163.03

Efficacy of CV-0000042, a Novel M4 Receptor Full Agonist, and CV-0000071, a Novel M4 Receptor Partial Agonist, in Preclinical In Vivo Models of Psychosis

Polina Stolyar,[1] Julianna Locantore,[1] Georgette Suidan,[1] Srinivas Chakilam,[1] Hanh Nguyen,[1] Philip Iredale[1]

[1]Cerevel Therapeutics, Cambridge MA, USA

Presenting Author: Polina Stolyar, Polina.Stolyar@Cerevel.com

SUMMARY

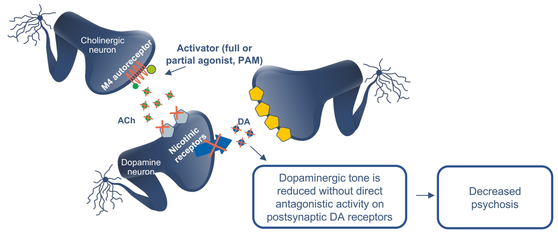
- This study evaluated the preclinical antipsychotic properties of M4 agonism using a novel M4 full agonist (CV-0000042) and a novel M4 partial agonist (CV-0000071)
- While both the full and partial agonists produced decreases in amphetamine-stimulated increases in ACh, only CV-0000042, the full agonist produced robust effects in both preclinical models of psychosis, aLMA, and CAR, leading to postulate that the degree of agonism may be an important factor in the selection of indication

ACKNOWLEDGMENTS: Thank you to Charles River (South San Francisco, CA, USA) for running locomotor activity and microdialysis studies. Also, thank you to Biotrial (Rennes, France) for running the CAR study.

Presented at Society for Neuroscience (SfN)
November 12-16, 2022 • San Diego, CA, USA

INTRODUCTION

- A primary role for the M4 muscarinic acetylcholine receptor (M4 mAChR) is as an inhibitory autoreceptor; the activation of M4 inhibits acetylcholine (ACh) release and regulates the transmission of dopamine (DA)
- Striatal M4 receptor activation inhibits D1-mediated increases in rodent locomotor activity
- Xanomeline, M4/M1–preferring agonist, showed promising clinical activity in psychosis-related indications but was hindered by side effects such as nausea, vomiting, and dyspepsia, likely driven by nonselective muscarinic agonism



OBJECTIVE

- To evaluate preclinical antipsychotic properties of M4 agonism while minimizing off-target side effects using the novel M4 full agonist CV-0000042 and novel M4 partial agonist CV-0000071

METHODS

DOSING

- CV-0000042 and CV-0000071 were dosed subcutaneously at 1 mL/kg for rat locomotor activity studies. Both compounds were dosed at 5 mL/kg for the mouse microdialysis study and at 2mL/kg for the rat conditioned-avoidance response study
- All animal experiments were conducted in accordance with institutions' Institutional Animal Care and Use Committee or equivalent ethics committee(s)

SPONTANEOUS LOCOMOTOR ACTIVITY (sLMA) FOR BOTH COMPOUNDS AND AMPHETAMINE-STIMULATED LOCOMOTOR ACTIVITY (aLMA) FOR CV-0000071

- Locomotor activity was recorded using a photobeam detection system. Experiments were carried out under normal lighting conditions. Male Sprague Dawley rats were habituated to the testing room for at least 60 minutes, after which rats undergoing aLMA were then habituated to the test chamber for 90 minutes; rats undergoing sLMA were not. At time zero, the rats (n=8) were injected with either vehicle or compounds alone for sLMA or in combination with vehicle or 1 mg/kg d-amphetamine for aLMA - vehicle, CV-0000042, CV-0000071, or haloperidol were administered subcutaneously while vehicle or amphetamine were administered interperitoneally. Post-study rats were euthanized via CO_2 and plasma and brain were collected and evaluated for compound exposure via HPLC with tandem mass spectrometry

AMPHETAMINE-STIMULATED LOCOMOTOR ACTIVITY (aLMA) FOR CV-0000042

- Locomotor activity was recorded via sets of 16 grid-forming photosensor beams in 5-min bins over a period of 60 minutes. Male Sprague Dawley rats (n=10/group) were randomized and habituated to the testing room for an hour prior to testing. All compounds were administered subcutaneously (CV-0000042, vehicle, or haloperidol 40 min prior to testing, and 0.5 mg/kg d-amphetamine or saline immediately before testing). Immediately after testing, the randomly selected n=4/group were euthanized via CO_2 asphyxiation, then blood was collected via cardiac puncture and spun down to plasma, which was analyzed for concentration of compound via LC-MS

CONDITIONED AVOIDANCE RESPONSE (CAR)

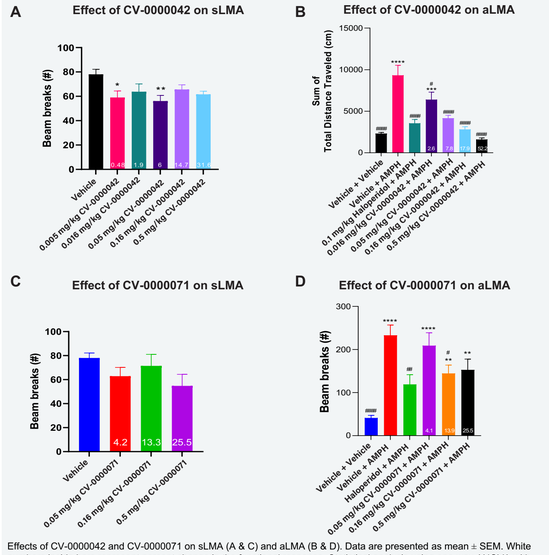
- Male Wistar rats were trained and tested in a computer-assisted, two-way active avoidance apparatus with a 4-min habituation followed by 30 trials separated by 20-30 second intervals. Each trial consisted of 10 seconds of white noise (conditioned stimulus [CS]) followed by a 10-second shock (unconditioned stimulus [US]) in the presence of sound. When the animal moved to the other compartment during the CS, the sound was terminated, no shock was delivered, and the response was recorded as an avoidance. When the animal did not change compartments during the US, the response was recorded as an escape failure. Training was carried out 5 days/week and only rats that reached a criteria of 80% avoidance in at least 2 consecutive days were used for compound experiments. Compounds were administered subcutaneously 30 minutes prior to testing. A crossover design was used with a minimum of a 48-hour washout period between treatments, during which animals were retrained to criteria; n=7-8 rats were used in the studies. A satellite group of rats (n=3) was used for plasma exposure analysis

MICRODIALYSIS

- I-shaped microdialysis probes (3-mm exposed polyacrylonitrile membrane [CRL, the Netherlands]) were inserted into the right striatum of adult male C57Bl/6 mice. One day after surgery, probes were perfused with aCSF containing 147 mM NaCl, 3.0 mM KCl, 1.2 mM $CaCl_2$, and 1.2 mM $MgCl_2$ at a flow rate of 1.5 µL/min. Microdialysis samples were collected for 30-minute periods by an automated fraction collector. After perfusion stabilization and collection of basal dialysate samples, mice received treatments (T = 0 min). Dialysate samples were collected for an additional 180 minutes following treatment. Concentrations of ACh were determined by HPLC with MS/MS detection using ACh-d9 as internal standard. Compounds were dosed at time zero in the same fashion as during the sLMA studies, with n=8/group

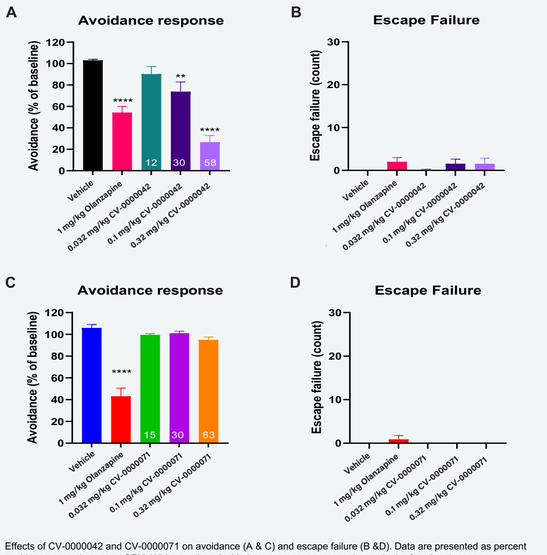
RESULTS

Figure 1. Locomotor activity.



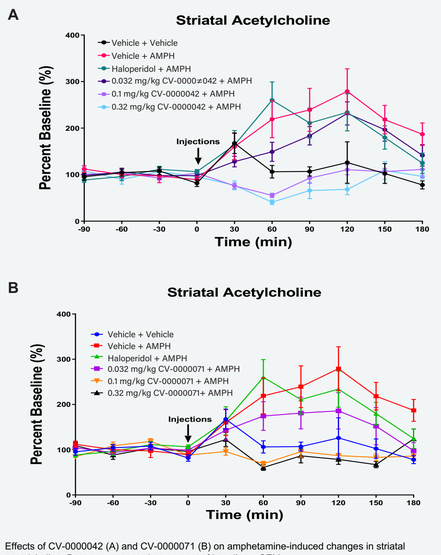
Effects of CV-0000042 and CV-0000071 on sLMA (A & C) and aLMA (B & D). Data are presented as mean ± SEM. White numbers inside bars represent percent change in the functional response. Statistical analysis using one-way ANOVA with Dunnett's multiple comparisons test; *,**,***,****P<0.05, P<0.01, P<0.001, P<0.0001 vs vehicle; #, ##, ###, ####P<0.05, P<0.01, P<0.001, P<0.0001 vs vehicle + AMPH group.

Figure 2. Conditioned avoidance response.



Effects of CV-0000042 and CV-0000071 on avoidance (A & C) and escape failure (B &D). Data are presented as percent avoidance or total counts ± SEM. White numbers inside bars represent percent change in the functional response. Statistical analysis using one-way ANOVA with Dunnett's multiple comparisons test; *,**,****P<0.01, P<0.0001 vs vehicle.

Figure 3. Microdialysis.



Effects of CV-0000042 (A) and CV-0000071 (B) on amphetamine-induced changes in striatal acetylcholine. Data are presented as percent of baseline ± SEM.

Full agonist CV-0000042

Partial agonist CV-0000071