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ATOMOXETINE REVERSES LOCOMOTOR HYPERACTIVITY, IMPAIRED NOVEL OBJECT RECOGNITION, AND PREPULSE INHIBITION IMPAIRMENT IN MICE LACKING PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE

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Abstract—Attention-deficit/hyperactivity disorder (ADHD) is a complex neurobehavioral disorder that is characterized by attention difficulties, impulsivity, and hyperactivity. A non-stimulant drug, atomoxetine (ATX), which is a selective noradrenaline reuptake inhibitor, is widely used for ADHD because it exhibits fewer adverse effects compared to conventional psychostimulants. However, little is known about the therapeutic mechanisms of ATX. ATX treatment significantly alleviated hyperactivity of pituitary adenylate cyclase-activating polypeptide (PACAP)-deficient (PACAP$^{-/-}$) mice with C57BL/6J and 129S6/SvEvTac hybrid background. ATX also improved impaired novel object recognition memory and prepulse inhibition in PACAP$^{-/-}$ mice with CD1 background. The ATX-induced increases in extracellular noradrenaline and dopamine levels were significantly higher in the prefrontal cortex of PACAP$^{-/-}$ mice compared to wild-type mice with C57BL/6J and 129S6/SvEvTac hybrid background. These results suggest that ATX treatment-induced increases in central monoamine metabolism may be involved in the rescue of ADHD-related abnormalities in PACAP$^{-/-}$ mice. Our current study suggests that PACAP$^{-/-}$ mice are an ideal rodent model with predictive validity for the study of ADHD etiology and drug development. Additionally, the potential effects of differences in genetic background of PACAP$^{-/-}$ mice on behaviors are discussed.

Key words: atomoxetine (ATX), attention-deficit/hyperactivity disorder (ADHD), hyperactivity, memory, pituitary adenylate cyclase-activating polypeptide (PACAP), prepulse inhibition.

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Abbreviations: 5-HT, serotonin; ADHD, attention-deficit/hyperactivity disorder; ANOVA, analysis of variance; ATX, atomoxetine; AUC, area under the curve; DA, dopamine; DAT, dopamine transporter; NA, noradrenaline; NET, noradrenaline transporter; PACAP, pituitary adenylate cyclase-activating polypeptide; PFC, prefrontal cortex; PPI, prepulse inhibition.

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INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders and is characterized by attention difficulties, impulsivity, and hyperactivity (Castellanos and Tannock, 2002; Durston, 2003; Greydanus et al., 2007). Previous studies estimated a 5.3% prevalence of ADHD in children worldwide (Polanczyk et al., 2007). ADHD is highly heritable, but the etiology of ADHD is heterogeneous and complex, and it involves multiple genes and environmental factors (Cortese, 2012; Akutagava-Martins et al., 2013). Several candidate genes for ADHD, such as dopamine (DA) transporter (DAT) 1, DA receptor 4, and serotonin (5-HT) transporter genes, were identified and extensively studied (Elia et al., 2012), but the exact mechanisms of ADHD are not fully understood.

Psychostimulants are the most commonly used drugs for the treatment of ADHD (Greenhill et al., 2002). However, therapy with these drugs is often associated with undesirable side effects, such as anorexia, insomnia, drug abuse, and weight loss (Kollins, 2003; Kolar et al., 2008; Heal et al., 2009). Atomoxetine (ATX), a selective presynaptic noradrenaline (NA) transporter (NET) inhibitor, is the first non-stimulant medication that is approved for ADHD with limited abuse potential and a relatively benign side effect profile (Kratochvil et al., 2003; Christman et al., 2004). The effects of ATX and amphetamine on the defining phenotypes of ADHD were studied extensively in rodents. ATX decreases hyperactivity in spontaneously hypertensive rats and DAT-deficient mice, which are commonly used ADHD rodent models (Sora et al., 1998; Gainetdinov et al., 1999; Sagvolden and Xu, 2008; Arime et al., 2011; Del'Guidice et al., 2014). Importantly, approximately 50% of patients who do not respond to psychostimulant medications are responsive to ATX, which indicates that ATX is a viable alternative (Newcorn et al., 2008). Therefore, it is important to understand the mechanisms of the clinical effects of ATX, which are likely distinctive from psychostimulants. Only a few animal models were developed to address the mechanisms of action of ATX (Arime et al., 2011; Del'Guidice et al., 2014), but the underlying mechanisms remain unclear. A new mouse model for ADHD is required to precisely elucidate the therapeutic mechanism of ATX at the molecular level.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a highly conserved pleiotropic neuropeptide that functions as a neurotransmitter, neuromodulator and neurotropic factor (Hashimoto et al., 2006, 2011; Vaudry et al., 2009). PACAP-deficient ($PACAP^{-/-}$) mice display psychomotor abnormalities, including hyperactivity, increased novelty-seeking behavior, and deficient prepulse inhibition (PPI) (Hashimoto et al., 2001; Tanaka et al., 2006; Hattori et al., 2012; Ago et al., 2013). Amphetamine ameliorated the hyperactivity and deficient PPI in $PACAP^{-/-}$ mice, which suggests that $PACAP^{-/-}$ mice mimic the fundamental behavioral characteristics of ADHD (Tanaka et al., 2006). This study investigated the effects of ATX on the behavioral abnormalities of $PACAP^{-/-}$ mice.

EXPERIMENTAL PROCEDURES

Animals

All animal care and handling procedures were performed according to the Guidelines for the Care and Use of Laboratory Animals of the Japanese Pharmacological Society, and the Animal Care and Use Committee of the Graduate School of Pharmaceutical Sciences, Osaka University approved all procedures. All efforts were made to minimize the number of animals used. The generation of $PACAP^{-/-}$ mice using a gene-targeting technique was reported previously (Hashimoto et al., 2001). The null mutation was successively backcrossed to Crlj:CD1 mice (Charles River, Tokyo, Japan) at least 10 times for the PPI and novel object recognition tests. We also mated male $PACAP^{+/-}$ mice with a 129S6/SvEvTac (Taconic, Germantown, NY) background with female $PACAP^{+/-}$ mice with a C57BL/6J background (Shimizu Laboratory Supplies, Kyoto, Japan) to generate C57BL/6J and 129S6/SvEvTac hybridized $PACAP^{-/-}$ mice (F1) for the open field test and *in vivo* microdialysis (Hattori et al., 2012). All $PACAP^{-/-}$ mice and wild-type littermates ($PACAP^{+/+}$) (control mice) were obtained from the intercrossing of animals that were heterozygous for the mutant PACAP gene ($PACAP^{+/-}$). All experiments were conducted using 8 to 9-week-old male mice that were group-housed (4 to 5 per cage) with a 12-h light–dark cycle (light on at 8:00 am) at a controlled room temperature (22 ± 1 °C). Pelleted food (CMF, Oriental Yeast, Osaka, Japan) and water were available *ad libitum*, except during behavioral testing. All experiments were conducted at the same time each day, and different mice were used in each experiment.

Drugs

ATX hydrochloride was provided by Mitsubishi Tanabe Pharma Corp., Yokohama, Japan and dissolved in saline (0.9% NaCl). The drugs were injected intraperitoneally just before testing.

Open field test

Locomotor activity in the open field was quantified using an Acti-Track infrared photocell beam detection system (Panlab, Barcelona, Spain). The mice were placed in the center of plastic activity monitoring boxes (45 cm width × 45 cm depth × 30 cm height) following an injection of ATX or an equal amount of corresponding vehicle solution and parameters indicative of locomotor activity, such as distance traveled, were assessed for 90 min. Each mouse was tested individually and had no contact with other mice. The box was cleaned between tests.

Novel object recognition test

Novel object recognition test was performed as previously reported (Ago et al., 2013). Each mouse was habituated by housing in the test cage (22-cm width × 38-cm depth × 20-cm height) for 2 days. Two identical objects