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TAK-063, A PHOSPHODIESTERASE 10A INHIBITOR, MODULATES NEURONAL ACTIVITY IN VARIOUS BRAIN REGIONS IN phMRI AND EEG STUDIES WITH AND WITHOUT KETAMINE CHALLENGE

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Abstract—TAK-063 is a selective phosphodiesterase 10A (PDE10A) inhibitor that produces potent antipsychotic-like and pro-cognitive effects at 0.3 mg/kg (26% PDE10A occupancy in rats) or higher in rodents through the balanced activation of the direct and indirect pathways of striatal medium spiny neurons (MSNs). In this study, we evaluated the specific binding of TAK-063 using *in vitro* autoradiography (ARG) and the modulation of brain activity using pharmacological magnetic resonance imaging (phMRI) and electroencephalography (EEG). [^{3}H]TAK-063 significantly accumulated in the caudate—putamen (CPu), ventral pallidum (VP), substantia nigra (SN), hippocampus (Hipp), and amygdala (Amy), but not in the frontal cortex (Fcx), brainstem (Bs), or cerebellum (Cb) in an ARG study using rat brain sections. [^{3}H]TAK-063 accumulation in the CPu was more than eighteen-fold higher than that in the Hipp and Amy. TAK-063 at 0.3 mg/kg increased the blood oxygenation level-dependent (BOLD) signal in the striatum and Amy, and decreased it in the Fcx in a phMRI study with anesthetized rats. TAK-063 at 0.3 mg/kg significantly reduced the ketamine-induced increase in EEG gamma power both in awake and anesthetized rats. TAK-063 at 0.2 mg/kg (35% PDE10A occupancy in monkeys) also reduced the ketamine-induced increase in EEG gamma power in awake monkeys. In line with the EEG data, TAK-063 at 0.3 mg/kg reversed the ketamine-induced BOLD signal changes in the cortex, Bs, and Cb in a phMRI study with anesthetized rats. These data suggest that TAK-063 at about 30% PDE10A occupancy modulates activities of multiple brain regions through activation of neuronal circuits in rats and monkeys.

Key words: PDE10A, TAK-063, phMRI, EEG.

INTRODUCTION

The connectivity of the corticostriatal circuit enables sensory inputs to be associated with the output functions such as motor and cognitive responses (Shepherd, 2013). The circuit consists of a cortical and a striatal component (Hersch et al., 1995; Bolam et al., 2000), and the medium spiny neurons (MSNs) in the striatum are the principal cells that receive inputs from cortical components. MSNs project in two different directions, namely the direct and indirect pathways (Gerfen and Surmeier, 2011). These two pathways are considered to have a competing effect on the striatal outputs and on the consequent modulation of thalamic and cortical functions (Silkis, 2001).

Phosphodiesterase 10A (PDE10A) is a dual-substrate enzyme that hydrolyzes both cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) (Fujishige et al., 1999; Soderling et al., 1999). PDE10A is highly expressed in MSNs of the mammalian striatum (Soderling and Beavo, 2000; Seeger et al., 2003; Xie et al., 2006), and regulates the output function of both the direct and indirect pathways (Siuciak et al., 2006). Dysfunction of the corticostriatal circuit has been implicated in various central nervous system (CNS) disorders including schizophrenia; thus, pharmacological inhibition of PDE10A and the resulting activation of the corticostriatal circuit could be a promising therapeutic approach for these disorders (Kehler and Nielsen, 2011; Kehler, 2013).

TAK-063 is a potent, selective, and orally active PDE10A inhibitor (Kunitomo et al., 2014; Harada et al., 2015). Similar to other PDE10A inhibitors such as MP-10 (Schmidt et al., 2008; Grauer et al., 2009), TAK-063 showed potent antipsychotic-like effects in some rodent models of schizophrenia such as MK-801-induced hyperactivity, and lower risks of side effects than that showed by the current antipsychotics (Suzuki et al.,

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Abbreviations: Amy, amygdala; ARG, autoradiography; AUC, area under the curve; BOLD, blood oxygenation level-dependent; Bs, brainstem; cAMP, cyclic adenosine monophosphate; Cb, cerebellum; cGMP, cyclic guanosine monophosphate; CNS, central nervous system; CPu, caudate—putamen; EEG, electroencephalography; Fcx, frontal cortex; FFT, Fast Fourier Transformations; Hipp, hippocampus; ic, internal capsule; MSNs, medium spiny neurons; NMDA, *N*-methyl-D-aspartate; PDE10A, phosphodiesterase 10A; phMRI, pharmacological magnetic resonance imaging; PSL, photostimulated luminescence; SN, substantia nigra; VP, ventral pallidum.

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2015). Interestingly, TAK-063, but not MP-10, showed potent antipsychotic-like effects in methamphetamine-induced hyperactivity and prepulse inhibition deficits in rodents. Characterization of underlying mechanisms of action revealed that TAK-063 can induce more balanced activation of the direct and indirect pathways than MP-10 did via its faster off-rate from PDE10A (Suzuki et al., 2016). In addition, TAK-063 enhanced various cognitive functions, such as recognition memory, attention, impulsivity, working memory, and executive function, in naïve rats or in the *N*-methyl-D-aspartate (NMDA) receptor antagonist-induced rodent models of schizophrenia (Shiraishi et al., 2016). Thus, it is plausible that TAK-063 can modulate the function of multiple brain regions through the augmentation of striatal outputs.

In this study, we evaluated the detailed binding pattern of TAK-063 using *in vitro* autoradiography (ARG) with rat serial brain sections, and then investigated the effects of TAK-063 on brain activity by pharmacological magnetic resonance imaging (phMRI) in rats and electroencephalography (EEG) in rats and monkeys. EEG has a good temporal resolution and directly measures the neuronal activity although it has limited spatial resolution restricted to the cortical region close to the surface of the brain (Gloor, 1985). phMRI, on the other hand, has a whole-brain coverage but suffers from limitation of low temporal resolution; the blood oxygenation level-dependent (BOLD) response in phMRI is only indirectly linked to a neuronal activity (Leslie and James, 2000). The three methods together therefore should be highly complementary and help to reveal a fuller picture of the effects of TAK-063 on the brain function. To gain a better insight into the pharmacological profile of TAK-063 as a drug for schizophrenia, we also investigated the effect of TAK-063 on the signal produced by ketamine, an NMDA receptor blocker, that is known to induce schizophrenia-like symptoms in multiple species (Littlewood et al., 2006b; Pinault, 2008; Hodkinson et al., 2012; Doyle et al., 2013; Gil-da-Costa et al., 2013). Herein we report preclinical evidence that TAK-063, by binding to PDE10A, can modulate the neuronal activity in multiple brain regions through activation of neuronal circuits.

EXPERIMENTAL PROCEDURES

Animals

A total of 158 rats and six monkeys were included in the experiment. Male Wistar (CLEA Japan, Inc., Tokyo, Japan) and Sprague Dawley (SD) rats (Charles River Laboratories Japan, Inc., Yokohama, Japan, and Charles River, UK) were kept under standard laboratory conditions (12:12 h light/dark cycle) with food and water available *ad libitum*. Female cynomolgus monkeys (Macaca fascicularis, Keari Co., Ltd., Osaka, Japan) were kept under standard laboratory conditions and fed once daily with water available *ad libitum*. The care and use of the animals and the experimental protocols were in accordance with the guideline of Institutional Animal Care and Use Committee (Takeda Pharmaceutical Company Limited, Osaka and Kanagawa, Japan) and UK Animals (Scientific Procedures) Act of 1986 and the Ethical Review Panel of King's College London.

Chemicals and radioligand

TAK-063 and MP-10 succinate were synthesized by Takeda Pharmaceutical Company Limited (Kanagawa, Japan) (Verhoest et al., 2009; Kunitomo et al., 2014). [^{3}H]TAK-063 (37.0 MBq/mL in ethanol) was synthesized by Sekisui Medical Co., Ltd. (Tokyo, Japan). The specific radioactivity and radiochemical purity of [^{3}H]TAK-063 were 665 GBq/mmol and 98.1%, respectively. Ketamine hydrochloride (Daiichi Sankyo Propharma Co., Ltd., Tokyo, Japan, or Tocris Bioscience, Abingdon, UK), (+)-MK-801 hydrogen maleate (Sigma–Aldrich, St. Louis, MO), propofol (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan), and isoflurane (Abbott Laboratories, Irving, TX) were obtained commercially. TAK-063 was suspended in 0.5% (w/v) methylcellulose in distilled water for oral (p.o.) or 0.5% (w/v) methylcellulose in saline for intraperitoneal (i.p.) administration to rats. TAK-063 was dissolved in the following vehicle and administered intravenously (i.v.) to monkeys: 5% (v/v) N,N-dimethylacetamide, 10% (v/v) Ethanol, 30% (v/v) PEG-400, and 55% (w/v) Sulfobutylether-β-cyclodextrin. Ketamine was dissolved in saline and administered subcutaneously (s.c.) to rats or intramuscularly (i.m.) to monkeys.

Regions of interest (ROI) of the brain

ROI were set and abbreviated as follows by referring to the brain atlas (Paxinos and Watson, 1997): frontal cortex (Fcx), caudate—putamen (CPu), ventral pallidum (VP), hippocampus (Hipp), internal capsule (ic), amygdala (Amy), substantia nigra (SN), brainstem (Bs), and cerebellum (Cb).

In vitro ARG with rat brain sections

A total of four male SD rats were included in the experiment. *In vitro* ARG was conducted as previously described (Harada et al., 2015). The brains of SD rats were frozen, and then sagittal or serial coronal brain sections at 10.5, 7.6, 5.3, 3.8, 0.4 mm posterior and 3.2 mm anterior to the bregma were cut in a cryostat (Leica Microsystems, Wetzlar, Germany). After pre-incubation twice for 5 min in buffer (50 mM Tris–HCl pH 7.5, 1.7 mM EDTA, 6 mM $MgCl_2$, 120 mM NaCl, and 0.1% BSA) at room temperature, the sections were incubated in binding buffer (50 mM Tris–HCl pH 7.5, 1.7 mM EDTA, 6 mM $MgCl_2$, 120 mM NaCl, 0.1% BSA, and 0.03% Triton X-100) containing 20 nM [^{3}H]TAK-063 for 60 min at room temperature. Adjacent sections were used for the blocking study by adding MP-10 (final concentration of 1 μM). The sections were washed twice for 5 min at 4 °C in pre-incubation buffer, and then rapidly rinsed in ice-cold distilled water. The sections were dried under a stream of cool air, and were exposed to BAS IP TR 2040E imaging plates (GE Healthcare UK Ltd., Buckinghamshire, UK) for 7 days. The imaging plates were analyzed using an image analyzer and software (FLA-7000 and Image Gauge, Fujifilm, Tokyo, Japan). Radioactivity in each