Methylone, a rapid-acting entactogen with robust anxiolytic and antidepressant-like activity

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Abstract

Serotonin reuptake inhibitors (SRIs) represent the first line pharmacological treatment for a variety of neuropsychiatric illnesses, including post-traumatic stress disorder (PTSD) and major depressive disorder (MDD). They are slow-acting antidepressants (SAADs) with a delayed onset of action, so most patients do not show significant effects until at least 4 weeks, and often up to 8 weeks, of continuous treatment. SRIs are also associated with impairing side-effects and, even when optimally delivered, 40% of the patients do not respond. Methylone (3,4-methylenedioxy-N-methylcathinone; also known as MDMC, bk-MDMA and M1) is a beta-ketone analog of MDMA and a rapid-acting entactogen (RAE) that improved symptoms in 81% of patients in a clinical case series of 21 individuals with severe PTSD (Kelmendi et al., 2022). In the current study, we employ the Forced Swim Test (FST), a classic and widely used screen for antidepressants, to explore the effect of methylone. Antidepressants consistently reduce immobility in the FST. Here, we investigate the antidepressant-like activity of methylone compared with the prototypical selective SRI (SSRI), fluoxetine, and with novel rapid-acting antidepressants such as ketamine, psilocybin, and MDMA. Results demonstrate that methylone produced a rapid, robust, dose-dependent antidepressant-like response in the FST. A single dose of methylone (15 mg/kg, IP) reduced immobility by 99% compared to controls ($p<0.001$) compared to a 54% reduction with three doses of fluoxetine (3 x 10 mg/kg, IP). At this dose, methylone also significantly increased swimming and not climbing behavior in the FST, consistent with serotonergic activity (Detke et al., 1995). In addition, the effect of a single dose of methylone persisted for at least 72 hours post-dose compared with fluoxetine, which had a behavioral response that only lasted for 1-hour post-dose. We also explored effects of methylone on monoamine transporter binding, uptake, and release. Taken together, and consistent with the recent clinical case series, our results suggest that methylone may have clinical utility in the treatment of PTSD, MDD and other central nervous system disorders.

Introduction

Methylone is an entactogen and a beta-ketone analog of MDMA currently in development for the treatment of PTSD. Methylone was synthesized over 25 years ago, but the literature describing its properties is relatively sparse, focused largely on *in vitro* studies or binge-dosing regimens that mimic its illicit use. Methylone shares some chemical and pharmacological properties with MDMA, but also has some differences. For example, methylone is a serotonin (5HT), norepinephrine (NE), and dopamine (DA) reuptake inhibitor and releaser like MDMA, but with 3-4x lower potency for inhibition of serotonin uptake (Baumann et al., 2012).

Clinical experience with methylone has been described in three studies, showing it is well-tolerated, produces a milder range of effects compared with MDMA (Poyatos et al., 2021) and alleviates symptoms of PTSD and MDD in two retrospective clinical case series (Kelmendi et al., 2022; Averill et al., under review). It is notable that MDD and anxiety show high rates of comorbidity with PTSD and SSRIs are used to treat all three disorders, suggesting the reported effects of methylone in PTSD may translate to therapeutic benefit in multiple CNS disorders.

The current study was undertaken to test whether methylone showed rapid-onset antidepressant and anxiolytic activity in preclinical models of depression and anxiety. Moreover, since SSRI antidepressants interfere with the clinical response to MDMA, we evaluated whether co-administration of an SSRI with methylone affected the behavioral antidepressant-like response.

Methods

Animals: Male Sprague Dawley rats weighing 180-200g on arrival, were used for all behavioral studies, which took place at Melior Discovery. Rats acclimated to their home cages for at least one week before testing, were maintained in a controlled environment on a 12h light/dark cycle, with no more than 2 rats per cage. Animals received *ad libitum* access to standard rodent chow and water and were assigned randomly to treatment groups. All animal use and procedures were in accordance with established protocols approved by the Melior IACUC committee, Melior Standard Operation Procedures (SOP), and Transcend Therapeutics.

Drug treatments: Methylone HCl (0.5-30 mg/kg) or Fluoxetine HCl (10 mg/kg) were formulated in sterile 0.9% saline vehicle before intraperitoneal (IP) administration. Control animals received saline vehicle.

Forced Swim Test (FST): All studies were performed and scored by an experimenter blind to treatment group according to standard protocols at Melior Discovery. Briefly, rats were placed in a circular plexiglass container filled with water. Water was maintained at 22-25ºC and was changed for every animal. Day 1 (Training) consisted of a 15 min acclimation trial, and Day 2 (Testing, 24 h later) consisted of the 5 min test. A time sampling procedure was employed where animals were observed every 5 seconds for the duration of the test session (60 counts or 5 minutes) and scored for immobility (defined as the failure to struggle), swimming (defined as a circular movement around the tank), or climbing (defined as an upwards escape behavior). Data are expressed as the percent of the testing session (e.g., the number of immobility counts divided by 60).

Open Field Test (OFT): All studies were performed by an experimenter blind to treatment group and according to standard protocols at Melior Discovery (Exton, PA). The OFT was used to assess both locomotor activity and anxiety-like behavior. After habituation to the testing room and drug injection, rats were assessed for 30 minutes in the OFT using an automated activity monitoring system (MedAssociates). Locomotor activity was measured by recording the total ambulatory distance traveled (cm), reported in 5-min bins for the duration of the 30 min testing period. Time spent in the center of the open field, a measure of anxiety, was also recorded for the 30 min testing period.

Statistical Analysis: All data are presented as the mean ± SEM. Differences between two groups were determined by unpaired t-test, differences between more than two groups were determined by one-way ANOVA and post-hoc multiple comparison test noted in Figure Legends. When there were two different variables (drug x time), differences were determined by two-way ANOVA and Bonferroni post-hoc multiple comparison test. A p-value ≤ 0.05 indicated statistical significance. All analyses were completed using Graphpad Prism software version 9.3.1 (San Diego, CA).

Results

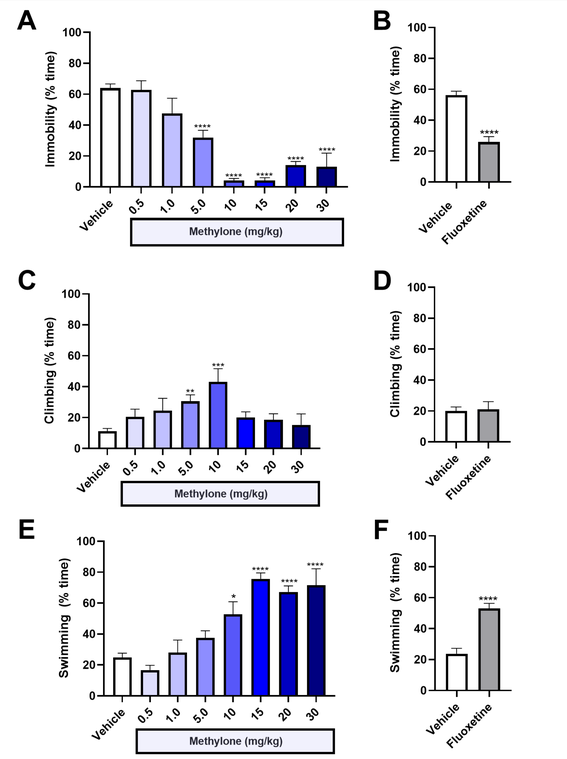

Figure 1. Methylone has a rapid-acting and robust antidepressant-like response in the Forced Swim Test. Methylone or Vehicle was administered 30 min prior to testing. Fluoxetine or Vehicle were administered 23.5, 5, and 1h prior to testing. Quantification of the percent time spent (A-B) immobile, (C-D) climbing, or (E-F) swimming during the 5-minute test session is shown. Data are presented as means ± SEM. *p<0.05, ***p<0.001, ****p<0.0001 vs. Vehicle control group; N=6-16 per group.

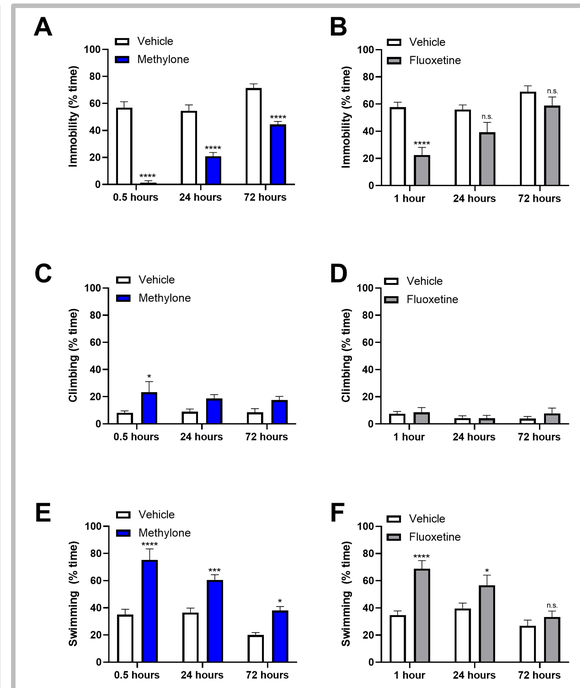

Figure 2. The effect of a single dose of methylone is long-lasting. Methylone or Vehicle was administered 30 min prior to forced swim testing. Fluoxetine or Vehicle were administered 23.5, 5, and 1h prior to testing. Animals were retested 24h or 72h later. Quantification of the percent time spent (A-B) immobile, (C-D) climbing, or (E-F) swimming during each 5-minute test session is shown. Data are presented as means ± SEM. *p<0.05, ***p<0.001, ****p<0.0001 vs. Vehicle control group; n.s. not significant; N=6-8 per group.

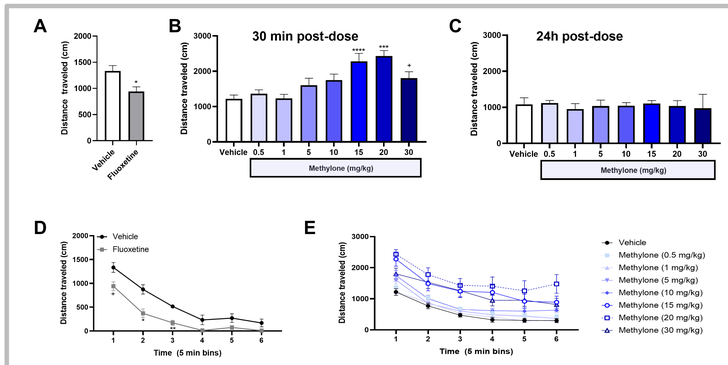

Figure 3. Observed antidepressant-like effects are not due to changes in locomotor activity. (A) Fluoxetine or Vehicle was administered 23.5, 5, and 1h prior to testing, and the total distance travelled in the OFT is shown for the first 5-minutes of the test, corresponding to the testing duration in the FST. (B) Methylone or Vehicle was administered 30 min prior to testing, and the total distance travelled in the OFT is shown for the first 5-minutes of the test. Total distance traveled is shown for the duration of the 30-minute OFT testing session in 5-minute time bins after (D) fluoxetine or (E) methylone treatment. Data are presented as means ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. Vehicle control group; N=6 for fluoxetine; N=7-13 per group for methylone.

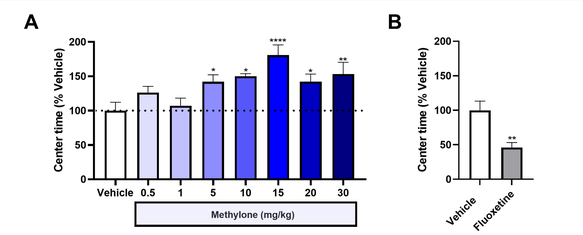

Figure 4. Methylone shows anxiolytic activity, increasing the time spent in the center of the open field test. The time spent in the center of the OFT is shown 30-minutes post-dosing with (A) methylone or (B) fluoxetine. Data are presented as means ± SEM.*p<0.05, **p<0.01, ****p<0.0001; N=6 for fluoxetine; N=7-13 per group for methylone.

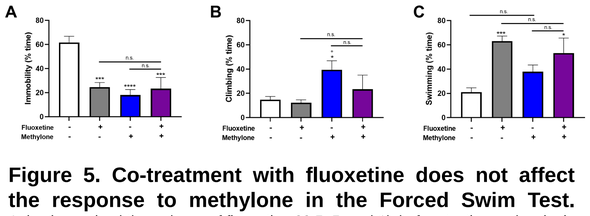

Figure 5. Co-treatment with fluoxetine does not affect the response to methylone in the Forced Swim Test. Animals received three doses of fluoxetine 23.5, 5, and 1h before testing and a single low dose of methylone (5 mg/kg, IP) 30 minutes before testing in the FST. Controls received fluoxetine only, methylone only, or saline vehicle. Quantification of the time spent (A) immobile, (B) climbing or (C) swimming are shown. Data are presented as means ± SEM. *p<0.05, ***p<0.001, ****p<0.0001 vs. Vehicle control group; $^{+}p\leq0.05$ vs. Fluoxetine alone, n.s., not significant; N=6-8 animals per group.

Conclusions

Key findings of the current study are:

1. Methylone produces a robust antidepressant-like effect in the FST that is long lasting at clinically meaningful doses (human equivalent dose: 100-150 mg).
2. Responses in the FST are not attributed to general increases in locomotor activity.
3. Methylone has an anti-anxiety effect in the OFT.
4. Fluoxetine does not affect the behavioral response to methylone in the FST, suggesting the potential for co-administration of methylone with SSRI antidepressants.

Taken together with published clinical studies, these results suggest that methylone may have a role in treating MDD, anxiety, and other disorders for which antidepressants are effective such as PTSD, with some potential advantages over MDMA.

References

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Acknowledgements

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