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Intranasal administration of rotenone in mice attenuated olfactory functions through the lesion of dopaminergic neurons in the olfactory bulb

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ABSTRACT

Many environmental chemicals are thought to affect brain function. It was reported that chemicals in the nasal cavity directly reach the brain through the connection between olfactory neurons and the olfactory bulb (OB). In this 'olfactory transport,' xenobiotics absorbed at the nasal mucosa reach the brain by bypassing some physical barriers and defenses, and thus olfactory transport is suspected to be a vulnerable mechanism of the brain against invasion threats of environmental chemicals. In this study, we focused on the neuronal toxicity of rotenone administered intranasally to mice. The results showed that the mice that were administered rotenone had attenuated olfactory functions. We also found that intranasally administered rotenone induced acute mitochondrial stress at the OB. The repeated administration of rotenone resulted in a decrease in the number of dopaminergic neurons, which are inhibitory interneurons in the OB. Taken together, our findings suggest that the inhalation of environmental toxins induces the neurodegeneration of cranial neurons through olfactory transport, and that olfactory dysfunction may be induced as an earliest symptom of neurodegeneration caused by inhaled neurotoxins.

1. Introduction

We are surrounded by numerous environmental chemicals that may be toxic to neurons. It has been shown that exposure to environmental chemicals can alter brain function through developmental retardation or neurodegeneration (Cannon and Greenamyre, 2011; Giordano and Costa, 2012). Many environmental neurotoxins were identified and environmental chemical-induced neurodegeneration models were established in research in which animals were exposed to suspected environmental chemicals (Binukumar et al., 2010). Neurodegenerative diseases that stem from purely genetic causes are rare (Bonifati et al., 2004; Zawia et al., 2009), and the precise causes of most sporadic neurodegenerative diseases are still unclear.

In the current toxicological research on neurodegeneration and in the design of animal models of neurodegenerative disease using neurotoxins, the chemicals are administered directly into the bodies of animals. However, the human body is usually exposed to environmental chemicals via three routes: ingestion, cutaneous contact, and inhalation. In addition, a previously unidentified and vulnerable route by which chemicals can reach the human brain has been reported. In this route, called 'olfactory transport,' chemicals in the nasal cavity reach the brain directly through passive diffusion from the extra-neural space to the cerebrospinal fluid and/or by the active axonal transport of olfactory neurons (Dhuria et al., 2010; Genter et al., 2009; Lucchini et al., 2012). The chemicals reach the brain via olfactory transport without being subjected to the body's detoxifying metabolism or blocking by the blood–brain barrier. Olfactory transport is thus thought to present an additional risk to the brain from environmental chemicals that are inhaled.

The olfactory information emitted from the olfactory neurons by odorants is transmitted to mitral cells in the olfactory bulb (OB), which is the primary center for olfactory information processing. In the OB, a large population of GABAergic inhibitory interneurons that are located in the glomerular and granule cell layers plays an important role in the initial processing of olfactory information (Mori et al., 1999; Parrish-Aungst et al., 2007). In addition, approx. 10–16% of all GABAergic neurons in the glomerular cell layer were reported to produce and release dopamine as well (De Marchis et al., 2007; Panzanelli et al., 2007; Parrish-Aungst et al., 2007).

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These cells function as periglomerular interneurons that form synaptic connections in the glomerulus with the olfactory neurons and the mitral cells. The dopaminergic interneurons are thought to be key elements in the control of odor detection, discrimination and learning (Cave and Baker, 2009).

During the progression of Parkinson's disease (PD), which is characterized by a loss of dopaminergic neurons at the substantia nigra, most patients suffer dysosmia as one of the earliest symptoms, followed by other non-motor symptoms and motor symptoms. Because dopaminergic neurons in the OB are related to the processing of olfactory information, the aberrant functioning of dopaminergic neurons in the OB is a possible cause of olfactory dysfunction in early PD. Prediger et al. (2012) hypothesized that the olfactory transport of environmental neurotoxins induces olfactory dysfunction by the lesion of dopaminergic neurons in the OB at the earliest stage of PD, and then this effect spreads out to other brain regions such as the substantia nigra. However, the relation between neurodegenerative disease and the olfactory transport of environmental neurotoxins has not been clarified. If the influence of environmental neurotoxins via olfactory transport in a short period is detected in the OB, this would indicate that the brain itself is facing a crisis of neurodegeneration through the inhalation exposure to the neurotoxins.

The survivability of dopaminergic neurons was shown to be susceptible to mitochondrial dysfunction (Anantharam et al., 2007; Ashrafi et al., 2014; Radad et al., 2006). Mitochondrial dysfunction leads to increased intracellular reactive oxygen species (ROS), which induces oxidative damage to the cells (Henchcliffe and Beal, 2008; Venditti et al., 2013). The ROS are also generated by dopamine metabolism in dopaminergic neurons. It was thus suggested that the increased ROS are related to the vulnerability of dopaminergic neurons (Segura-Aguilar et al., 2014).

Rotenone is an inhibitor of mitochondrial respiration chain complex-I (Marella et al., 2008; Talpade et al., 2000). During the complex-I inhibition by rotenone, an incomplete chain reaction of electron transport at the mitochondria generates an excess amount of intracellular ROS (Testa et al., 2005). Thus, dopaminergic neurons show high sensitivity to rotenone-induced cytotoxicity mediated by ROS. It was reported that the chronic subcutaneous administration of rotenone to rodents reduced the number of dopaminergic neurons in the substantia nigra (Betarbet et al., 2000; Fleming et al., 2004; Greenamyre et al., 2003; Sherer et al., 2003; Zhu et al., 2004), where dopaminergic neurons are particularly sensitive to the mitochondrial ROS in the brain (Damier et al., 1999; Hirsch et al., 1988).

In the present study, to investigate the vulnerability of the brain via olfactory transport, we examined the influence of intranasally administered rotenone on the dopaminergic neurons in the mouse OB. Since the OB is the portal of the brain for both the processing of olfactory information and the olfactory transport of xenobiotics, we also administered an olfactory-related behavior test and conducted an electrophysiological analysis of output neurons in the OB. We investigated the effects of rotenone on dopaminergic neurons in the OB by performing biochemical, immunohistochemical and molecular-biological analyses of tyrosine hydroxylase (TH), a key enzyme in the production of dopamine.

2. Materials and methods

2.1. Ethics statement

All animal experiments were conducted in accordance with the Asahikawa Medical University Guidelines for the Use of Laboratory Animals and were approved by the committee of Asahikawa Medical University for Animal Care and Use (approval ID: 14090).

2.2. Mice

The mice (20- to 25-week-old female BALB/c mice) were housed at room temperature (24–26 °C) under a 12-h light/dark cycle. Rotenone (Sigma–Aldrich, St. Louis, MO) was dissolved in dimethyl sulfoxide (DMSO) to make 0.1 M stock solution. For administration of rotenone to mice, rotenone stock solution was diluted with water. Intranasal administrations of rotenone or vehicle (3.2% DMSO) were performed under light anesthesia by the inhalation of diethyl ether as follows. The mouse was anesthetized and then laid on its back. Next, rotenone diluent (0.35 mg/kg) or vehicle was injected slowly into the right side of the nasal cavity with a micropipette. The dose of rotenone was determined by considering prevention for systemic toxicity of intranasally administered rotenone. These single administrations were given once a day for the indicated periods.

To minimize the influence of rotenone on the olfactory neurons, rotenone was injected into only the right side of the nasal cavity. For the chemical ablation of olfactory neurons, 7 μL of zinc aqueous solution (5% zinc sulfate, 0.1% carboxymethyl cellulose; Nacalai, Kyoto, Japan) was injected into right side of the nasal cavity.

2.3. Avoidance of butyric acid

The odor preferences of the mice were tested in a plexiglas Y-maze apparatus (long-arm length: 45 cm, short-arm length: 40 cm, arm width: 10 cm) as described (Utsugi et al., 2014). A 20-μL aliquot of butyric acid (undiluted or 25% diluent; Wako) or water absorbed in a piece of filter paper (1×1 cm^2; GE Healthcare, Little Chalfont, UK) in a Petri dish was placed at the end of each short arm. Clean air was supplied from the end of each short arm of the maze to the end of the long arm. A mouse was initially placed at the end of the long arm, and the duration that the animal spent in each short arm during a 4-min test period was recorded. The 'preference ratio' was defined as the ratio of the duration spent in the butyric acid arm to the duration in both short arms.

2.4. Electrophysiological analysis of mitral cells

Mice were deeply anesthetized with pentobarbital sodium (150 mg/kg) and processed by cardiac perfusion with ice-cold sucrose-based Ringer's solution (234 mM sucrose, 2.5 mM KCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 0.5 mM $CaCl_2$, 10 mM $MgCl_2$, 11 mM glucose, pH 7.4) oxygenated with 95% O_2 and 5% CO_2 mixed gas. Forebrains were dissected out and cut into parasagittal slices at a thickness of 200 μm with a microslicer (Dosaka EM, Kyoto, Japan). The slices were incubated in normal Ringer's solution (125 mM NaCl, 2.5 mM KCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 11 mM glucose, pH 7.4) saturated with the O_2/CO_2 gas at 37 °C for 30 min and equilibrated in the same solution at room temperature until use.

Electrophysiological experiments were performed at room temperature. Each forebrain slice was placed in a homemade recording chamber filled with normal Ringer's solution saturated with the O_2/CO_2 gas. Individual cells were observed by infrared differential interference contrast video microscopy through an E600FN microscope (Nikon, Tokyo). Glass electrodes were filled with CsCl intracellular solution (115 mM CsCl, 15 mM NaCl, 2 mM $MgCl_2$, 2 mM Na_2ATP, 0.5 mM EGTA/CsOH, 10 mM HEPES, 10 mM tetraethylammonium chloride, pH 7.2/CsOH). The electrode resistance was 5.6 ± 0.1 MΩ ($n = 51$; mean ± SE). The seal resistance achieved with the tips of the glass electrodes on the cell surface was 9.8 ± 0.8 GΩ ($n = 50$). The series resistance that occurred by the formation of a whole-cell patch configuration was 30 ± 1.8 MΩ ($n = 51$).