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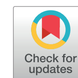
Effect of ghrelin on the motor deficit caused by the ablation of nigrostriatal dopaminergic cells or the inhibition of striatal dopamine receptors



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ABSTRACT

Ghrelin plays roles in a wide range of central functions by activating the growth hormone secretagogue receptor (GHSR). This receptor has recently been found in the substantia nigra (SN) to control dopamine (DA)-related physiological functions. The dysregulation of DA neurons in the SN pars compacta (SNc) and the consequent depletion of striatal DA are known to underlie the motor deficits observed in Parkinson's disease (PD). In the present study, we further investigated the role of the SN-ghrelin system in motor function under the stereotaxic injection of AAV-CMV-FLEX-diphtheria toxin A (DTA) into the SN of dopamine transporter (DAT)-Cre (DAT^{SN}::DTA) mice to expunge DA neurons of the SNc. First, we confirmed the dominant expression of GHSR1a, which is a functional GHSR, in tyrosine hydroxylase (TH)-positive DA neurons in the SNc of control mice. In DAT^{SN}::DTA mice, we clearly observed motor dysfunction using several behavioral tests. An immunohistochemical study revealed a dramatic loss of TH-positive DA neurons in the SNc and DAT-labeled axon terminals in the striatum, and an absence of mRNAs for TH and DAT in the SN of DAT^{SN}::DTA mice. The mRNA level of GHSR1a was drastically decreased in the SN of these mice. In normal mice, we also found the mRNA expression of GHSR1a within GABAergic neurons in the SN pars reticulata (SNr). Under these conditions, a single injection of ghrelin into the SN failed to improve the motor deficits caused by ablation of the nigrostriatal DA network using DAT^{SN}::DTA mice, whereas intra-SN injection of ghrelin suppressed the motor dysfunction caused by the administration of haloperidol, which is associated with the transient inhibition of DA transmission. These findings suggest that phasic activation of the SNc-ghrelin system could improve the dysregulation of nigrostriatal DA transmission related to the initial stage of PD, but not the motor deficits under the depletion of nigrostriatal DA. Although GHSRs are found in non-DA cells of the SNr, GHSRs on DA neurons in the SNc may play a crucial role in motor function.

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1. Introduction

Ghrelin is synthesized and acylated in the stomach by ghrelin O-acyltransferase (GOAT), which is required for activation of the growth hormone secretagogue receptor (GHSR) [1,2]. The GHSR is located in both the central nervous system and the periphery [3–6]. Central GHSRs play roles in a wide range of physiological functions including food intake, rewarding effect and memory performance [3,4,7,8]. In particular, in the substantia nigra pars compacta (SNc),

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ghrelin could activate dopamine (DA) neurons and increase the DA concentration in the striatum via the specific blockade of KCNQ channel function [9]. Furthermore, ghrelin is considered to act on SNc neurons to increase the concentration of tyrosine hydroxylase (TH) in the midbrain as well as DA turnover in the dorsal striatum [5,10]. In addition, it has been reported that ghrelin provides neuroprotective effects in DA neurons [5,10,11].

Progressive degeneration of DA neurons in the SNc and the resultant depletion of DA from the striatum underlie the motor behavior deficits observed in Parkinson's disease (PD) patients and in animals that have sustained damage to the nigrostriatal pathway [12]. In our recent studies, we found that the expression level of endogenous ghrelin receptors was dramatically decreased in DA neuron-differentiated from PD-specific iPSCs (Suda et al., Molecular Brain, in press). Furthermore, the inhibition of GHSRs in DA neurons induced the initial dysfunction of DA neurons, leading to extrapyramidal disorder under PD (Suda et al., Molecular Brain, in press).

In this study, we therefore examined whether phasic activation of the SN-ghrelin system could improve the motor deficits. For this purpose, we performed the stereotaxic injection of AAV-CMV-FLEX-diphtheria toxin A (DTA) into the SN of dopamine transporter (DAT)-Cre (DAT^{SN}::DTA) mice using a Flex-switch system to expunge DA neurons of the SNc. Furthermore, we used the mice treated with a classic D_2 receptor antagonist haloperidol as an initial PD model with the temporal dysregulation of DA transmission. We investigated the effect of phasic activation of the endogenous ghrelin system in the SN by microinjection of ghrelin into the SN on the recovery from the motor deficits under the conditions with the ablation of the nigrostriatal DA network using DAT^{SN}::DTA mice and the haloperidol-induced transient reduction in DA transmission.

2. Material and methods

2.1. Animals

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, as adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Animal Research Committee of Hoshi University. Male B6. SJL-*Slc6a3*$^{tm1.1(cre)Bkmm}$/J (DAT-Cre) mice (8–10 weeks old, Jackson Laboratory, Bar Harbor, ME, USA) and C57BL/6J mice (8–10 weeks old, Jackson Laboratory), weighing 20–23 g were used in this study. All mice were housed at up to 6 mice per cage and kept in a temperature-controlled room (24 ± 1 °C) maintained on a 12 h light-dark cycle (light on at 8 a.m.). Food and water were available *ad libitum*.

2.2. Drugs

Ghrelin (Peptide Institute, Inc., Osaka, Japan) and haloperidol (Serenace, Dainippon Pharmaceutical. Co., Ltd) were used in this study.

2.3. Stereotaxic intranigral virus injection

Stereotaxic injections were performed under isoflurane (3%) anesthesia using small-animal stereotaxic instruments (RWD Life Science, San Diego, CA, USA). Virus (AAV-CMV-FLEX-DTA) was bilaterally injected into the SN (from bregma: AP -3.0 mm, ML ±1.2 mm, DV -4.3 mm), as previously described [13] at a rate of 0.25 μL/min for 4 min.

2.4. Cannula implantation into the SN and microinjection

For cannula implantation into the SN of DAT^{SN}::DTA or C57BL/6J mice for ghrelin treatment, mice were placed in a stereotaxic apparatus and the skull was exposed the day before bilateral microinjection. A small hole was then made in the skull using a dental drill. A guide cannula (EIM-54; Eicom Co., Kyoto, Japan) was implanted into both sides of the SN (from bregma: AP -3.0 mm, ML ±1.2 mm, DV -4.3 mm). The bilateral injection was performed with two sets of a glass micropipette and an air pressure injector system (Micro-syringe Pump-Model ESP-32; Eicom Co.) at a rate of 0.25 μL/min for 4 min and was started at the same time.

2.5. Balance beam test

The apparatus consisted of a 1m-long bar (28 or 11 mm in diameter) with a black escape box on one end (O'HARA & Co., LTD., Tokyo, Japan). Following habituation trials, the mice were acclimated to enter the escape box on the 28 mm-diameter bar for 2 days before testing. The latency to reach the box on the 11 mm-diameter bar was then measured (cut off time = 60s). The test was performed 2–10 days after the injection of AAV-CMV-FLEX-DTA in DA neurons of the SN.

2.6. Rotarod test

Ten days after bilateral virus (AAV-CMV-FLEX-DTA) injection into the SN of DAT-Cre mice, rotarod test (4 rpm for 120 s maximum) was performed. The test was performed 30 min after bilateral intra-SN injection of ghrelin (1 nmol/site) in DAT^{SN}::DTA mice. In another test condition, mice treated with haloperidol were individually placed on a slowly rotating rod (4 rpm/min), and subjected to continuous acceleration at 20 rpm/min; the time at which the mouse fell off the rod was recorded (for 128 s maximum). Mice were treated with haloperidol (0.5 mg/kg, s.c.) or saline given 30 min before ghrelin treatment. The test was performed 30 min after bilateral intra-SN injection of either saline vehicle or ghrelin (1 nmol/site, 0.25 μl min^{-1} for 4 min) using the auto-injector connected with guide cannula. Time spent on the rod was measured.

2.7. Horizontal bar test for the evaluation of catalepsy

Catalepsy was evaluated using the horizontal bar test as described previously [14]. Briefly, animals were placed so that both forepaws were over a horizontal bar 5 cm above the floor, and the amount of time (s) the animal maintained this position was recorded for up to 60 s. Catalepsy was considered to have finished when a forepaw touched the floor or when the mouse climbed on the bar. Mice were treated with haloperidol (0.5 mg/kg, s.c.) or saline given 30 min before ghrelin treatment. The test was performed 30 min after bilateral intra-SN injection of either saline vehicle or ghrelin (1 nmol/site, 0.25 μl min^{-1} for 4 min) using guide cannula.

2.8. Immunohistochemistry

Mice were deeply anaesthetized with 3% isoflurane and intracardially perfusion-fixed with freshly prepared 4% paraformaldehyde. Coronal brain sections (8 μm) were incubated in blocking solution for 1 h at room temperature, and then incubated for 48 h at 4 °C with primary antibodies; anti-TH (ImmunoStar, WI, USA) or anti-DAT (Millipore, MA, USA). The antibody was then rinsed and incubated with an appropriate secondary antibody for 2 h at room temperature. Fluorescence of immunolabelling was detected using a light microscope (BX-61; Olympus, Tokyo, Japan)

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