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Effect of Varenicline on Detrusor Overactivity in Rat Model of Parkinson's Disease Induced by Intranigral 6-Hydroxydopamine

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Abstract

Background: Parkinson disease (PD) is a progressive neurodegenerative disorder characterized by both motor and non-motor symptoms. Bladder dysfunction is the common non-motor symptom of PD, most often presenting with detrusor overactivity (DO). Treatment of DO is currently limited, poorly tolerated and sometimes ineffective. Bladder responses are not only mediated by muscarinic cholinergic receptors (mAChR) but also by nicotinic cholinergic receptors (nAChR). However, nicotinic receptor subtypes and functions in the bladder are not clearly identified. Purpose: This study aimed at investigating the effect of varenicline, an alpha7 full agonist and alpha4beta2/alpha3 partial agonist, on detrusor strips in rat PD model induced by substantia nigra injection of 6-hydroxydopamine. Method: The detrusor activity was studied in an isolated organ bath system. Results: In PD group, the detrusor activity was increased, whereas varenicline decreased the DO. Conclusion: Alpha7 nAChR agonists may have therapeutic potential in treatment of bladder overactivity in PD.

Keywords

Nicotinic Cholinergic Receptors, Overactive Bladder, Varenicline, Parkinson Disease, Alpha7 Nicotinic Cholinergic Receptor Agonists

1. Introduction

Although Parkinson's disease (PD) is characterized by motor symptoms, it is also associated with non-motor symptoms. Among the non-motor symptoms,

bladder dysfunction is a common disorder with an incidence 55% - 80% [1] [2] [3]. Recognition of non-motor symptoms is important in the clinical follow-up of PD. In PD, bladder dysfunction can be overactive or obstructive [4] and detrusor overactivity (DO) is frequently encountered in urodynamic evaluations [5].

The data concerning the relationship between the stage of dopaminergic degeneration and bladder dysfunction are still inconsistent [3] [6]. Unlike motor disorders, bladder dysfunction in PD is generally unresponsive to L-DOPA therapy [7]. Anticholinergics which are commonly prescribed as a first-line treatment for overactive bladder [8] may have potential adverse effects [9] [10].

It has been suggested that PD pathology is not only associated with dopaminergic degeneration in the brain, but different neurotransmitters may also play a role in different brain regions [1]. Bladder responses have been modulated both by muscarinic (mostly M3 and M2) receptors [11] and nicotinic cholinergic receptors (nAChRs) (mostly alpha (α) 7, α3 and beta (β) 4 subtypes) by getting involved in bladder contraction and relaxation [12] [13]. It has been reported that stimulation of α7 nAChRs may reduce detrusor muscle contractility, as well as cause the release of a soluble factor that inhibits bladder reflexes [14]. Varenicline is an α7 full agonist and α4β2 /α3 partial agonist [15]. This study aimed at investigating the effect of varenicline, on isolated detrusor muscle activity in intranigral 6-hydroxydopamine (6-OHDA)-induced rat PD model.

2. Methods

2.1. Subjects

Thirty male Sprague-Dawley rats (10 - 12 weeks old, 250 - 260 g) were housed under standard circumstances of a 12-hour light/dark cycle, temperature (21˚C - 24˚C), humidity (55%), and free access to water and food. All experiments were conducted between 09:00 and 17:00. This study was approved by the Pamukkale University Experimental Animal Ethics Committee and all experimental procedures were performed in accordance with the requirements of the guidelines.

2.2. Stereotaxic Surgery and Experimental Design

The experimental groups were as follows: Group 1: Control (Control + Saline): Saline solution was injected into substantia nigra pars compacta (SNc), Group 2: 6-OHDA (6-OHDA + Saline), Group 3: Varenicline (6-OHDA + Varenicline). The rats were anesthetized with 80 mg/kg intraperitoneal (i.p.) ketamine (Ketalar; Pfizer, Lüleburgaz, Turkey) and 8 mg/kg ip xylazine (Alfazyne; Alfasan Int., B.V., Woerden, Holland, Netherlands). 6-OHDA (8 microg/4 micro L) was injected into right-side SNc according to Paxinos & Watson [16] at the following coordinates (mm): AP: −4.8 from bregma, L: 2.4 from midsagittal line, DV: 8 from skull surface. Desipramin (30 mg/kg) and pargyline (10 mg/kg) were injected i.p. in order to protect the noradrenergic neurons. Induction of 6-OHDA lesion was tested after 3 weeks, using Apomorphine (Sigma-Aldrich Co., St.

Louis, Missouri, USA) 0.5 mg/kg-induced contralateral rotation in separate rats not included in the isolated organ bath studies. All measures were taken to maximize animal refinement; dextromethorphan (5 mg/kg, s.c.), penicillin (100 000 U, i.m.) and sterile saline (2 ml/rat, s.c. twice a day) was injected to each rat for 3 consecutive days after surgery. Varenicline was injected 1 mg/kg i.p. daily for two weeks after stereotaxic surgery (Figure 1).

2.3. Isolated Organ Bath Studies

Under deep anesthesia at the end of the sixth week, the bladder was carefully excised through a vertical lower abdominal incision. Full-thickness intact longitudinal strips (almost 2 × 10 mm) were prepared and mounted under 2 g resting tension in an organ bath, containing 20 mL of Krebs solution (25 mM $NaHCO_3$, 1.22 mM KH_2PO_4, 118.3 mM NaCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 4.7 mM KCl, and 11.1 mM glucose), gassed with 95% O_2 and 5% CO_2 at 37˚C. Isometric tension changes were recorded using isometric force transducer and recorded by 8-channel transducer data acquisition system using a software program (MP35 Biopac Systems, Inc.).

The isolated detrusor muscle was contracted by a single concentration of KCl (120 mmol/L) and cumulative carbachol, final concentration: (10^{-7} - 3 × 10^{-5} M). The relaxing effect of cumulative oxybutynin (10^{-8} M - 3 × 10^{-6} M) concentrations were tested in submaximal-carbachol-precontracted detrusor and the inhibition (percent) of the maximum contraction was expressed as the oxybutynin relaxation. Before and after oxybutynin application, amplitude (mg) was measured as the contractions at the peak, and frequency (Hz) were measured as contractions per minute.

3. Statistical Analysis

The data were normally distributed and statistically evaluated using one-way analysis of variance (ANOVA). When any significance was detected, the Tukey post-hoc multiple comparison test was applied to determine significant differences among the groups. P values < 0.05 were accepted as statistically significant. The data are presented as mean ± SEM. The analyses were performed using IBM SPSS 21 (IBM Corp., Armonk, New York, USA) for Windows.

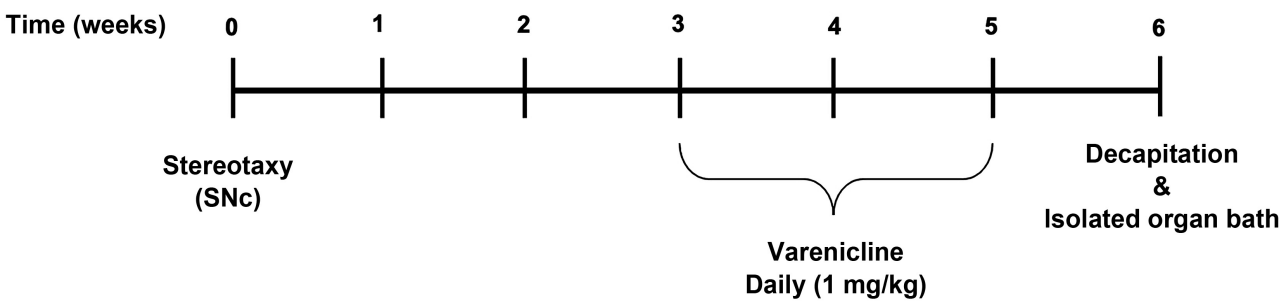

Figure 1. Timeline of the experimental procedures.

4. Results

4.1. Contractile Responses

Carbachol (10^{-7} - 3×10^{-5} M) and KCl (120 mmol/l) induced contractions in detrusor strips of the experimental groups.

One-way ANOVA results showed that there were significant differences in 10^{-6} M ($F_{2,27} = 4.410$; P = 0.022), 3×10^{-6} M ($F_{2,27} = 8.551$; P = 0.001), 10^{-5} M ($F_{2,27} = 14.705$; P = 0.000) and 3×10^{-5} M ($F_{2,27} = 23.259$; P = 0.001) concentrations of carbachol between groups. Cumulative contractile response was significantly higher at 10^{-5} M carbachol in 6-OHDA group compared to control (P = 0.040). Varenicline at 10^{-6} M (P = 0.020), 3×10^{-6} M (P = 0.001), 10^{-5} M (P = 0.000) and 3×10^{-5} M (P = 0.001) doses were lowered the contractility in comparison to 6-OHDA (Figure 2).

Maximal carbachol responses were significantly higher in the 6-OHDA group compared to the control (30%) and varenicline (59%). On the other hand, KCl-induced maximal contractions were significantly ($F_{2,21} = 4.932$; P = 0.014) lower (37%) in varenicline group compared 6-OHDA (Table 1).

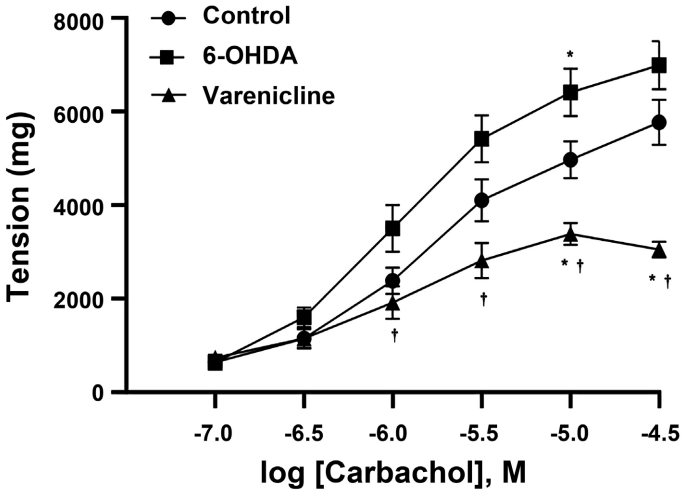

Figure 2. Carbachol-induced cumulative contractile responses of detrusor strips obtained from 3 experimental groups (n = 6 - 10 rats/group). Values represent means ± SEM. *P < 0.05, versus Control. †P < 0.05, Varenicline versus 6-OHDA.

Table 1. Carbachol and KCl-induced contractile responses of detrusor strips.

	Carbachol				KCL
	EC50	pD2	E_{max} (mg)	Hill slope	E_{max} (mg)
Control	1.7×10^{-6}	5.77	5771.56 ± 507.92	0.98	2728.76 ± 289.88
6-OHDA	1.05×10^{-6}	5.98	7503.82 ± 482.60[a]	1.01	3309.17 ± 322.26
6-OHDA + Varenicline	1.02×10^{-6}	6.07	3051.44 ± 174.40[b]	1.49	2076.26 ± 196.38[c]

pD2 for carbachol denotes –log EC50, and Emax is the tissue's maximum contractile response. The milligram maximal tension developed in response to a single depolarizing concentration (120 mmol/l) of KCl is defined as KCl-induced contraction. [a]P = 0.0096 (6-OHDA vs Control), [b]P = 0.001 (Varenicline vs 6-OHDA), [c]P = 0.0028 (Varenicline vs 6-OHDA). Values are mean ± SEM (n = 6 - 10 rats/group).

The hill slope for control and 6-OHDA are close to 1.0. However, in varenicline, the hill slope is above 1.0 (1.49) indicating the positive cooperativity of varenicline to its binding site.

4.2. Relaxant Responses

In carbachol precontracted detrusor strips, significance between -group differences were obtained for oxybutynin at 3×10^{-7} M ($F_{2,21} = 4.053$; P = 0.032), 10^{-6} M ($F_{2,21} = 3.827$; P = 0.038), 3×10^{-6} M ($F_{2,21} = 6.058$; P = 0.008), 10^{-5} M ($F_{2,21} = 10.577$; P = 0.001) and 3×10^{-5} M ($F_{2,21} = 12.159$; P = 0.001). The relaxant effect of oxybutynin was significantly reduced by 6-OHDA. On the other hand, the relaxant responses at 10^{-7} M (P = 0.052), 3×10^{-7} M (P = 0.046), 10^{-6} M (P = 0.036), 3×10^{-6} M (P = 0.011), 10^{-5} (P = 0.000) and 3×10^{-5} M (P = 0.000) were found to be reduced in 6-OHDA compared to control. Moreover, in varenicline group, relaxant effects of oxybutynin at doses of 3×10^{-6} M (P = 0.029), 10^{-5} (P = 0.031) and 3×10^{-5} M (P = 0.023) were significantly higher compared to 6-OHDA (Figure 3).

There were significant group differences in amplitude of phasic contractions. Before oxybutynin ($F_{2,24} = 9.39$; P = 0.001), after oxybutynin ($F_{2,24} = 3.762$; P = 0.038), and percent decrease in amplitude ($F_{2,24} = 3.910$; P = 0.034) were significant. The amplitude of phasic contractions (Figure 4) before oxybutynin in 6-OHDA group were significantly higher than control (P = 0.008) and Varenicline (P = 0.001) groups. Table 2 shows that oxybutynin reduced the amplitude significantly (P < 0.001). However, the effect of oxybutynin was higher than 6-OHDA compared to control and varenicline. On the other hand, oxybutynin also increases the frequency in all groups, but the effect was significant (P < 0.001) only in 6-OHDA group. In terms of frequency, no significance was found between groups.

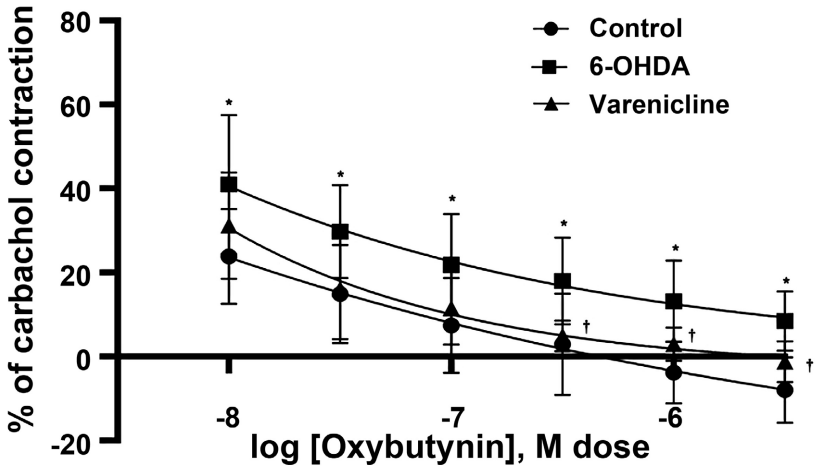

Figure 3. Oxybutynin-induced cumulative relaxant responses of detrusor strips obtained from 3 experimental groups (n = 8 - 10 rat/each group), that were precontracted with submaximal carbachol. Values represent means ± SEM. *P ≤ 0.05, vs Control. †P < 0.05, Varenicline vs 6-OHDA.

Table 2. Comparison of detrusor muscle contractions in terms of amplitude and frequency.

Experimental Groups	Amplitude (mg)			Frequency (Hz)	
	Before Oxy	After Oxy	%Decrease	Before Oxy	After Oxy
Saline (Control)	3654.88 ± 291.53	1257.53 ± 110.17*	63.79 ± 4.24	1.83 ± 0.27	2.22 ± 0.28
6-OHDA + Saline	6218.78 ± 887.57[a]	1011.87 ± 70.32*	82.16 ± 1.84[a]	1.76 ± 0.30	2.81 ± 0.27*
6-OHDA + Varenicline	3029.62 ± 392.11[b]	873.7 ± 129.69*	65.29 ± 8.27	1.86 ± 0.27	2.24 ± 0.35

Oxy: oxybutynin, 6-OHDA: 6-hydroxydopamine. [a]P < 0.05 vs control, [b]P < 0.05 vs 6-OHDA. *P < 0.001 after vs before oxy. Amplitude: mg contractions at the peak; Frequency: Hz contractions per minute. n = 8 - 10 rats/each group.

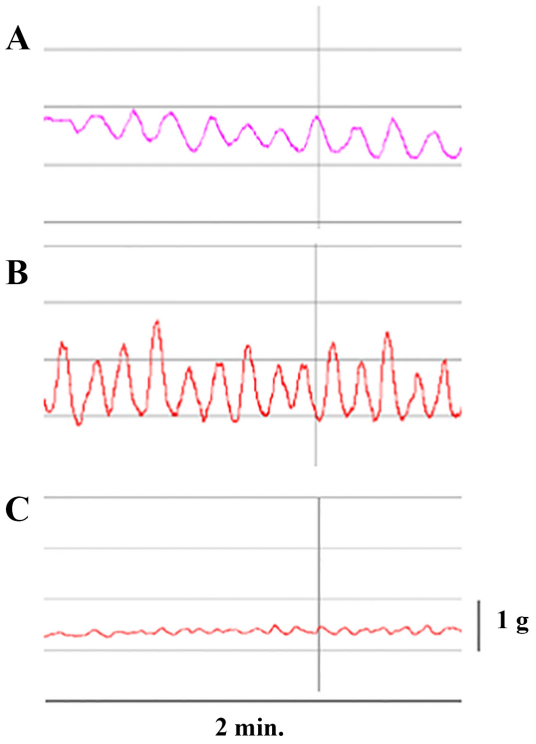

Figure 4. Phasic contractions of detrusor muscle: A: Control, B: 6-OHDA, C: Varenicline.

5. Discussion

Varenicline, which has both agonistic ($\alpha 7$) and partial agonistic ($\alpha 4\beta 2$ and $\alpha 3$) effects on different subtypes of nAChRs, may have both peripheral and central effects on carbachol-induced contractions on detrusor muscle strips. In this study, rats with Parkinson's disease were given varenicline i.p. for two weeks. Varenicline is actively transferred from the bloodstream to the brain in rats, according to microdialysis studies [17].

Overactive bladder, which is one of the common non-motor symptoms of PD, negatively affects the quality of the patient's life. The causes and treatments for this condition are still controversial. Animal models of PD induced by intranigral injection of 6-OHDA shows similar bladder overactivity as seen in PD patients [18]. This study shows that 6-OHDA increased the contractile responses of detrusor muscle similar to the DO that is clinically observed in PD. Interes-

tingly, our data showed that varenicline decreased the 6-OHDA-induced contractile responses.

The most common muscarinic receptor subtype that mediates detrusor contraction in response to muscarinic agonists is M3 [11], whereas the M2 receptor is not directly involved in normal bladder contraction [19]. Carbachol is a well-known muscarinic agonist and has nicotinic activity particularly in autonomic ganglia. Moreover, carbachol acts via G-protein coupled receptors (GPCR) particularly the Gq leading to generation of inositol 1,4,5-trisphosphate (IP3), diacylglycerol, and GTPase, RhoA, Rhokinase C and protein kinase C [20]. In the bladder carbachol increases IP3 levels and inhibits cAMP formation [11].

It is noteworthy to mention that carbachol produced more contractility than KCL in this study. This effect is expected because carbachol effect is via G-proteins, and the GPCR agonists are known to induce higher contractility than KCl. KCL-induced contractions are via increasing calcium (Ca^{2+}) influx through voltage-operated Ca^{2+} channels, and consequently activation of Ca^{2+} dependent myosin light chain (MLC) kinase, and increase in MLC phosphorylation [20]. In our study, carbachol and KCl responses were increased in the 6-OHDA group, whereas this effect was decreased by varenicline. However, the effect of varenicline was more evident on carbachol contractility than KCL. Varenicline may probably have an effect on action on enzymes in the phosphatidylinositol signaling pathway.

Both cholinergic, muscarinic and nAChRs receptors are involved in bladder detrusor activity. nAChRs regulate bladder function and are involved in both bladder contraction and relaxation. $\alpha 7$ nAChR is more likely involved in the regulation of detrusor functions [12]. $\alpha 7$ nAChRs could reduce bladder contractility by reducing ATP, increasing nitric oxide synthase and by an undefined urothelium derived inhibiting factor [14] [21] [22]. Accordingly, these $\alpha 7$ nAChR mediated activities could be involved in the effect of varenicline in 6-OHDA induced DO.

Although phasic contractions were observed in all three groups in this study, the amplitudes of these contractions were significantly increased in 6-OHDA group. Varenicline reduced the amplitudes in 6-OHDA group. After oxybutynin, there was no difference among all three groups. Interestingly oxybutynin increased the frequency in all groups but the effect was significantly higher only in 6-OHDA group.

An altered dopamine basal ganglia-frontal circuit is involved in the brain pathology that causes bladder dysfunction, most commonly evident as overactivity [1]. Neuronal nAChRs subtypes are broadly distributed in the brain [23]. The most common subunits are $\alpha 4$, $\alpha 7$, $\beta 2$, and 90% of them are in the $\alpha 4\beta 2$ form [24]. SNc neuronal firing and striatal dopamine activates dopamine-GABAergic direct pathway, inhibits the basal ganglia output nuclei, and also inhibit the micturition reflex. In Parkinson's disease because of this pathway patients may result in DO and increased urinary urgency/frequency [1] [25]. Varenicline may affect

DO via both its $\alpha 7$ full agonistic and $\alpha 4\beta 2$ partial agonistic effect.

6. Conclusion

Pharmacological agents that could selectively activate urothelial $\alpha 7$ receptors may have beneficial effects against DO in PD. Also, pharmacological agents with $\alpha 4\beta 2$ nAChR partial agonistic properties can be effective in neurodegenerative diseases such as PD with a central effect on non-motor symptoms. Results of the present study revealed that varenicline is effective in treating PD related phasic contractions with increased amplitude. Further studies are required to elaborate the molecular mechanisms involved in the therapeutic effect of varenicline in the treatment of DO.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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