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Time-dependent effects of olanzapine treatment on the expression of histidine decarboxylase, H1 and H3 receptor in the rat brain: The roles in olanzapine-induced obesity

Meng He[a,b], Qingsheng Zhang[b], Chao Deng[b], Tiantian Jin[b], Xueqin Song[c], Hongqing Wang[b], Xu-Feng Huang[b,c,*]

[a] School of Chemistry, Chemical Engineering and Life Sciences, Wuhan University of Technology, Wuhan, Hubei, China
[b] Illawarra Health and Medical Research Institute and Centre for Translational Neuroscience, School of Medicine, University of Wollongong, NSW 2522, Australia
[c] Department of Psychiatry, The First Affiliated Hospital, Zhengzhou University, Zhengzhou 450052, China

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ABSTRACT

Antipsychotic treatment, particularly olanzapine and clozapine, induces severe obesity. The Histamine H1 receptor is considered to be an important contributor to olanzapine-induced obesity, however how olanzapine modulates the histaminergic system is not sufficiently understood. This study examined the effect of olanzapine on key molecules of the histaminergic system, including histidine decarboxylase (HDC), H1 receptor (H1R) and H3 receptor (H3R), in the brain at different stages of olanzapine-induced obesity. During short-term treatment (8-day), olanzapine increased hypothalamic HDC mRNA expression and H1R binding in the arcuate nucleus (Arc) and ventromedial hypothalamus (VMH), without changing H3R binding density. HDC mRNA and Arc H1R binding were positively correlated with increased food intake, feeding efficiency and weight gain. When the treatment was extended to 16 and 36 days, H1R binding was increased not only in the hypothalamic Arc and VMH but also in the brainstem dorsal vagal complex (DVC). The H1R bindings in the Arc, VMH and DVC were positively correlated with weight gain induced by olanzapine treatment. However, the expression of HDC and H3R mRNA was not increased. These results suggest that olanzapine time-dependently modulates histamine neurotransmission, which suggested the different neuronal mechanisms underlying different stages of weight gain development. Treatment targeting the H1R may be effective for both short- and long-term olanzapine-induced weight gain.

1. Introduction

Weight gain has been identified as one of the major side effects induced by second generation antipsychotic (SGA) drug treatment. The histamine H1 receptor (H1R) binding affinity is considered to be a main indicator of SGA-induced weight gain (reviewed in He et al., 2013). According to the literature, the order of weight gain liability of SGAs is: clozapine ~ olanzapine > quetiapine ≥ risperidone > ziprasidone ~ aripiprazole (Nasrallah, 2008). The order of binding affinity of antipsychotics on the H1 receptor is approximately: clozapine > olanzapine > quetiapine > risperidone > ziprasidone > aripiprazole (Kim et al., 2007; Kroeze et al., 2003).

Histaminergic neurons, located at the tuberomammillary nucleus (TMN) in the posterior hypothalamus, project to brain areas including the hypothalamus and brainstem dorsal vagal complex (DVC). Histamine is synthesized by the oxidative decarboxylation of histidine by the enzyme histidine decarboxylase (HDC) (Haas et al., 2008). Histamine is stored in axon varicosities or released into the synaptic cleft (Haas et al., 2008). Both the synthesis and release of histamine are controlled by the presynaptic H3 receptors (Haas et al., 2008). Hypothalamic histamine regulates food intake and energy expenditure by activating the postsynaptic H1Rs in the hypothalamic paraventricular nucleus (PVN), ventromedial (VMH) and arcuate nucleus (Arc) (Ookuma et al., 1993; Umehara et al., 2010). Previous studies have reported that olanzapine and clozapine affect the H1R expression in the hypothalamus (Han et al., 2008; He et al., 2014b; Humbert-Claude et al., 2012). However, how olanzapine modulates the brain histaminergic neurotransmission, and its potential associations with weight gain, is not completely understood.

HDC is highly expressed in the TMN where neuronal histamine is produced (Krusong et al., 2011). Intracerebroventricular injection of the HDC inhibitor, alpha-fluoromethylhistidine, blocks the synthesis of

* Corresponding author at: IHMRI, University of Wollongong NSW 2522, Australia.
E-mail address: xhuang@uow.edu.au (X.-F. Huang).

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histamine, and attenuates the suppressive effect of histidine on food intake (Yoshimatsu et al., 2002). HDC knock-out mice have an increased body weight and epididymal adipose tissue (Yoshimatsu et al., 2002). Therefore, it is important to examine the effect of olanzapine on HDC expression to determine the role of the histaminergic system on olanzapine-induced obesity.

The H3 receptor (H3R) is highly expressed in the hypothalamus (Pillot et al., 2002). H3R antagonists (*e.g.* NNC 38-1049) have been reported to decrease food intake (Malmlof et al., 2005). H3R knock-out mice have mild weight gain (Takahashi et al., 2002). Previous studies have shown that the H3R mediates food intake partly by regulating histamine synthesis and release (Ishizuka et al., 2008; Malmlof et al., 2005). Since olanzapine treatment may modulate the histaminergic system in the hypothalamus, it is possible that olanzapine may indirectly affect H3R expression, although olanzapine has a low H3R affinity.

Moreover, evidence from both clinical and animal studies have suggested that olanzapine-induced weight gain occurs in three stages: (1) rapid body weight increase in the early stage (humans: first 3 months; rats: first 2 weeks); (2) reduced rate of weight gain in the middle stage (humans: 3–18 months; rats: weeks 3–4); (3) body weight plateau and the high body weight is maintained with continued olanzapine treatment in the late stage (human: > 18 months, rats: week 5) (Allison and Casey, 2001; He et al., 2014b; Huang et al., 2006; Norrby et al., 2012). The evidence suggest that neural mechanisms may differ in different stages of olanzapine-induced weight gain. Therefore, it is suggested that olanzapine may modulate histamine neurotransmission in a time-dependent manner during different stages of obesity development, which could have clinical significance.

2. Methods

2.1. Animals and drugs

Female Sprague-Dawley (SD) rats (weight 200–225 g) were obtained from the Animal Resources Centre (Perth, WA, Australia). The rats were housed under environmentally controlled conditions (22 °C on a 12 h light-dark cycle, lights on 0700 h). Rats were allowed *ad libitum* access to standard laboratory chow diet (3.9 kcal/g; 10% fat, 16 protein and 74 carbohydrate by calories) and water throughout the studies. All animal experiments were approved by the Animal Ethics Committee, University of Wollongong, and complied with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (2004). Olanzapine (Zyprexa) was obtained from Eli Lilly, Indianapolis, IN, USA.

2.2. Animal treatment

In order to examine the effects of olanzapine on the hypothalamic HDC, H1R, and H3R expression in different stages of olanzapine-induced weight gain, female SD rats were randomly divided into three groups and treated with olanzapine for 8 days, 16 days and 36 days to represent the three stages of olanzapine-induced weight gain (He et al., 2014b). Briefly, rats (n = 12/group) were fed sweet cookie dough pellets (62% carbohydrate, 22% protein, 6% fibre, 10% vitamins and minerals) mixed with either olanzapine (1 mg kg^{-1}) or placebo three times daily at eight-hourly intervals at 0700 h, 1500 h and 2300 h (equivalent to 3 mg kg^{-1} day^{-1}) for 8, 16 and 36 days (He et al., 2014b). Food intake and body weight were measured every 48 h. In this study, female rats were used because previous studies reported that olanzapine treatment increased food intake, weight gain and adiposity in female rats (Huang et al., 2006; Weston-Green et al., 2011), which is consistent with the clinical findings of female patients as a risk factor for antipsychotic-induced weight gain/obesity (Roumestan et al., 2008). The dosage of oral olanzapine was chosen based on the previous dose-dependent study (Weston-Green et al., 2011). It was calculated as being similar to the clinically relevant dosage of 10 mg day^{-1} (based on body surface area of different species) (Reagan-Shaw et al., 2008), and mimicked olanzapine-induced obesity in clinical settings.

2.3. Histological procedures

At the end of each experiment, rats were sacrificed using carbon dioxide (CO_2). Six brains from each group were used for quantitative RT-PCR studies for mRNA expression and other six brains from each group were used for quantitative receptor autoradiography. For RT-PCR, rats were sacrificed 2 h after the last drug administration to investigate the immediate effect of olanzapine on gene expression (Huang et al., 2006). For the receptor binding study, rats were sacrificed 48 h after the last drug treatment to ensure the requirement of drug washout period (Han et al., 2008; Weston-Green et al., 2008). Brains were cut at 14 μm coronal sections at −18 °C and thaw-mounted onto Polysine™ Microscopeslides (Menzel GmbH & Co. KG, Braunschweig, Germany).

2.4. Receptor binding autoradiography and quantification

2.4.1. Histamine H1 receptor binding and quantification

Previous studies in humans and rodents have demonstrated that H1Rs are rich in the cortical regions (the prefrontal, cingulate, and primary motor cortex M1), middle brain areas (the ventral tegmental area), hypothalamus, and the limbic regions (hippocampus HIP, caudate putamen CPu, and medial posterodorsal amygdala MeP) (Hu et al., 2010; Iwabuchi et al., 2005; Jin and Panula, 2005). In the hindbrain, H1Rs are abundant in the brainstem DVC (Bhuiyan et al., 2011; Poole et al., 2008). The present study examined the effect of H1R binding density in the prefrontal cortex (PFC), M1, cingulate cortex (Cg), CPu, accumbens nucleus, core (AcbC), Arc, PVN, VMH, DMH, LHA, HIP, MeP and brainstem DVC of the rats treated with olanzapine for 8, 16 and 36 days.

The H1R binding autoradiography was performed using the procedures described previously (Hu et al., 2010). Briefly, slide sections were left to thaw and dry at room temperature before the binding procedures were carried out. Sections were incubated with [^{3}H]pyrilamine (specific activity 27.0 Ci/mmol, Amersham Biosciences UK Limited) in 50 mM sodium potassium phosphate buffer (pH 7.4) for 1 h. The non-specific binding was detected by adding 10 μM triprolidine (Sigma, NSW, Australia) using the same buffer. After incubation, the sections were washed in ice-cold buffer (4 × 2 min), dipped in ice-cold distilled water and dried under a stream of cold air to remove excess buffer salts. All of the slides were exposed to Amersham high performance autoradiography film (GE healthcare, Pallards Wood, UK) for 3 months. The films were developed using standard procedures. Quantification of the autoradiographic images was performed using a computer-assisted image analysis system, Multi-Analyst, which is connected to a GS-690 Imaging densitometer (Bio-Rad, Hercules, California) (Han et al., 2008). The [^{3}H]-microscales from Amersham were used as standards (Zavitsanou et al., 2002). The specific binding values were obtained by subtracting non-specific binding values from the total binding values. Brain regions were identified based on the rat brain atlas (Paxinos and Watson, 2007).

2.4.2. Histamine H3R binding and quantification

Previous studies have shown that H3R are distributed in the cortical areas (PFC, M1 and Cg), nucleus accumbens, hypothalamic VMH and PVN, and CPu and MeP (Pillot et al., 2002; Pollard et al., 1993). In the DVC, H3R is low (Pillot et al., 2002). The present study examined the effects of olanzapine treatment on H3R binding density in the hypothalamic Arc, PVN, VMH, DMH, and LHA, as well as PFC, Cg, M1, CPu, AcbC and MeP.

The procedure for the H3R autoradiography was based on standard procedures from (Anichtchik et al., 2000; Le et al., 2009) using [^{3}H]-NAMH (specific activity 84.4 Ci/mmol, PerkinElmer (Waltham, MA)).