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Research paper

Preclinical predictors that the orthosteric mGlu2/3 receptor antagonist LY3020371 will not engender ketamine-associated neurotoxic, motor, cognitive, subjective, or abuse-liability-related effects

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ABSTRACT

The novel mGlu2/3 receptor antagonist, LY3020371, has been shown to produce antidepressant-like effects comparable to that of the clinically-effective antidepressant ketamine. In the present study, we investigated whether LY3020371 would be predicted to be free of the side-effects and safety pharmacology issues associated with ketamine. In contrast to ketamine, LY3020371 produced small increases in locomotion and did not impair motor performance on an inverted screen. Ketamine, but not LY3020371, increased dopamine efflux in the nucleus accumbens of rats. Ketamine also produced cognitively-impairing effects in rats in a T-maze and in a psychomotor vigilance task and altered theta synchrony between the hippocampus and mPFC, whereas LY3020371 had either no significant impact or lesser effects in these assays. In mice, ketamine, but not LY3020371, negatively affected spontaneous alternation in a Y-maze. Rats were trained to discriminate LY3020371 from vehicle where 30 mg/kg produced 100% drug-appropriate responding and the ED_{50} for LY3020371 was 9.4 mg/kg, i.p. In rats discriminating LY3020371, neither *d*-amphetamine nor phencyclidine fully substituted for LY3020371 (35–45%) and the mGlu2/3 receptor agonist LY354740 partially attenuated the discriminative stimulus effects of LY3020371. These are the first data to demonstrate the discriminative stimulus effects of an mGlu2/3 receptor antagonist. Some alterations were suggested to occur in the density of mGlu2/3 receptor binding sites in the drug discrimination rats relative to their age-matched non-drug-exposed controls. In preclinical toxicology studies of 14 day dosing of doses up to 1000 mg/kg, i.v. in rats and up to 500 m/kg, i.v. in Cynomologous monkeys, LY3020371 produced uM plasma exposures without producing critical toxicological findings. It is concluded that LY3020371 does not recapitulate the motor, cognitive, subjective, neurochemical, electrophysiological, or toxicological findings reported with ketamine. Thus, LY3020371 possesses both the efficacy signatures of a rapidly-acting antidepressant and a safety profile enabling proof of concept studies in patients.

Since its introduction in 1998 (Ornstein et al., 1998a,b), the mGlu2/3 receptor antagonist, LY341495 (Kingston et al., 1998), has been the principle research tool for studying the functional significance of this class of metabotropic glutamate receptors. A new selective antagonist of mGlu2/3 receptors, the bi-cyclohexane amino acid, LY3020371, was recently described (Smith et al., 2012; Chappell et al., 2016). This molecule demonstrates low nM affinity and full orthosteric antagonism of mGlu2 and mGlu3 receptors in human cloned systems that translate to functional blockade of mGlu2/3 agonist-driven effects in native rat and human tissue preparations (Witkin et al., 2016). Since this novel antagonist displayed the physiochemical and pharmacological properties for an ideal small molecule antagonist, we provided a characterization of its effects on behavior, neurochemistry, electrophysiology, and metabolomics profile. In this in vivo work, we focused on biological outputs shown historically to be predictors of antidepressant activity

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; (*S*)-3,4-DCPG, (*S*)-3,4-dicarboxyphenylglycine; LY341495, (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid; mGlu, metabotropic glutamate; LY354740.H_2O, bicyclo[3.1.0]hexane-2,6-dicarboxylic acid, 2-amino-, (1*S*,2*S*,5*R*,6*S*)-(9CI)·H_2O; LY379268, 2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid, 4-amino-, (1*R*,4*R*,5*S*,6*R*)-(9CI); LY404039, agonist (−)-(1*R*,4*S*,5*S*,6*S*)-4-amino-2-sulfonylbicyclo [3.1.0] hexane-4,6-dicarboxylic acid; MGS0039, 2-amino-3-[(3,4-dichlorophenyl) methoxy]-6-fluoro-, (1*R*,2*R*,3*R*,5*R*,6*R*)-(9CI)-Bicyclo[3.1.0]hexane-2,6-dicarboxylic acid; LY459477, (1*S*,2*S*,4*R*,5*R*,6*S*)-2-amino-4-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid; NBQX, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-(9CI)-benzo[*f*]quinoxaline-7-sulfonamide.

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comparable to that of ketamine (Witkin et al., 2017). The rationale for this therapeutic area emphasis was based upon the hypothesis that blockade of mGlu2/3 receptors would provide rapid-onset and large-effect size efficacy in treatment-resistant depressed (TRD) patients (c.f., Chaki et al., 2004; Alt et al., 2006; Witkin and Eiler, 2006; Pałucha-Poniewiera et al., 2010; Fukumoto et al., 2016).

The data supporting the potential efficacy of LY3020371 in TRD rests on the biological commonalties existing between mGlu2/3 receptor antagonists and ketamine, a drug well-documented for its antidepressant efficacy as predicted by Trullas and Skolnick (1990) and originally confirmed by the clinical work of Berman et al. (2000) and Zarate et al. (2006) (see Abdallah et al., 2015). The biological overlap in the effects engendered by both mGlu2/3 receptor antagonism and blockade of NMDA receptors, via ketamine, include the induction of glutamate release, amplification of AMPA receptor function, downstream impact upon mTor pathways, and the ultimate generation of synapses and functional enhancement by those morphological changes and behavioral changes that are consistent with an antidepressant action (Li et al., 2010; Dwyer et al., 2012, 2013; Witkin et al., 2016, 2017; however, see also Popp et al., 2016). In our characterization of the antidepressant potential of LY3020371 (Witkin et al., 2017) and LY341495 (Witkin et al., 2016), we demonstrated that both ketamine and LY3020371 increased the probability of dopamine cell firing in the ventral tegmental area, increased multiple antidepressant-relevant neurotransmitters in the medial prefrontal cortex, and enhanced oxygen availability in the anterior cingulate cortex of rats (Witkin et al., 2017). LY3020371 as well as ketamine also enhanced wakefulness and produced behavioral effects in the forced-swim assay like that produced by conventional antidepressants; these effects were directly related to drug concentrations in cerebral spinal fluid (Witkin et al., 2017). In alignment with the known AMPA receptor-dependent effects of both ketamine and mGlu2/3 receptor antagonists (c.f., Alt et al., 2006; Maeng et al., 2008; Witkin et al., 2016, 2017), we also provided metabolomics data that independently predicted AMPA receptor GluA2 amplification by ketamine and by LY3020371 (Witkin et al., 2017).

In addition to its beneficial effects in TRD patients, ketamine produces a host of side-effects that critically limit its long-term utility as a therapeutic agent. These effects include motor incoordination, psychotomimetic effects typical of dissociate anesthetics, abuse liability, memory impairment, and nerve cell loss (Iadarola et al., 2015; Kim et al., 2016). Since ketamine and LY3020371 produce other biological effects in common with one another as just summarized (Witkin et al., 2017), it is imperative to provide, in addition, preclinical predictors of side effect potential. We hypothesize that LY3020371 will not produce preclinical behavioral, neurochemical, or toxicological effects like ketamine. We make this conjecture since mGlu2/3 receptor antagonists do not block the NMDA receptor ion channel like ketamine and thereby do not directly impact pyramidal nerve cell firing via consequent inhibition of fast-spiking GABA interneurons as shown with NMDA receptor antagonists. Based upon the data presented in the present report, we conclude that preclinical markers of ketamine-like motor impairment, memory impairment, subjective effects, abuse liability, and brain lesions are not found after dosing with LY3020371 or its oral prodrug LY3027788 in rats or Cynomologous monkeys.

1. Materials and methods

All work was done in accordance with "The principles of laboratory animal care" (NIH publication No. 85-23, revised 1985). Furthermore, an internal Animal Care and Use Committee approved all research protocols and monitored compliance.

1.1. Locomotor activity

Locomotor activity was measured with a 20 station photobeam activity system (San Diego Instruments, San Diego, CA, USA) with seven photocells per station. Locomotor activity was recorded as the number of ambulations, where ambulation was defined as the breaking of adjacent photobeams. Male SD rats were placed individually into polypropylene cages (40.6 × 20.3 × 15.2 cm, no bedding). A habituation period occurred for the first 60 min the rats were in the chambers. Rats were then removed, weighed, injected (i.v., tail vein) with vehicle, LY3020371, ketamine, or 3 mg/kg *d*-amphetamine and returned to the locomotor arena. Consecutive breaks of the photobeams were recorded for the next 30 min. Data were analyzed by one-way ANOVA followed by Dunnett's test with alpha set at 0.05.

1.2. Inverted-screen test

Male, Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were used and weighed 90–110 g. The rats were dosed with test compound and returned to their home cage. Twenty five min after pre-treatment, animals were tested on the inverted screen and were scored after 60 s as follows: 0 = climbed over, 1 = hanging on to screen, 2 = fell off.

1.3. Dopamine and dopamine metabolite efflux in the shell of the nucleus accumbens and associated locomotor activity

Male Sprague Dawley rats (Taconic Farms, Inc., Albany, NY) with body weights of 250 to 300 g were used for microdialysis studies. After acclimatization for at least 1 week, rats were anesthetized with isoflurane to allow stereotaxic implantation of microdialysis guide cannulas (BioAnalytical Systems (BAS), West Lafayette, IN MD2250 or MD2251) fixed in place using 3 bone screws and Trim II dental acrylic (Henry Schein, Inc., Denver, PA). Surgeries were vendor performed by Taconic Farms, Inc. as described below.

During the surgery, rats were maintained under anesthesia with isoflurane (3%) and placed in a stereotaxic apparatus. A single burr hole was made and a microdialysis guide cannula was inserted into a specific brain region and cemented to the skull with dental acrylate. Stereotaxic coordinates for the shell of the nucleus accumbens cannula were as follows: A (anterior to bregma), 1.7 mm; L (lateral from the midsagittal suture, right side) −0.8 mm, and V (ventral from the skull surface), −6.0 mm (Paxinos and Watson 1986). Vendor surgeries were performed 3 to 7 days prior to shipment to our facility and allowed to acclimate for a period of 4 to 5 days prior to study initiation.

<24 h before the experiment the guide in the cannula was replaced with the microdialysis probe which was affixed in place with glue while gently restraining the rat. Dialysis probes from BioAnalytical Systems (BAS; West Lafayette, IN MD2200) of the pin-type made of polyacrylonitrile, (MWCO = 30.000 Da) and a dialysis tubing length of 2 mm were used. Rats were acclimated to the test chamber (*SmartFrame* Open Field System, Kinder Scientific, Poway California, USA) the day before the microdialysis assessment. The open field system consisted of a square rack configuration of 32 photobeams arranged in an 16 × 16 formation, at a height of 5 cm. The input tube of the dialysis probe is connected to a syringe pump (BeeHive and BabyBee, BAS) which delivers an artificial cerebrospinal fluid containing 150 mM NaCl, 3 mM KCl, 1.7 mM CaCl2 and 0.9 mM MgCl2 (pH 6.0) to the probe at a rate of 0.8 μL/min overnight. Syringes are refilled the next morning with aCSF and the perfusion rate increased to 1.5 μL/min and the output tubes from the rats are attached to a refrigerated fraction collector (HoneyComb, BAS). After a period of about 2 h in order to establish stable baseline values, collection of 30 min fractions is started. Four baseline samples are collected before injection of any drugs.

LY3020371 was dissolved in distilled water and pH adjusted to 6 to 8 with 5 N NaOH and administered (1 mL/kg; i.p. route) at 1, 3, or 10 mg/kg. *S*-(+)-Ketamine HCl, (Lot number 021K12931, Sigma-Aldrich, St. Louis, MO), was dissolved in 0.9% physiological saline and administered (1 mL/kg; s.c. route) at 25 mg/kg. The vehicle control group for both compounds was 0.9% physiological saline. The dose range of drugs was chosen based on their potency to affect a monoamine change