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Differential DAergic Control of D1 and D2 Receptor Agonist Over Locomotor Activity and GABA Level in the Striatum

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The basal ganglia, a group of nuclei, are associated with a variety of functions, including motor control. The striatum, which is the major input station of the basal ganglia in the brain, is regulated in part by dopaminergic input from the substantia nigra. The striatum is made up 96% of medium spiny neurons which are GABAergic cells. GABAergic cells are known to contain DA receptors which divide into two main branches- the D1 receptor (D1R)-expressing direct pathway and the D2 receptor (D2R)-expressing indirect pathway. The role of these two efferent pathways has not been clear in control of motor behaviors. To establish the influence of the different DA subtypes on GABAergic systems in the striatum, D1 selective receptor agonist (SKF 38393) and D2 selective receptor agonist (Quinpirole) were administered to mice. SKF 38393 and quinpirole were administered intraperitoneally in a volume of 0, 1, 5, 10 (mg/kg) and motor activity was assessed for 60 min immediately after the injection of DA agonists. Mice were sacrificed after behavioral test and the striatum in the brain were dissected for analysis of GABA level with HPLC. Both SKF 38393 and quinpirole dose-dependently increased locomotor activity but, GABA level in the striatum was clearly different in two agonists. These findings provide insight into the selective contributions of the direct and indirect pathways to striatal GABAergic motor behaviors.

Key words: basal ganglia, striatum, D1 & D2 agonist, locomotor, GABA

INTRODUCTION

The basal ganglia, a group of nuclei, are associated with a variety of functions, including movement, reward, and motivational processes (Percheron et al., 1994; Mink, 1996; DeLong and Wichmann, 2007). The striatum, which is the major input station of the basal ganglia in the brain, is regulated in part by dopaminergic input from the substantia nigra and projects to the output nuclei of the basal ganglia via two pathways that work together to modulate behavior (Mink, 1996; Hikosaka et al., 2000). The striatum is made up 96% of medium spiny neurons which are GABAergic cells (Yelnik et al., 1991). The GABAergic cells have DA receptors which divide into two main branches- the D1 receptor (D1R)-expressing direct pathway and the D2 receptor (D2R)-expressing indirect pathway (Bateup et al., 2010). The direct pathway projects to the substantia nigra pars reticulata (SNpr) and the indirect pathway projects to the internal globus pallidus (GPe) and the STN (Gerfen et al., 1990). Patients with Parkinson's disease (PD) which is severe movement disorder show the loss of striatal dopamine and rats with lesions of the nigrostriatal dopamine pathway caused by 6-hydroxydopamine (6-OHDA) serve as a

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model of PD (Steg and Johnels, 1993; Redgrave et al., 2010). Thus, the nigrostriatal dopamine pathway in the basal ganglia is important for motor control and classical models of proposed two efferent pathways have not been clear in part of GABAergic striatal motor behavior. Here, we have explored whether GABAergic cells in the striatum are affected by specific excitation of D1 and D2 dopamine receptor subtypes and modulate locomotor activity.

MATERIALS AND METHODS

Animals

Male C57BL/6J male mice 7 weeks of age were obtained from the Orient Co. (Kyungki-Do, Korea), and they were housed for 1 week under a 12 : 12 h light-dark cycle in a temperature-controlled and humidity-controlled room. All animal care and testing conditions were in accordance with the IACUC (Institutional Animal Care and Use Committee) in College of Medicine, the Catholic University of Korea.

SKF 38393 and quinpirole treatment

Hydrochloride salts of SKF 38393 and quinpirole (LY 171555) were obtained from Sigma Aldrich (St. Louis, MO, USA). All drug doses were calculated as the free base and the drugs were dissolved in 0.9% saline vehicle. All the mice were received intraperitoneally.

Behavioral measurements

The locomotor activity was measured in a rectangular container (40×40×45 cm) that was equipped with a video camera above the centre of the floor as described previously (Chae et al., 2004). The walls and floor were made of clear plastic and they were painted with white. The locomotor activity was monitored by a video-tracking system using the SMART program (PanLab, Barcelona, Spain). Mice were allowed to adapt themselves for 1 h in the container and the distance they travelled was recorded every 10 min throughout a 1-h baseline and for 1 h after treatment. Locomotor activity was measured in cm.

Sample collections and GABA measurements

At the end of behavioral measurements, the striatums were dissected from the mice and all samples were frozen at –70 °C. GABA analysis was done using high performance liquid chromatography (HPLC). The mobile phase consisted of 40% acetonitrile in 20 mM sodium acetate buffer (pH 4.5 with glacial acetic acid) with a flow rate of 0.7 mL/min. Samples underwent pre-column derivatization with o-phthaldialdehyde (OPT), and were detected with a fluorescence detector (excitation 350 nm, emission 450 nm). Area was used to calculate results from a standard curve, with an internal standard (commercial γ-aminobutyric acid).

Statistical analysis

The experimental results are expressed as means±s.e. The behavioral data were analyzed using the SPSS program (Version 13.0). Statistical differences among groups were analyzed by one-way analysis of variance followed by the Tukey's post-hoc and LSD technique. $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The present study explored whether locomotor activity and GABAergic cells in the striatum are affected by specific excitation of dopamine D1 and D2 receptor subtypes.

There was a dose-dependently (0, 1, 5 or 10 mg/kg) significant increase in the locomotor activity of the mice after treatment with SKF 38393 or quinpirole as seen in Fig. 1. Significant increases in activity were observed 10 min after injection of SKF 38393. The higher dose (SKF 38393 10 mg/kg) significantly increased activity at every time-point sampled over the 1hr test period [$F(3,19)=18.307$, $p<0.001$]. SKF 38393 is a synthetic compound

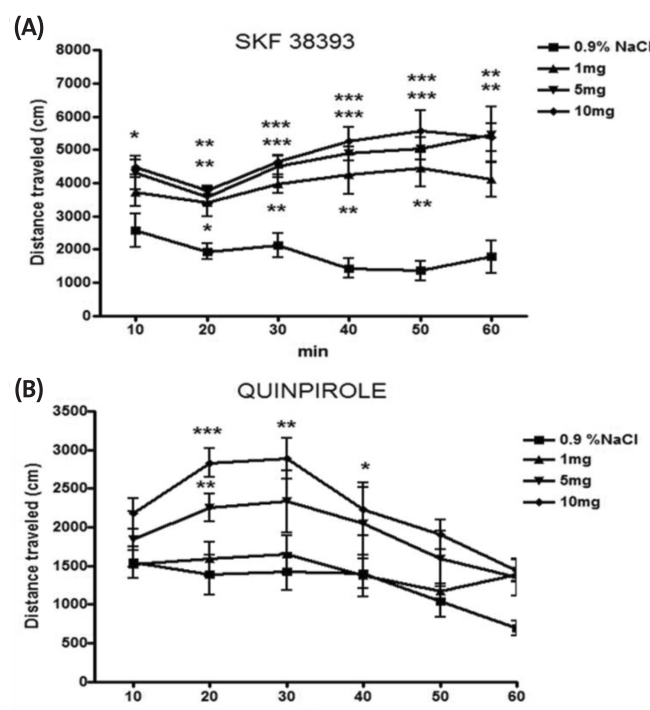

Fig. 1. Time-course of the effects of (A) the dopamine D1 receptor agonist SKF 38393 in a dose of 0, 1, 5, 10 mg/kg, (B) the dopamine D2 receptor agonist quinpirole in a dose of 0, 1, 5, 10 mg/kg measuring throughout 1 hr after treatment. The data are expressed as means (±S.E.M.). All data were analyzed statistical significance ($p<0.05$) by Tukey's test. *$p<0.05$, ** $p<0.01$, ***$p<0.001$ compared to vehicle group.

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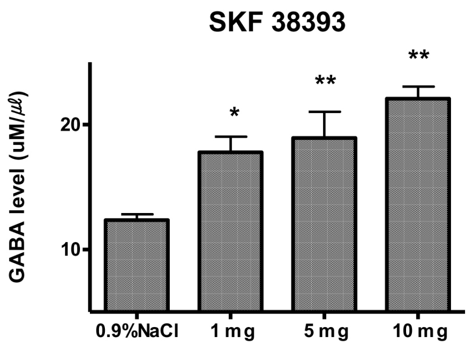

Fig. 2. GABA levels obtained from the striatum of the dopamine D1 receptor agonist SKF 38393 in a dose of 0, 1, 5, 10 mg/kg. The data are expressed as means (±S.E.M.). All data were analyzed statistical significance ($p<0.05$) by Tukey's test. *$p<0.05$, **$p<0.01$ compared to vehicle group.

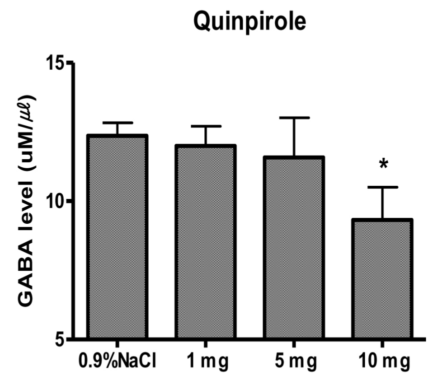

Fig. 3. GABA levels obtained from the striatum of the dopamine D2 receptor agonist quinpirole in a dose of 0, 1, 5, 10 mg/kg. The data are expressed as means (±S.E.M.). All data were analyzed statistical significance ($p<0.05$) by LSD test. *$p<0.05$ compared to vehicle group.

which acts as a selective D1/D5 receptor partial agonist. Most of previous studies have reported that dopamine D1 receptor agonists are stimulating locomotor activity (Bruhwyler et al., 1991; Mazurski and Beninger, 1991). We observed a significant increase in locomotor activity of the mice injected with the dopamine D1 receptor agonist,SKF 38393, compared to control injected with vehicle. The results confirm previous studies indicating that D1 agonist predominantly increase locomotor activity (Molloy and Waddington, 1985 and 1987; Mazurski and Beninger, 1991). Also, dopamine D2 receptor agonist, quinpirole, significantly increases locomotor activity at 20 min after injecting quinpirole in dose dependent manner [$F(3,17)=14.48$, $p<0.001$]. But, the role of dopamine D2 receptors in the locomotor activity of adult animals is ambiguous. For example, administration of quinpirole led to dose-dependent (0.05~1.0 mg/kg) increase of locomotion consistent with the present result (Brown et al., 2002; Stuchik et al., 2007). However, in other studies, the effects of quinpirole in rodents have been shown to depend on dose and time after injection. At lower doses (0.1 mg/kg), decreases in activity are observed (Horvitz et al., 2001; Schindler et al., 2002) and at higher doses (0.5-10 mg/kg), increases in activity can be seen at 60 min after injection (Horvitz et al., 2001). The doses in the current study were in high range and activity was measured immediately following injection. Although biphasic pattern of quinpirole on locomotor inhibition followed by excitation is not unclear, the results confirm that higher doses of D2 receptor agonist increase locomotor activity.

GABA levels in the striatum were clearly different in two agonists. GABA level induced by SKF 38393 was increased in the striatum [$F(3,16)=11.827$, $p<0.001$] (Fig. 2) and GABA level induced by quinpirole was decreased [$F(3,16)=2.05$, $p=0.15$] (Fig. 3). Thus, dopamine D1 receptor agonist appeared to exert increase in GABA release and dopamine D2 receptor agonist appeared to inhibit GABA release in the striatum. The study of Harsing and Zigmond (1997) demonstrated that the overflow of GABA evoked by electrical field stimulation (8 Hz) was increased two-fold by SKF-38393 (10 microM), and electrically evoked GABA overflow was reduced 50% by quinpirole (10 microM) in the striatum. Thus, our results support previous studies indicating that dopamine agonist can exert an excitatory influence on GABA release via D1 receptor and an inhibitory influence on GABA release via D2 receptor within the striatum. The striatum, which is the major input station of the basal ganglia in the brain, is regulated in part by dopaminergic input from the substantia nigra. The striatum is made up 96% of medium spiny neurons which are GABAergic cells (Yelnik et al., 1991). Reduced dopamine innervation of the striatum results in hypokinesia and difficulty in initiating different motor patterns and enhanced striatal dopamine activity give rise to hyperkinesia (Blair, 2003; Grillner et al., 2005). These results provide inkling that the striatum has a important role in the selection of motor behavior (Grillner et al., 2005). The GABAergic cells have DA receptors which divide into two main branches- the D1 receptor (D1R)-expressing direct pathway and the D2 receptor (D2R)-expressing indirect pathway (Bateup et al., 2010). The direct pathway projects to the substantia nigra pars reticulata (SNpr) and the indirect pathway projects to the internal globus pallidus (GPe) and the STN (Gerfen et al., 1990). The striatal medium spiny output neurons are inhibitory (GABAergic). Striatal neurons are activated by dopaminergic innervations via D1 type receptors, it provides strong inhibition to the SNpr and thereby disinhibits thalamic neurons in a motor center which are responsible for releasing locomotor. In the indirect pathway from the striatum via the GPe and the STN, striatal neurons activated by dopaminergic innervations via D2 type receptors inhibit thalamus' neurons in a motor center that affect motor behavior (DeLong and Wichmann, 2007). Actually, most of previous experiments have reported that dopamine D1 receptor agonists are stimulating locomotor activity (Bruhwyler et al., 1991; Mazurski and Beninger, 1991), however, the role of dopamine D2 receptors in the locomotor activity is ambiguous (Horvitz et al., 2001; Brown et al., 2002; Schindler et al., 2002; Stuchik et al., 2007). Thus, this behavioral hypothesis is needed to be further investigated. Parkinsonism resulted from the loss of nigrostriatal dopamine is a movement disorder characterized by tremor, hypokinesia, rigidity, and postural instability (Gerfen et al., 1990; DeLong and Wichmann, 2007). The study on PD has been greatly facilitated in the 1-methyl4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) primate model of the disease by using metabolic imaging and electrophysiological studies. The MPTP model has suggested that neuronal discharge is increased in the STN, GPi, and SNr but decreased in the GPe, thus resulting in excessive inhibition of components of the motor circuit in the thalamus, cortex, and brainstem. These aspects of the model are generally supported by lesioning and inactivation studies, which have shown that inactivation of the STN or GPi increases the metabolic activity in cortical motor areas and improves bradykinesia and tremor in patients with PD. However, lesions of the thalamus do not lead to significant bradykinesia or akinesia, and lesions of the GPi do not result in dyskinesias (DeLong and Wichmann, 2007). Although this issue is complex and still unsettled, nigrostriatal GABAergic pathway in the basal ganglia is important for motor control. Both SKF 38393 and quinpirole dose-dependently increased locomotor activity but, GABA level in the striatum was clearly different in two agonists. Our results provide insight into the selective control of the direct and indirect pathways to striatal GABAergic motor behaviors. Specific GABAergic motor control of the direct and indirect pathways in the basal ganglia requires further investigations which will contribute to therapeutic understanding of motor function disorders.

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