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Implication of $mGlu_5$ receptor in the enhancement of morphine-induced hyperlocomotion under chronic treatment with zolpidem

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ABSTRACT

Long-term exposure to zolpidem induces drug dependence, and it is well known that the balance between the GABAergic and glutamatergic systems plays a critical role in maintaining the neuronal network. In the present study, we investigated the interaction between GAB_{AA} receptor α1 subunit and $mGlu_5$ receptor in the limbic forebrain including the N.Acc. after treatment with zolpidem for 7 days. $mGlu_5$ receptor protein levels were significantly increased after treatment with zolpidem for 7 days, and this change was accompanied by the up-regulation of phospholipase Cβ1 and calcium/calmodulin-dependent protein kinase IIα, which are downstream of $mGlu_5$ receptor in the limbic forebrain. To confirm that $mGlu_5$ receptor is directly involved in dopamine-related behavior in mice following chronic treatment with zolpidem, we measured morphine-induced hyperlocomotion after chronic treatment with zolpidem in the presence or absence of an $mGlu_5$ receptor antagonist. Although chronic treatment with zolpidem significantly enhanced morphine-induced hyperlocomotion, this enhancement of morphine-induced hyperlocomotion was suppressed by treating it with the $mGlu_5$ receptor antagonist MPEP. These results suggest that chronic treatment with zolpidem caused neural plasticity in response to activation of the mesolimbic dopaminergic system accompanied by an increase in $mGlu_5$ receptor.

1. Introduction

Benzodiazepines are used as sedatives, sleeping aids, and anti-anxiety drugs. However, the long-term use of benzodiazepines induces psychological and physical dependence. Many studies have supported the notion that the mesolimbic dopaminergic transmission system, which originates in the ventral tegmental area and projects predominantly to the nucleus accumbens, plays important role in producing psychological dependence (Vanderschuren and Kalivas, 2000). Accumulating evidence suggests that the rewarding effects and behavioral sensitization induced by psychostimulants may be accompanied by long-lasting neural plasticity that may participate in structural modifications in the dopaminergic neuronal system (Shibasaki et al., 2011). On the other hand, even though benzodiazepines induce psychological dependence without increasing the release of dopamine (Invernizzi et al., 1991; Shibasaki et al., 2013a), the mechanisms by which benzodiazepines induce psychological dependence are not fully understood.

γ-Aminobutyric acid type A ($GABA_A$) receptor consists of a five-subunit complex (2α, 2 β and 1 γ subunit), which contains Cl^- channels, and the activation of $GABA_A$ receptor by GABA results in an increase in Cl^- influx. Especially, the activation of $GABA_A$ receptor containing the α1 subunit induces sleep, sedation, abuse potential and anti-epilepsia (Tan et al., 2011). It has been reported that zolpidem, a $GABA_A$ receptor α1 subunit agonist, induces physical and psychological dependence in humans (Pitchot and Ansseau, 2009). It is also reported that subunit selective benzodiazepines sparing α1 may devoid of addiction liability (Tan et al., 2010). Furthermore, stimulation of $\alpha1GABA_A$ receptors is sufficient, but not necessary, for mediation of the abuse potential of these drugs (Rowlett et al., 2005). Especially, it is reported that 30 mg/kg of zolpidem for 4 days or 10 days has been used for physical dependence study (Mirza and Nielsen, 2006; Perrault et al., 1992). Moreover, our recent study demonstrated that chronic treatment with zolpidem enhanced morphine-induced hyperlocomotion by increasing dopamine response through the up-regulation of KCC2 in the limbic forebrain (Shibasaki et al., 2013a). It has been reported that $GABA_A$ receptors might be involved in the acquisition of morphine-induced sensitization (Zarrindast et al., 2007). Moreover, our previous study indicated

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that GABAergic receptors are involved in morphine-induced locomotor sensitization (Narita et al., 2003). The $GABA_A$ receptor-dependent rewarding mechanism is sensitive to drug dependence with a dopamine-independent system operating in opioid-naïve animals and a dopamine-dependent system operating in morphine-dependent or morphine-withdrawn animals (Laviolette et al., 2004).

The balance between the GABAergic and glutamatergic systems is important for maintaining the neuronal network. It has been shown that an imbalance of these systems causes abnormal neuronal activity, and is related to Alzheimer's disease and schizophrenia (Kehrer et al., 2008; Sun et al., 2009). Furthermore, metabotropic glutamate (mGlu) receptors interact with GABAergic signaling (Semyanov and Kullmann, 2000). $mGlu_5$ receptor is involved in synaptic plasticity and drug dependence (Aoki et al., 2004). Generally, $mGlu_5$ receptor is classified as a group I mGlu receptor, which is one of the three major groups of a family of eight mGlu receptor subtypes, and is coupled to the Gq family of G proteins (Conn and Pin, 1997). The activation of $mGlu_5$ receptor results in phospholipase C (PLC)-catalyzed phosphoinositide hydrolysis, which leads to the production of inositol trisphosphate (IP_3) and diacylglycerol (DAG). The production of IP_3 promotes the release of intracellular Ca^{2+} from its stores within the endoplasmic reticulum. The increase in Ca^{2+} influx from either extracellular or intracellular sources and the production of DAG are essential elements for the stimulation of various forms of protein kinase C (PKC). Our recent report suggested that chronic treatment with zolpidem induced the up-regulation of phosphorylated-PKCγ (Shibasaki et al., 2013a).

Morphine is known as a prototypical μ-opioid receptor agonist, which serves as the standard analgesic for severe pain. In clinical fields, it is reported that the patients with a history of alcohol dependence were easy to be addicted to opioids under the pain control for chronic pain conditions (Ballantyne and LaForge, 2007; Hojsted and Sjogren, 2007). Moreover, $GABA_A$ α1 subunit relates to ethanol-enhancing effects (Rudolph et al., 1999). Our previous study demonstrated that $mGlu_5$ receptor/PKC cascade regulates morphine-induced rewarding effects (Aoki et al., 2004). Thus, these changes by chronic treatment with zolpidem may influence the vulnerability to opioid abuse under chronic pain control. While it has been hypothesized that chronic treatment with zolpidem activated the $mGlu_5$ receptor cascade, it is not yet clear how zolpidem influences the $mGlu_5$ receptor cascade in mesolimbic dopaminergic neurons. Therefore, the present study was designed to investigate the influence of the $GABA_A$ receptor α1 subunit agonist zolpidem on the $mGlu_5$ receptor cascade and response to morphine.

2. Materials and methods

2.1. Animals

The present study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University. Experiments were performed using male ICR mice (Tokyo Animal Science Laboratories, Tokyo, Japan), weighing 20–22 g at the beginning of the experiments, which were housed in groups of six at a temperature (23 ± 1 °C)- and humidity (55%)-controlled room. They were maintained under 12-h light-dark cycle with laboratory mouse food and water available ad libitum.

2.2. Locomotor activity

The locomotor activity of mice was measured by an ambulometer (ANB-M20, O'Hara, Co., Ltd., Tokyo, Japan) as described previously (Shibasaki et al., 2013a). Briefly, a male ICR mouse was placed in a tilting-type round activity cage of 20 cm in diameter and 19 cm in height. Any slight tilt of the activity cage caused by horizontal movement of the animal was detected by microswitches. Total activity counts in each 10 min segment were automatically recorded for 30 min prior to injection and for 180 min after morphine administration.

To investigate alteration of morphine-induced hyperlocomotion after chronic treatment with zolpidem, mice were administered with zolpidem (30 mg/kg, i.p.) or vehicle once a day for 7 days. Morphine (10 mg/kg, s.c.)-induced hyperlocomotion was measured 24 h after final administration of zolpidem. In antagonist test, some animals were pre-treated with the $mGlu_5$ receptor antagonist MPEP 10 min before chronic treatment with zolpidem for 7 days.

2.3. Western blotting

To determine the alteration of protein levels in the mouse brain, samples are prepared 24 h after final administration of zolpidem (30 mg/kg, i.p.) or vehicle once a day for 7 days. As acute treatment group, mouse was administered with zolpidem (30 mg/kg, i.p.), and 1 h or 24 h after administration, mouse was sacrificed. Tissues from the limbic forebrain including the nucleus accumbens (N.Acc.), lower midbrain including the ventral tegmental area (VTA), striatum and prefrontal cortex were retrieved from the mouse, and fractions were prepared by centrifugation with lysis buffer (10 mM Tris–HCl pH-7.5, 150 mM NaCl, 0.5 mM EDTA, 10 mM NaF, 0.5% Triton X-100) containing a phosphatase inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, USA). The homogenate was centrifuged at 1000*g* for 10 min, and the pellet was used as the nuclear fraction for CaMKII, CaMKIV, p-CREB and p-STAT. The supernatant was centrifuged at 20,000*g* for 35 min; the pellet was used as the membrane fraction for Glu1 receptor, NR2A, NR2B, $mGlu_5$ receptor and PLCβ1, and the supernatant was used as the cytosol fraction for TH and GAD65/67. The protein concentration was quantified by the Lowry protein assay (Bio-Rad Laboratories, GmbH). Briefly, equal amounts of protein (12 mg) were separated by SDS-PAGE (10%) and then transferred to PVDF membranes (ATTO, Tokyo, Japan). After samples were blocked with TBS containing 5% milk and 0.1% Tween for 1 h at room temperature, they were incubated with each primary antibody diluted (Glu1 receptor; 1:5000, NR2A; 1:5000, NR2B; 1:5000, $mGlu_5$ receptor; 1:3000, PLCβ1; 1:5000, CaMKII; 1:5000, CaMKIV; 1:5000, p-STAT3; 1:5000, p-CREB (ser 133); 1:5000, TH; 1:2000 and GAD65/67; 1:5000) in TBS containing 5% milk 0.1% Tween at 4 °C overnight. The membrane was then washed three times in TBS containing 0.05% Tween, and incubated with horseradish peroxidase-conjugated second antibody (Southern Biotechnology Associates, Birmingham, AL, USA) diluted 1:10,000 in TBS containing 5% milk and 0.1% Tween at room temperature for 2 h. After incubation, the membranes were washed three times in TBS containing 0.05% Tween. Proteins were visualized by an enhanced chemiluminescence detection system (Pierce ECL Western Blotting Substrate, Thermo Fisher Scientific Germany Ltd. & Co. KG). Equal loading was confirmed by the use of antibody for b-actin (Applied Biological Materials Inc., Canada) diluted 1:50,000. The results of western blotting were analyzed by a FluorChem3 system (Laboratory & Medical Supplies, Tokyo, Japan). All results were normalized by β-actin.

2.4. RNA preparation and semiquantitative analysis by reverse transcription-polymerase chain reaction (RT-PCR)

To determine the alteration of mRNA levels, samples from mouse brain were prepared 24 hr after the final administration