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Effects of Chronic, Systemic Treatment with the Dopamine Receptor Agonist R-Apomorphine in Partially Lesioned Rat Model of Parkinson's Disease: An Electrophysiological Study of Substantia Nigra Dopamine Neurons

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Abstract

Previous studies have suggested that R-apomorphine (R-APO), a non-selective dopamine (DA) receptor agonist, has neuroprotective effects in the experimental models of Parkinson's disease (PD). In this study, we investigated the effects of chronic, systemic treatment with R-APO in the firing activity of substantia nigra pars compacta (SNc) DA neurons in 6-hydroxydopamine (6-OHDA) partially lesioned rats. In the 6-OHDA-lesioned rats treated with vehicle, injection of 6-OHDA (20.1 μg) into the striatum produced a partial lesion causing 41% loss of tyrosine hydroxylase-immunoreactive (TH-ir) neurons in the SNc. In the partially lesioned rats, chronic, systemic treatment of R-APO (10 mg/kg/day, s.c., 11 days) attenuated loss of TH-ir neurons in the SNc. The partial lesion of the nigrostriatal pathway and R-APO treatment did not change the firing rate and firing pattern of DA neurons in the SNc of rats. In contrast, the R-APO treatment increased the number of spontaneously active DA neurons of the SNc in the partially lesioned rats, while the lesion decreased the number of spontaneously active DA neurons. In addition, the chronic R-APO treatment decreased the responsiveness of the DA neurons to intravenously administrated R-APO in the partially lesioned rats. These results indicate that chronic, systemic R-APO treatment has the neuroprotective effect, and reverses the decrease in the number of spontaneously active DA neurons in the SNc whereas the treatment induces a reduction in the sensitivity of DA receptors in the SNc to R-APO stimulation in this model.

Key Words: apomorphine, substantia nigra, tyrosine hydroxylase, extracellular recording, Parkinson's disease

Introduction

The degeneration of dopamine (DA) neurons in the substantia nigra pars compacta (SNc) is the primary pathology of Parkinson's disease (PD). This degeneration is currently believed to be due to a complex interplay of factors including oxidative stress, mitochondrial dysfunction and defects in the ubiquitin-proteasome system. Until today, drug treatments for PD only alleviate most of the major signs and symptoms of the disease in a majority of patients. However, functional disability continues to worsen over time.

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Although the advent of $_L$-DOPA therapy has been associated with the prolongation of survival in patients with PD, no drug has yet been identified that definitively slows or stops the progression of PD.

There is circumstantial evidence to indicate that DA receptor agonists not only have symptomatic relief of PD and decrease in the rate of motor complications induced by $_L$-DOPA, they also possess neuroprotective efficacy (5). R-apomorphine (R-APO) is a non-selective D_1/D_2 receptors agonist acting both pre- and post synaptically. Systemic administration of R-APO has been found to improve motor function in the striatal 6-hydroxydopamine (6-OHDA)-lesioned rats (26). Immunocytochemical and neurochemical studies have also shown that R-APO at higher concentrations exerts a neuroprotective effect which protects striatal DA depletion and ameliorates tyrosine hydroxylase (TH) positive neuron loss in 6-OHDA, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine-induced animal models of PD (11, 15). Additionally, a study has shown that continuous subcutaneous infusion of R-APO following MPTP injection rescued striatal DA terminals in mice (3). *In vitro* studies have shown that R-APO chelates iron, reduces oxidation of polyunsaturated fatty acids, scavenges free radicals in mitochondrial fractions, and protects pheochromocytoma (PC12) cells against oxidative stress induced either by hydrogen peroxide or 6-OHDA (12, 13, 24). These properties have been implicated in inhibition of brain mitochondrial lipid peroxidation and protein oxidation (13). Furthermore, R-APO has been shown to induce trophic factors in mesencephalic DA neurons in culture (16). However, the effect of R-APO on DA neuronal activity in the SNc is still unknown.

The anti-Parkinsonian effects of R-APO are largely dependent on a number of factors such as duration of treatment and the model used to mimic PD. PD is usually replicated in mice by systemic administration of MPTP or in rats by injection of 6-OHDA into different parts of the nigrostriatal pathway. However, the main difficulty using MPTP toxicity as a model of PD is that it is an acute or sub-acute process, whereas PD is a slow progressive illness. Therefore, the aim of the present study was to investigate whether chronic, systemic treatment with R-APO (11 days) produces better neuroprotective effect in the intrastriatal 6-OHDA rat model which mimics the slow progress and early stage of PD (26, 27) by using immunocytochemical and electrophysiological methods. In particular, we examined changes in the firing rate and firing pattern of DA neurons in the SNc and their response to the non-selective DA receptor agonist R-APO after chronic treatment with R-APO.

Materials and Methods

Animals and Experimental Groups

Male Sprague-Dawley rats, weighing 250-340 g, were used in this study. Rats were housed in standard cages in groups of a maximum of five animals in a temperature-controlled (23 ± 1°C) environment on a 12 h light/dark cycle with free access to food and water. All experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996, and according to the guideline of the Institutional Animal Care Committee of the University. All efforts were made to minimize the number of animals used and their suffering. The experiments were performed on three groups of rats: sham-lesioned rats treated with vehicle (n = 13), 6-OHDA-lesioned rats treated with vehicle (n = 15) and 6-OHDA-lesioned rats treated with R-APO (n = 9).

Drugs

Desipramine hydrochloride, 6-OHDA hydrochloride and R-APO hydrochloride were from Sigma-Aldrich, MO, USA. Desipramine was prepared in 0.9% saline; 6-OHDA and R-APO were prepared in 0.9% saline containing 0.02% ascorbic acid. These drugs were prepared on the day of the experiment.

6-OHDA Injections in the Striatum and R-APO Treatment

To produce a PD model with partial lesion of nigrostriatal DA neurons, 6-OHDA (20.1 µg/3 µl) was injected over 5 min unilaterally into the striatum (26, 27). Stereotaxic infusion followed the coordinates of the Paxinos and Watson atlas (20): AP 1.0 mm anterior to bregma, L 3.0 mm from the midline, D 5.0 mm from the dura. The injection was made at a rate of 1.0 µl/min using a glass micropipette connected to a 5 µl microsyringe (Hamilton). Following injection, the micropipette was left in place for 10 min before being retracted to allow complete diffusion of the drug. In the same manner, sham-lesioned rats received an injection of 3 µl of saline containing 0.02% ascorbic acid into the striatum. 6-OHDA-lesioned rats were treated with injections of desipramine (25 mg/kg, i.p.) 30 min before surgery in order to protect noradrenergic terminals from 6-OHDA toxicity. R-APO-treated rats received 10.0 mg/kg/day R-APO s.c. for eleven consecutive days (26). The vehicle-treated rats received an equal volume of saline containing 0.02% ascorbic acid. In R-APO-treated rats, the initial administration of R-APO started 15 min before 6-OHDA injection.

In Vivo *Electrophysiological Recordings and Drug Administration*

After 11 days of 15 to 20 days treatment in the three groups, rats were anaesthetized with 4% chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic frame. Body temperature was maintained at 37 ± 0.5°C, heart rate and papillary diameter were monitored throughout the experiment. The extracellular recordings of DA neurons in the SNc were performed using glass microelectrodes preloaded with 1% Pontamine Sky Blue in 0.5 M sodium acetate. Their impedance typically ranged between 10-15 MΩ. Glass microelectrodes were positioned using the following coordinates: AP 4.8-5.3 mm posterior to bregma, L 1.9-2.1 mm from the midline, D 7.1-7.3 mm from the dura (20). The neuronal firings were amplified, bandpass-filtered using a pre-amplifier (AVB-11A, Nihon Kohden, Japan), displayed on an oscilloscope (VC-11, Nihon Kohden, Japan) and stored in a computer equipped with the CED 1401/Spike 2 analysis system (Cambridge Electronic Design, England) for off-line analysis. Neurons which met the following criteria were included for off-line analysis in this study: (i) a slow regular, irregular or bursting firing pattern, displayed firing rates within the range of 0.5-8 spikes/s and a wide action potential (> 2.4 ms) (14); (ii) the recorded neurons location was histologically confirmed from the SNc. The baseline activity of identified neurons was recorded for 10-20 min before any treatment.

The firing pattern of DA neurons in the SNc was analyzed and the following parameters were calculated: firing rate, mean interspike interval (ISI) coefficient of variation (a parameter reflecting the degree of regularity of neuronal firing, which is defined as the ratio between standard deviation and mean ISI), number of single spike within burst, number of bursts and percentage of the neurons exhibiting burst-firing (14, 25). In this analysis, burst firing was defined as having, in addition to a number of shorter two-spike burst doublets, a minimum of two three-spike bursts with decreasing amplitude and increasing duration in each burst. Burst firing was characterized as a series of 2-7 spikes and their onset and termination were defined as an ISI less than 80 ms and greater than 160 ms, respectively (14). These parameters regarding the firing pattern were determined by 500 consecutive spikes.

For systemic administration, the non-selective DA receptor agonist, R-APO, was injected i.v. in doubling doses (0.5-16.0 μg/kg) at approximately 2 min intervals. Working solutions (0.1 ml) were injected intravenously at appropriate dosage concentrations in the course of 30 s. Only one neuron was observed per animal during the procedure of the systemic administration.

Histological and Immunocytochemical Examinations

At the end of each experiment, the recording site was marked by the ejection of Pontamine Sky Blue (- 20 μA, 15 min). The rat was given an overdose of urethane and perfused with saline followed by 4% paraformaldehyde; the brain was immediately removed and post-fixed in the same fixative for 4 h. They were then placed in phosphate buffered saline with 20% sucrose overnight. The brains were frozen and cut into 30 μm thick coronal sections using a cryostat. Cresyl violet staining of the sections mounted on gelatin-coated slides was used to determine the location of the recording sites.

The serial sections of the SNc and ventral tegmental area (VTA) were examined for immunocytochemical staining of TH, as previously described (25), to determine the extent of DA neurons degeneration in the SNc. After TH staining, counting of TH immunoreactive (TH-ir) neuron bodies in the SNc was carried out on 3 representative sections per animal. A neuron was considered when it was intact, round with clear nucleus and/or cytoplasm. The number of TH-ir neurons was expressed as the average of the counts obtained from the 3 representative sections. The full extent of the structure in each section was examined in all groups. Only sections in which the medial and lateral parts of the SNc and VTA were clearly separated by the medial terminal nucleus of the accessory optic tract level were selected for analysis of TH-ir neuron number. This approach has been used by others to ensure that comparable rostrocaudal levels of the SNc are sampled between animals (25). Only rats with less than 50% loss of TH-ir neurons in the SNc were used to analyze the TH immunocytochemical and electrophysiological data.

Data Treatment and Statistical Analysis

All data were expressed as means ± S.E.M., or as percentage of baseline firing rate. Comparisons between different groups of TH-ir neuron number, the firing rates, the number of burst, the number of single spike per burst and the changes in the firing rate induced by R-APO administration and ED_{50} values were analysed using one way ANOVA. *Post-hoc* multiple comparisons were made using the Tukey's test or Dunnett's test as appropriate. The mean ISI coefficients of variation were analyzed using the non-parametric analysis Mann-Whitney *U* test. The proportions of different firing patterns between different groups were compared using the χ^2 test. ED_{50}, which is the dose of R-APO that produces half-maximal effect, is calculated using nonlinear re-

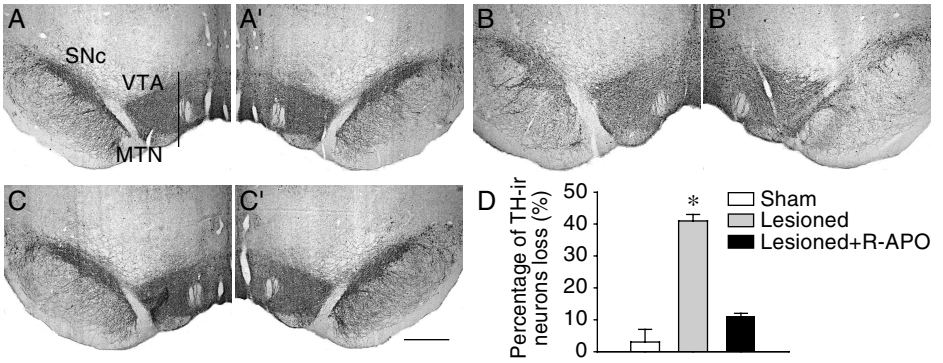

Fig. 1. Photomicrographs of TH immunocytochemical staining showing the lesioned (right) and unlesioned sides of the SNc in a sham-lesioned rat treated with vehicle (A, A'), 6-OHDA-lesioned rat treated with vehicle (B, B') and 6-OHDA-lesioned rat treated with R-APO (C, C'). Histogram showing the percentage of TH-ir neuron loss in the SNc of sham-lesioned rats treated with vehicle (n = 6), 6-OHDA-lesioned rats treated with vehicle (n = 6) and 6-OHDA-lesioned rats treated with R-APO (n = 6; D). MTN, medial terminal nucleus; VTA, ventral tegmental area. *$P < 0.001$ in comparison with the values of sham-lesioned rats treated with vehicle. Scale bar, A, A'-C, C' = 500 µm.

gression equation $E = E_{max}/(1+[ED_{50}/X]^n)$, where E is the effect induced by a certain dose of R-APO, E_{max} is the maximal effect, X is the dose of R-APO and *n* is the factor slope of the dose-effect curve (4). Statistical analyses were performed using SPSS 11.0 for Windows and the level of significance was determined as $P < 0.05$.

Results

Effect of R-APO Treatment on the Number of TH-ir Neurons in the SNc

In the sham-lesioned rats treated with vehicle (n = 6), the 6-OHDA-lesioned rats treated with vehicle (n = 6) and the 6-OHDA-lesioned rats treated with R-APO (n = 6), TH-ir neurons loss in the SNc on the injected side was 3 ± 4%, 41 ± 2% and 11 ± 1%, respectively, compared with the uninjected side (Figs. 1A, A'-C, C'). In the 6-OHDA-lesioned rats treated with vehicle, the percentage of TH-ir neurons loss was significantly higher than that of the sham-lesioned rats treated with vehicle ($P < 0.001$, n = 6; Tukey's test; Figs. 1A, A', B, B' and D), which was similar to a partial lesion of the nigrostriatal pathway as seen in patients with preclinical PD. In the 6-OHDA-lesioned rats treated with R-APO, no significant differences were found in the number of TH-ir neurons in the SNc compared with sham-lesioned rats treated with vehicle ($P > 0.05$, n = 6; Tukey's test; Figs. 1A, A', C, C' and D) indicating that the administrated R-APO had a neuroprotective action *in vivo* against 6-OHDA toxicity.

Effect of R-APO Treatment in the Firing Activity of DA Neurons in the SNc

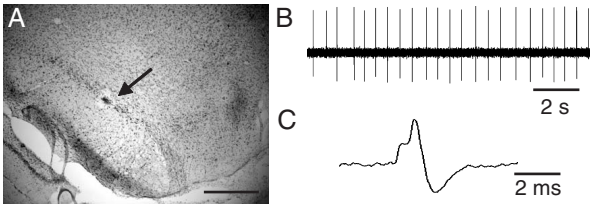

Fig. 2. Histological photomicrograph showing the recording site (arrow) in the SNc marked with iontophoretically injected Pontamine Sky Blue in sham-lesioned rat treated with vehicle (A). The representative sample of the spontaneous firing activity illustrating the firing rate of DA neuron (B) and the corresponding extracellular action potential waveform (C) in sham-lesioned rat treated with vehicle. Scale bar, A = 500 µm.

All of recording sites used in the sham-lesioned rats treated with vehicle, the 6-OHDA-lesioned rats treated with vehicle and the 6-OHDA-lesioned treated with R-APO were verified to be within the SNc (Fig. 2A). The recorded neurons located in the SNc displayed the electrophysiological characteristics of DA neurons in this study corresponding to the criteria presented in previous reports (Figs. 2B and C) (4, 14).

In the sham-lesioned rats treated with vehicle, the firing rate of DA neurons in the SNc ranged from 0.97 to 6.84 spikes/s with a mean of 3.39 ± 0.25 spikes/s (n = 38; Fig. 3A). In 500 consecutive spikes, the number of bursts varied from 8 to 114 with a mean of 41.00 ± 12.09 bursts (Fig. 3B), and the average number of single spike per burst was 3.07 ± 0.26 (Fig. 3C). The percentage of neurons exhibiting burst-firing was 21% (Fig. 3D), and the mean ISI coefficient of variation was 0.39 ± 0.03 (Fig. 3E).

In the 6-OHDA-lesioned rats treated with vehicle, DA neurons in the SNc had a firing rate

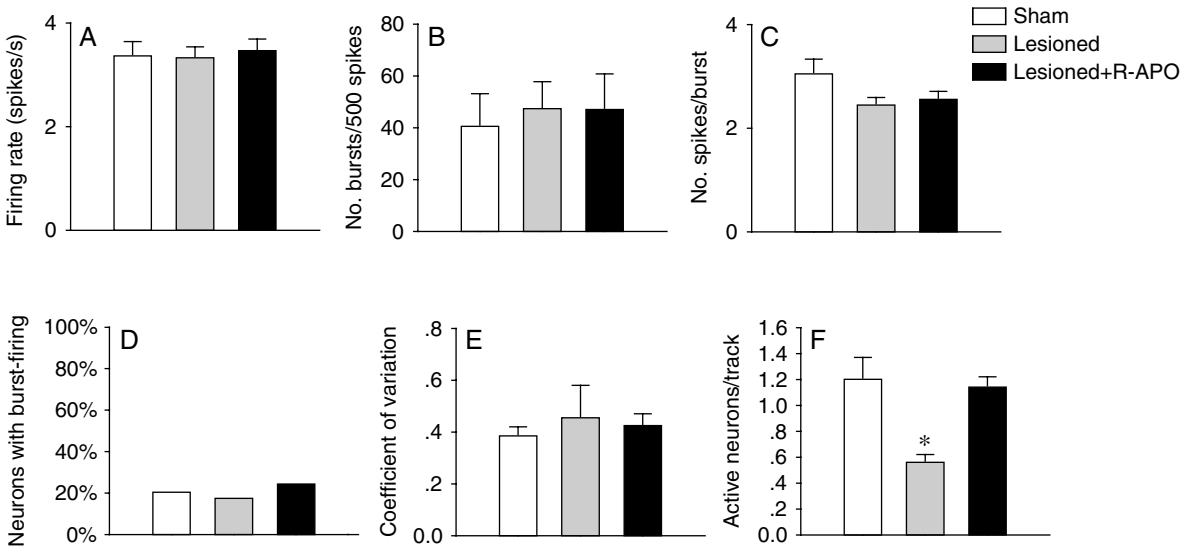

Fig. 3. Histograms showing the distribution of mean firing rate (A), the number of bursts (B), single spikes per burst (C), the percentage of the neurons with burst-firing (D) and mean ISI coefficient of variation (E) of DA neurons recorded in sham-lesioned rat treated with vehicle (n = 38), 6-OHDA-lesioned rat treated with vehicle (n = 43) and 6-OHDA-lesioned rat treated with R-APO (n = 43), and the number of spontaneously active neuron per track (F) in the SNc in sham-lesioned rat treated with vehicle (n = 8), 6-OHDA-lesioned rat treated with vehicle (n = 10) and 6-OHDA-lesioned rat treated with R-APO (n = 7). *$P < 0.001$ in comparison with the values in sham-lesioned rat treated with vehicle rats.

ranging from 1.08 to 6.45 spikes/s; the mean firing rate increased slightly, but not significantly, to 3.35 ± 0.19 spikes/s compared with the sham-lesioned rats treated with vehicle ($P > 0.05$, n = 43; Tukey's test; Fig. 3A). The number of bursts out of 500 consecutive spikes varied from 16 to 99; the mean number of bursts increased slightly to 47.88 ± 9.87 ($P > 0.05$; Tukey's test; Fig. 3B). The number of single spike per burst was 2.47 ± 0.12, which did not change significantly compared with the sham-lesioned rats treated with vehicle ($P > 0.05$; Tukey's test; Fig. 3C). The percentage of burst-firing neurons decreased to 18% but the changes were not significant ($P > 0.05$; χ^2 test; Fig. 3D). The mean ISI coefficient of variation was 0.46 ± 0.12 ($P > 0.05$; Mann-Whitney *U* test; Fig. 3E).

In the 6-OHDA-lesioned rats treated with R-APO, the firing rate of DA neurons varied from 1.01 to 6.35 spikes/s. The mean firing rate of these neurons was 3.49 ± 0.20 spikes/s and did not change significantly compared with the sham-lesioned rats treated with vehicle ($P > 0.05$, n = 43; Tukey's test; Fig. 3A). The number of bursts per 500 spikes ranged from 11 to 114; the mean number of bursts increased slightly to 47.55 ± 13.25 ($P > 0.05$; Tukey's test; Fig. 3B). The number of single spike per burst, 2.58 ± 0.13, was similar to that obtained in the sham-lesioned rats treated with vehicle ($P > 0.05$; Tukey's test; Fig. 3C). 25% of the neurons showed a burst-firing pattern, similar to the sham-lesioned rats treated with vehicle ($P > 0.05$; χ^2 test; Fig. 3D). The mean ISI coefficient of variation increased, although not significantly, to 0.43 ± 0.04 ($P > 0.05$; Mann-Whitney *U* test; Fig. 3E). These results indicated that the firing rate of DA neurons and degree of regularity of the neuronal firing did not change after chronic R-APO treatment.

In the sham-lesioned rats treated with vehicle (n = 8), the number of spontaneously active DA neurons in the SNc per track were 1.21 ± 0.16 (Fig. 3F). In the 6-OHDA-lesioned rats treated with vehicle (n = 10), the number of spontaneously active DA neuron in the SNc per track decreased significantly to 0.57 ± 0.05 compared with the sham-lesioned rats treated with vehicle ($P < 0.001$; Tukey's test; Fig. 3F). In the 6-OHDA-lesioned rats treated with R-APO (n = 7), the number of spontaneously active DA neurons in the SNc per track were 1.15 ± 0.07 and, thus, did not change significantly compared with the sham-lesioned rats treated with vehicle ($P > 0.05$; Tukey's test; Fig. 3F) indicating that chronic R-APO treatment recovered the number of spontaneously active DA neuron in the SNc per track.

Effect of R-APO Treatment in Response of DA Neurons in the SNc to DA Receptor Stimulation

In the sham-lesioned rats treated with vehicle, the 6-OHDA-lesioned rats treated with vehicle and the 6-OHDA-lesioned rats treated with R-APO, systemic administration of the non-selective DA receptor agonist R-APO (0.5-16.0 μg/kg, i.v.) dose-dependently inhibited the firing rate of all DA neu-

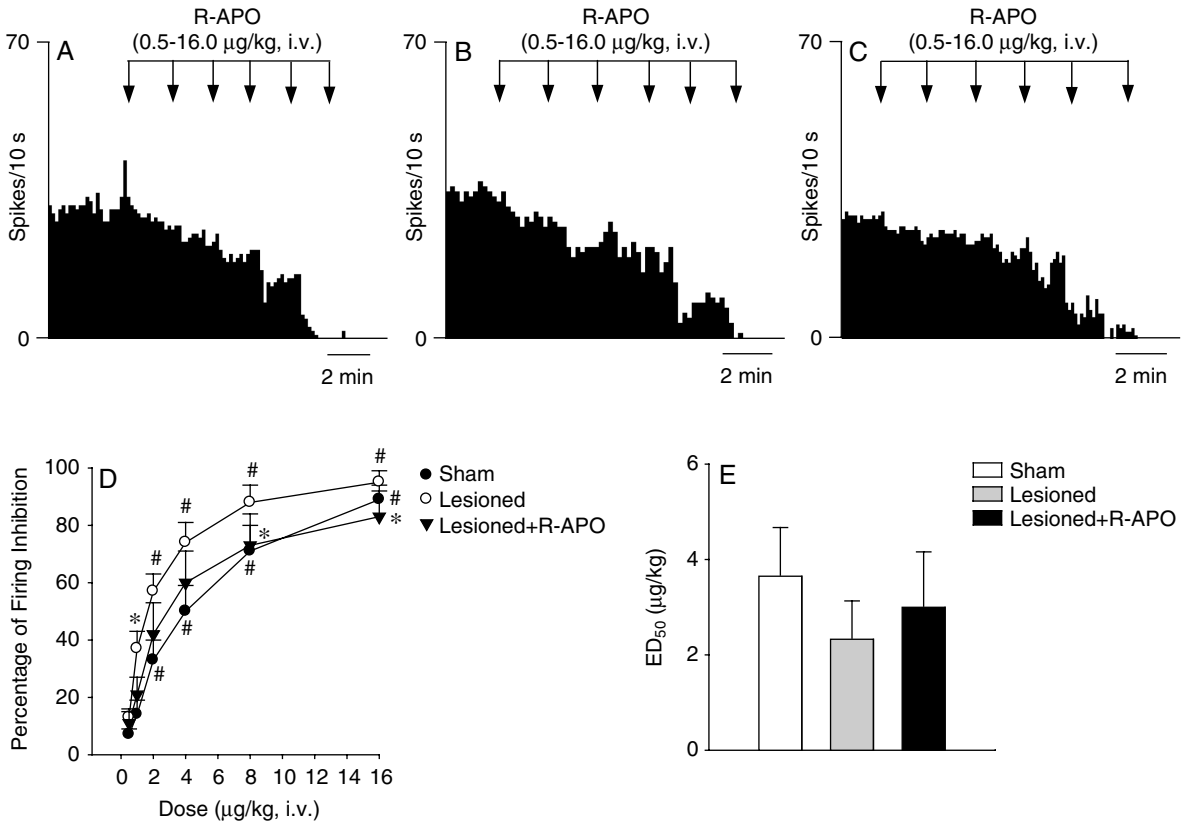

Fig. 4. Firing rate histograms showing the effect of systemic administration of the nonselective DA receptor agonist R-APO on SNc DA neurons in sham-lesioned rats treated with vehicle (n = 10), 6-OHDA-lesioned rats treated with vehicle (n = 8) and 6-OHDA-lesioned rat treated with R-APO (n = 9). The administration of R-APO (0.5-16.0 μg/kg, i.v.) dose-dependently inhibited the firing rate of the neurons in the SNc of all the three groups. Dose-response curves showing the effects of the administered cumulative doses of R-APO on the firing rate of DA neurons in the SNc of the three groups (D). Histogram showing ED_{50} values of sham-lesioned rats treated with vehicle (n = 7), 6-OHDA-lesioned rats treated with vehicle (n = 8) and 6-OHDA-lesioned rat treated with R-APO (n = 7; E). Arrows indicate time of i.v. administration of R-APO. $^{*}P < 0.01$, $^{\#}P < 0.001$ in comparison with the baseline firing rate.

rons examined in the SNc as compared to the baseline firing rate, respectively (sham-lesioned rats treated with vehicle, $P < 0.001$, n = 10, $F_{6, 63} = 14.31$; 6-OHDA-lesioned rats treated with vehicle, $P < 0.001$, n = 8, $F_{6, 49} = 10.90$; 6-OHDA-lesioned rats treated with R-APO, $P < 0.001$, n = 9, $F_{6, 56} = 3.89$; one-way ANOVA; Figs. 4A-D). This inhibition was significant at doses higher than 2 μg/kg for the neurons of the SNc in the sham-lesioned rats treated with vehicle and in the 6-OHDA-lesioned rats treated with vehicle, respectively (sham-lesioned rats treated with vehicle, $P < 0.001$; 6-OHDA-lesioned rats treated with vehicle, $P < 0.01$; Dunnett's test; Figs. 4A, B and D) indicating that the response of DA neurons in the SNc to stimulation of R-APO did not change after partial lesion of the nigrostriatal pathway in rats. However, *post-hoc* analysis revealed that the inhibition of the firing rate produced by R-APO reached statistical significance at doses higher than 8 μg/kg for the neurons of the SNc in the 6-OHDA-lesioned rats treated with R-APO ($P < 0.01$; Dunnett's test; Figs. 4C and D). These results indicate that the chronic R-APO treatment markedly decreased the response of DA neurons in the SNc to the stimulation of the intravenous administration of R-APO in the partially lesioned rats.

Baseline firing rate of DA neurons in the SNc for ED_{50} values of R-APO in the sham-lesioned rats treated with vehicle, the 6-OHDA-lesioned rats treated with vehicle and the 6-OHDA-lesioned rats treated with R-APO were 3.88 ± 0.53 (n = 7), 2.34 ± 0.78 (n = 8) and 3.28 ± 0.45 (n = 7) spikes/s, respectively. Baseline firing rate had no significant difference between the three groups ($P > 0.05$, $F_{2, 19} = 1.60$; one-way ANOVA). ED_{50} values for R-APO were 3.67 ± 1.00 μg/kg in sham-lesioned rats treated with vehicle, 2.35 ± 0.78 μg/kg in 6-OHDA-lesioned rats treated with vehicle and 3.02 ± 1.14 μg/kg in 6-OHDA-lesioned rats treated with R-APO. The ED_{50} values from the sham-lesioned rats treated with vehicle, the 6-OHDA-lesioned rats treated with vehicle and the 6-OHDA-lesioned rats treated with R-APO were not statistically different ($P > 0.05$, $F_{2, 19} = 0.52$; one-way

ANOVA; Fig. 4E). Chronic R-APO treatment did not reverse ED_{50} value of the 6-OHDA-lesioned rats compared with the 6-OHDA-lesioned rats treated with vehicle ($P > 0.05$, n = 7; Tukey's test; Fig. 4E), and this effect was consistent with the change of firing rate in the three groups.

Discussion

Our data showed that DA neuron loss in the SNc was 41% compared to the unlesioned side, which mimic the preclinical stage in PD. The result is similar to that by Yuan *et al*. who showed that the number of TH-ir neurons decreases to about 50% in the SNc of the lesioned rats (27). Therefore, this model allows us to test the efficacy of treatment with DA receptor agonist R-APO. A number of studies have shown that the DA receptor agonists have neuroprotective effects in tissue culture and in animal models of PD. Neuroprotective mechanism of R-APO may contribute to reducing the oxidation of polyunsaturated fatty acids and scavenging free radicals and chelates iron (12, 13, 24). In addition, R-APO may have anti-inflammatory and neurotrophic actions (10, 18). The present study indicates that chronic R-APO treatment has a significant protection of TH-ir neurons in the SNc in the partially lesioned rat model of PD. These results are in agreement with a previous work that reports neuroprotective effect of R-APO in the model (26).

In the present study, no differences in the firing rate and firing pattern were observed between the sham-lesioned rats treated with vehicle, the 6-OHDA-lesioned rats treated with vehicle and the 6-OHDA-lesioned rats treated with R-APO. In contrast, we found that chronic R-APO treatment increased the number of spontaneously active DA neurons in the SNc of the 6-OHDA-lesioned rats, while the lesion resulted in a decrease in the number of spontaneously active DA neurons in the SNc. The number of DA neuron loss, striatal DA levels and sensitivity of the DA neurons autoreceptors in the SNc are responsible for the firing activity of the neurons in partially 6-OHDA-lesioned rats. Several studies have reported the extraordinary capacity of the DA system to compensate for extensive damage in 6-OHDA-lesioned rats (18, 29). Thus, rats that have lost 90% or more of striatal DA levels exhibit recovery of behavioral function within l-2 months following the lesion (6, 30). In a similar manner, patients with PD do not show profound motor impairment until the loss of brain DA levels exceeds 80% (29, 30). The compensatory changes in the neurochemistry of this system are likely to contribute to the behavioral recovery in rats including an increase in DA synthesis within the remaining DA terminals (1), increased DA release (28), increased sensitivity of striatal neurons to DA (9) and a decrease in the removal of DA from the synapse due to the lesion-induced loss of DA uptake sites on DA terminals (29).

Previous studies have shown that the lesion-induced loss of DA neurons is accompanied by a corresponding decrease in the number of spontaneously active DA neurons in the SNc (4, 17). Although the lesion resulted in a moderate to severe loss of striatal DA levels, the relative proportion of DA neurons exhibiting spontaneous activity as well as their basal firing rate and firing pattern were not altered from control values (4, 17). Alterations in firing activity of the DA neurons were observed in rats only when the extent of striatal tissue DA depletion was greater than 95%, which also corresponded to the lesion size from which rats typically fail to exhibit behavioral recovery (4, 6, 17). It is postulated that lesion-induced increase in striatal activity would cause a feedback activation of DA neuron firing (19). Another candidate might be an offsetting influence to this striatonigral feedback which cause a substantial increase in the sensitivity of D_2 autoreceptors (21). In this way, the remaining DA neurons would maintain the wide dynamic range of electrophysiological responsivity that may be necessary for the normal function of the extrapyramidal motor system. Therefore, from our data and previous reports, partial lesion of the nigrostriatal pathway and chronic R-APO treatment could not change the firing rate and firing pattern of DA neurons in the SNc. Regarding the number of spontaneously active neurons per electrode track, our data showed that the injection of 6-OHDA into the striatum resulted in a decrease in the number of spontaneously active neurons, whereas chronic R-APO treatment increased the number of spontaneously active neurons in the partially lesioned rats. These results are in agreement with the number of TH-ir neurons in the SNc suggesting neuroprotective effects of R-APO in this region. In addition, the reduction in the decrement of active neurons may also be for a reversion of the loss.

Administration of low doses of R-APO inhibits nigral DA neurons by acting at D_2 receptors located on the dendrites of these neurons (2). The present study indicates that administration of R-APO (0.5-16.0 μg/kg, i.v.) dose-dependently inhibited the firing rate of SNc DA neurons in the three groups. However, the difference of inhibitory response in these neurons occurred with the increase of dose of R-APO. In the sham-lesioned rats treated with vehicle and the 6-OHDA-lesioned rats treated with vehicle, the initial dose of R-APO inducing the inhibitory response in the DA neurons was similar indicating that the DA receptor sensitivity to R-APO stimulation did not change in this model. These findings are in agreement

with a previous report that shows no differences in the firing inhibition of SNc DA neurons to R-APO in partially medial forebrain bundle-lesioned rats (4), and with an other study that has demonstrated that the up-regulation of D_2 receptors does not occur until the striatum is largely denervated (8). However, these results are in contrast with that reported by Pucak and Grace who described an increased sensitivity of SNc DA neurons to R-APO in the intraventricular infusion of 6-OHDA producing DA-depleted rats (21). Two reasons may account for this discrepancy. First, up-regulation of D_2 receptor relates to the degree of lesion in nigrostriatal pathway. Second, R-APO has selectivity for D_2-like receptors located in dendritic terminals (2). Injection of 6-OHDA into different parts of the brain might produce diverse degrees of nigrostriatal dendrite degeneration which display different response to R-APO. In the 6-OHDA-lesioned rats treated with R-APO, the initial dose of R-APO inducing inhibitory response of the neurons to the administration of R-APO was markedly higher than that of the sham-lesioned rats treated with vehicle and the 6-OHDA-lesioned rats treated with vehicle indicating that the DA receptor sensitivity was decreased after the chronic R-APO treatment. A more likely explanation is that the partial lesion of the nigrostriatal pathway results in the down-regulation of D_2 receptors which decreases the responsiveness of D_2 autoreceptors in the SNc to R-APO. The results are supported by the findings that the persistent stimulation of D_2 receptors produces a reduction in D_2 autoreceptor sensitivity and the receptor binding sites in the ventral tegmental area and the striatum (5, 7, 22). In addition, repeated R-APO treatment has been found to cause behavioral supersensitivity in association with reduced D_2 receptor binding in the striatum (5, 7, 22). Our data showed that the ED_{50} values for R-APO in the 6-OHDA-lesioned rats treated with R-APO were not statistically different compared with the sham-lesioned rats treated with vehicle and the 6-OHDA-lesioned rats treated with vehicle, respectively. A previous study has found a relationship between ED_{50} for R-APO and the firing rate of DA neurons in which the faster cells require more R-APO to reach 50% inhibition (23). In our data, the firing of DA neurons showed no differences in the three groups, which is consistent with these studies.

In summary, the present study indicates that the chronic, systemic treatment with R-APO produces significant neuroprotective effects in the striatal infusion of 6-OHDA producing partially DA-depleted rats. It also provides cellular evidence that the firing rate and firing pattern of DA neurons in the SNc do not change in this model whereas R-APO treatment recovers the decreased number of spontaneously active neurons per electrode track. In addition, the treatment decreases the responsitivity of DA neurons in the SNc to R-APO stimulation in partially lesioned rats suggesting down-regulation of D_2 receptors in this region after the chronic treatment with R-APO.

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