Methylone: Distinct Pharmacological and Mechanistic Effects Compared with MDMA

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Abstract

Post-traumatic stress disorder (PTSD) is a debilitating psychiatric illness affecting 12 million adults in the United States in a given year. Available treatments are limited. Selective serotonin reuptake inhibitors (SSRIs) represent first-line pharmacological options. However, despite the established modest efficacy of SSRIs, these treatments are sub-optimal. The therapeutic response is slow - most patients do not show significant effects until at least 4 weeks (and often up to 8 weeks) of continuous treatment, and even when optimally delivered, 30-40% of patients do not respond at all. MDMA-assisted psychotherapy has shown promise in recent clinical trials and may soon become an available treatment for PTSD. But its outreach may be limited due to the cardiovascular side-effects and an inability to co-administer with SSRIs. Methylone is the beta-ketone analog of MDMA. However, methylone shows distinct pharmacological and subjective effects. Initial clinical studies of methylone include two published Phase 1 studies and two retrospective clinical case series demonstrating that methylone is well-tolerated and may alleviate symptoms of PTSD and MDD. Unpublished data show methylone is active in a preclinical model of PTSD, and a recently published report shows robust, fast-acting, and long-lasting antidepressant-like activity in the Forced Swim Test (FST) as well as anxiolytic activity, measured by increased center time in the Open Field Test. An SSRI did not reduce methylone's activity, a notable distinction from MDMA. Here we explore methylone's underlying mechanism of action as it relates to efficacy and safety. In vitro studies were conducted using rat brain synaptosomes. We demonstrated that methylone blocked reuptake and facilitated release at monoamine transporters (i.e., SERT, NET, DAT). Results showed that methylone's relative affinities for the different transporters were distinct from MDMA. Specifically, methylone had less effect on serotonin and dopamine transporters. To determine whether these sites of action were specific, the agonist/antagonist activity of methylone (vs. MDMA) was measured using a high throughput beta-arrestin-based screen of 168 different G-protein coupled receptors (GPCRs). Methylone showed no agonist or antagonist activity at any GPCRs while MDMA showed activity at 7 GPCRs. Previous work has shown that MDMA is a 5HTR2B agonist, which may have cardiovascular safety implications. In contrast, we found that methylone showed no activity at this receptor. Finally, we examined the downstream gene expression changes induced by methylone and MDMA using RNAseq in brain areas relevant to PSTD and MDD. Rats were treated with methylone or MDMA and sacrificed 8 hours later. Drug-induced gene expression was compared between methylone and MDMA-treated groups, further highlighting the differences between these structurally similar drugs. Work is ongoing to understand what underlies methylone's lack of SSRI interaction observed in preclinical behavioral studies. Together, this work demonstrates that methylone shares important therapeutic features with MDMA but also has distinct pharmacological and mechanistic properties that may have significant therapeutic implications in the treatment of PTSD.

Introduction

Methylone is an entactogen and beta-ketone analog of MDMA currently in development for the treatment of PTSD. Methylone was synthesized over 25 years ago, but its representation in the literature is relatively sparse, focused largely on *in vitro* studies or binge-dosing regimens that mimic its recreational use. Methylone shares some chemical and pharmacological properties with MDMA, but also shows some key differences.

Recent work highlights both anxiolytic and antidepressant-like effects of methylone in animal models (Warner-Schmidt et al., 2023) as well as beneficial effects in a model of PTSD (Yu et al., 2022). Clinical experience with methylone has now been described in five studies, showing it is well-tolerated, not hallucinogenic, produces a milder range of effects compared with MDMA (Poyatos et al., 2021, 2022, 2023) and alleviates symptoms of PTSD and MDD in two retrospective clinical case series (Kelmendi et al., 2022; Averill et al., 2023).

The current study was undertaken to investigate the mechanism of action of methylone compared with MDMA. Both methylone and MDMA are monoamine reuptake inhibitors and releasers, but their relative potencies for the different transporters is distinct. Furthermore, off-target effects of the two compounds may underlie the differences in observed clinical experience, i.e., methylone is described as an overall 'softer' experience than MDMA.

Work is ongoing to distinguish these two compounds and to understand the mechanism of action underlying their therapeutic benefit for PTSD and other CNS disorders.

Results

Figure 1. Effects on release of norepinephrine (NE), serotonin (5HT), and dopamine (DA) from rat brain synaptosomes.

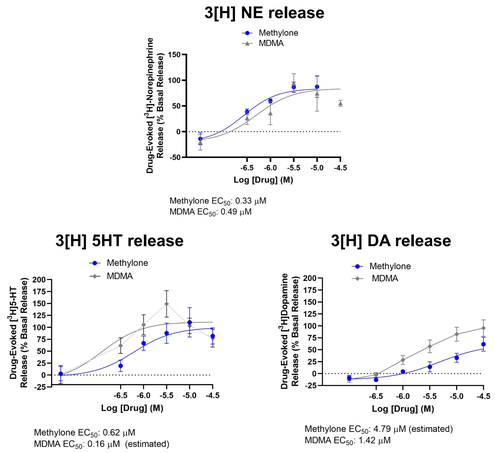
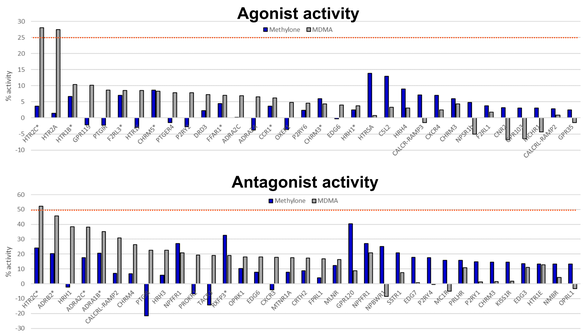


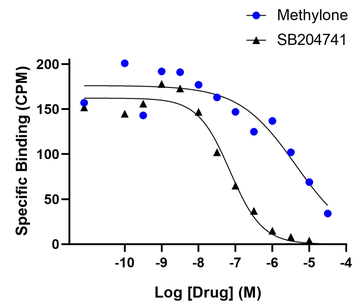
Figure 2. Agonist/Antagonist activity at 168 different G-protein coupled receptors (GPCRs).



Top 20 GPCRs for Methylone and MDMA (10uM) are shown; *indicates GPCRs on both 'top20' lists. Red dashed line shows threshold for agonist/antagonist activity.

- Methylone showed no agonist/antagonist activity; MDMA met threshold for 5HT2A, 5HT2C activity.

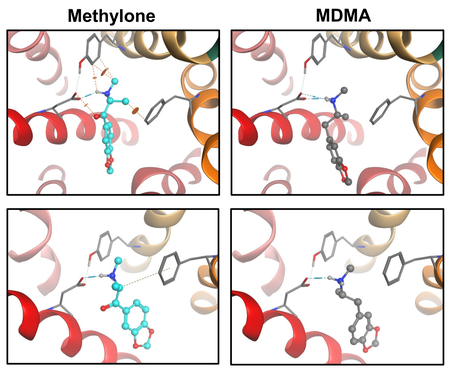
Figure 3. Competitive radioligand binding to 5HT2B receptors.



- **Methylone shows weak affinity for 5HT2B (K_i = *3749 nM*)**
- SB204741 positive control is a potent 5HT2B antagonist (K_i = *63 nM*)
- MDMA reported to bind to 5HT2B receptors (K_i = *500 nM*)[1]

1. Setola et al., 2003, *Mol Pharmacol*, 63(6):1223-9.

Figure 4. Docking to human 5HT2C, 5HT2A receptors.



- 5HT2C (top): Methylone's different shape (vs. MDMA) does not fit well, generating steric clashes indicated by the orange disks and resulting in poor or no binding to the receptor.
- 5HT2A (bottom): MDMA fits well, but due to conformational issues, methylone does not.

Study Design

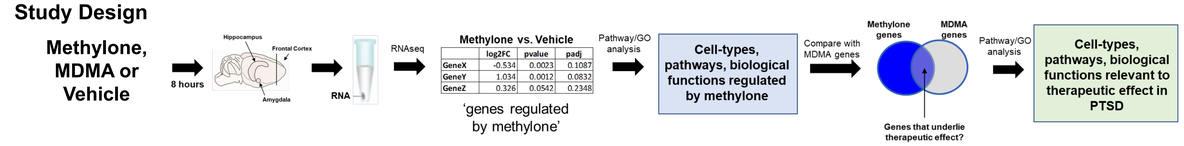


Figure 5. Number of genes regulated by methylone or MDMA in key brain areas related to PTSD.

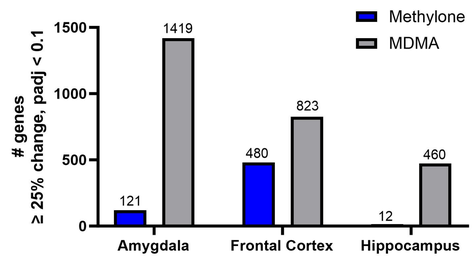
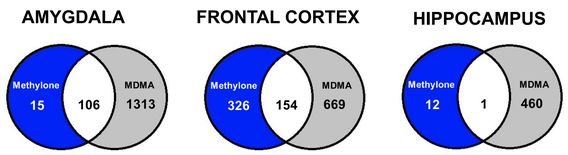


Figure 6. Overlapping (and non-overlapping) gene expression changes following methylone or MDMA administration.



- Function of overlapping genes include neuronal plasticity and conduction, which likely support therapeutic effects.
- Non-overlapping MDMA genes appear to be regulated (at least in part) to 5HT2A/2C receptor activation.

Conclusions

- In clinical and preclinical studies, methylone and MDMA show potential efficacy for treating PTSD but with notable differences in their mechanisms of action and observed clinical effects.
- Both act on monoamine transporters, but with different relative potencies. Methylone also appears to have no off-target effects versus MDMA, which acts of 5HT2A, 5HT2B, 5HT2C receptors and others reported in the literature.
- Gene expression studies suggest that MDMA regulates more gene targets than methylone, likely due to activation of 5HT receptors (and others). Overlapping gene changes may reflect those relevant for therapeutic efficacy.

References

Averill et al., 2023, *Annals of Clinical Case Reports, in press*
Kelmendi et al., 2022, *Annals of Clinical Case Reports,* 7
Poyatos et al., 2021, *Biology (Basel),* 10(8):788
Poyatos et al., 2022, *Int J Mol Sci,* 23(23):14636
Poyatos et al., 2023, *Frontiers Pharmacology,* 14:1122861
Warner-Schmidt et al., 2023; *Frontiers Psychiatry,* 13:1041277
Yu et al., 2022, poster at the SFN annual meeting, San Diego, CA