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Brain regions and monoaminergic neurotransmitters that are involved in mouse ambulatory activity promoted by bupropion

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ABSTRACT

Bupropion (BUP), a substituted phenyl-ethylamine, has been utilized for the treatment of depression and for smoking cessation, however, one concern is that BUP may increase a risk of psychosis similar to other substituted phenyl-ethylamine amphetamine (AMPH) and methamphetamine (MetAMPH). BUP promotes ambulation in mice and causes behavioral sensitization on the ambulation-promoting effect when repeatedly administered as well as AMPH and MetAMPH. The present study aimed to elucidate brain regions and monoaminergic neurotransmitters that are involved in the ambulation-promoting effect of BUP. c-Fos-like immunoreactivity (c-Fos-IR) mapping in brain in combination with measuring ambulatory activity was conducted to determine brain region(s) that is involved in the ambulatory effect of BUP. Three kinds of statistical analyses for c-Fos-IR in 24 brain regions consistently showed that c-Fos-IR in the Caudate putamen (CPu) is positively correlated with the ambulatory response to BUP. In addition, multiple regression analysis indicated that the ambulatory response is a function of c-Fos-IR not only in the CPu but also in the lateral septum nucleus (LS), median raphe nucleus (MnR), lateral globus pallidus (LGP), medial globus pallidus (MGP), locus coeruleus (LC) and ventral hypothalamic nucleus (VMH). Effects of BUP on monoaminergic neurotransmitters in the CPu were examined using *in vivo* microdialysis method, as the pharmacological experiments indicated that monoaminergic neurotransmitters, dopamine (DA) in particular, mediate the ambulatory response to BUP. Response of DA in the CPu to BUP was parallel to the ambulatory response, showing that DA in the CPu is involved in the ambulatory response to BUP. The present study also suggests that other brain regions such as the LC, the origin nucleus of norepinephrine (NE) neurons, and another neurotransmitter NE may also play some roles for the ambulatory response to BUP, however, further studies are needed to elucidate the roles.

1. Introduction

Bupropion (BUP), a substituted phenyl-ethylamine, was found to possess therapeutic benefits as an atypical antidepressant medication [18], smoking-cessation medication [18] and drug abuse-cessation medication [10,14,46]. Clinical use of BUP for the treatment of depression and for smoking-cessation was approved by the Food and Drug Administration (FDA) in the United States. Daily dose of 450 mg is recommended for the treatment of depression. The original immediate release formulation of BUP is dosed three times daily, followed by introduction of the sustained-release formulation that is dosed twice daily and the extended-release formulation that is dosed once daily [29].

Though BUP has been extensively utilized for the treatment of depression and for smoking cessation, one concern is that BUP may increase a risk of psychosis [29]. Several studies report that BUP may cause or worsen psychosis [6,21,25,27,62]. BUP inhibits the dopamine (DA) transporter (DAT) and the norepinephrine (NE) transporter (NET), and is an antagonist at the neuronal nicotinic acetylcholine receptors (nAChRs) [48,50]. The IC_{50} of BUP for inhibiting DA uptake is higher than the IC_{50} values for inhibiting NE uptake and nAChRs function [16]. This pharmacological property also suggests that BUP can have the potential for precipitating psychosis. However, whether or not BUP increase a risk of psychosis and neuronal mechanisms underlying the potential of BUP to increase the risk have not been elucidated sufficiently [29].

BUP promotes ambulatory activity in mice [54,55,59]. Repeated administration of BUP to the same mouse augments the ambulation-promoting effect (behavioral sensitization) [58]. These ambulatory effects of BUP are similar to those of amphetamine

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(AMPH) and methamphetamine (MetAMPH) [22,30], other substituted phenyl-ethylamine, that have the potential to produce psychosis-like mental disorder (amphetamine psychosis) [26]. Mechanism underlying the locomotion-promoting effect of AMPH in mice is elucidated well [7,24,36,51,64], whereas mechanisms underlying the ambulation-promoting effect of BUP have poorly been elucidated.

The present study aimed to elucidate brain region(s) and neurotransmitter(s) that are involved in the ambulation-promoting effect of BUP.

Mapping c-Fos-like immunoreactivity (c-Fos-IR), which indicates neuronal activation, is useful for determining brain regions that are activated by CNS acting drugs and involved in specific behaviors [43,66]. BUP increases c-Fos-IR in various rat brain regions [8], whereas effects of BUP on c-Fos-IR in mouse brain have not been studied. The present study conducted c-Fos-IR mapping in combination with measuring ambulatory activity to determine mouse brain region(s) that is involved in the ambulatory response to BUP.

BUP inhibits dopamine (DA) transporter (DAT) and norepinephrine (NE) transporter (NET) and enhances the extracellular DA and NE levels in rat brain [11,33]. On the other hand, effects of BUP on monoaminergic neurotransmitters in mouse brain have not been elucidated well. The present study examined whether or not monoaminergic neurotransmitters mediate the ambulatory response to BUP using drugs that modulate monoaminergic neurotransmission. Drugs used were α-methyl-*p*-tyrosine (AMPT), reserpine (RES), chlorpromazine (CPZ), fluphenazine (FLU), SCH12679 (SCH), spiperone (SPI), haloperidol (HAL) and pimozide (PIM). AMPT and RES deplete monoaminergic neurotransmitters in brain [17,41,61]. CPZ, FLU, SCH, SPI, HAL and PIM antagonize DA receptors (DARs) and their affinities for DARs are much higher than those for NE receptors [13,47]. Then, the present study examined effects of BUP on monoaminergic neurotransmitters in brain region(s) that was identified by the c-Fos-IR study using *in vivo* microdialysis in order to elucidate relationships between responses to BUP of monoaminergic neurotransmitters in the brain region(s) and of the ambulatory activity.

2. Materials and methods

2.1. Subjects

Male ICR strain mice (Clea Japan, Tokyo, Japan) aged 7–15 weeks and weighing 35–45 g at the start of experiments were used. Mice were housed in aluminum cages (three mice/cage) with a stainless-steel mesh top and paper bedding. Commercial solid food (Clea Japan) and tap water were provided ad libitum. The cages were placed in a room artificially illuminated by fluorescent lamps on a 12-h light:12-h dark schedule (light period: 07:00–19:00) and a room temperature of 25 ± 1 °C. All experiments were conducted during the light phase.

All experiments were performed in accordance with the guidelines of the Ethics Committee for Experimental Animals of the National Institute for Environmental Studies, Japan.

2.2. Drugs

Bupropion HCl (BUP), chlorpromazine HCl (CPZ), fluphenazine 2HCl (FLU), SCH12679 ((R)-(−)-Phenyl−2,3,4,5-tetrahydro-1*H*-7,8-dimethoxy-3-benzazepine) maleate (SCH) and spiperone (SPI) (Sigma-Aldrich, Tokyo, Japan) were prepared in 0.9% NaCl (Nacalai Tesque, Kyoto, Japan) solution (saline). Haloperidol (HAL) (Sigma-Aldrich) was prepared in 0.1% acetic acid (Nacalai Tesque) solution. Pimozide (PIM) (Sigma-Aldrich), α-methyl-*p*-tyrosine (AMPT) (ICN Biomedicals, Solon, OH, USA), and reserpine (RES) (Sigma-Aldrich) were mixed with a small amount of polyoxyethylene sorbitan monooleate (Tween 80) (Nacalai Tesque) and diluted in saline. Doses of BUP, CPZ, FLU, and SCH were expressed as the salt weights.

AMPT was intraperitoneally administered to mice, and other drugs were subcutaneously administered. The administration volume was 1 ml/100 g body weight regardless of the type of drug and dosage.

2.3. Measurement of mouse ambulatory activity

Ambulatory activity was measured using an ambulometer (SAM-10; O'Hara and Co., Tokyo, Japan) [55–59,54,52,53,57] (Appendix A). Each activity cage (20 cm in diameter) is supported by a fulcrum in the center of the bottom; the fulcrum tilts according to movement of the mouse in the activity cage. The tilting movement of the activity cage activates three micro-switches that surround the cage. The number of activations of micro-switches during a set time is recorded, and the result is printed out.

After adapting mice to the activity cages for 30 min, saline, 5 mg/kg or 10 mg/kg of BUP was administered to the mice, followed by measuring the ambulatory activity for 60 min. Combined administration of saline or 50 mg/kg AMPT plus saline or 2, 4, or 8 mg/kg RES was performed on mice at the same time one day before measuring the ambulatory response to 10 mg/kg BUP. Drugs that preferentially antagonize DARs were administered after 30 min of adaptation, 10 min later followed by measuring the ambulatory response to 10 mg/kg BUP.

2.4. Immunocytochemistry for c-Fos

2.4.1. Preparation of brain samples

The following procedures were performed in three separate experiments on mice given saline (n = 8) or BUP (n = 11). We obtained seven brain samples from mice given saline and 10 brain samples from mice given BUP. One brain sample from each of the animal groups was excluded because of unsuccessful transcardial perfusion of fixation.

Individual mice were placed in activity cages, and 30 min later, saline was administered, followed by measurement of ambulatory activity for 60 min. After the measurement, they were returned to their home cages. This procedure was repeated every day for 3 days to reduce stress to the mice (acclimation procedure), because stress induces c-Fos-IR in various brain regions [3,23]. On the 4th day, individual mice were placed in activity cages. After 30 min of adaptation, saline or 10 mg/kg BUP was administered, and ambulatory activity was measured for 60 min. Immediately after the end of the ambulatory measurements, the mice were deeply anesthetized with pentobarbital (Nembutal®, Sumitomo Dainippon Pharma Co., Ltd., Osaka, Japan) and perfused transcardially with saline containing heparin (Wako Pure Chemical Ind. Ltd., Osaka, Japan) followed by Speh's fixative (4% paraformaldehyde, 0.2% saturated picric acid, and 0.05% glutaraldehyde in 0.1 M phosphate buffer pH 7.4), which is a modification of Zamboni's fixative [65]. Brains were removed and post-fixed in the same fixative overnight at 4 °C. They were soaked in 0.1 M phosphate buffer (pH 7.4) containing 25% sucrose for cryoprotection until they had completely sunk. Brains were individually frozen using methyl butane cooled by dry ice and stored at −80 °C.

2.4.2. Immunocytochemistry for c-Fos in brain samples

Coronal sections of brains from the olfactory bulb to the midbrain were cut at a thickness of 50 μm using a cryostat. Immunocytochemistry was performed on free-floating sections in four separate batches. Each batch included samples derived from mice administered either saline or BUP to ensure that the extent of