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Respiratory activity in the 6-hydroxydopamine model of Parkinson's disease in the rat

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Respiratory disturbances accompany Parkinson's disease. Weakness of the respiratory muscles or lowering of central respiratory drive might be responsible for respiratory disability. Striatal injection of 6-hydroxydopamine (6-OHDA) simulates motor symptoms of Parkinson's disease in the rat. Present study investigated whether unilateral infusion of 6-OHDA into the striatum may evoke respiratory disorders and therefore be a model for the study of the respiratory aspects of Parkinson's disease. Two weeks after the infusion the animals were anesthetized, vagotomized, paralyzed and artificially ventilated. Neural respiratory activity in the vehicle and 6-OHDA treated groups of animals was assessed from the peak amplitude of the phrenic and hypoglossal bursts, frequency of bursts and minute activity during baseline ventilation and acute intermittent hypoxia composed of five 1.5 minute long episodes of 11% oxygen introduced every 3 minutes. An impairment of dopaminergic pathways by 6-OHDA evoked separate effects on phrenic and hypoglossal activity. Under baseline conditions the respiratory parameters taken from the integrated phrenic nerve activity unchanged, while the pre-inspiratory part of the hypoglossal activity (pre-I HG) was reduced both in terms of its onset and amplitude. 6-OHDA did not affect the phrenic response to acute intermittent hypoxia but it increased the hypoglossal response (Fig. 2). Hypoxia activated the pre-I HG in both experimental groups. Although the pre-I HG increased strongly during hypoxic stimulation, the ratio of the pre-inspiratory hypoglossal amplitude to the inspiratory hypoglossal amplitude never achieved similar values as in the sham group. This ratio decreased significantly during secondary decline of the hypoxic respiratory response. A decline of the hypoxic response was more intense in the hypoglossal activity than in the phrenic activity and moved into hypoxic apnoea more frequently in the Parkinson's disease model. The results indicate a differential modulation of the phrenic and hypoglossal neural output with increased chemical drive when dopaminergic pathways were impaired by 6-OHDA suggesting that such a mechanism may contribute to respiratory insufficiency in Parkinson's disease. An involvement of a modified mechanism of dopamine efflux and of serotonin and orexin during hypoxia is suggested in the observed changes in the hypoglossal activity in the 6-OHDA model of PD**.**

Key words: 6-hydroxydopamine**,** phrenic and hypoglossal nerve activity, acute intermittent hypoxia, rat

INTRODUCTION

Parkinson's disease (PD) is a neurogenerative disorder characterized by neurologic features including bradykinesia, tremor and rigidity. Clinical symptoms of Parkinson's disease are associated with a selective loss of dopaminergic neurons in several brain areas mainly in the substantia nigra pars compacta, and with a reduction of dopamine release in the striatum (Hornykiewicz 1975). In addition to the extrapyramidal motor disorders, an important part of the PD syndrome is formed by disturbances in breathing pattern and pulmonary ventilation. Clinical observations of PD patients imply an impairment of respiratory muscle function (Estenne et al. 1984, Tzelepis et al. 1988) as well as pulmonary and upper airway obstruction (Hovestadt et al. 1989, Sabate et al. 1996). It is suggested (Izquierdo-Alonso et al. 1994) that a dysfunction of upper airway muscles is the main cause of the respiratory abnormalities reported in patients with Parkinson's disease. It is however unclear whether the weakness of the respiratory muscles or a change in the neural drive to the respiratory muscles is responsible for

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respiratory disturbances in PD. Furthermore, the respiratory response to hypoxia in PD patients is diminished and interpreted as a probable consequence of reduced sensitivity of peripheral chemoreceptors to low oxygen concentration in the blood (Serebrovskaya 1998). The neural mechanisms underlying the respiratory aspects of Parkinson's disease still remain poorly described.

Experimental animal models replicate dopamine deficit in Parkinson's disease. Unilateral injection of 6-hydroxydopamine (6-OHDA), a structural analog of dopamine, into the nigrostriatal system in the rat results in unilateral selective destruction of nigrostriatal dopaminergic neurons and creates various motor symptoms of PD (Ungerstedt 1968). This model is one of the most reproducible tools used for investigating the mechanisms of motor and biochemical effects of the dopamine neurons loss.

Animal model of PD has not yet been introduced to the research of pathophysiology of respiratory alternations attributable to dopamine deficit. This study was designed to address the question whether 6-OHDA lesions in the nigrostriatal dopaminergic system of the rat could generate similar respiratory symptoms as observed in parkinsonian patients. The specific objective of this study was to investigate whether the 6-OHDA model of PD has an impact on neural respiratory activity to the diaphragm, the upper airway muscles and on the respiratory response to acute intermittent hypoxia in a setting when ventilation of the lungs is accomplished by mechanical means and, due to paralysis of the muscles does not depend on muscle function. For this purpose phrenic and hypoglossal nerve activity, as a nervous output to the diaphragm and the upper airway muscles respectively, was studied during eupneic ventilation and during acute intermittent hypoxia in control animals and following unilateral 6-OHDA infusion. We have chosen the unilateral 6-OHDA model of Parkinson's disease because it is widely used for the study of dopamine denervation pathophysiology, its neuroanatomical correlates and mechanisms of dopaminergic neurotransmission (Tadaiesky et al. 2008). Evident motor impairment in the unilateral model is very well defined behaviorally (Schwarting and Huston 1996). The bilateral model of PD is less frequently applied because bilateral 6-OHDA injections are traumatic, cause aphagia and adipsia, and high mortality rate (Ungerstedt 1971). PD patients exhibit more or less asymmetrical neuronal degeneration and lateralized clinical symptoms (Djaldetti et al. 2006, Hobson 2012) including postural asymmetry.

The experiments revealed a difference in the response to acute intermittent hypoxia of the phrenic and hypoglossal activity suggesting uneven neural hypoxic drive to the diaphragm and the muscles of the upper respiratory tract in 6-OHDA model of Parkinson's disease.

METHODS

Animals and 6-OHDA injection

All experimental procedures followed the European Communities Council Directive 86/609/EEC for the care and use of laboratory animals and were approved by the Local Ethics Committee for Animal Experimentation. Adult male Wistar rats weighing 240–260 g were used in the present study. Animals were anesthetized with thiopental (Sandoz, Kundl-Rokuska, Austria) at a dose of 90 mg/kg intraperitoneally. The head of the rat was mounted in a stereotaxic apparatus. A small hole in the skull was drilled to introduce the needle of Hamilton syringe into the right striatum according to the stereotaxic coordinates (AP 1.0, L 2.8, and D 5.0) of the Paxinos and Watson (2007) Atlas. Thirty minutes before 6-OHDA infusion desipramine (Sigma Chemicals, Poznan, Poland) was administered intraperitoneally at a dose of 25 mg/kg to prevent the uptake of 6-OHDA by noradrenergic terminals (Fulceri et al. 2006). Six-hydroxydopamine was dissolved into a concentration of 4 μg/μl in 0.9% NaCl containing 0.1% ascorbic acid. Six microliters of 6-OHDA solution were injected into the striatum over a period of 6 minutes. The needle was left in place after the injection for another 6 minutes and then slowly withdrawn. Sham-lesioned animals received into the striatum 6 μl of vehicle instead of 6-OHDA solution. Following the procedure, the animals were housed under laboratory conditions in 12/12 hour light/dark cycle during two weeks after lesioning. Food and water were available *ad libitum*.

Electrophysiological experiment

Fourteen days after unilateral injection of 6-OHDA or vehicle the animals were anesthetized intraperitone-

ally with 800 mg/kg of urethane (Sigma Chemicals, Poznan, Poland) and 70 mg/kg of α-chloralose (Fluka, Neu-Ulm, Germany). Following the tracheostomy, rats were paralyzed with pipecuronium bromide (Arduan, Gedeon-Richter, Budapest, Hungary) at initial dose of 0.08 mg/kg, supplemented every hour, and artificially ventilated (7025 Rodent Ventilator, Ugo Basile, Comerio, Italy) with oxygen enriched air to keep the oxygen pressure in arterial blood not less than 100 mmHg.

A femoral artery was cannulated to monitor blood pressure and to measure arterial blood acid-base as well as of oxygen and carbon dioxide. A femoral vein was cannulated to administer supplemental anesthesia and fluids as required. Rectal temperature was maintained throughout the experiment at 37–38°C by means of external heating. Arterial blood pressure was measured with a BP-2 Columbus Instruments (Columbus, OH, USA) monitor whereas the arterial partial pressure of O_2 and CO_2 and pH – using a Blood Gas Assembly (AVL Compact2, Graz, Austria).

Vagus nerves were isolated in the neck and cut to eliminate an entrainment of the respiratory activity with lung inflation induced by respiratory pump. The C5 phrenic nerve root and the hypoglossal nerve were transected in the neck. Central ends of the whole phrenic nerve and the main hypoglossal trunk were placed on bipolar silver electrodes for recording. The activities of both nerves were amplified, and filtered (5–2 500 Hz) using a NeuroLog system (Digitimer Ltd., Wewelyn, UK) and integrated with the time constant of 70 ms. Raw and integrated nervous activities and arterial blood pressure were digitized using a CED Power 1401 data acquisition interface, recorded on a computer and analyzed using the Spike 2 software (Cambridge Electronic Design, CED, Cambridge, UK).

After encountering apnoea in the phrenic and hypoglossal activity ($PaCO_2$ about 39 mmHg in both groups of experiments) by an increase of the respiratory pump rate, parameters of mechanical ventilation were set in this way that $PaCO_2$ was kept at about 42–43 mmHg. The oxygen was added to the inlet of respirator to keep PaO_2 at about 100–103 mmHg. After these manipulations the animals recovered for about 20 minutes and respiratory pattern and arterial blood pressure accomplished stabilized values. At the end of experiment the respiratory response to 7% CO_2 in O_2 was evaluated.

The phrenic and hypoglossal activity and the effects of acute intermittent hypoxia were studied in two experimental groups: (1) control group of sham-lesioned animals (n=10); (2) 6-OHDA group (n=7).

Acute intermittent hypoxia protocol consisted of five episodes of ventilation with 11% oxygen in nitrogen. Each hypoxic exposure lasted about 1.5 minutes and was administered in 3-minute intervals. The respiratory variables and blood pressure were continuously recorded before, during and after intermittent hypoxia. Arterial blood samples were taken prior to and during the first, third and fifth hypoxic exposure.

Histology

At the end of each experiment rats were perfused transcardially with saline followed by 4% formaldehyde. The brains were removed, fixed in 4% formaldehyde, frozen and cut into 50-μm coronal sections. Tissue sections were stained with hematoxiline and eosine and analyzed by light microscopy to localize the injection. The lesion sites were marked on the corresponding cross-section of stereotaxic atlas of the rat brain (Paxinos and Watson 2007). The acquired data with confirmed striatal injections were included into the analysis.

Analysis of data

We assumed that in anesthetized, paralyzed and artificially ventilated rats, maintenance of a similar $PaCO_2$ and PaO_2 in arterial blood created equal baseline conditions for an adequate comparison of the pattern of the phrenic and hypoglossal neurograms between sham-operated and 6-OHDA-treated rats. Several indices of the respiratory output were estimated from the recorded neural signals. The frequency (f Phr) of integrated phrenic bursts expressed fictive respiratory rate. The peak integrated phrenic amplitude (A Phr) and the minute phrenic activity (M Phr) were neural correlates to the tidal volume and minute ventilation. The inspiratory time, Ti, the expiratory time, Te, and the respiratory cycle time, Tc, were calculated from the integrated phrenic nerve activity. The respiratory duty cycle was measured by dividing the inspiratory time, Ti, by the respiratory cycle time, Tc.

The hypoglossal peak amplitude (A HG), the minute activity (M HG) as well as the pre-inspiratory hypoglossal activity (pre-I HG) calculated from the hypo-

Table I

Baseline respiratory parameters in sham and 6-OHDA group

Parameter	Sham	6-OHDA	*T*-test
Inspiratory time, *Ti* (s)	0.32±0.04	0.39±0.02	*P*=0.13
Expiratory time, *Te* (s)	0.84±0.06	0.81±0.04	*P*=0.62
Resp. cycle length, *Tc* (s)	1.16±0.05	1.20±0.04	*P*=0.54
Freq. of breathing (breaths/min)	51.7±2.28	50.1±1.82	*P*=0.51
Duty cycle *Ti/Tc*	27.6±3.30	32.8±1.92	*P*=0.33
T pre-I HG (s)	0.32±0.07	0.16±0.01	*P*=0.17
A pre-I /A HG	0.18±0.03	0.15±0.04	*P*=0.56

(T pre-I HG) duration of the pre-inspiratory hypoglossal activity in seconds; (A pre-I /A HG) the ratio of the pre-inspiratory hypoglossal amplitude to the inspiratory hypoglossal peak amplitude. Data are presented as mean ± SE.

glossal neurogram indicated the upper airways respiratory output. The area under the curve of the integrated signal of the phrenic (PHR Area) and hypoglossal (HG Area) activities was calculated using Spike 2 software. Amplitude of the pre-inspiratory hypoglossal activity (A pre-I HG) was indicated by the level of the integrated hypoglossal activity at the time point corresponding to the phrenic activity onset. The time between the onset of the integrated hypoglossal activity and that of the phrenic activity showed the duration of the pre-inspiratory hypoglossal activity (T pre-I HG). All calculated parameters were assessed at the baseline and measured every 30 seconds during each hypoxic episode. Changes were expressed as a percentage of the averaged parameter of nerve activity (% of baseline) immediately preceding each hypoxic episode of intermittent hypoxia and reported as the means ± SE. The duration of the pre-I HG was calculated relative to the duration of the respiratory cycle measured between the onsets of integrated hypoglossal bursts (T pre-I/Tc), while amplitude of the pre-I HG was presented as a fraction of the peak inspiratory hypoglossal amplitude (A pre-I/A HG). Variability in frequency of the phrenic burst (CV-f) was quantified with the coefficient of variation (mean/SD). Statistical analysis was carried out using non parametric statistics Kruskal-Wallis Anova followed by the Whitney-Mann *U* test for comparison of the respiratory parameters between groups. For comparison within the group Wilcoxon's signed-ranks test or Student *T*-test was used. Values of less than 0.05 were considered to indicate statistical significance.

Table II

Effect of intermittent hypoxia on arterial blood pressure in the control and 6-OHDA group

	Control				6-OHDA			
	0 s	30 s	End	R	0 s	30 s	End	R
H1	86.8±6.3	61.7±6.5	36.1±6.1	46.2±4.3	86.6±17.5	51.8±10.1	27.5±6.7	38.7±9.2
H5	75.2±8.3	54.7±9.1	38.1±5.9	37.7±5.2	81.3±22.1	64.4±13.9	27.0±6.8	34.4±7.9

Mean ± SE values of arterial blood pressure (mmHg) were calculated during the 1st (H1) and 5th (H5) hypoxic episode of intermittent hypoxia in the control and 6-OHDA-lesioned rats. (0 s) before hypoxia; (30 s) time point of 30 s from the onset of hypoxia; (End) just before the end point of hypoxia; (R) at 15 s of recovery from hypoxia. No significant changes in arterial blood pressure were present between the control and 6-OHDA group.

RESULTS

Histological analysis of the brain sites of 6-OHDA injections revealed that 6-OHDA lesions were performed in the dorsolateral striatum.

Baseline phrenic and hypoglossal activity, arterial blood gases and arterial blood pressure in sham and 6-OHDA-lesioned animals

In the sham and the 6-OHDA groups the respiratory pattern of the phrenic and hypoglossal nerves activities did not exhibit significant difference when the baseline partial oxygen and carbon dioxide pressure in arterial blood was maintained at comparable levels by the means of mechanical ventilation.

The baseline respiratory parameters calculated for the control animals and for the animals that have been injected with 6-OHDA are outlined in Table I. It was found that the baseline phrenic bursts frequency (fictive respiratory rate) was similar in both groups. No significant differences in the Ti, Te, Tc, and Ti/Tc were noted. The coefficient of variation of the respiratory frequency did not change. The pre-inspiratory hypoglossal activity was present under the baseline conditions in both experimental groups (Table I). In the 6-OHDA group the pre-inspiratory hypoglossal activity was insignificantly reduced in terms of the onset and amplitude.

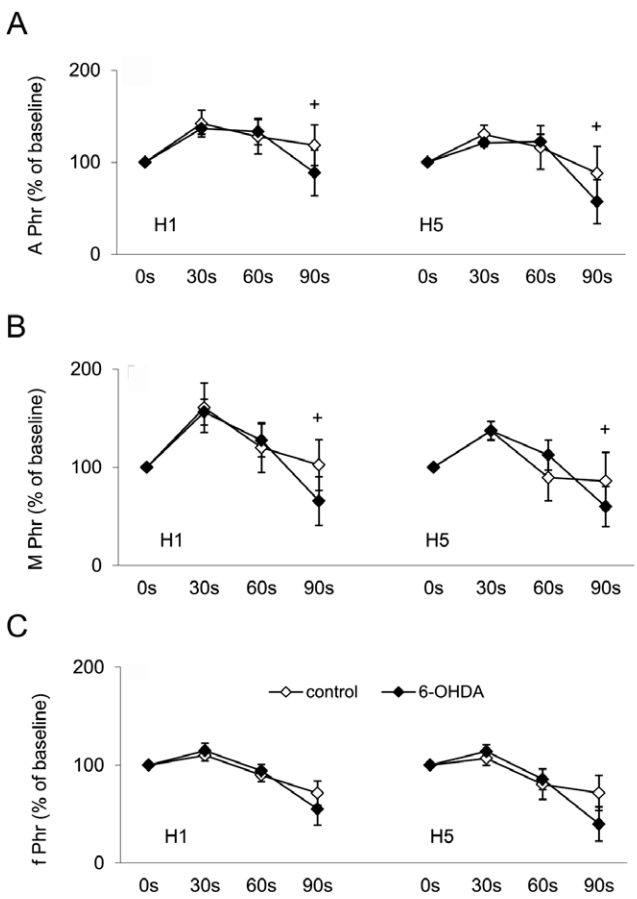

Fig. 1. Effect of acute intermittent hypoxia on phrenic activity. (A) phrenic amplitude (A Phr); (B) minute phrenic activity (M Phr) and (C) frequency of phrenic bursts (f Phr) were measured at 30, 60 and 90 seconds from the start of the first (H1) and the last episode (H5) of intermittent hypoxia. Changes are expressed as a percent of baseline phrenic activity (0 s) before each hypoxic exposure. Values are mean ± SE. + denotes significant difference (P<0.05 Wilcoxon) between the values at 60 and 90 seconds of hypoxia in the 6-OHDA-lesioned group.

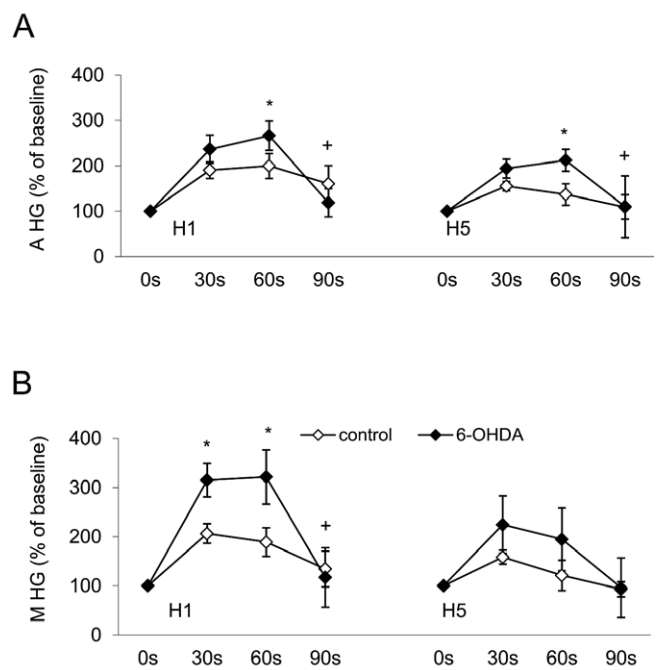

Fig. 2. Effect of acute intermittent hypoxia on hypoglossal nerve activity. (A) hypoglossal amplitude (A HG); (B) minute hypoglossal activity (M HG) of the first (H1) and the last episode (H5) of intermittent hypoxia. Changes are expressed as a percent of baseline hypoglossal activity (0 s) before each hypoxic exposure. Values are mean ± SE. Asterisk denotes significant difference (P<0.05) in the response between the control and lesioned animals. + denotes a significant difference (P<0.05) between the values at 60 and 90 seconds of hypoxia in the 6-OHDA-lesioned animals.

The apneic threshold was comparable in both groups (Pa CO_2 38.9±0.31 mmHg in the control group and 39.3±0.6 mmHg in the 6-OHDA group; P=0.56). The baseline parameters of the arterial blood in the sham group were as follows: Pa O_2 100.9±3.8 mmHg Pa CO_2 42.1±1.24 mmHg and pH 7.32±0.01; in the 6-OHDA group: Pa O_2 102.8±2.0 mmHg, Pa CO_2 42.9±0.8 mmHg and pH 7.31±0.05; P=0.39, P=0.56, P=0.59, respectively.

We did not find any significant differences in responses to 7% CO_2 between the control and 6-OHDA group. The ratio between the baseline phrenic minute activity and the CO_2 response was 1.38±0.26 in the sham-operated animals and 1.30±0.14 in the 6-OHDA-treated animals (P=0.74). As for the baseline hypoglossal minute activity and the CO_2 response the ratio was 1.92±0.32 in the control group and 1.85±0.22 in the 6-OHDA-treated animals (P=0.42).

Arterial blood pressure was at a similar level in both groups (Table II).

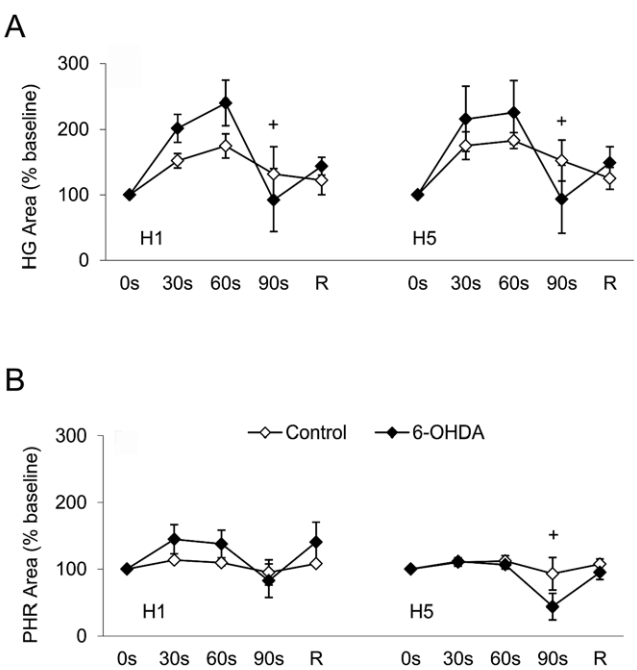

Fig. 3. Effect of acute intermittent hypoxia on area below integrated signal of the phrenic (PHR Area) and hypoglossal (HG Area) nerve activity during the first (H1) and the last episode (H5) of intermittent hypoxia. Changes are expressed as a percent of baseline hypoglossal activity (0 s) before each hypoxic exposure. Values are mean ± SE. Asterisk denotes significant difference (P<0.05) in the response between the control and the 6-OHDA-lesioned animals.

Respiratory response to hypoxia in sham and 6-OHDA-lesioned animals

In both experimental groups the respiratory response to each hypoxic episode of acute intermittent hypoxia consisted of the initial period of hypoxic augmentation turned into secondary hypoxic depression of the respiratory activity. A pattern of gradually diminished increments of the phrenic and hypoglossal activity during subsequent episodes of hypoxia was maintained in both control and 6-OHDA groups.

Hypoxic stimulation

Figure 1 depicts the hypoxic changes in the peak amplitude, minute activity and frequency of integrated phrenic bursts during the initial and final hypoxic episode of the intermittent hypoxia in both experimental groups. The striatal 6-OHDA lesion did not change the magnitude and profile of hypoxic phrenic response to acute intermittent hypoxia. Figure 2 shows corresponding integrated hypoglossal burst amplitude and minute activity during hypoxic response. The hypoglossal activity responded to hypoxia to a greater extent following 6-OHDA treatment than in the control group. Phrenic and hypoglossal response to intermittent hypoxia reached the peak of stimulatory effect within 30 seconds from the onset of each hypoxic episode in control conditions. The hypoxic stimulation of the phrenic activity attained a much lower level than that of the hypoglossal activity (P<0.01, Wilcoxon's test) for the peak amplitude and minute activity in control group and following 6-OHDA treatment.

During the first episode of hypoxia A HG increased to 199.7±27.5% in the control group and to 266.4±32.3% in 6-OHDA group. The difference between these results was significant (P<0.05). M HG rose to 206.3±19.8% in control and to 321.5±55.3% in 6-OHDA group (P<0.05). The profile of hypoxic hypoglossal response demonstrated maximal effect for the A HG and M HG at the time point of 30 seconds and declined thereafter. In 6-OHDA-treated animals, the stimulatory hypoglossal hypoxic response attained a plateau from the time point of 30 seconds to the time point of 60 seconds.

During the fifth episode of intermittent hypoxia A HG was significantly greater (212.3±24.3%) in 6-OHDA group than in the control group (155.2±10.0%; P<0.05). M HG amounted to 158.4±14.3% in control and to

224.5±58.3% in 6-OHDA group and the difference between groups did not reach statistical significance. An increase of A HG contributed mostly to a greater increment of M HG during hypoxia in 6-OHDA group while increases in frequency of bursts during hypoxia were comparable in both groups.

Figure 3 depicts changes in the area under the phrenic (PHR Area) and hypoglossal (HG Area) integrated bursts of discharge during the course of hypoxia in both experimental groups. This parameter reflects similar neural respiratory capability as the one calculated for the amplitude and minute activity of the phrenic and hypoglossal activities shown in Figure 1 and 2. The HG Area during 30 seconds of hypoxic exposures of intermittent hypoxia increased more during hypoxia in the 6-OHDA group than in the sham group (P<0.05). The PHR Area during hypoxic episodes showed a similar response in both groups.

During hypoxic episodes of intermittent hypoxia the pre-I HG began to discharge earlier in neural expiration. The duration of the pre-inspiratory activity in the control group increased from 114±33 ms to 256±7 ms (P=0.59) at 30 s of hypoxia in control and from 63±15 ms to 154±28 ms (P<0.01) in 6-OHDA group with high increments of its amplitude in both groups. Figure 4 depicts the pre-inspiratory amplitude in relation to the peak amplitude of the hypoglossal activity (A pre-I/A HG) and the duration of the pre-inspiratory activity in relation to the respiratory cycle length (T pre-I/Tc) during the 1st and the 5th hypoxic episode of intermittent hypoxia. The hypoxic increase of the A pre-I/A HG ratio and the T pre-I/Tc ratio was statistically different from the baseline values throughout intermittent hypoxia in both groups. It was found that following 6-OHDA treatment the pre-inspiratory hypoglossal amplitude increased relatively less than the inspiratory hypoglossal amplitude during the initial fazes of the hypoxic response, therefore the A pre-I/A HG ratio was lower. The T pre-I/Tc ratio also did not approach the values obtained in the sham group.

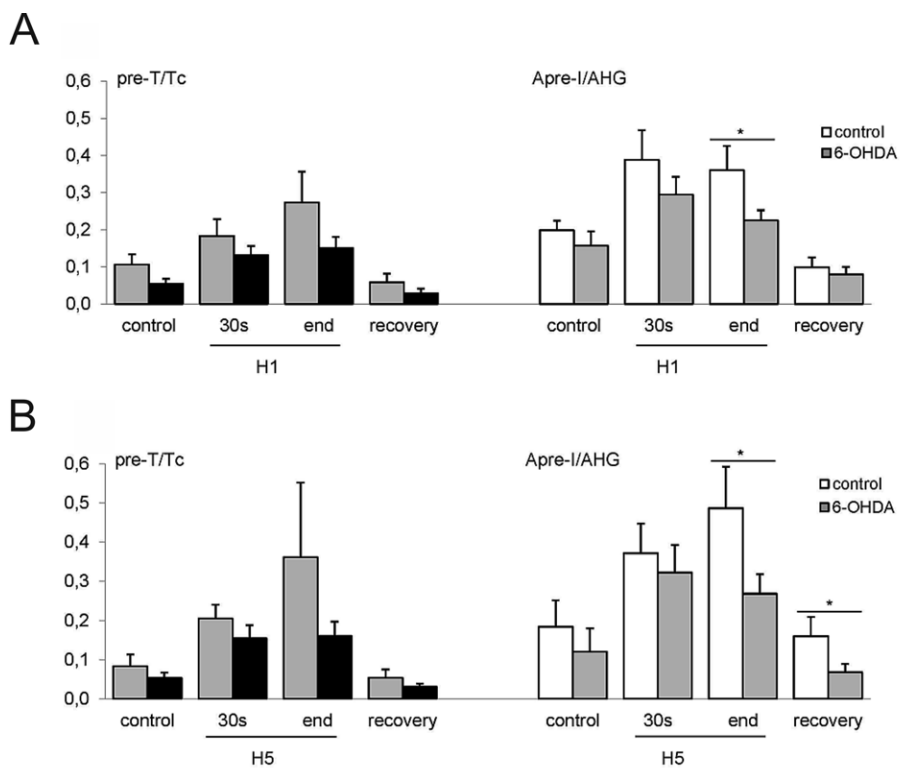

Fig. 4. Duration and amplitude of the pre-inspiratory hypoglossal activity before (control), during acute intermittent hypoxia (30 s and before the end of hypoxic episode) and during recovery. (pre-T/Tc) duration of the pre-inspiratory hypoglossal activity relative to the respiratory cycle length; (A pre-I /AHG) amplitude of the pre-inspiratory hypoglossal activity relative to the amplitude of the inspiratory hypoglossal activity. Panel A – the first hypoxic episode H1. Panel B – the last episode of intermittent hypoxia H5. Values are mean ± SE. Asterisk denotes significant difference (P<0.05) in the response between the control and the 6-OHDA-lesioned animals.

Decline of hypoxic response

A decrement of excitatory effects during the course of each hypoxic episode was more prominent in 6-OHDA-treated group (see Fig. 1 and 2). In that phase the respiratory rate slowed down significantly ($P<0.05$) relative to the peak of hypoxic stimulation. At the end of the hypoxic test the A Phr and M Phr fell bellow the baseline value. Hypoglossal activity excited to higher levels by hypoxia in 6-OHDA-lesioned animals decreased to the baseline. In the control animals the magnitude of Phr and HG hypoxic response measured between time point corresponding to the peak of hypoxic stimulation and the hypoxic decline at the end of hypoxia demonstrated a mild decrease, while in 6-OHDA group the decline of HG response was significant ($P<0.05$). In both groups the profile of the Phr and HG Area followed that of the Phr and HG minute activity. Contribution of the respiratory rate to the hypoxic respiratory decline was evident ($P<0.05$) during recurrent episodes of hypoxia. At the same time, the pre-I HG still had a tendency to discharge earlier during expiration while its amplitude in relation to the hypoglossal amplitude decreased significantly ($P<0.05$) in comparison with the same time point of a response in the control group. Immediately after withdrawing the hypoxic mixture from the respirator the pre-I HG decreased to prehypoxic values. Hypoxic depression up to neural apnoea was more frequently observed in the 6-OHDA group appearing in two out of ten experiments in the control group and in four out of seven experiments in the 6-OHDA group.

At the end point of lung ventilation with hypoxic mixture partial oxygen pressure, PaO_2, in the arterial blood decreased to 36.9±1.93 mmHg in the control and 38.2±1.79 mmHg in 6-OHDA group ($P=0.986$). In the same time $PaCO_2$ decreased to 37.9±0.78 mmHg and to 38.1±1.20 mmHg in the control and 6-OHDA group ($P=0.499$), respectively.

In both experimental groups the respiratory effects of each hypoxic exposure were accompanied by a progressive fall in arterial blood pressure that slowly approached to prehypoxic values when hypoxic ventilation was removed (Table II). Hypoxic hypotension was similar in time course and magnitude for both groups. There was a poor correlation between the magnitude of hypoglossal hypoxic stimulation, depression and hypoxic apnoea versus the amount of hypoxic hypotension therefore changes in the blood pressure could not account for augmented hypoxic respiratory response in 6-OHDA treated animals.

DISCUSSION

Phrenic vs. hypoglossal activity following 6-OHDA treatment during baseline ventilation

This paper gives the first description of the respiratory activity in the 6-OHDA model of Parkinson's disease implemented to study respiratory disorders due to central dopamine system impairment. Detailed analysis of variables of neural respiration in anesthetized and artificially ventilated animals two weeks after striatal injection of 6-OHDA has revealed that the parameters of the respiratory pattern: Tc, Ti, Te, and the duty cycle calculated from the phrenic neurogram did not alter significantly when mechanical ventilation of the lungs maintained gas exchange at a comparable baseline level, as in the control animals. At the same time, the hypoglossal neurogram demonstrated insignificantly lower pre-inspiratory activity – its time of occurrence moved to late neural expiration and the amplitude was reduced in proportion to the peak inspiratory amplitude of a given hypoglossal burst following 6-OHDA treatment.

Impairing dopamine in this model of PD does not affect phrenic activity but it modulate the pre-inspiratory hypoglossal activity at the baseline ventilation. This respiratory effect of 6-OHDA is consistent with a suggestion (Estenne et al. 1984) that in PD patients the diaphragmatic activity during rest breathing remains unchanged, whereas other motor outputs are affected.

Response to hypoxia

With the increased hypoxic drive more differences occurred in the profile of biphasic phrenic and hypoglossal response to intermittent hypoxic episodes. Hypoxic stimulation of the phrenic activity attained similar level in both groups, while the hypoglossal response to the first hypoxic episode was significantly higher. This tendency sustained throughout the subsequent episodes of hypoxia. The hypoxic phrenic response is influenced by a background level of gas exchange. Antecedent hypercapnia (Tin et al. 2012) decreased while hyperoxia (Pokorski et al. 2005) increased the hypoxic phrenic or diaphragmatic EMG

response. It is highly probable that the same concerns the hypoglossal activity. Baseline conditions in the sham-operated and 6-OHDA-treated groups were comparable both in terms of the respiratory activity and the baseline $PaCO_2$, PaO_2 and pH. We are therefore convinced that the contribution of these factors to changes in the magnitude of hypoxic response or the relations between phrenic and hypoglossal responses can be disregarded in the present study. It would be worth to explore whether preceded hyperoxic or hypercapnic ventilation modulates responsiveness of the respiratory system to hypoxia following 6-OHDA treatment in the same way as in intact animals.

Secondary decline of the hypoxic phrenic and hypoglossal response during the course of hypoxia was deeper, particularly for hypoglossal activity. More depressed hypoglossal activity during the second part of the response to hypoxia and neural apnoea more often evoked in the 6-OHDA group may have functional consequences in the increased probability of upper airway collapse.

Episodic or intermittent hypoxia stimulates the hypoglossal activity to a greater extent than the phrenic activity (Bradford et al. 2005). Phrenic and hypoglossal activity responds to several stimuli and chemicals with different intensity (Weiner et al. 1982). For instance, exogenous dopamine in healthy animals inhibits the hypoglossal activity while only depressing the phrenic activity (van Lunteren et al. 1984). A reverse effect could take place when dopamine level decreased in 6-OHDA model.

Although activated in concert during respiration the respiratory pump muscles and upper airway muscles are driven by specified spinal and medullary population of motoneurons, respectively. Phrenic and hypoglossal motoneurons in turn receive their respiratory inputs mostly from separated premotoneurons (Peever et al. 2002, Koizumi et al. 2008) and, moreover, have distinct interconnections with other systems such as, for instance, a multisynaptic projection from substantia nigra to hypoglossal motoneurons (Fay and Norgren 1997). Hypoglossal activity participates in maintaining upper airway patency by regulation of tongue position and stiffness. Extrinsic and intrinsic tongue muscles innervated by hypoglossal nerve facilitate the dilation of the upper airways (Bailey and Fregosi 2004) opposing the effects of negative pressure generated by diaphragm and other respiratory muscles during inspiration. In Parkinson's disease tongue muscles are affected and striatal dopamine depletion has influence on tongue function both in PD and following 6-OHDA lesion (Ciucci et al. 2011). Anatomical and functional properties of phrenic and hypoglossal respiratory outputs may therefore determine their sensitivity to hypoxia following the impairment of nigrostriatal dopamine system by 6-OHDA lesion.

Hypoglossal pre-inspiratory activity

In the 6-OHDA-treated rats under the baseline conditions the hypoglossal pre-inspiratory activity was reduced in terms of duration and amplitude probably because of increased threshold for triggering this activity (Sica et al. 1984). During hypoxia both peak amplitude of the inspiratory part of the hypoglossal activity and the pre-inspiratory hypoglossal activity heightened together. Because the pre-inspiratory activity increased relatively more than the inspiratory amplitude, the ratio of the pre-inspiratory amplitude to the inspiratory amplitude of the hypoglossal activity (A pre-I/A HG) in a given hypoglossal burst was enhanced. This enhancement of the A pre-I/A HG ratio, however, did not approach the corresponding values in the sham-operated group and became significantly lower just prior to the hypoxic neural depression or apnoea.

It is suggested (Lee and Fuller 2000) that pre-inspiratory hypoglossal activity is responsible for the maintenance of the upper airway's patency. If it is so, variations in the onset and the amplitude of the pre-I HG and the pattern of hypoglossal bursts as a whole and a decreased ratio of the pre-inspiratory to inspiratory hypoglossal activity at the end of hypoxic trial can develop a different ability to dilate the upper airways under the 6-OHDA conditions.

The mechanism of recruitment and control of the pre-I activity in hypoglossal motoneurons is not fully recognized yet. This activity is influenced by reflexes from the lungs (Saito et al. 2002) and by chemical drive (Lee and Fuller 2010). Vagotomy and a comparable gas exchange both during the baseline and the hypoxic ventilation in the sham and the 6-OHDA groups argue for an involvement of some other factors in eliciting a difference in the magnitude of the pre-I

HG under the baseline condition and during hypoxia. Neuroanatomical and electrophysiological studies reported that the pre-inspiratory hypoglossal activity as well as the inspiratory hypoglossal might be driven by the pontine Kölliker-Fuse (KF) nucleus neuronal pool (Ezure and Tanaka 2006) because the KF nucleus contains a group of neurons indicated as premotor to hypoglossal motoneurons (Kuna and Remmers 1999). The KF neurons get a projection from the NTS neurons excited by hypoxia (Song at al. 2011) and sends projection to the medial forebrain bundle being a part of the nigrostriatal pathways that are affected by striatal 6-OHDA injection. The reported changes in the pre-inspiratory hypoglossal activity following 6-OHDA lesion may engage the KF neurons.

Neurotransmitter mechanisms related to the modulation of neural respiratory activity following depletion of striatal dopamine by 6-OHDA

The model of PD used in the present study is based on depletion of dopamine in the nigrostriatal system caused by the neurotoxin 6-hydroxydopamine. Besides the nigrostriatal dopaminergic system, dopamine is present in number of brain stem regions involved in the control of respiration (Goiny et al. 1991) and dopaminergic axonal projections are found in the vicinity of medullary respiratory neurons (Sun et al. 1994). Peripherally, dopamine is present in the carotid bodies – the main sensors of oxygen tension (Niemi and Ojala 1966). It alters neural respiratory activity and modulates the respiratory hypoxic response (Hsiao et al. 1989, Lalley 2008) through D_2 dopaminergic receptors (Huey et al. 2000). In general dopamine is considered to have a depressing effect on respiration (Bolme et al. 1977, Henson et al. 1992, Güner et al. 2002) through peripheral chemoreceptors mechanism. Central inhibitory dopaminergic neuromodulation of respiration (Nielsen and Bisgard 1983) is a challenging topic because agonists and antagonists of dopamine receptors evoke divergent respiratory effects (for review see Lalley 2008) indicating complexity of dopaminergic mechanism. Hypoxia itself stimulates dopamine release in the carotid bodies (Gonzalez et al. 1995), in the respiratory brainstem neurons (Goiny et al. 1991) and in the striatum (Wang et al. 1995). Our research would seem to indicate the existence of a modified response of the neural respiratory outputs to hypoxia in the 6-OHDA model of PD. However, consequences of striatal dopamine depletion evoked by 6-OHDA at the level of respiratory neurons and carotid bodies have not been studied yet. In the D_2 dopamine receptor knock-out mouse, an impairment of dopaminergic neurotransmission concerns whole nervous system. In these circumstances hypoxia evoked divergent changes in dopamine release, catecholamine content and chemoreceptor activity in the carotid bodies associated by unchanged or augmented hyperventilatory hypoxic response (Huey et al. 2003, Prieto-Lloret et al. 2007), whereas attenuation of this response was not found. Similarity of the hypoxic respiratory effects in D_2 receptor lacking animals and that observed in the current study may suggest that in the 6-OHDA model an inhibitory impact of D_2 receptors on respiration is weakened. It cannot be excluded also that following 6-OHDA injection an increase in the hypoxic stimulation of the hypoglossal activity and further augmentation of the hypoxic response decline, a phase postulated to be a central depressant effect of hypoxia (Bisgard and Neubauer 1995), are linked with modification of the mechanisms of the hypoxic dopamine efflux at central or peripheral level. There is a degree of plasticity in 6-OHDA model. It can be speculated that some compensatory mechanism, possibly supersensitivity of D_2 receptors (Prieto et al. 2009), is activated to maintain hypoxic drive to the diaphragm at unaltered level.

Besides the fact that striatal 6-OHDA injection impairs dopaminergic neurons and pathways alternations in the serotoninergic and other neurotransmitter systems have been reported both in Parkinson's disease (Hornykiewicz 1975) as well as in animal models of this disease (Blandini et al. 1996, Reader and Dewar 1999, Scholtissen et al. 2006). In Parkinson's disease serotonin and complex interactions with numerous serotonin receptor subtypes are involved in motor and non-motor symptoms. In the dopamine-depleted brain by 6-OHDA alteration of the striatal level of serotonin as well as electrophysiological properties of serotoninergic neurons and function of serotonin receptor subtypes in several structures of the brain takes place (Di Matteo et al. 2008, Huot et al. 2011). In the respiratory system serotonin is an important neuromodulator of respiratory rhythm and pattern of motor activity. Hypoglossal motoneurons receive potent excitatory serotoninergic drive *via* 5-TH-2A receptors particularly significant for their functioning (Kubin et al.

1992). During hypoxia serotonin is released in the dorsomedial medulla where the NTS and hypoglossal neurons are present and through 5-TH-2A receptors contributes to hypoxic ventilatory and airway response (Kanamaru and Homma 2009). Intermittent hypoxia elicits post hypoxic long-term facilitation of the respiratory activity that is serotonin dependent (Bach and Mitchell 1996). Changes in the serotoninergic system paralleled with dopaminergic impairment might shape the hypoglossal hypoxic response and explain the mechanism of preferential hypoxic stimulation of the hypoglossal activity relative to the phrenic activity following striatal 6-OHDA treatment.

Finally, the results of the present study suggest that it is also worth to focus on a possible role of a neuropeptide, orexin. Both the orexin and the dopamine systems are functionally and anatomically interconnected (Bubser et al. 2005). Orexin contributes to upper airway patency. Orexin receptors are expressed (Volgin et al. 2002) in hypoglossal motoneurons and injection of orexin into Kölliker-Füse nucleus where a population of hypoglossal pre-motoneurons is located (Gestreau et al. 2005) exerts an excitatory effect on pre-I HG activity (Dutschmann et al. 2007). The known impairment of dopaminergic neurons in Parkinson's disease is accompanied by the increasing loss of orexin neurons which is correlated with the disease progression (Thannickal et al. 2007). In a similar way, 6-OHDA injections can participate in the damage of orexinergic neurons (Cui et al. 2010). It is possible that an orexinergic mechanism underlies the changes in the pre-inspiratory HG activity under baseline conditions and during hypoxic trial following 6-OHDA treatment.

Hypoxic response in Parkinson's disease and 6-OHDA model of Parkinson's disease

Descriptions of the ventilatory response to hypoxia in PD patients can be found in only a few papers that report a variety of results – from augmented (Feinsilver et al. 1986) through normal (Seccombe et al. 2011) to depressed hypoxic ventilatory response (Serebrovskaya et al. 1998, Onodera et al. 2000). Another dissimilarity among the studies concerns the sensitivity to carbon dioxide which indicates a reduced (Seccombe et al. 2011), normal (Onodera et al. 2000) and increased ventilatory response to hypercapnia (Feinsilver et al. 1986). PD patients selected to the aforementioned studies had a mild to moderate clinical disability score. Patients examined by Feinsilver and coworkers (1986) did not show restrictive or obstructive lung disease, had normal end tidal CO_2 while the end tidal O_2 was not indicated, and demonstrated augmented ventilatory response to isocapnic hypoxia. In Seccombe and coauthors (2011) and Onodera and colleagues (2000) studies most of the patients presented normal lung function and arterial blood gases within a normocapnic and normoxic limits. In spite of generally comparable baseline ventilation, one group of patients manifested normal ventilatory response to twenty minutes of 15% hypoxia (Seccombe et al. 2011), while another group (Onodera et al. 2000) showed a significantly lower hypoxic ventilatory response to isocapnic hypoxia. On the other hand, in Serebrovskaya and coauthors (1998) studies the end tidal O_2 was 10% lower and the end tidal CO_2 was elevated by 15% during air breathing. During isocapnic 11% hypoxia lasting 5–6 minutes ventilatory response was notably depressed when severity of hypoxia increased. In other study (Serebrovskaya et al. 2003), progressive isocapnic intermittent hypoxia evoked 48% lower ventilatory response. Thus, it is possible that in the case of PD patients chosen by Serebrovskaya and others (1999, 2003) hypoxic ventilatory response was attenuated because breathing with air was already below a normal range. Diversity of the ventilatory responses to hypoxia and hypercapnia in quoted human PD studies suggests that although there are significant associations between the state of general motor impairment and abnormalities in respiratory function, some other factors should be taken into consideration to anticipate the direction of the ventilatory response to chemical stimuli. The results of the present study correspond more to that of Feinsilver and colleagues (1986) and Seccombe and others (2011) and contrast to that of Serebrovskaya and coauthors (1998, 2003). However, we used a poikolocapnic hypoxia, therefore CO_2 level could shape additionally the respiratory response. On the other hand, an increment in the hypoxic hypoglossal response in 6-OHDA group was attained when both oxygen and carbon dioxide levels in baseline and during the course of hypoxia were similar to that in the control group. Serebrovskaya and coworkers (1998) and Onodera and others (2000) strongly believe that in PD patients not only respiratory muscles are weaker but also chemosensitivity to hypoxia is impaired leading to depressed hyperventilatory hypoxic response,

even in the early stage of PD. However, following unilateral 6-OHDA lesion of the striatum there was no sign of respiratory hyposensitivity to hypoxia. Even though 6-OHDA treatment did not change the magnitude of hypoxic excitation of the phrenic activity, simultaneous increase in hypoxic stimulation of the hypoglossal activity and late decline of this response (more emphasized in the hypoglossal activity) suggested rather an increase at neural level in respiratory responsiveness to hypoxia following the 6-OHDA treatment.

There is no data in literature on the effects of the upper airways impairment in Parkinson's disease on ventilatory response to hypoxia. In several studies (Feinsilver et al. 1986, Serebrovskaya et al. 1998, 2003, Onodera et al. 2000, Seccombe et al. 2011) the functional state of the upper airways in PD patients was either normal or without clear disability, hence we assume that hypoxic response in these PD patients was not related to the upper airways impairment.

The design of the current study on 6-OHDA model was homogenous comparing to that in the human studies, and what was important, in our study chemical input to the respiratory complex was comparable between the animals of both groups. The lack of reciprocal message to the respiratory centers from the respiratory muscles, the diaphragm and the upper airways allowed us to estimate a change in mere central respiratory drive to these muscles. The information that after an impairment of nigrostriatal dopaminergic pathways by 6-OHDA, the respiratory control system generates modified respiratory drive only to hypoglossal motoneurons – what can be seen in altered pre-inspiratory hypoglossal activity and augmented hypoglossal hypoxic response – can have a compensatory meaning, assuming that the upper airways are affected by 6-OHDA.

A discrepancy between ventilatory response to hypoxia in PD patients and respiratory response in the rat model of PD might come from distinct experimental conditions, the characteristic of hypoxic stimulus and recordings of different respiratory outputs. Level of consciousness and spontaneous breathing *versus* muscle paralysis and artificial ventilation might be of importance. When subjects breathe spontaneously lung ventilation is a consequence of specific activation of the diaphragm, intercostal and other respiratory muscles by central respiratory drive. On the other hand lung ventilation depends on mechanical properties and efficiency of respiratory muscles. One of the features of Parkinson's disease is a characteristic change in posture and rigidity of the muscles including the rib cage muscles. In parkinsonian patients changes in mouth pressure (Cardoso and Pereira 2002) suggest a weakness of respiratory muscles (Haas et al. 2004). It is possible that intercostal muscles that share both postural and respiratory function may impact on the magnitude of ventilatory response to hypoxia in PD patients. In case of paralyzed animals the respiratory muscles are relaxed and the neuromuscular loop is opened, the phrenic nerve activity is not transformed into the ventilatory motor function and expresses a neural demand for ventilation at given circumstances. It is important to establish whether the 6-OHDA treatment in freely moving, conscious animals with probably evoked weakness of the muscles would cause similar changes in the ventilatory response to hypoxia as in anesthetized, artificially ventilated rats.

CONCLUSIONS

Unilateral infusion of 6-OHDA into the nigrostriatal region affected separately the pattern of activity of the phrenic and the hypoglossal neural output in anesthetized, paralyzed and artificially ventilated rats. The lack of changes in the phrenic activity and no difference in the phrenic response to hypoxia accompanied by the increased hypoglossal response, reduced the pre-inspiratory hypoglossal activity under the baseline conditions and lower excitation of the pre-inspiratory hypoglossal activity throughout hypoxia suggest a differential modulation of these two respiratory outputs by striatal 6-OHDA injection. An altered hypoglossal response to recurrent episodes of hypoxia in 6-OHDA-lesioned animals might be a consequence of modified mechanisms of dopamine efflux during hypoxia, leading to less accented inhibitory effects of dopamine on breathing. A role of serotonin and orexin neurotransmission in the effects of the 6-OHDA treatment on the hypoglossal activity is suggested.

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