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TAK-137, an AMPA receptor potentiator with little agonistic effect, produces antidepressant-like effect without causing psychotomimetic effects in rats

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ABSTRACT

Ketamine produces a rapid-onset antidepressant effect in patients with treatment-resistant depression (TRD), although it concurrently causes undesirable psychotomimetic side effects. Accumulating evidence suggests that ketamine produces antidepressant effects via activation of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA-R), with consequent activation of the mammalian target of rapamycin (mTOR) pathway and up-regulation of brain-derived neurotrophic factor (BDNF). We previously reported that TAK-137, an AMPA-R potentiator with little agonistic effect, had potent procognitive effects with lower risks of bell-shaped dose-response and seizure induction. In this study, we characterized the potential of TAK-137 as a novel antidepressant in rats. In rat primary cortical neurons, TAK-137 increased the phosphorylated form of Akt, extracellular signal-regulated kinase, mTOR, and p70S6 kinase, and dose-dependently increased the expression level of BDNF protein. The antidepressant-like effects of ketamine and TAK-137 were assessed on the day after final administration using the novelty-suppressed feeding test in rats. A single intraperitoneal administration of ketamine shortened the latency to feed. Under these conditions, oral administration of TAK-137 for 3 days shortened the feeding latency. Ketamine induced hyperlocomotion and reduced prepulse inhibition, which may be associated with psychotomimetic effects, while TAK-137 did not. TAK-137 may be a safer and rapid-onset therapeutic drug for the treatment of major depressive disorder, including TRD.

1. Introduction

Major depressive disorder (MDD) is one of the most prevalent psychiatric diseases, posing a considerable economic and social burden (McLaughlin, 2011; Sartorius, 2001). Despite the wide use of antidepressant medications, many patients do not achieve relief of depressive symptoms (Philip et al., 2010). A large-scale study, Sequenced Treatment Alternatives to Relieve Depression (STAR*D), showed that approximately 50% of patients with MDD responded to the first treatment, but only 30% reached a full remission (Gaynes et al., 2009). Furthermore, there is a delay of weeks or months in the onset of action even in the responders. Therefore, more effective medications are required based on a better understanding of the biological basis underlying MDD.

A single intravenous sub-anesthetic dose of ketamine, an *N*-methyl-D-aspartate receptor (NMDA-R) antagonist, had a rapid and sustained antidepressant effect in patients with treatment-resistant depression (TRD) (Berman et al., 2000; Murrough et al., 2013), although its abuse potential and dissociative properties limit the use of ketamine in clinical practice (Krystal et al., 1994). Two major hypotheses for the mechanisms of action of antidepressant activity of ketamine are via disinhibition of pyramidal neurons and via a metabolite of R-ketamine (2*R*,6*R*)-hydroxynorketamine (HNK)-mediated activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA-Rs). In

Abbreviations: AMPA-R, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ANOVA, analysis of variance; AUC, area under the curve; BDNF, brain-derived neurotrophic factor; ERK, extracellular signal-regulated kinase; GABAergic, gamma-aminobutyric acid-ergic; HBT1, 2-(((5-methyl-3-(trifluoromethyl)-1H-pyrazol-1-yl)acetyl)amino)-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxamide; HNK, hydroxynorketamine; MDD, major depressive disorder; MTOR, mammalian target of rapamycin; NBQX, an AMPA receptor antagonist; NMDA-R, *N*-methyl-D-aspartate receptor; NSF, novelty-suppressed feeding; PPI, prepulse inhibition; SD, Sprague-Dawley; STAR*D, Sequenced Treatment Alternatives to Relieve Depression; TAK-137, 9-(4-Phenoxyphenyl)-3,4-dihydropyrido[2,1-c][1,2,4]thiadiazine 2,2-dioxide; TRD, treatment-resistant depression; WKY, Wistar Kyoto

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the first hypothesis, ketamine is considered to produce paradoxical increases in glutamate release through the inhibition of NMDA-R on gamma-aminobutyric acid-ergic (GABAergic) interneurons (disinhibition of pyramidal neurons), resulting in AMPA-R activation (Duman, 2014). In the second hypothesis, (2*R*,6*R*)-HNK is considered to exert the antidepressant-like effects through early and sustained activation of AMPA-Rs (Zanos et al., 2016; Zanos et al., 2018) although some controversial observations regarding the antidepressant-like effects of (2*R*,6*R*)-HNK have been made (Shirayama and Hashimoto, 2018; Yamaguchi et al., 2018). In line with both of these proposed mechanisms, the antidepressant-like effects of ketamine in multiple animal models of depression were blocked by pretreatment with an AMPA-R antagonist NBQX (Koike et al., 2011; Maeng et al., 2008). Therefore, AMPA-R activation may play a key role in the antidepressant-like effect of ketamine. Moreover, the alterations of AMPA-R subunits have also been implicated in the pathophysiology of MDD. Reduced expression level of AMPA-R GRIA1 and GRIA3 mRNA were reported in perirhinal cortex (Beneyto et al., 2007), CA1, and dentate gyrus (Duric et al., 2013) in post-mortem tissues from depressed patients. AMPA-R activation may be a promising therapeutic approach as a novel therapy for the treatment of MDD, including TRD (Alt et al., 2006; Skolnick, 2008).

Preclinical characterization of the mechanisms of action of ketamine revealed that its antidepressant-like effects required activation of the mammalian target of rapamycin (mTOR) in the prefrontal cortex (Li et al., 2010) and activity-dependent brain-derived neurotrophic factor (BDNF) release in cortical neurons (Lepack et al., 2014). mTOR regulates axon growth, dendritic arborization, and synaptogenesis, which are crucial for synaptic plasticity (Cao et al., 2009). BDNF is known to promote neuronal survival and growth, and plays an important role in neuronal plasticity (Bathina and Das, 2015). Importantly, upregulation of mTOR and BDNF during the course of ketamine exerting its antidepressant-like effects was inhibited by NBQX (Lepack et al., 2014; Zhou et al., 2014). Moreover, AMPA-R activation has been demonstrated to increase neuronal BDNF expression and activate the mTOR signaling pathway (Jourdi et al., 2009). In line with these data, AMPA-R activation by AMPA-R potentiators has been shown to produce rapid antidepressant-like effects in animal models of depression (Fukumoto et al., 2014; Knapp et al., 2002). Therefore, ketamine may produce antidepressant activity via increases in synaptic plasticity through AMPA-R-mediated mTOR activation and BDNF release.

We recently discovered a novel AMPA-R potentiator with little agonistic effect, TAK-137 (Kunugi et al., 2019). TAK-137 had potentiator activities comparable to LY451646 in Ca^{2+} influx and AMPA-R-mediated currents in primary cultured hippocampal neurons, while it had much lower agonistic effects on them than LY451646. Preclinical characterization using rats and monkeys revealed that TAK-137 showed potent procognitive effects with lower risks for bell-shaped dose-response and seizure induction compared to LY451646 (Kunugi et al., 2019).

In this study, we explored the antidepressant-like effect of TAK-137 in rats to determine whether TAK-137 triggers activation of the mTOR signaling pathway and the production of BDNF in rat primary cortical neurons. The potential side effects of TAK-137 also were examined at doses exerting antidepressant-like effects.

2. Materials and methods

2.1. Animals

Female Sprague-Dawley (SD) rats and male Wistar Kyoto (WKY) rats were supplied by Charles River Laboratories Japan, Inc. (Yokohama, Japan) and maintained at the Takeda Pharmaceutical Company Limited. The animals were housed in a light-controlled room (12-h light/dark cycle, with lights on at 7:00 AM) and were habituated for > 1 week prior to experiments. The care and use of the animals and the experimental protocols used in this research were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company Limited. Animal care was in compliance with the Guide for Care and Use of Laboratory Animals. All efforts were made to minimize suffering and to reduce the number of animals used.

2.2. Drugs

TAK-137, 9-(4-Phenoxyphenyl)-3,4-dihydropyrido[2,1-c][1,2,4]thiadiazine 2,2-dioxide, was synthesized by Chemical Development Laboratories, Takeda Pharmaceutical Company Limited, and was suspended in 0.5% (w/v) methylcellulose in distilled water. Ketamine (Ketalar™) was purchased from Daiichi Sankyo Co., Limited (Tokyo, Japan) and diluted in 0.9% saline.

2.3. In vitro study

2.3.1. Preparation of rat primary neurons

Cultures of primary neurons were prepared from the cerebral cortex of E19 embryonic SD rats (Charles River Laboratories Japan, Inc.). Rat embryos were decapitated and the brains were removed. Cerebral cortices were dissected in ice-cold Hanks' Balanced Salt Solutions under a microscope and then triturated into cells using a neural cell dispersion kit (Sumitomo Bakelite, Tokyo, Japan). The dissociated cells were suspended in Neurobasal medium containing B27 supplement (Thermo Fisher Scientific, Waltham, MA), 2 mM L-glutamine (Lonza, Basel, Switzerland), 100 U/mL penicillin (Lonza), and 100 μg/mL streptomycin (Lonza) and 20 μg/mL gentamicin sulfate (Lonza). The cells were plated on poly-L-lysine-coated 12-well plates (Sumitomo Bakelite) at a density of 5.5×10^5 cells/well for analysis of the mTOR-related pathway and on poly-L-lysine-coated 96-well plates (Sumitomo Bakelite) at a density of 5×10^4 cells/well for BDNF protein measurement, and were cultured in a humidified CO_2 incubator with 5% CO_2 at 37 °C for 5-6 days and then used for the experiments.

2.3.2. Analysis of phosphorylation signals within the mTOR-related pathway

Primary cortical neurons were treated with AMPA (3 μM) and TAK-137 (0.1 and 1 μM) in culture medium and then cultured in a humidified CO_2 incubator with 5% CO_2 at 37 °C for 10 min for Akt and 60 min for extracellular signal-regulated protein kinase (ERK), mTOR, and p70S6 kinase (p70S6K). The cells were washed once with phosphate buffered saline and were collected using lysis buffer (50 mM Tris-HCl (pH 7.5)), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 2 mM EDTA, protease inhibitor cocktail (Roche, Mannheim, Germany), and a phosphatase inhibitor cocktail (Roche). Phosphorylated and total protein levels were measured using Alpha Screen Surefire Assay Kits (Perkin Elmer, Waltham, MA). For mTOR, total protein levels were measured using Pathscan Sandwich ELISA Kit (Cell Signaling Technology, Danvers, MA). Values were expressed as raw values minus background.

2.3.3. BDNF production in primary neurons

Primary cortical neurons were treated with TAK-137 in the presence or absence of AMPA (1 μM) and cultured in a humidified CO_2 incubator for 24 h. The cells were washed once in phosphate buffered saline and were resuspended in 60 μL of lysis buffer (20 mM Tris-HCl at pH 8.0, 137 mM NaCl, 10% glycerol, 1% NP-40, 1% protease inhibitor cocktail [Sigma-Aldrich, St. Louis, MO]). Concentrations of BDNF protein were measured using BDNF ELISA Kits (Promega, Fitchburg, WI).

2.4. Behavioral study

2.4.1. Novelty-suppressed feeding (NSF) test

Five-week-old male WKY rats were purchased and acclimated for about 6 weeks prior to the experiment. Animals were food-deprived and transferred to the test room overnight prior to testing. On the day of testing, each rat was placed in one corner of an open area

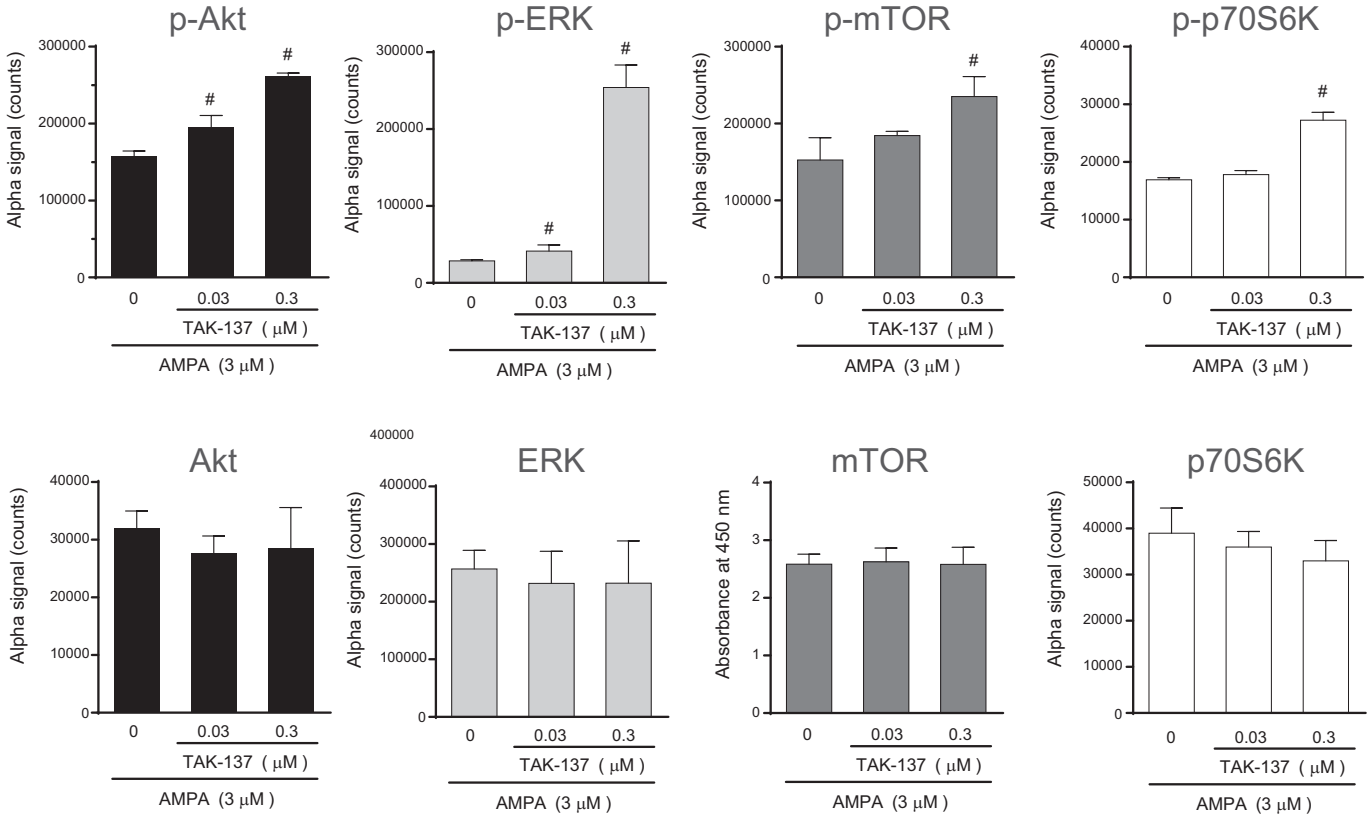

Fig. 1. Effect of TAK-137 on the mTOR signaling pathway in rat primary cortical neurons.
Cells were treated with AMPA (3 μM) and TAK-137 (0, 0.03, or 0.3 μM) for 10 min for Akt or 60 min for ERK, mTOR, and p70S6K, and were collected using lysis buffer. Values are expressed as the mean ± standard deviation ($n = 4$). Phosphorylated and total protein levels were measured using Alpha Screen Surefire Assay Kits, except total mTOR protein levels, which were measured using a Pathscan Sandwich ELISA Kit. Statistical significance was determined using one-way ANOVA followed by post-hoc Dunnett's multiple comparison tests with significance set at $^{\#}P \leq 0.05$ versus the vehicle-treated group.

(length × width × height: 60 × 60 × 30 cm) with a food pellet (CE-2; CLEA Japan, Tokyo) positioned in the center. The animal was removed immediately after feeding, or after 15 min had elapsed, whichever came first. The amount of time to take the first bite was recorded as the latency to feeding under 30 lx illumination. TAK-137 at 0.3 mg/kg, p.o. was administered for 1 or 3 days. A single dose of ketamine at 10 mg/kg, i.p. was administered. Testing was performed at 1 day after the last administration.

2.4.2. Measurement of locomotor activity

Eleven-week-old male WKY rats were placed separately into locomotor chambers (length × width × height: 24 × 37 × 30 cm) for > 60 min for habituation. The animals were removed, treated with either vehicle or test compounds, and quickly returned to the chamber. Locomotor activity was monitored with a SUPERMEX spontaneous motor analyzer (Muromachi Kikai CO., Ltd., Tokyo, Japan). Activity counts were acquired in 1-min bins and then data accumulated during the 3-h period following administration were analyzed.

2.4.3. Prepulse inhibition (PPI) of acoustic startle in rats

Experiments were performed using 11-week-old male WKY rats. Eight SR-lab acoustic startle chambers (San Diego Instruments, Inc., San Diego, CA) were used to assess PPI of acoustic startle in rats. TAK-137 (0.3 mg/kg, p.o.) and ketamine (10 mg/kg, i.p.) were administered to rats 2 h before testing and immediately before testing, respectively. During test sessions, individual animals were placed in the startle chamber and background noise (70 dB) was played. After a 5-minute acclimation period, each animal was presented with 54 trials with variable intertrial intervals (7 − 23 s). Three types of trials were administered: 1) a pulse only trial of 118 or 82 dB presented for 40 milliseconds, during which the startle response was recorded for 40 milliseconds beginning at the onset of a pulse; 2) prepulse trials consisting of 118 dB presented for 40 milliseconds, preceded 100 milliseconds earlier by a 20-millisecond prepulse of 76 or 82 dB, during which the startle response was recorded for 40 milliseconds beginning at the onset of the 118 dB stimulus; and 3) a no stimulus trial in which only the background noise was administered. Rats with an average maximum startle amplitude of ≤25 in the pulse only trials at 118 dB were excluded from data analyses. The percentage of PPI of the 76 dB or 82 dB prepulse was calculated using the following formula: [(average maximum startle on pulse-only trials at 118 dB − average maximum startle on prepulse trials)/average maximum startle on pulse-only trials at 118 dB × 100].

2.5. Statistical analysis

Statistical analyses were performed using GraphPad Prism 6.04 (GraphPad Software, Inc., San Diego, CA). Student's *t*-tests (for homogeneous data) or the Aspin-Welch test (for nonhomogeneous data) were performed to assess the statistically significant differences between 2 groups. Differences yielding P values ≤0.05 were considered statistically significant. In experiments with multiple doses of test compounds and the locomotor assay, statistical significance was analyzed using the analysis of variance (ANOVA). Post-hoc comparisons were made using Dunnett's test or Bonferroni's test when significant main effects were observed with significance set at $P \leq 0.05$.

3. Results

3.1. TAK-137 dose-dependently activated the mTOR signaling pathway and induced BDNF protein production in primary cortical neurons

AMPA-R-mediated mTOR activation and subsequent rapid synaptogenesis in the medial prefrontal cortex may play a key role in the mechanisms underlying the antidepressant effects of ketamine (Li et al., 2010). Thus, we investigated whether TAK-137 triggers activation of the mTOR signaling pathway in rat primary cortical neurons. As TAK-137 requires a receptor agonist to promote function of AMPA-R (Kunugi et al., 2019), this study was carried out with a low concentration (3 μM) of AMPA. TAK-137 significantly increased the levels of the phosphorylated Akt and ERK at 0.03 and 0.3 μM, and increased mTOR and p70S6K levels at 0.3 μM without influencing the levels of their total protein (Fig. 1).

BDNF is a key mediator for antidepressant effect by regulating neurogenesis, neuronal maturation, survival, and synaptic plasticity (Mondal and Fatima, 2018). In rat primary cortical neurons, TAK-137 at 0.1 and 1 μM in the presence of AMPA (1 μM) increased the BDNF protein levels approximately 2- and 5-fold, respectively, relative to vehicle. In contrast, TAK-137 in the absence of AMPA produced a significant but relatively small increase ($\sim < 2\times$ compared to vehicle) in the BDNF protein levels at 1 μM (Fig. 2).

3.2. TAK-137 shortened the latency to feed in the NSF test in rats

Antidepressant-like effects of ketamine and TAK-137 were evaluated by the NSF test using food-deprived rats. In this test, antidepressant-like effects are evaluated by measuring the latency to bite a food pellet in the novel environment (Marcussen et al., 2008). Chronic, but not acute, treatment with current antidepressants such as fluoxetine have been reported to shorten the latency to feed in the NSF test (Marcussen et al., 2008). Ketamine at 10 mg/kg, i.p. has been widely used to characterize its antidepressant-like activity in rats; plasma concentration of ketamine at 10 mg/kg, i.p. in rats was comparable to that used to detect antidepressant effect in patients with TRD in clinical studies (Shaffer et al., 2014; Zhou et al., 2014). TAK-137 at ≥0.1 mg/kg, p.o. induced potent cognitive enhancement in multiple cognitive domains in rats. Thus, ketamine at 10 mg/kg, i.p. and TAK-137 at 0.3 mg/kg, p.o. were used in the present experiments. Ketamine significantly reduced the latency to feed at 1 day after treatment (Fig. 3A). Under these conditions, 1-day treatment of rats with TAK-137 at 0.3 mg/kg, p.o. tended to shorten the latency to feed, but 3-day treatment significantly did it on the day after the final TAK-137 administration (Fig. 3B).

3.3. TAK-137 did not affect locomotor activity or PPI in rats

NMDA-R antagonist-induced hyperlocomotion and disruption of PPI are regarded as a surrogate marker for psychotic symptoms (Ginski and Witkin, 1994; van den Buuse, 2010). Ketamine produced a psychotomimetic effect in healthy individuals (Vollenweider et al., 2000). Thus, we investigated the potential side effects at a dose where they produced antidepressant-like effect in the NSF test. To evaluate locomotor activity following ketamine challenge, two-way ANOVA of mean activity counts showed significant effects for treatment ($F_{1,\ 14} = 10.66$, $p = 0.0056$) and time ($F_{47,\ 658} = 7.415$, $p < 0.0001$), and a significant interaction between these two factors ($F_{47,\ 658} = 3.945$, $p < 0.0001$) (Fig. 4A). Ketamine at 10 mg/kg, i.p. significantly increased accumulated activity counts during the 180 min following treatment in rats (Fig. 4B). To evaluate locomotor activity following TAK-137 challenge, two-way ANOVA of mean activity counts showed a significant effect for time ($F_{47,\ 658} = 1.681$, $p = 0.0036$) but not treatment ($F_{1,\ 14} = 0.1560$, $p = 0.6988$), and there was no significant interaction between these two factors ($F_{47,\ 658} = 1.133$, $p = 0.2552$) (Fig. 4C). TAK-137 at 0.3 mg/kg, p.o. did not increase accumulated activity counts during the 180 min following treatment (Fig. 4D). In the PPI test, ketamine at 10 mg/kg, i.p. significantly reduced the percentage of PPI at both the 76 and 82 dB prepulse intensities in WKY rats (Fig. 4E), whereas TAK-137 at 0.3 mg/kg, p.o. did not affect the percentage of PPI (Fig. 4F).

4. Discussion

Accumulating data indicated that ketamine produced its antidepressant activity by activation of AMPA-R through disinhibition of pyramidal neurons or by direct stimulation by the active metabolite (2*R*,6*R*)-HNK. Thus, activation of AMPA-R could be an alternative approach to producing potent antidepressant-like effects with a fast onset of action. AMPA-R potentiators can be categorized into two classes: low- and high-impact modulators. As low-impact AMPA-R potentiators do not induce BDNF production (Carmichael, 2012), we focused on an AMPA-R potentiator with the potential to induce BDNF release. LY451646, which can induce BDNF production, can induce bell-shaped dose-responses for various pharmacological effects and is associated with an increased risk of seizures. We hypothesized that the agonistic property of this molecule was related to bell-shaped responses and discovered the novel AMPA-R potentiator, TAK-137, which induced lesser agonistic effects (Kunugi et al., 2019). TAK-137 and LY451646 bind to the ligand binding domain of AMPA-R, and both inhibited

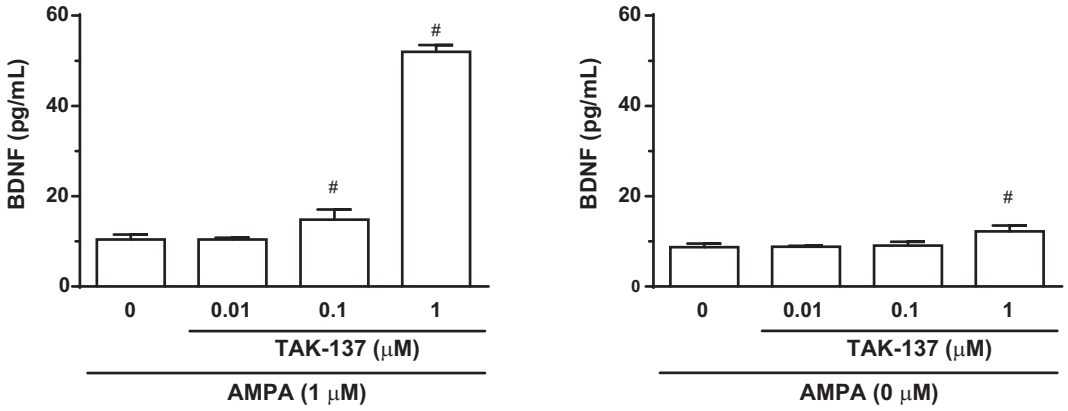

Fig. 2. Effect of TAK-137 on BDNF protein expression in rat primary cortical neurons.
Cells were treated with vehicle or TAK-137 (0.01, 0.1, or 1 μM) in the presence or absence of AMPA (1 μM) for 24 h and then were collected using lysis buffer. Values are expressed as the mean ± standard deviation ($n = 3$). Statistical significance was determined using one-way ANOVA followed by post-hoc Dunnett's multiple comparison tests with significance set at $^{\#}P \leq 0.05$ versus the vehicle-treated group.

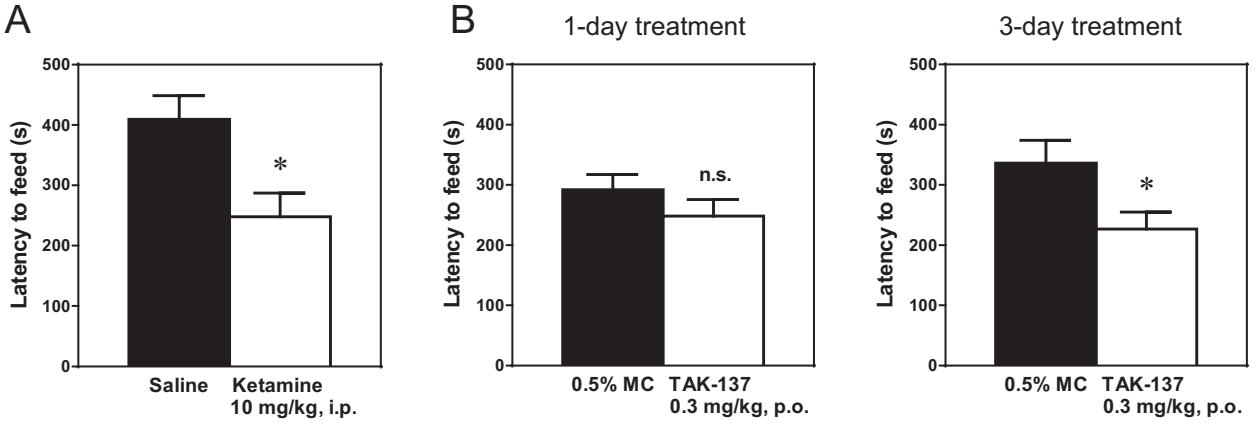

Fig. 3. Effect of ketamine and TAK-137 on latency to feed in the NSF test.
The latency to feed in the NSF test in the rats treated with (A) single treatment of ketamine (10 mg/kg, i.p.) ($n = 6$), (B, left panel) single ($n = 13$), or (B, right panel) 3-day ($n = 9$) treatment of TAK-137 (0.3 mg/kg, p.o.) was compared with that in vehicle-treated animals ($^{*}P \leq 0.05$, Student's *t*-test). The latency to feed was scored manually and is indicated as the mean ± SEM. n.s., not significant.

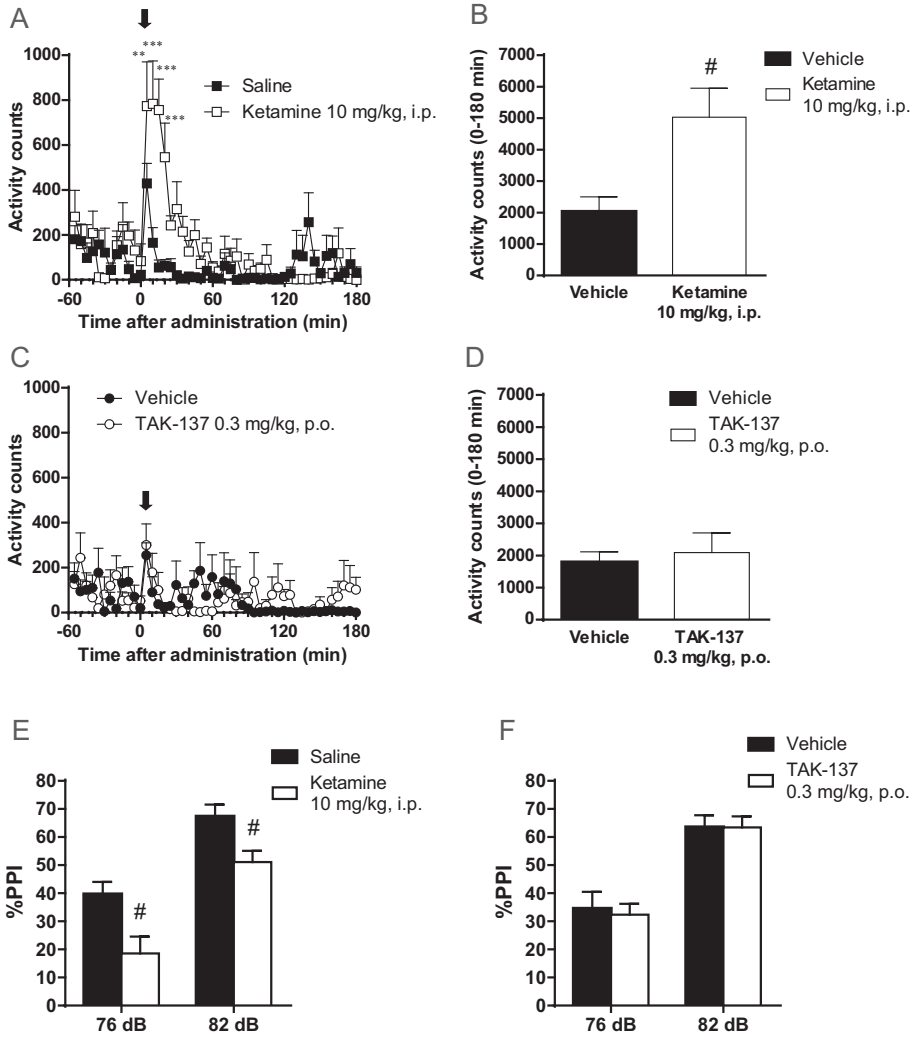

Fig. 4. Effect of ketamine and TAK-137 on locomotor activity and PPI.
Time course of locomotor activity induced by ketamine at 10 mg/kg, i.p. (A) or TAK-137 at 0.3 mg/kg, p.o. (C) was measured in 5-min intervals. The arrow indicates the drug injection time. A two-way repeated measures ANOVA followed by post-hoc Bonferroni's multiple comparison tests, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ as compared with control group. Total locomotor activities during the 180-min period following administration of ketamine (B) or TAK-137 (D) were calculated and are indicated as the mean ± SEM ($n = 6$). Statistical significance was determined using Aspin-Welch test with significance set at $^{\#}P \leq 0.05$.
PPI of the startle reflex after prepulse stimuli at two intensity levels (76 and 82 dB) was compared following administration of vehicle ($n = 8$ for ketamine-treated group or $n = 9$ for TAK-137-treated group) and (E) ketamine (10 mg/kg, i.p.) ($n = 7$) or (F) TAK-137 (0.3 mg/kg, p.o.) ($n = 9$). Values are expressed as the mean % PPI ± SEM. Statistical significance was determined using Student's *t*-test with significance set at $^{\#}P \leq 0.05$.

binding between [^{3}H]-HBT1 and the ligand binding domain with K_i values of 0.061 and 0.363 μM, respectively. In the presence of agonists, TAK-137 and LY451646 induced Ca^{2+} influx in GluA1i CHO cells with $LogEC_{50}$ values of −5.98 ± 0.021 and −6.10 ± 0.014 M, respectively, and in primary cultured hippocampal neurons with $LogEC_{50}$ (E_{max}) values of −6.36 ± 0.020 (126%) and −6.30 ± 0.193 (148%) M, respectively. Importantly, in the absence of AMPA, LY451646, but not TAK-137, robustly induced Ca^{2+} influx in primary neurons with a $LogEC_{50}$ (E_{max}) value of −5.23 ± 0.033 (86%) M, while the $LogEC_{50}$ (E_{max}) for TAK-137 was −5.64 ± 0.019 (10%) M. Consistent with our

hypothesis, TAK-137 had a wider exposure margin between cognitive improvement and no seizures than LY451646 in rats, as evidenced by the results showing that TAK-137 had 116-fold (AUC_{brain}) and 43.7-fold (brain C_{max}) exposure margins, while LY451646 had 3.1-fold (AUC_{brain}) and 7.5-fold (brain C_{max}) exposure margins. However, the lower agonistic effect on AMPA-R may lead to lower efficacy in antidepressant-like effects. In the present study, we characterized the antidepressant-like properties of TAK-137.

First, we characterized activation of the mTOR signaling pathway and BDNF production by TAK-137 in rat cortical primary neurons. Significant reductions in the mTOR signaling pathway were observed in the prefrontal cortex of MDD patients (Jernigan et al., 2011). Increased BDNF expression was found in the post-mortem brains from depressed subjects treated with antidepressant (Chen et al., 2001). Hence, these are also clinically well-characterized molecules in the pathophysiology of MDD, in addition to ketamine. Importantly, AMPA-R potentiators have been found to activate the mTOR pathway and BDNF production (Jourdi et al., 2009; Lauterborn et al., 2000). As expected, TAK-137 significantly elevated the phosphorylated forms of Akt, ERK, mTOR, and p70S6K in the presence of AMPA (3 μM) within 1 h. TAK-137 also elevated BDNF expression level in the presence of AMPA (1 μM) at 24 h after treatment. These results clearly demonstrated that TAK-137 was effective at stimulating the mTOR signaling pathway and BDNF production in cortical primary neurons that mimicked the effect of ketamine.

The current antidepressants take several weeks to achieve their therapeutic effects, while ketamine exerts a rapid antidepressant effect in patients with depression. Twenty-eight-day, but not single, administration of current antidepressants, and single treatment with ketamine, can produce antidepressant-like activity in the NSF test (Li et al., 2010; Marcussen et al., 2008). TAK-137 also was effective in the NSF test, suggesting promise for the compound for having the antidepressant-like activity. Ketamine is known to induce hyperlocomotion following acute treatment, which might interfere with an assessment of its antidepressant-like effect in preclinical behavior studies (Fig. 4A and B). Thus, we decided to select paradigm(s) in which the efficacy of ketamine could be detected after its plasma clearance (on 1 day after administration). Ketamine at 10 mg/kg, i.p. reduced latency to feed at 24 h after treatment, which mimicked the rapid antidepressant effect of ketamine in depressed patients. In this assay, administration of TAK-137 at 0.3 mg/kg, p.o. for 3 days significantly reduced feeding latency when animals were tested 24 h after the last dose administration. We previously demonstrated that repeated administration of TAK-137 for 4 days increased proliferation of progenitor cells (Kunugi et al., 2019), which may, in part, contribute to this antidepressant-like effect. Taken together, these findings suggest that TAK-137 produces antidepressant-like effects in rats, which is consistent with previous findings with other AMPA-R potentiators, such as CX546 and S47445, which also have been shown to be effective in the NSF test (Fukumoto et al., 2014; Mendez-David et al., 2017). The limitation of this study is that the antidepressant-like activity of TAK-137 was characterized using only the NSF test. Additional studies using other stress models such as learned helplessness or the chronic unpredictable mild stress model would further support the potential of TAK-137 as a novel antidepressant.

An adverse event of ketamine is its psychotomimetic effect, which limits its clinical usefulness (Browne and Lucki, 2013). The molecular mechanism underlying psychotic symptoms remains largely unknown, but moderate disinhibition via blockade of NMDA-R on GABAergic interneurons may be involved in psychotic symptoms (Farber, 2003). At the dose at which antidepressant-like effects were produced in the NSF test, ketamine but not TAK-137 caused a hyperlocomotor response and PPI reduction. Although additional assessments of TAK-137 in other models of psychotomimetic side effects are necessary to clarify relative margins between efficacy and safety, compounds such as TAK-137 could be promising antidepressant agents with reduced risks of psychotomimetic-like effects than ketamine.

AMPA-R activation can cause NMDA-R activation by removal of Mg^{2+} block upon strong depolarization. Therefore, AMPA-R activation could counteract the cognitive impairment induced by NMDA-R antagonists such as MK-801 and ketamine, possibly through the opposite direction of glutamate signals on the same neurons. AMPA-R potentiators reversed ketamine-evoked impairment in cognitive function, providing a promising therapeutic potential for the treatment of schizophrenia (Ranganathan et al., 2017). Importantly, for an antidepressant effect ketamine-evoked NMDA-R inhibition and AMPA-R activation may occur in different time frames on different types of neurons, such as GABAergic interneurons and postsynaptic neurons, respectively. Their cellular sites of action should be carefully considered in the interpretation of therapeutic outcome.

5. Conclusions

In conclusion, TAK-137, an AMPA receptor potentiator with little intrinsic activity, produced strong activation of the mTOR signaling pathway and increased BDNF protein production in primary cortical neurons in the presence of AMPA. Furthermore, TAK-137 produced an antidepressant-like behavioral effect similar to that of ketamine in a NSF test. These data support that enhancement of physiologically released glutamate-induced AMPA-R activation by TAK-137 is sufficient to produce antidepressant-like effect. Another AMPA-R potentiator with little agonistic effect, TAK-653 is currently under evaluation in clinical trials.

Declaration of Competing Interest

The authors are employees of Takeda Pharmaceutical Company Limited. The authors declare no other conflict of interests.

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