

Inhibition of NADPH oxidase within midbrain periaqueductal gray decreases pain sensitivity in Parkinson's disease via GABAergic signaling pathway

Wenwen ZHANG^{1*}, Lin SUN^{2*}, Xudong YANG³, Ruimin WANG⁴, Hushan WANG¹
**These authors contributed equally to this work.*

¹Department of Anesthesiology, The First Hospital of Jilin University, Changchun, Jilin, China, ²Department of General Gynecology, The First Hospital of Jilin University, Changchun, Jilin, China, ³Department of Endoscopy, Changchun Hospital of Traditional Chinese Medicine, Changchun, Jilin, China, ⁴Department of Operation Room, The Second Hospital of Jilin University Changchun, Jilin, China

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Summary

Hypersensitive pain response is observed in patients with Parkinson's disease (PD). However, the signal pathways leading to hyperalgesia still need to be clarified. Chronic oxidative stress is one of the hallmarks of PD pathophysiology. Since the midbrain periaqueductal gray (PAG) is an important component of the descending inhibitory pathway controlling on central pain transmission, we examined the role NADPH oxidase (NOX) of the PAG in regulating exaggerated pain evoked by PD. PD was induced by central microinjection of 6-hydroxydopamine to lesion the left medial forebrain bundle of rats. Then, Western Blot analysis and ELISA were used to determine NOXs and products of oxidative stress (i.e., 8-isoprostaglandin F_{2a} and 8-hydroxy-2'-deoxyguanosine). Pain responses to mechanical and thermal stimulation were further examined in control rats and PD rats. In results, among the NOXs, protein expression of NOX4 in the PAG of PD rats was significantly upregulated, thereby the products of oxidative stress were increased. Blocking NOX4 pathway in the PAG attenuated mechanical and thermal pain responses in PD rats and this was accompanied with decreasing production of oxidative stress. In addition, inhibition of NOX4 largely restored the impaired GABA within the PAG. Stimulation of GABA receptors in the PAG of PD rats also blunted pain responses. In conclusions, NOX4 activation of oxidative stress in the PAG of PD rats is likely to impair the descending inhibitory GABAergic pathways in regulating pain transmission and thereby plays a role in the development of pain hypersensitivity in PD. Inhibition of NOX4 has beneficial effects on the exaggerated pain evoked by PD.

Key words

Mechanical sensitivity • Thermal sensitivity • Neurodegeneration • NADPH • GABA

Corresponding author

Hushan Wang, Department of Anesthesiology, The First Hospital of Jilin University, 71 Xinmin Street, Changchun, Jilin 130021, China. Email: wanghushan@aol.com

Introduction

Parkinson's disease (PD) is characterized by the loss of central dopaminergic (DA) neurons and the presence of α -synuclein-containing aggregates in the substantia nigra pars compacta (Samii *et al.* 2004). Notably, epidemiological studies indicate that a high frequency of hypersensitive pain is presented in PD patients (Beiske *et al.* 2009, Wasner and Deuschl 2012). Also, behavioral studies show that sensitivity to pain is increased in patients (Djaldetti *et al.* 2004, Nandhagopal *et al.* 2010). Central nervous mechanisms are considered to play an important role in processing abnormalities in pain response in PD patients (Conte *et al.* 2013). Non-dopaminergic neurotransmission has been implicated in influencing descending pain pathways at central levels (Conte *et al.* 2013). Nonetheless, treatment options for these amplified pain sensations have been limited, partly due to our poor understanding of the

neural mechanisms responsible for PD-induced pain. Thus, it is significant to investigate the pathophysiology of hyperalgesia in PD.

Chronic oxidative stress is one of the hallmarks in PD (Rekatsina *et al.* 2020, Simon *et al.* 2020). Studies in human PD patients and animal models of experimental PD show that activation of glial cells and elevation of reactive oxygen species (ROS) are common features of the PD brain (Hassanzadeh and Rahimmi 2018, Rekatsina *et al.* 2020, Tansey *et al.* 2007, Wang *et al.* 2015). Chronic production of oxidative stress and releases of pro-inflammatory cytokines (PICs) by astrocytes and microglia stimulated by oxidative stress leads to the exacerbation of DA neuron degeneration in the substantia nigra pars compacta (Tansey *et al.* 2007, Wang *et al.* 2015). Also, peripheral immune system is involved in the pathogenesis of PD. Infiltration and accumulated immune cells from the periphery are identified in and around the affected brain regions of PD patients (Tansey *et al.* 2007, Wang *et al.* 2015). Moreover, oxidative stress and inflammatory processes have been suggested as promising interventional targets for PD and even other neurodegenerative diseases (Rekatsina *et al.* 2020, Tansey *et al.* 2007). A better understanding of the role of oxidative stress in development of PD will provide new insights into the pathological processes and help to establish general therapeutic strategies as well as effective interventions to alleviate hypersensitive pain observed in PAD patients.

As a component of the descending pain modulatory network, the midbrain periaqueductal gray (PAG) has an inhibitory or excitatory control on pain transmission via the rostral ventromedial medulla, projecting to the spinal and medullary dorsal horn (Behbehani 1995, Carrive and Morgan 2012, Craig 1995). Accordingly, in the present study, we examined the underlying mechanisms by which the changes in neural substrate activity in the PAG are engaged in PD-induced pain.

GABA is a main inhibitory neurotransmitter in the central nerve system in control of neuronal excitability. After GABA releases from presynaptic terminals, GABA transporters play a role in regulating a rapid removal of extracellular GABA (Borden 1996, Richerson and Wu 2003), which thereby leads to ending of inhibitory synaptic transmission. Thus, this mechanism is responsible for GABA spillover to neighboring synapses (Borden 1996, Overstreet and Westbrook 2003) and GABA homeostasis (Borden 1996, Semyanov *et al.*

2004). In contrast, under certain pathological and physiological conditions the abnormal levels of GABA are observed (Allen *et al.* 2004, Wu *et al.* 2007). Previous studies suggest that ROS signal pathways are activated in the brain of rats with excitatory neuronal activities and this alters expression of GABA via PICs (Rekatsina *et al.* 2020, Su *et al.* 2015).

Therefore, in this study, we determined the levels of NADPH oxidase (NOX1-4) within the PAG tissues of PD rats and control rats. We also examined 8-isoprostaglandin F_{2α} (8-iso PGF_{2α}, a product of oxidative stress) and 8-hydroxy-2'-deoxyguanosine (8-OHdG, a key biomarker of protein oxidation). Based on the previous evidence that NOX4 is highly expressed in the central nervous system in the process of various nociception (Kallenborn-Gerhardt *et al.* 2013), we hypothesized that protein expression of NOX4 is upregulated in the PAG of PD rats and blocking NOX4 in the PAG attenuates amplified products of oxidative stress and thereby attenuates mechanical and thermal pain responses in PD rats. We further hypothesized that the levels of GABA in the PAG of PD rats are decreased and blocking NOX4 restores the impaired GABAergic inhibitory pathways thereby alleviating pain responses.

Methods

Animals

All animal protocols were in accordance with the guidelines of the International Association for the Study of Pain and approved by the Animal Care and Use Committee of Jilin University. Male Sprague-Dawley (200-250 g) were housed in individual cages with free access to food and water and were kept in a temperature-controlled room (25 °C) on a 12/12 h light/dark cycle.

Induction of PD by 6-hydroxydopamine (6-OHDA) lesions

Animals were anesthetized by sodium pentobarbital (60 mg/kg, i.p., Sigma Co.) and then placed in a David Kopf stereotaxic instrument. Injections of 6-OHDA (7 μg/2μl/each location, dissolved in saline containing 0.02 % ascorbic acid) were made at two locations into the left medial forebrain bundle. Stereotaxic coordinates for the lesions were 3.3 mm rostral to the interaural line, 1.4 mm left of the midline, and 6.5 and 6.8 mm ventral to the dural surface. The 6-OHDA solution was administered through a cannula using a microinjection pump at a rate of 1 μl/min. The

cannula was left in place for 5 min after completion of each injection and then slowly retracted. Equivalent injections of saline were made in control animals. Note that thirty minutes prior to surgery, rats received an intraperitoneal injection of desipramine hydrochloride (20 mg/kg, i.p., Sigma Co.) to protect noradrenergic neurons and fibers.

Rotation behavior test

Two weeks after 6-OHDA injection, rats injected with 6-OHDA and control rats with saline injection were placed in a cylindrical container (300 mm diameter). Methamphetamine (3 mg/kg, i.p.) was injected to trigger rotational behavior. The rotational behavior was counted at 10-min intervals for 60 min after methamphetamine administration. Animals with > 7 turns/min of rotational behavior were included in the study, as dopaminergic neurons and fibers of those rats are destroyed after 6-OHDA lesions (Ishida Y *et al.* 1998).

PAG cannulation and drug infusion

Three days were allowed before the experiments. Rats were implanted with a stainless steel guide cannula (0.8 mm o.d.) with sodium pentobarbital (60 mg/kg, i.p.) and the guide cannula was secured to the skull. Stereotaxic coordinates for the dorsolateral PAG (dl-PAG) were 7.6 mm posterior to the bregma, 0.65 mm lateral to the midline, and 4.2 mm ventral to the brain surface.

Following this, cannula was connected to an osmotic minipump (Alzet pump brain infusion kit, DURECT Inc., Cupertino, CA) with polycarbonate tubing. The pumps were placed subcutaneously between the scapulae, and loaded with vehicle [artificial cerebrospinal fluid (aCSF)] as control, NOX4 inhibitor GKT137831 (10 μ M, Cayman Chem. Co), agonist of GABA_A receptor muscimol (100 μ M, Sigma Co.), respectively. Then, they were delivered at 0.25 μ l per hour (Alzet Model 1003D/3 day-delivery DURECT Inc., Cupertino, CA). This intervention allowed animals to receive continuous PAG infusion via the osmotic minipumps before the experiments and brain tissues were taken out.

Examination of pain sensitivity

Rats were placed in individual plastic boxes to acclimate for > 30 min in order to quantify the mechanical sensitivity of the hind paw. Mechanical

withdrawal threshold (PWT) of rat hind paw responding to the stimulation of von Frey filaments was examined. A series of calibrated von Frey filaments (ranging from 0.5 to 18.0 g) were applied perpendicularly to the plantar surface of the hind paw with a sufficient force to bend the filaments for 60s or until paw withdrew. If a response was seen, the filament of next lower force was given. Without a response, the filament of next greater force was used. To avoid injury during tests, the cutoff strength of the von Frey filament was 18 g. The tactile stimulus producing a 50 % likelihood of withdrawal was determined using the "up-down" method (Chaplan *et al.* 1994). Each test was repeated twice at roughly 2 min intervals. The mean value was used as the force producing a withdrawal response.

Rat withdrawal latency (PWL) to a radiant heat was measured to examine thermal hyperalgesia. Rats were positioned separately in cages on an elevated glass platform and allowed for 30 min acclimation. Each hind paw received three stimuli with a 10 min interval, and the mean of the three withdrawal latencies was defined as PWL. The heat was maintained at a constant intensity. To prevent tissue damage, the cut-off latency was set at 20 s. All the behavioral tests were performed in a blind style.

At the end of the experiments, 2 % Evans blue in 0.25 μ l was infused through the cannula. Then, the animals were anesthetized by sodium pentobarbital and intra-cardiacally perfused with physiological saline followed by 4% of paraformaldehyde solution. The midbrain was sectioned and the location of injection sites was verified by histological examination of blue dye according to the atlas of Swanson (Swanson 1998). The rats with microinjection site was localized within the dl-PAG were included for data analysis.

ELISA measurements

The rats were first euthanized by overdose sodium pentobarbital (120 mg/kg, i.p.) and then the dorsolateral regions of PAG were dissected under an anatomical microscope. Total protein of the PAG tissue was then extracted by homogenizing sample in ice-cold radioimmunoprecipitation assay buffer with protease inhibitor cocktail kit. The lysates were centrifuged and the supernatants were collected for measurements of protein concentrations using a bicinchoninic acid assay reagent kit. The levels of 8-iso PGF₂ α and 8-OHdG were examined using an ELISA assay kits (obtained from Promega Co. and Abcam Co.) according to the provided description. Briefly, polystyrene 96-well microtiter

immunoplates were coated with affinity-purified rabbit primary antibodies. Parallel wells were coated with purified rabbit IgG for evaluation of nonspecific signal. After overnight incubation, plates were washed. Then, the diluted samples and 8-iso PGF2 α /8-OHdG standard solutions (100pg/ml-100ng/ml) were distributed in each plate. The plates were washed and incubated with anti-8-iso PGF2 α /8-OHdG galactosidase. Then, the plates were washed and incubated with substrate solution. After incubation, the optical density was measured using an ELISA reader with a wave length of 575 nm. In the similar way, the levels of GABA were determined (LDN Diagnostics, Inc. Colorado Springs, CO) according to the provided description.

Western blot analysis

Similar to the ELISA, the dl-PAG tissues were removed and total protein was extracted. The lysates were centrifuged and the supernatants were collected. After being denatured, the supernatant samples containing

20 μ g of protein were loaded onto gels and electrically transferred to a polyvinylidene fluoride membrane. The membrane was incubated with respective primary antibodies (at 1:500, Abcam and/or Antibodies online Com): rabbit anti-NOX1/ anti-NOX2/anti-NOX3/anti-NOX4. The membranes were washed and incubated with an alkaline phosphatase conjugated anti-rabbit secondary antibody (1:1000). The immunoreactive proteins were detected by enhanced chemiluminescence. The bands recognized by the primary antibody were visualized by exposure of the membrane onto an x-ray film. The membrane was stripped and incubated with anti- β -actin to show equal loading of the protein. Then, the film was scanned and the optical densities of primary antibodies and β -actin bands were determined using the Scion Software (National Institute of Health). Values for densities of immunoreactive bands/ β -actin band from the same lane were determined. Each of the values was then normalized to a control sample.

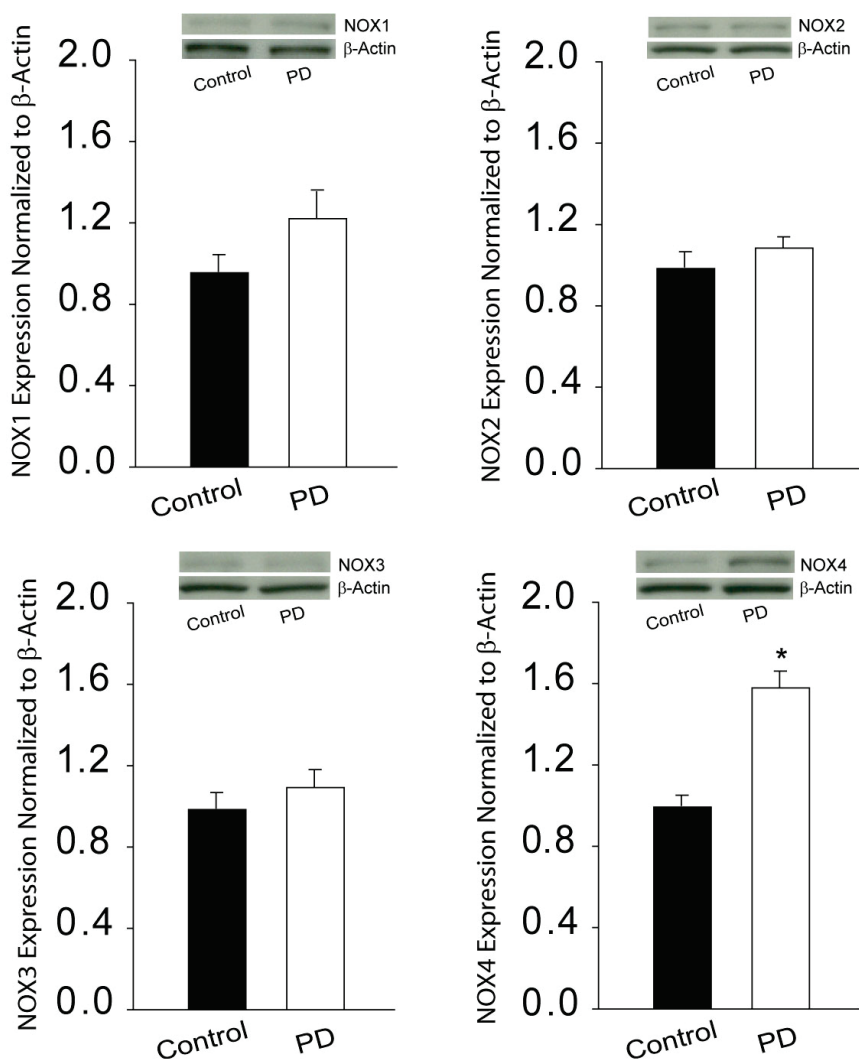


Fig. 1. The protein levels of NOXs expression in the PAG. Representative bands and averaged data, showing that protein levels of NOX1, NOX2 and NOX3 were not altered significantly in the dl-PAG of PD rats, but NOX4 was amplified in the dl-PAG of PD rats. Insignificant difference was seen between PD rats and control rats for NOX1, NOX2 and NOX3 ($P > 0.05$). * $P < 0.05$, PD rats vs. control rats. $n = 6-8$ in each group.

Statistical analysis

All data were analyzed using a one-way analysis of variance since we compared each data set of among different groups of animals. As appropriate, Tukey's post hoc analyses were utilized to determine differences between groups. Values were presented as means \pm standard error (SE). For all analyses, differences were considered significant at $P < 0.05$. All statistical analyses were performed by using SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL USA).

Results

Expression of NOXs

We first examined the protein levels of NOX1, NOX2, NOX3 and NOX4 in the PAG of PD rats and control rats. Figure 1 shows that no significant differences in expression of NOX1, NOX2 and NOX3 were observed among two groups ($P > 0.05$, PD rats vs. control rats for each of NOXs, $n = 6-8$ in each group).

In contrast, NOX4 in the PAG was amplified in PD rats as shown in Figure 1D ($P < 0.05$, PD rats/ $n = 8$ vs. control rats/ $n = 6$).

Effects of NOX4 inhibition on product levels of oxidative stress

In order to determine the effectiveness of blocking NOX4 pathway by infusing GKT137831 into the PAG, we further examined products of oxidative stress in the PAG of control rats, PD rats and PD rats with GKT137831 as shown in Figure 2A,B. These figures demonstrate that 8-iso PGF 2α and 8-OHdG were increased in the dl-PAG of PD rats ($n = 15$) as compared with control rats ($n = 12$, $P < 0.05$, PD vs. controls). In addition, infusion of NOX4 inhibitor GKT137831 attenuated increases of both 8-iso PGF 2α and 8-OHdG in the PAG ($P < 0.05$, PD rats vs. PD rats with infusion of inhibitor/ $n = 12$). Note that no significant differences in 8-iso PGF 2α and 8-OHdG were observed between control rats and PD rats infused with GKT137831 ($P > 0.05$).

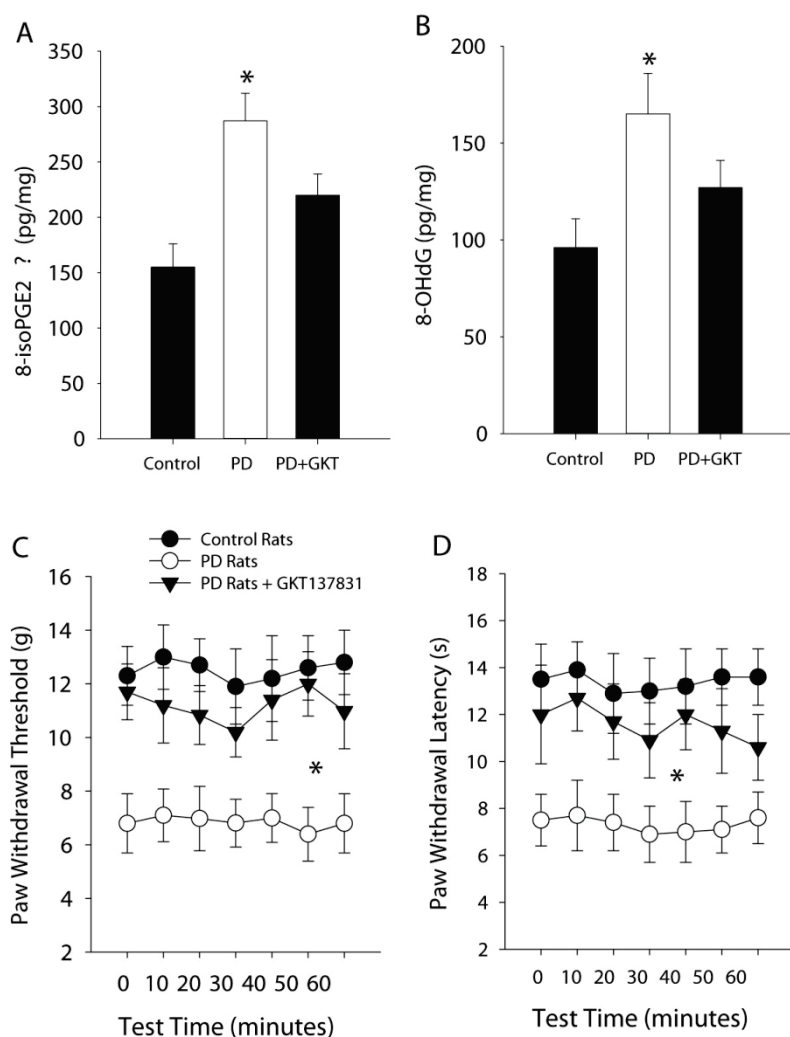


Fig. 2. The effects of NOX inhibition on products of oxidative stress and pain response. **A, B**) 8-iso PGF 2α and 8-OHdG were increased in the dl-PAG of PD rats ($n = 15$) as compared with control rats ($n = 12$). Infusion of NOX4 inhibitor GKT137831 (GKT) attenuated increases in 8-iso PGF 2α and 8-OHdG in the dl-PAG ($n = 12$). * $P < 0.05$, PD rats vs. control rats and PD rats with infusion of GKT137831. Note that no significant differences in 8-iso PGF 2α and 8-OHdG were observed between control rats and PD rats infused with GKT137831 ($P > 0.05$). **C, D**) Effects of blocking NOX4 signal pathway in the dl-PAG on pain responses to mechanical and thermal stimuli. Mechanical and thermal hyperalgesia was lower in PD rats ($n = 15$) as compared with control animals ($n = 10$). Infusion of GKT137831 into the PAG attenuated hypersensitive pain responses in PD rats ($n = 12$). * $P < 0.05$ vs. control rats and PD rats that received infusion of GKT137831 over a 60 min-testing time.

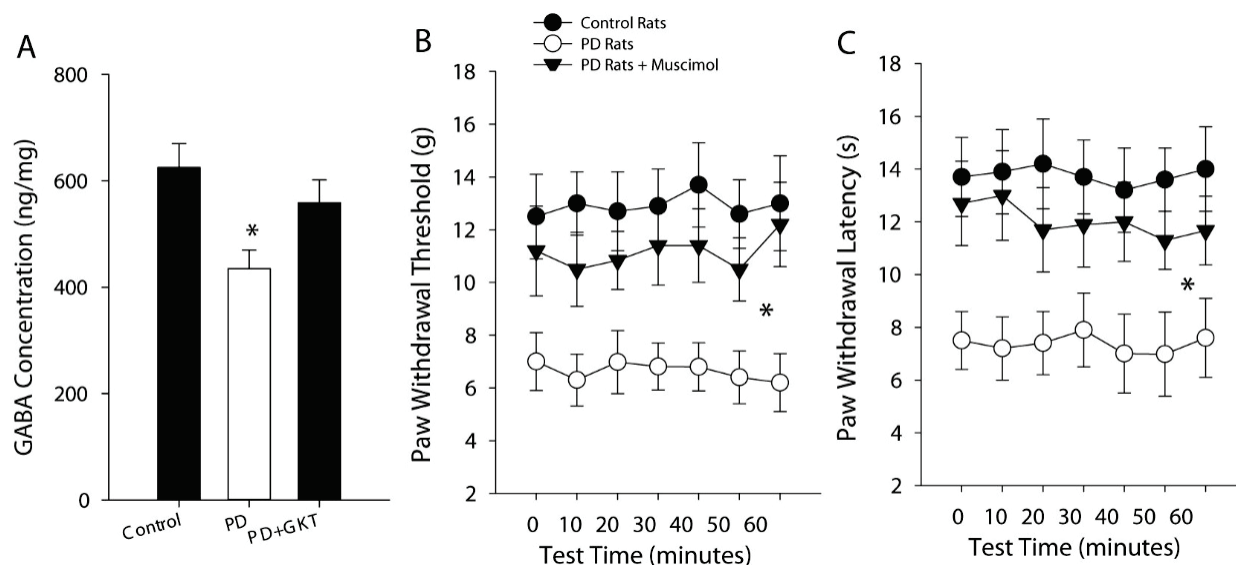


Fig. 3. The involvement of GABA in the effects of NOX4. **A)** The levels of GABA in the dl-PAG were significantly diminished in PD rats ($n=12$) as compared with control rats ($n=10$). Infusion of NOX4 inhibitor GKT137831 (GKT) largely restored impaired GABA. $*P < 0.05$ vs. control rats and rats with infusion of GKT137831 ($n=12$). **B, C)** Effects of stimulation of GABA α receptor in the dl-PAG on pain responses to mechanical and thermal stimulation. Mechanical and thermal hyperalgesia appeared in PD rats ($n=10$) as compared with control animals ($n=8$). Infusion of GABA α receptor agonist, muscimol into the dl-PAG attenuated hypersensitive responses in PD rats ($n=12$). $*P < 0.05$ vs. control rats and PD rats with infusion of muscimol over a 60 min-testing time.

Effects of NOX4 inhibition on pain responses to mechanical and thermal stimuli

Figure 2 C,D show that PWT and PWL appeared to be less in PD rats over 60 min of test time ($n=15$, $P < 0.05$ vs. control rats) as compared with control rats ($n=10$). We further examined the effects of blocking NOX4 using GKT137831 in the dl-PAG on PWT and PWL in PD rats ($n=12$ in each group). Figure 2 C,D further demonstrate that PWT and PWL were significantly increased during a 60 min-period of the test with a 10 min-interval after blocking NOX4 ($P < 0.05$ vs. PD rats). Note that there were no differences in PWT and PWL between controls and PD rats with NOX4 blocking ($P > 0.05$ vs. control rats).

In addition, in five PD rats with microinjection site was not localized within the dl-PAG, GKT137831 failed to alter either PWT or PWL. i.e., PWT was 7.2 ± 2.3 g ($P > 0.05$ vs. PD rats) and PWL was 7.9 ± 3.1 s ($P > 0.05$ vs. PD rats).

Involvement of GABA in the effects of NOX4

Figure 3A demonstrates that the levels of GABA were significantly decreased in the dl-PAG of PD rats compared with control animals. The levels of GABA were 625 ± 45 ng/mg in control rats ($n=10$) and 435 ± 35 ng/mg in PD rats ($n=12$, $P < 0.05$ vs. control rats). With infusion of GKT137831 the lessened GABA was restored to 559 ± 43 ng/mg ($n=12$, $P < 0.05$ vs.

PD rats), but no significant differences were observed in the GABA levels between control animals and PD animals with NOX blocking ($P > 0.05$ vs. control rats).

We further examined the effects of stimulation of GABA α receptor by infusion of muscimol in the dl-PAG on PWT and PWL in PD rats. Figure 3 B,C show that PWT and PWL were significantly increased during a 60 min-period of the test with a 10 min-interval after stimulation of GABA α receptor ($P < 0.05$ vs. PD rats). No significant differences in PWT and PWL were observed between control rats and PD rats with muscimol ($P > 0.05$ vs. control rats). This result suggests the engagement of GABA in hypersensitive mechanical and thermal responses in PD rats.

Discussion

Nigrostriatal lesions induced by 6-OHDA in rats are widely used to study PD (Blandini *et al.* 2008, Schwarting and Huston 1996). It has been reported that 6-OHDA injected into the medial forebrain bundle of rats leads to extensive destruction of dopaminergic neurons of the substantia nigra pars compacta (Deumens *et al.* 2002). A unilateral lesion of the nigrostriatal pathway also causes rotational behavior toward the lesioned side after administration of methamphetamine (Inden *et al.* 2004, Maegawa *et al.* 2015). In the present study, the same rat model of PD was used and consistent with prior studies

we have observed that rotational behavior appeared > 7 turns/min after methamphetamine in 6-OHDA-injected rats.

Evidence has suggested that antinociception is mediated partly by descending pathways arising from the midbrain PAG (Klemm 2004, Millan 2002). Early studies showed that electrical stimulation or opioids microinjected into the PAG produced profound long-lasting antinociception (Klemm 2004, Millan 2002). In particular, activated neuronal cells are identified in PAG of PD rats evoked by 6-OHDA (Maegawa *et al.* 2015), suggesting neural substrates are likely present within the PAG in engagement of the abnormalities in pain response observed in PD. Furthermore, previous studies showed that PIC mediators appear in the PAG, and activation of PICs in the PAG plays a role in modulating pain response or is involved in morphine withdrawal response (Benamar *et al.* 2008, Hao *et al.* 2011). PIC signal pathways in the PAG is likely to play a role in regulating amplified pain response in a rat model of PD. It should be noted that the releases of PICs by astrocytes and microglia are stimulated by oxidative stress thereby leading to the subsequent pathophysiological responses (Hassanzadeh and Rahimmi 2018, Tansey *et al.* 2007, Wang *et al.* 2015). Thus, our current study examined specifically the effects of ROS signal pathways within the PAG on mechanical and thermal pain in PD rats induced by 6-OHDA.

One source of ROS production is the enzyme family of NOX. The rodent genome encodes four genes that contain the catalytic NOX subunit, namely NOX1, NOX2, NOX3, and NOX4 (Altenhofer *et al.* 2012). This electron-transferring subunit is constitutively inactive in resting cells and generates ROS after inflammatory stimuli (Salvemini *et al.* 2011). Whereas NOX2 activation is predominantly associated with innate immunity mediated host defense and NOX1 with blood pressure control and related vascular mechanisms (Gavazzi *et al.* 2006, Lam *et al.* 2010), NOX4 is expressed in the central nervous system in the process of various nociception (Kallenborn-Gerhardt *et al.* 2013). Evidence has also identified activation of NOX4 as a causative factor that contributes to inflammatory role in the peripheral nervous system (Kallenborn-Gerhardt *et al.* 2012). Nonetheless, in the current study, we determined the effects of PD on expression levels of NOX1, NOX2, NOX3 and NOX4 in the dl-PAG of rats. We observed that NOX4 was upregulated among NOXs after development of PD and this was likely to lead to

increases of ROS generation in the PAG. Consistent with this notion, in the current study, we found that 8-iso PGF2 α and 8-OHdG were increased in the dl-PAG of PD rats and blocking NOX4 in this brain region attenuated amplification of these products of oxidative stress. Notably, we observed that inhibition of NOX4 signal pathways in the PAG alleviated hypersensitive pain responses to mechanical and thermal stimuli in PD rats.

Interestingly, results of our present study further demonstrated that the levels of GABA were significantly decreased in the dl-PAG of PD rats. Prior studies showed antinociceptive effects evoked by stimulation of this region of PAG (Behbehani 1995, Carrive and Morgan 2012). This supports our notion that activation of NOX4 within the PAG plays a de-inhibitory role in regulating the descending pain pathways. When NOX4 signal pathways are blocked in the dl-PAG, the impaired GABA descending pain pathways are largely restored because we have observed that chronic infusion of NOX4 inhibitor alleviated pain responses in PD rats, accompanied with increasing GABA levels in the PAG. Furthermore, our results found that mechanical and thermal hyperalgesia in PD rats was attenuated following stimulation of GABA α receptors in the dl-PAG by infusion of muscimol, suggesting that NOX4 signal influences GABAergic transmission within this region of PAG of PD rats and thereby amplifies pain response.

We speculate that there are some possibilities that NOX4 signal can alter GABAergic pathway. 1) activation of NOX4 inhibits the releases of GABA in the dl-PAG since a lower level of GABA was observed in this region in our current study, 2) activation of NOX4 is likely to damage neurons of the dl-PAG thereby leading to a reduction in GABA, 3) the amplified NOX4 observed in our study is likely to alter activities of ion channels on neurons of the dl-PAG and decrease the GABA levels or attenuate GABAergic transmission. Additional studies need to determine a precise mechanism responsible for how NOX4 signal pathways alter GABAergic pathway within the dl-PAG in involvement of hypersensitive pain responses in PD.

We have shown that 1) the levels of NOX4 expression are upregulated in the dl-PAG of PD rats, 2) inhibition of NOX4 signal pathway in this brain region alleviates hypersensitive pain responses to mechanical and thermal stimuli in PD rats, 3) inhibition of NOX4 in this brain region increases GABA levels, 4) activation of GABA in the dl-PAG attenuates PD-induced pain. We concluded that NOX4 signal pathways are augmented in

the dl-PAG of PD rats and thereby de-inhibit GABAergic mediated-descending regulation in pain transmission. These abnormalities are likely to contribute to the development of mechanical and thermal hypersensitivity in PD rats. Results of this study provided a base for the mechanisms responsible for PD-induced pain. This

further offers promising clues to target central nerve system for the development of new therapeutic strategies for managing pain sensitivity in PD patients.

Conflict of Interest

There is no conflict of interest.

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