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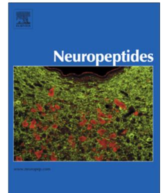

Hypothalamic orexin prevents hepatic insulin resistance induced by social defeat stress in mice

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ABSTRACT

Depression is associated with insulin resistance and type 2 diabetes, although the molecular mechanism behind the pathological link remains unclear. Orexin, a hypothalamic neuropeptide regulating energy and glucose homeostasis, has been implicated in the endogenous antidepressant mechanism. To clarify whether orexin is involved in the coordination between mental and metabolic functions, we investigated the influence of orexin deficiency on social interaction behavior and glucose metabolism in mice subjected to chronic social defeat stress. Chronic stress-induced glucose intolerance and systemic insulin resistance as well as social avoidance were ameliorated by calorie restriction in an orexin-dependent manner. Moreover, orexin-deficient mice maintained under ad libitum-fed conditions after defeat stress exhibited hyperinsulinemia and elevated HOMA-IR (homeostasis model assessment for insulin resistance), despite normal fasting blood glucose levels. In a pyruvate tolerance test to evaluate hepatic insulin sensitivity, chronic stress-induced abnormal glucose elevation was observed in orexin-deficient but not wild-type mice, although both types of mice were susceptible to chronic stress. In addition, insulin-induced phosphorylation of Akt in the liver was impaired in orexin-deficient but not wild-type mice after chronic stress. These results demonstrate that the central physiological actions of orexin under ad libitum-fed conditions are required for the adaptive response to chronic defeat stress, which can prevent the development of hepatic insulin resistance but not social avoidance behavior. Moreover, calorie restriction, a paradigm to strongly activate orexin neurons, appears to prevent the persistence of depression-like behavior *per se*, leading to the amelioration of impaired glucose metabolism after chronic stress; therefore, we suggest that hypothalamic orexin system is the key for inhibiting the exacerbating link between depression and type 2 diabetes.

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1. Introduction

Depression and type 2 diabetes are both highly prevalent chronic diseases, and frequently comorbid (Mezuk et al., 2008; Renn et al., 2011). Furthermore, patients with comorbid depression and type 2 diabetes have been shown to have an increased risk of cardiovascular complications and a higher mortality rate than patients with diabetes alone (Nouwen et al., 2011). Although it is well established that depression and type 2 diabetes are associated, the primary cause remains unclear (Knol et al., 2006; Mezuk et al., 2008; Nouwen et al., 2011); however, increasing evidence suggests that depression is positively associated with insulin resistance as the main cause of type 2 diabetes (Timonen et al., 2006; Silva et al., 2012). Moreover, animal studies demonstrated that chronic restraint stress resulted in reduced insulin sensitivity without an increase in body weight gain (Uchida et al., 2012), and an antidepressant selective serotonin reuptake inhibitor (SSRI) restored insulin resistance in low-birth-weight rats regarded as both a pre-depressive and pre-diabetic model (Buhl et al., 2010). Therefore, blockade of the pathological link between depression and insulin resistance appears to be valuable to reduce the future risk of diabetes (Pan et al., 2012).

Chronic social defeat stress (CSDS) is a mouse model of chronic stress-induced psychiatric disorders, including major depression and anxiety (Krishnan et al., 2007). In particular, the social avoidance observed in this model is considered depression-like behavior, because it can be normalized by chronic, but not acute, treatment with SSRI (Berton et al., 2006). Furthermore, CSDS induces long-lasting metabolic abnormalities via sympathetic nervous system activation (Chuang et al., 2010b). The combination of CSDS and high-fat feeding resulted in the dysregulation of lipid metabolism (Chuang et al., 2010a); however, the influence of CSDS on glucose metabolism and adaptive mechanisms for preventing metabolic abnormalities during chronic stress have not been fully understood.

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Neuropeptides function as slow-acting neuromodulators (van den Pol, 2012). Some neuropeptides, such as neuropeptide Y and orexin, that modulate the stress response or adaptation further play crucial roles in the hypothalamic regulation for maintenance of whole-body glucose and energy homeostasis (Alldredge, 2010; Scott et al., 2011; Blouet and Schwartz, 2010). Therefore, abnormal functions of hypothalamic neuropeptides may underlie the synergistic development of depression and metabolic disorders during chronic stress.

Orexin (hypocretin) neurons producing orexin-A and -B are localized in the lateral, perifornical and posterior hypothalamus and act as an interface of systems of emotion, reward, energy/glucose homeostasis and arousal (Sakurai and Mieda, 2011; Tsuneki et al., 2008; Tsuneki et al., 2012). In depressive patients, daily oscillation of orexin A levels in the cerebrospinal fluid (CSF) is reported to be dampened (Salomon et al., 2003), and suicidal patients with major depressive disorders showed reduced CSF orexin A levels (Brundin et al., 2007). Also, mice subjected to CSDS exhibit diminished levels of orexins in the midbrain dopamine system and hypothalamus (Nocjar et al., 2012), whereas calorie restriction (CR) exerts an antidepressant-like effect by increasing the activity of orexin neurons in mice after CSDS (Lutter et al., 2008). In narcoleptic patients with orexin deficiency, depressive symptoms are strikingly overrepresented (Fortuyn et al., 2011), and the frequency of obesity and type 2 diabetes is increased (Honda et al., 1986; Schuld et al., 2000). We therefore hypothesized that orexin plays an important role in the adaptive response to chronic stress for preventing the development of both psychiatric and metabolic abnormalities in parallel.

The aim of the present study was to clarify how orexin is involved in the regulation of glucose metabolism under chronic stress conditions. To this end, we investigated the influences of orexin deficiency using preproorexin-deficient (*Orexin*$^{-/-}$) mice or activation of the orexin system induced by CR on social interaction behavior, glucose tolerance, systemic insulin sensitivity, hepatic glucose production, and insulin signaling in the liver.

2. Materials and methods

2.1. Materials

Human regular insulin Novolin R was provided by Novo Nordisk (Copenhagen, Denmark). Anti-insulin receptor substrate 1 (IRS1) antibody and anti-IRS2 antibody were purchased from Millipore (Temecula, CA). Anti-insulin receptor β subunit antibody and anti-Akt1 antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-Akt (Ser^{473}) antibody and anti-β-actin monoclonal antibody were from Cell Signaling Technology (Beverly, MA). All other reagents were purchased from Sigma–Aldrich Japan (Tokyo, Japan) or Wako Pure Chemical Industries (Osaka, Japan), unless otherwise indicated.

2.2. Animals

Mice were housed at 23–25 °C under a 12:12 h light–dark cycle (lights on from 0700 to 1900 h) with free access to normal chow diet (PicoLab Rodent Diet 20; Japan SLC, Hamamatsu, Japan) and water. *Orexin*$^{-/-}$ mice were generated as described previously (Chemelli et al., 1999). *Orexin*$^{+/-}$ mice (N11 backcross to C57BL/6J) were crossed, and the *Orexin*$^{-/-}$ offspring were mated to obtain the male *Orexin*$^{-/-}$ mice used in this study. Male C57BL/6J mice were purchased from Japan SLC and used as wild-type (WT) controls. Male ICR (CD-1) mice were also from Japan SLC. All experimental procedures used in this study were approved by the Committee for Animal Experiments at the University of Toyama.

2.3. Chronic social defeat stress (CSDS)

The CSDS test was performed as shown in Fig. 1A, according to previously described methods (Krishnan et al., 2007; Chuang et al., 2010b) with slight modification. In brief, a male WT or *Orexin*$^{-/-}$ mouse of 2–3 months old was intruded into the home cage of an aggressive male ICR mouse of 2–4 months old, and exposed to defeat stress with physical attack by the resident ICR mouse for 10 min. The intruding mouse was further exposed to subsequent 24-h stress with aversive sensory stimulation in the home cage of the resident mouse, where the intruding and resident mice were separated with a transparent plastic partition plate with small holes. The CSDS stimulation was repeated for 10 consecutive days, using a different ICR mouse every day. Similar handling was applied to non-stressed controls without using ICR mice. To evaluate the behavioral consequences, the social interaction test was performed at day 11 and 26, and the social interaction ratio (percentage) of time spent in the interaction zone in the absence versus presence of unfamiliar ICR mice during 150-s of observation was calculated. Defeated mice with a social interaction ratio <100 and >100 were considered 'susceptible' and 'unsusceptible' to CSDS, respectively. Unsusceptible mice were not included in the following experiments. The amount of food intake was measured from day 14 to 16. Thereafter, mice were randomly selected and subjected to either ad libitum feeding (AL) or the CR regimen. The CR group was food restricted (70% of ad libitum intake), based on the average food intake for three days. Consequently, eight subgroups were prepared: control WT-AL group, control WT-CR group, susceptible WT-AL group, susceptible WT-CR group, control *Orexin*$^{-/-}$-AL group, control *Orexin*$^{-/-}$-CR group, susceptible *Orexin*$^{-/-}$-AL group, and susceptible *Orexin*$^{-/-}$-CR group.

2.4. Glucose tolerance test, insulin tolerance test, and pyruvate tolerance test

On experimental day 27 (Fig. 1; 17 days after the last defeat stress), mice were fasted for 5 h and then injected with either glucose (2 g/kg, i.p.), insulin (0.75 U/kg, i.p.), or pyruvate (2 g/kg, i.p.) for the respective tolerance tests. Five-hour fasting was chosen according to a recently established method (Andrikopoulos et al., 2008). Blood samples were collected from the tail vein at the indicated times. Blood glucose levels were measured using a FreeStyle Freedom glucose meter (Nipro, Osaka, Japan).

2.5. Measurements of serum parameters

On experimental day 29 (Fig. 1; 19 days after the last defeat stress), mice were fasted for 5 h and then blood samples were collected from the orbital sinus of mice at 1300 h. The samples were kept at room temperature for 1 h and then centrifuged at 900*g* for 30 min. The supernatants were obtained as serum samples and stored at −80 °C until use. Serum levels of insulin and leptin were measured by ELISA kits (Takara Bio, Shiga, Japan). Insulin sensitivity was assessed by homeostasis model assessment of insulin resistance (HOMA-IR), calculated as follows: HOMA-IR = (fasting serum glucose in mg/dl) × (fasting serum insulin in μU/ml)/405. Serum levels of corticosterone under ad libitum-fed conditions were measured using a corticosterone EIA kit (Cayman Chemical, Arbor, MI).

2.6. Western blot analysis

On experimental day 31 (Fig. 1; 21 days after the last defeat stress), mice were fasted for 5 h and then intraperitoneally (i.p.) injected with insulin (0.75 U/kg) or phosphate-buffered saline. Thirty minutes after injection, the liver tissue was rapidly isolated,

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