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The central mechanism of risperidone-induced hyperprolactinemia

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ABSTRACT

Risperidone is known to increase prolactin secretion in treating mental illness patients. This side-effect is thought to be mediated via central signaling pathway. However, the exact pathway involved between risperidone and hyperprolactinemia are still unknown. Therefore, we have treated mice with risperidone and investigated the central mechanisms. The present study showed that in risperidone treated group, the level of the serum prolactin significantly increased, which was consistent with increased positive prolactin staining in pituitary gland. Elevated c-fos expression was observed in the arcuate hypothalamic nucleus (Arc) where we found 65% c-fos positive neurons co-localised with neuropeptide Y (NPY) in mice treated with risperidone. In addition, the results from in situ hybridization showed that the NPY mRNA in the Arc was significantly increased, whereas the tyrosine hydroxylase (TH) mRNA dramatically decreased compared with control group in the paraventricular hypothalamic nucleus (PVN). These findings revealed that risperidone may mediate the transcriptional regulation of Arc NPY and TH in the PVN. Furthermore, risperidone induced a decreased dopamine synthesis in the PVN and thus reduced the dopamine-induced inhibition of prolactin release, ultimately lead to hyperprolactinemia. Therefore, insights into these neuronal mechanisms open up potential new ways to treat schizophrenia patients in order to ameliorate hyperprolactinemia.

1. Introduction

Atypical antipsychotic drugs can be of great benefit in treating a range of mental disorders, including schizophrenia, bipolar disorder and Alzheimer's disease, but most of them are associated with some adverse effects, ranging from obesity (Allison and Casey, 2001; Allison et al., 1999; Wirshing et al., 1999) to decreased bone density and hence leads to osteoporosis (Meaney et al., 2004; Wang et al., 2014). Meanwhile, one of the atypical antipsychotics, risperidone, was found to induce hyperprolactinemia that refers to increased serum prolactin concentrations, which is primarily responsible for the development of galactorrhoea, gynaecomastia, menstrual abnormalities, sexual dysfunction and in the longer-term osteoporosis (Bishop et al., 2012; Cookson et al., 2012). In particularly, one clinical study has reported that hyperprolactinemia occurred in 91% of patients treated with risperidone (Montgomery et al., 2004), by which we can assume that risperidone is strongly associated with abnormal prolactin secretion causing potential adverse effects.

Prolactin is a single-chain polypeptide consisting of 199 amino acids and is secreted by anterior pituitary gland lactotroph cells. Prolactin stimulates mammary glands producing milk. Prolactin is also involved in maternal behaviour and stress-related alterations in the hypothalamic–pituitary–adrenal axis (Slattery and Neumann, 2008). Prolactin has other actions in the central nervous system, including stimulating proliferation of oligodendrocyte precursor cells (Gregg et al., 2007). It is known that hypothalamic dopamine inhibits prolactin secretion by acting on dopamine D2 receptors to cause inhibition of the lactotrophs by increasing potassium conductance and thereby hyperpolarizing the cell membrane (Cookson et al., 2012).

Human body homeostasis is regulated by complex neurological mechanisms that link brain to various organs. Previous studies have demonstrated that hypothalamic areas (Insel et al., 2014; Sharan and Yadav, 2014; Sumiyoshi et al., 2013) produce key neurotransmitters and hormones responsible for neuroendocrine regulation (Karl et al., 2004; Kim et al., 2014; Lin et al., 2009). Risperidone affects hypothalamic regulation causing weight gain, glucose dysregulation and dyslipidemia (Ronsley et al., 2015). Moreover, recent studies have indicated that risperidone treatment decreases bone density by indu-

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cing hyperprolactinemia (Hamner, 2002; Torre and Falorni, 2007). However, we still do not know the underline mechanism of risperidone-induced hyperprolactinemia.

Anatomically, hypothalamus is comprised of different nuclei whose can be activated depending on the type and strength of the stimuli they receive. Hypothalamic Arc, PVN, ventromedial (VMH) and dorsomedial (DMH) hypothalamic nuclei are involved in the regulation of body metabolism. A close interplay between the hypothalamic neural networks and prolactin secretion takes place via three neuronal pathways including an autonomic outflow from the brain, hypothalamic neuroendocrine regulation via pituitary, and secretion of the hypothalamic neuromodulators (Sharan and Yadav, 2014). In particular, there are two major neuronal populations that play an important role in the regulation of energy homeostasis in the Arc. The Arc neurons that co-localised the NPY and agouti-related protein (AgRP) act to increase appetite, whereas the neurons that co-expressed cocaine- and amphetamine-related transcript (CART) and proopiomelanocortin (POMC) decrease appetite (Loh et al., 2015). The neurons from these two populations in the Arc projecting to other hypothalamic areas are closely involved in appetite control, for instance, the Arc NPY acts on Y1 receptors in the PVN and mediates its actions on controlling feeding through the dopamine systems (Shi et al., 2013) and the CART that abundantly exist in the Arc carry out their functions independently aside of the sympathetic systems (Elias et al., 1998). Since atypical antipsychotics, risperidone acts in the NPY system via the 5-hydroxytryptamine (5-HT) receptor in the Arc to up-graduate of producing prolactin in pituitary gland (Cookson et al., 2012; Lian et al., 2015). Thus, in this study we will unravel these actions that occur in the hypothalamus and its central mechanisms by which they affect serum prolactin secretion in order to provide new information to ameliorate hyperprolactinemia side-effects of risperidone.

2. Materials and methods

2.1. Animals

At 10 weeks of age, 24 female mice (C57BL/6J) were divided into two groups: risperidone treatment group (n = 12), control group (n = 12). Six mice of each group were collected serum, brains and pituitary for immunohistochemistry experiments. The remained 12 mice (n = 6 per group) were collected brains for in situ *hybridization*. Six transgenic mice (n = 5) expressing GFP at NPY neurons were purchased from Jackson Laboratory were collected brains for double labelling immunohistochemistry. Five female mice (C57BL/6J) were collected brains for double labelling.

2.2. Ethic and animal care

All animal experimental protocols were approved by the Third Military Medical University Animal Care Committee according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication number 8023). All 34 female mice were used for all experiments and were lodged in conditions of controlled temperatures of 22 °C (room temperature, RT) and illumination (12:12 h light–dark cycle, lights on at 07:00 h). Mice were provided ad libitum access to water and standard chow diet (8% calories from fat, 21% calories from protein, 71% calories from carbohydrate, 2.6 kcal/g; Gordon's Speciality Stock Feeds, Yanderra, NSW, Australia).

2.3. Determination of serum prolactin levels in response to risperidone treatment

At 10 weeks of age, 24 female mice (C57BL/6J) were divided into two groups: risperidone treatment group (n = 12), control group (n = 12), and transferred to individual cage for 3 days for environmental acclimatization. Approximately, at the similar time of each day for 4 weeks, one group of mice was intraperitoneally injected with risperidone (2 mg/kg body weight) and control group was treated with PBS. The drug doses were based on previous rodent studies (Kapur et al., 2003; Mishra and Mohanty, 2010). Because the half-life of risperdone in rodents is about 4–6 times faster than in humans, we have used 2 mg/kg risperdone in the present study to allow clinically comparable doses. After 30 min of treatment, we injected 2% sodium pentobarbital (120 mg/kg body weight) to narcotize mice and collected serum from each group (n = 6 per group) from the left ventricle of heart. The radioimmunoassay prolactin kit (BPE32246m, Hu Shang Biological Technology Co., Ltd., Shanghai, China) was used to measure serum prolactin levels, according to protocol of radioimmunoassay prolactin kit. The remained 12 mice (n = 6 per group) were used for in situ *hybridization*. The levels of prolactin were calculated through excel based on OD which detected by ultraviolet spectrophotometer.

2.4. The expression of prolactin in the pituitary in response to risperidone treatment

Right after serum collecting as described above, the 6 mice of each group were perfused with saline and then 4% paraformaldehyde. Mice brains and pituitary were immediately isolated after dislocation and placed in 4% paraformaldehyde PBS solution for 30 min. The pituitary were embedded in paraffin and cut into 7 μm tissue sections using a microtome. Sections were deparaffinized, rehydrated, and incubated in 3% H_2O_2 for 10 min. Sections were then rinsed in Tris PBS and blocked with 5% normal goat serum in 1 × PBS for 1 h. Pituitary tissue sections were incubated with Anti-Prolactin antibody (1:2500) overnight (ab183967; Abcam Ltd., Cambridge, UK). After 3 times rinsing, slides immersed in a peroxidase conjugated goat anti-rabbit IgG (1:1000) (PV-9001, ZSGB-BIO) for 20 min at room temperature. Sections were washed in Tris-PBS solution and treated with diaminobenzidine for 5 min. After cover slipping, the images of prolactin expression in pituitary gland, a defined area (4 × 4 grid reticule), was acquired under the 100 objective of a Zeiss Axiophot microscope equipped with the ProgRes digital camera (Carl Zeiss Imaging Solutions GmbH, Munich, Germany). Cells with positive staining were counted, and the average of three sections was used as the final count for each mouse.

2.5. Immunoreactivity of c-fos positive neurons in the Arc and p-Erk1/2 in the PVN

The brain of 12 mice (6 mice of each group) obtained along with pituitary was immersed in PBS solution, containing 30% sucrose for overnight. After cutting the brain into coronal sections of 30 μm thickness by microtome (Menzel-Glaser, Braunschweig, Germany), we detected the c-fos and phosphorylated extracellular signal-regulated kinases 1 and 2 (p-Erk1/2) immunoreactivities using immunohistochemistry method described previously (Shi et al., 2013). The primary antibodies of rabbit-anti-mouse c-fos (1:4000, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and rabbit-anti-mouse p-Erk1/2 (1:4000, Cell Signaling Technology Inc., Danvers, MA, USA) were used in PBS containing 0.1% Triton X-100. Brain sections were incubated 3 h with secondary antibodies, goat anti-rabbit (Sigma-Aldrich, St Louis, MO, USA), which diluted at 1:250 in PBS. The c-fos immunoreactivity in the Arc and p-Erk1/2 immunoreactive neurons in the PVN were detected and counted under a Zeiss Axiophot microscope (Carl Zeiss). And the data represented as the average number of stained nuclei within the brain nuclei of interest.

2.6. Double labelling of NPY and c-fos immunoreactivity in the hypothalamic neurons of GFP-NPY mice

Six transgenic mice (n = 5) expressing GFP at NPY neurons were purchased from Jackson Laboratory. GFP-NPY mice were treated with risperidone at 4 mg/kg body weight, *i.p.*, from their age of week 10 for