), 8–10 weeks of age, weighing 200–250 g were injected *ip*, with a single dose of vehicle, molsidomine and L-DOPA.

Measurement of blood pressure

The rats were anesthetized with thiopental (70 mg/kg) by *ip* injection. The left carotid artery was cannulated with polyethylene tubing (1.5 mm o.d.) filled with heparin solution in saline to facilitate pressure measurements using Datamax apparatus (Columbus Instruments, Ohio, USA). In all groups, SBP and DBP were measured after the introduction of anesthesia in rats *i.e.* prior to administration of the studied drugs at a time point 0 min (control pressure), and subsequently after drugs' administration. In groups receiving L-DOPA, BP measurement started before benserazide administration and lasted 90 min while in the remaining groups, BP measurement began before molsidomine/vehicle injection and lasted 60 min. The statistical analysis was performed on the data from the 60-min interval, starting from the moment preceding molsidomine or saline administration.

Statistics

Analysis of the time-dependent changes in SBP and DBP was carried out using a repeated measures ANOVA followed (if significant) by the Bonferroni test for *post-hoc* comparisons. The significance of differences in SBP or DBP between sham-operated and 6-OHDA-lesioned groups 24 h after the penultimate dose of vehicle, was estimated using Student's *t*-test for independent samples. Statistical analysis of SBP or DBP in 4 groups of 6-OHDA-lesioned rats, 24 h after the penultimate doses of vehicle and the studied drugs, was performed using a two-way ANOVA followed (if significant) by the Bonferroni test for *post-hoc* comparisons.

The p values of less than or equal to 0.05 were considered to indicate statistical significance. The statistical analysis was done using STATISTICA 10.0 Software (Statsoft, Inc, USA).

Results

The effects of chronic administration of molsidomine and L-DOPA on BP in unilaterally 6-OHDA-lesioned rats

Fig. 1A, D shows the time course of SBP and DBP changes measured in unilaterally sham-operated and 6-OHDA-lesioned rats treated chronically with saline, molsidomine, L-DOPA and molsidomine +L-DOPA. A repeated measures ANOVA performed for SBP and DBP in sham-operated and 6-OHDA-lesioned groups demonstrated only a significant effect of time (Fig. 1A, $F_{(6,72)} = 9.561$; 1B, $F_{(6,72)} = 10.556$; $p < 0.0001$). However, the same analysis carried out in 4 groups of 6-OHDA-lesioned rats separately for SBP and DBP revealed an overall treatment effect of molsidomine (Fig. 1C, $F_{(1,22)} = 45.867$, $p < 0.00001$; Fig. 1D, $F_{(1,22)} = 20.599$ $p < 0.0001$), a lack effect of L-DOPA treatment and no interaction of molsidomine and L-DOPA. This analysis also showed an effect of time (Fig. 1C, $F_{(6,132)} = 47.304$, $p < 0.0001$; Fig. 1D, $F_{(6,132)} = 36.794$, $p < 0.0001$) and an interaction of time × molsidomine treatment (Fig. 1C, $F_{(6,132)} = 21.642$, $p < 0.0001$; Fig. 1D, $F_{(6,132)} = 14.973$, $p < 0.0001$), but no interactions of time × L-DOPA, and time × molsidomine × L-DOPA.

As shown in Fig. 1A, B, in sham-operated and 6-OHDA-lesioned rats treated with vehicle, SBP and DBP decreased slightly during the last 20 min of the 1-h measurement compared to values of these parameters at time point 0 min. *Post-hoc* comparison of BP in 4 groups of 6-OHDA-lesioned rats (Fig. 1C, D) showed that L-DOPA did not affect SBP and DBP. However, molsidomine, administered alone or in combination with L-DOPA, decreased significantly both SBP and DBP already after 10 min as compared to their values before drug injection as well as when compared to the corresponding time points in 6-OHDA-lesioned groups treated with saline or L-DOPA alone (Fig. 1C, D). The time-dependent declines in SBP and DBP did not differ in a statistically significant manner between the group treated with molsidomine alone and that receiving molsidomine jointly with L-DOPA, however, a more pronounced declining tendency was visible in the latter group.

SBP and DBP 24 h after chronic treatment with molsidomine and L-DOPA in unilaterally 6-OHDA-lesioned rats

Table 1 presents values of SBP and DBP in unilaterally sham-operated and 6-OHDA-lesioned rats, measured 24 h after the penultimate (13th) dose of vehicle and the studied drugs. SBP and DBP did not differ significantly between sham-operated and 6-OHDA-lesioned groups (SBP $t = 0.615$, $df = 12$; $p > 0.05$; DBP $t = -0.077$, $df = 12$, $p > 0.05$). A two-way ANOVA performed separately for SBP and DBP in 4 groups of 6-OHDA-lesioned rats demonstrated the lack of treatment effects for molsidomine (SBP $F_{(1,22)} = 2.039$, $p > 0.05$; DBP, $F_{(1,22)} = 1.667$, $p > 0.05$) and L-DOPA (SBP $F_{(1,22)} = 0.005$, $p > 0.05$; DBP, $F_{(1,22)} = 0.379$, $p > 0.05$) and no interaction between these drugs (SBP $F_{(1,22)} = 3.674$, $p = 0.07$; DBP, $F_{(1,22)} = 1.511$, $p > 0.05$). The latter analysis indicates no effects of the studied drugs on BP 24 h after their administration (Table 1).

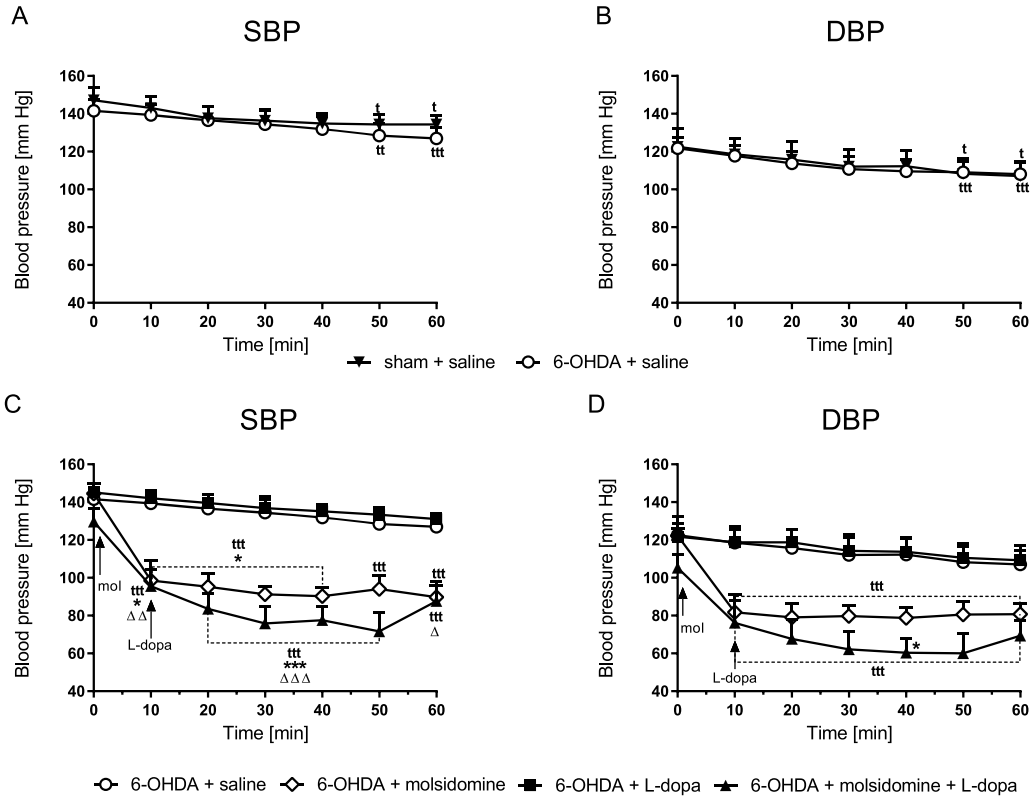

Fig. 1. Systolic (SBP) and diastolic (DBP) blood pressure in unilaterally sham-operated and 6-OHDA-lesioned rats treated chronically *ip* with saline (A, B) or with molsidomine (mol) and L-DOPA, alone or in combination (C, D). Data are presented as the mean ± SEM, the number of animals per group n = 6–8. Statistically significant differences in SBP or DBP were calculated using a repeated measures ANOVA, followed (if significant) by the Bonferroni test for *post-hoc* comparison, $^{t}p < 0.05$, $^{ttt}p < 0.001$ *vs.* time point 0 min; $^{*}p < 0.05$, $^{***}p < 0.001$ *vs.* corresponding time points in 6-OHDA + saline-treated group and $^{\Delta\Delta\Delta}p < 0.001$ *vs.* 6-OHDA + L-DOPA-treated group.

Table 1
The effect of chronic, systemic treatment with molsidomine (mol) and L-DOPA, alone or in combination, on systolic (SBP) and diastolic (DBP) blood pressure measured 24 h after the penultimate doses of the studied drugs.

Experimental groups	Mean SBP [mmHg]	Mean DBP [mmHg]
Sham + saline	147.17 ± 6.58	121.67 ± 5.89
6-OHDA + saline	141.63 ± 6.04	122.63 ± 9.71
6-OHDA + mol (2 mg/kg)	144.50 ± 5.57	122.17 ± 3.84
6-OHDA + L-DOPA (25 mg/kg)	152.50 ± 3.18	127.17 ± 5.68
6-OHDA + mol + L-DOPA	132.83 ± 7.29	108.50 ± 5.88

Experiments were performed on one group of unilaterally sham-operated and 4 groups of 6-OHDA-lesioned rats which received vehicle or 6-OHDA (8 μg/4 μl) into the left MFB. Systemic administration of vehicle or the studied drugs started 2 weeks after surgery and lasted the following 2 weeks. The data are presented as the mean ± SEM, the number of rats per group was n = 6 for a sham + saline, 6-OHDA + mol-, 6-OHDA + L-DOPA-, 6-OHDA + mol + L-DOPA- and n = 8 for 6-OHDA + saline-treated group.

The effects of a single dose of molsidomine and L-DOPA on BP in intact rats

Fig. 2A, B illustrate the time course of SBP and DBP changes measured in intact rats treated with a single dose of saline or the studied drugs. A repeated measures ANOVA performed for each set of data presented in these figures separately for SBP and DBP revealed an overall acute treatment effects of molsidomine (Fig. 2A, $F_{(1,20)} = 31.160$, $p < 0.0001$; Fig. 2B, $F_{(1,20)} = 25.622$, $p < 0.0001$) and L-DOPA (Fig. 2A, $F_{(1,20)} = 5.578$, $p < 0.05$; Fig. 2B, $F_{(1,20)} = 8.368$, $p < 0.01$) but no interaction between these drugs. Furthermore, this analysis showed an effect of time (Fig. 2A, $F_{(6,120)} = 53.802$, $p < 0.0001$; Fig. 2B, $F_{(6,120)} = 29.938$, $p < 0.0001$), and an interaction of time × acute molsidomine treatment (Fig. 2A, $F_{(6,120)} = 46.274$, $p < 0.0001$; Fig. 2B, $F_{(6,120)} = 26.269$, $p < 0.0001$) but no interactions of time × acute L-DOPA (Fig. 2A, $F_{(6,120)} = 1.998$, $p > 0.05$; Fig. 2B, $F_{(1,120)} = 2.150$, $p = 0.052$), and time × acute molsidomine × L-DOPA.

Post-hoc analysis demonstrated that a single dose of L-DOPA did not evoke a statistically significant decrease in SBP and DBP in the intact rats. Molsidomine administered at a single dose, alone or in combination with L-DOPA, reduced SBP and DBP already after 10 min compared to values of these parameters before drug injection as well as when compared to the corresponding time points in the control group treated with saline (Fig. 2A, B). The time-dependent declines in SBP and DBP, in group receiving molsidomine jointly with L-DOPA and in that treated with molsidomine alone persisted at the same levels (Fig. 2A, B).

Discussion

Alterations in BP manifested by orthostatic hypotension (OH) and supine hypertension (HT), are highly prevalent in parkinsonian patients [24–27]. Treatment of these symptoms constitutes a serious therapeutic problem. Therefore, research of BP changes in various animal models of PD appears to have a great theoretical and practical importance. In the present study, for the first time an analysis of SBP and DBP was carried out in anesthetized unilaterally 6-OHDA-lesioned rats treated chronically with molsidomine and L-DOPA, alone and in combination. The unilateral lesion of the nigrostriatal dopaminergic system was chosen as a rat model of PD in this experiment, because the extensive bilateral lesion induced by 6-OHDA infusion into the MFB resulted in aphagia and adipsia what required a tube feeding of rats [31]. On the contrary to bilateral lesion, the unilaterally 6-OHDA-lesioned rats are vital and do not require special feeding. The major advantages of the unilateral model of PD include an easy

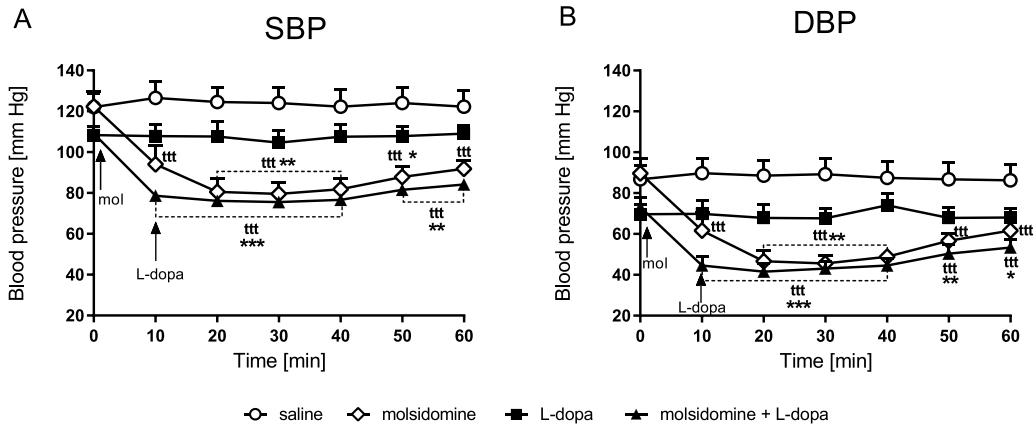

Fig. 2. The effects of single doses of molsidomine (mol) and L-DOPA, administered alone or in combination, to intact rats on systolic (SBP) and diastolic (DBP) blood pressure (A, B). Control group received a single injection of saline instead of drugs. Data are presented as the mean ± SEM, the number of animals per group n = 6. Statistically significant differences in SBP or DBP in the Bonferroni *post-hoc* test, $^{ttt}p < 0.001$ *vs.* time point 0 min; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* corresponding time points in saline-treated group (A, B).

quantitative assessment of motor deficit (rotation behavior) and its usefulness in the pharmacological screening of drugs that have effects on DA and its receptors [30,31]. Furthermore, the unilateral rat model of PD expresses some subtle signs of cardiovascular autonomic dysfunction [32]. Consequently, in these rats a greater decrease in heart rate (HR) was observed at night compared to controls whereas during a day declines in HR were similar in both groups [32]. It is also worth underlining that the used dose of 6-OHDA in our study, besides the drastic loss of DA in the ipsilateral nigrostriatal system [22], significantly reduced the level of noradrenaline (NA) in the ipsilateral STR, SN and other brain structures [33], despite pretreatment with the NA transporter blocker desipramine that inhibits 6-OHDA uptake into the noradrenergic neurons [28]. In PD, loss of noradrenergic neurons in the locus coeruleus and consequently, NA content in different brain structures preceded degeneration of DA neurons in the SNc [34]. Moreover, parkinsonian patients with OH were reported to show the loss of noradrenergic innervation in the heart and the decreased NA level in the plasma [25–27]. An additional feature of the unilateral lesion characterized by us was almost 25% decline in nNOS protein level in the ipsilateral SN suggesting the reduced NO production in this structure [16].

Referring to BP changes in the anesthetized unilaterally sham-operated and 6-OHDA-lesioned rats, measured in the present study 4 weeks after surgery, some slight but significant decreases in SBP and DBP were observed in the end phase of the 1-h lasting measurement. As similar decreases in BP occurred in both groups, it is believed that they could be due to anesthesia. This assumption seems to be confirmed by results of the previous study by Horn et al. [4] who reported that microinjection of NO donor, sodium nitroprusside (SNP) into the PVN decreased BP and renal nerve discharge in the anesthetized rats and these effects were blocked by pretreatment with bicuculline, a $GABA_A$ receptor antagonist [3]. These data suggest that NO produced endogenously in the PVN, acting locally *via* GABAergic neurotransmission, modulates BP. GABA is known to exert tonic inhibitory effect on the sympathetic nervous system in the PVN. Thus, GABA interneuron-induced inhibition of PVN spinally projecting vasopressin-containing neurons that synapse with renal sympathetic neurons results in a decrease in sympathetic activity in the kidney and renal vasodilatation [35]. Most anesthetics potentiate function of $GABA_A$ receptors [36], therefore it is likely that thiopental, used in our study, acting *via* these receptors in the PVN could cause a slight lowering of BP in the sham-operated and 6-OHDA-lesioned rats. But also other mechanisms may contribute to the decline of BP. Notably, the decreases in BP and heart rate were observed in unanesthetized rats injected bilaterally with 6-OHDA into the SNc [37]. In these rats, after intravenous infusion of an α-adrenergic antagonist, smaller declines in SBP and DBP were observed than in sham-operated animals treated in the same way, suggesting an impairment of vascular sympathetic activity in that PD model [37]. In contrast to bilateral infusion of 6-OHDA into the SNc, unilateral injection of this neurotoxin into the left STR increased BP in unanesthetized rats [38]. As the increase in BP was visible only in the left side-lesioned rats but not in the right side-lesioned ones, the authors of this study conclude that this effect was due to an asymmetrical cardiovascular response to unilateral lesion [38]. However, it cannot be excluded that the increased BP in the latter study could be associated with the compensatory activity of the noradrenergic system which is unlikely to be damaged after microinfusion of 6-OHDA into the STR due to poor noradrenergic innervation of this structure. The above data show that 6-OHDA, depending on the site of its infusion in the brain and presence or absence of anesthesia during the BP measurement evokes hypotensive or hypertensive effects.

In the present study, chronic administration of L-DOPA to 6-OHDA-lesioned rats maintained BP at the same level as in those receiving saline. L-DOPA is metabolized to DA in the brain, because its peripheral transformation is blocked by pretreatment with benserazide, the selective inhibitor of peripheral DOPA decarboxylase. Thus, L-DOPA affects BP predominantly acting centrally in the brain *via* the formed DA which activates DA D_1 and D_2 receptors. Activation of striatal DA receptors by agonists induced hypertension in intact anesthetized rats [8], hence, one would expect that L-DOPA will also increase BP. However, the lack of changes in BP in our study suggests that the replenishment of DA by the administered L-DOPA in 6-OHDA-lesioned rats was not sufficient to enhance BP. It is also likely that the unilateral damage of the noradrenergic pathway plays some role here.

In contrast to L-DOPA, chronic treatment with molsidomine alone distinctly decreased SBP and DBP already after 10 min, and these effects were slightly more strongly expressed when molsidomine was administered in combination with L-DOPA. NO donated by molsidomine participates in the lowering of BP by acting directly *via* vasodilatation and indirectly in the central nervous system *via* modulation of vascular sympathetic tone. Intraperitoneally administered molsidomine affects both these mechanisms. Regarding the centrally mediated decline of BP in anesthetized rats in our study, NO donated by molsidomine can

produce it, *via* the GABA-mediated neurotransmission in the PVN [3,35] as described above. On the other hand, it has been demonstrated in unanesthetized rats that microinjection of the NO donor SNP, into the PVN or mPFC increased BP [4–6]. Furthermore, in conscious rats, microinjection of L-glutamate into these structures caused increases in BP which were similar to that evoked by NO donors [4–6]. The latter effects indicate an important contribution of glutamatergic transmission in the PVC and mPFC to the regulation of BP.

In PD and its animal model, disturbances in the GABAergic transmission in the basal ganglia are an inherent consequence of the dopaminergic deafferentiation of the STR that finally leads to the inhibition of glutamatergic pathway projecting from the thalamus to the cortex and to the decreased release of Glu [39]. The lower level of Glu in the mPFC may reduce NMDA receptor-mediated NO synthesis and, in fact, in unilaterally 6-OHDA-lesioned rats a 50% decrease in NOS activity was observed in the ipsilateral frontal cortex [12]. L-DOPA partially normalized glutamatergic transmission from the thalamus to the cortex in unilaterally 6-OHDA-lesioned rats, but NO produced by Glu-mediated NO synthesis, had no effect on BP measured in our study. In the light of these data, we can hypothesize that deficiency of NO in the brain in PD may contribute to essential hypotension in patients with a distinct sympathetic dysfunction but in patients with a subtle sympathetic dysfunction it may cause hypertension. Therefore, an application of NO donors seems to be reasonable in PD patients with supine HT. In line with this suggestion, it has been demonstrated that a low dose of the NO donor nitroglycerin decreased HT but did not worsen OH in the morning [40]. The declines in the BP observed directly after chronically administered molsidomine, alone or in combination with L-DOPA, were transient and disappeared within 24 h. Thus, the action of molsidomine is different from the typical antihypertensive drugs that produce a prolonged decline in SBP. As molsidomine administered jointly with L-DOPA also caused a transient but not prolonged hypotensive effect, the combined therapy with NO donors and L-DOPA in the treatment of motor disturbances, postulated by us, may be also beneficial, at least in some PD patients, in decreasing supine HT at night without worsening OH in the morning.

Conflict of interest

None declared.

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