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Research Report

# Association of caffeine to MDMA does not increase antinociception but potentiates adverse effects of this recreational drug

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DA, dopamine
DAT, dopamine transporter
5-HT, serotonin
SERT, serotonin transporter
MDMA, 3,4-methylenedioxy-methamphetamine or ecstasy
METH, methamphetamine

ABSTRACT

Ecstasy (MDMA) street tablets often contain several other compounds in addition to MDMA, particularly caffeine. Then, it becomes necessary to study the consequences of caffeine plus MDMA combination. MDMA (1 mg/kg) elicited an analgesic response both at the spinal and supraspinal levels. However, when associated, MDMA and caffeine did not show any synergistic interaction. When caffeine was administered prior to MDMA, a potentiation of locomotor activity was observed, which consisted in an increase in maximal values and in a prolonged time of activity. In the neurotoxicity studies, a hyperthermic effect of MDMA was observed. Although caffeine alone failed to alter body temperature, it potentiated MDMA-induced hyperthermia. This association also significantly increased MDMA lethality (from 22% to 34%). Following administration of MDMA to rats, there was a persistent decrease in the number of serotonin transporter sites in the cortex, striatum and hippocampus, which was potentiated by caffeine co-treatment. This MDMA toxicity in rats was accompanied by a transient dopaminergic impairment in the striatum, measured as decreased [$^3$H]WIN35428 binding sites, by 31% 3 days after treatment, which was not modified by caffeine. A transient down-regulation of 5-$HT_2$ receptors occurred in the cortex of MDMA-treated rats, whose recovery was slowed by co-treatment with caffeine. In conclusion, the association of MDMA with caffeine does not generate any beneficial effects at the antinociceptive level. The acute effects stemming from this association, in tandem with the final potentiation of serotonergic terminals injury, provide evidence of the potentially greater long-term adverse effects of this particular recreational drug combination.



## 1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) is a recreational drug that achieved popularity in the United States about two decades ago, but which continues to be highly popular in Europe, particularly at dance parties ("raves"). Ecstasy street tablets from the illicit market often contain several other compounds in addition to MDMA, particularly caffeine in varying amounts. In a recent paper, Klingler et al. (2005) determined that about 10% of all pills analyzed were supplemented with this xanthine derivative. Moreover, beverages containing caffeine ("energy drinks") are frequently

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consumed while taking ecstasy in order to reduce drowsiness and fatigue.

The acute effects of MDMA in the central nervous system are complex, with several molecular sites of action. MDMA has major effects on serotonin (5-HT) pathways since it causes an acute and rapid increase in extracellular 5-HT in the striatum, frontal-cortex and hippocampus via the release of 5-HT and the inhibition of its uptake (Green et al., 2003). In addition, dopamine (DA) and noradrenalin have also been implicated. MDMA binds to all three of the monoamine presynaptic transporters, exhibiting its highest affinity for the 5-HT transporter (SERT). It also binds to several classical receptors, its highest affinity being for 5-HT2, α2-adrenergic, M1 and H1 receptors. MDMA itself induces DA release (Schmidt et al., 1987; Steele et al., 1987; White et al., 1994; Cadoni et al., 2005), although the acute increase in serotonergic neurotransmission that concomitantly occurs markedly amplifies the concentration of extracellular DA (Gudelsky and Nash, 1996) through an activation of postsynaptic 5-HT2A\2C receptors (Sprague et al., 1998).

Administration of a neurotoxic regimen of MDMA to rats results in inhibition of tryptophan hydroxylase, decreased cerebral tissue concentrations of 5-HT and 5-hydroxy-indoleacetic acid (Shankaran and Gudelsky, 1998; Wallace et al., 2001). A neurotoxic damage to presynaptic serotonergic nerve endings also occurs (Battaglia et al., 1987, 1988).

While the effects of repeated administration of high doses of MDMA on 5-HT nerve fibers and terminals have been studied extensively, little is known about its effects on dopaminergic and serotonergic receptor density. Psychosis, and especially paranoid psychosis, has been the most frequent disorder associated with MDMA use in humans (McGuire et al., 1994). Since DA and 5-HT changes have been associated with psychiatric diseases, it would be interesting to determine the possible relationship between modifications of $D_2$ and 5-$HT_2$ receptor density and the degree of neurotoxicity in a regimen schedule of MDMA in rats that simulates chronic ingest.

On the other hand, caffeine is a very popular psychostimulant among young adults. This drug acts as a nonselective $A_1$ and $A_2$ adenosine receptor antagonist and as a phosphodiesterase inhibitor. It increases DA release from striatal nerve terminals (Okada et al., 1997). Le Donne and Sonsalla (1994) pointed out that activation of adenosine $A_1$ receptors can protect against methamphetamine (METH)-induced neurotoxicity in mice. Furthermore, METH-induced decrements in neostriatal DA content and tyrosine hydroxylase activity in mice were potentiated by concurrent treatment with caffeine.

At the antinociceptive level, O'Regan and Clow (2004), in a study performed in humans, suggested that MDMA, at least in the short term, may cause serotonin-mediated alterations in pain sensitivity. Moreover, the authors also demonstrated an association between pain tolerance and MDMA usage. Crisp et al. (1989) studying the antinociceptive effect of MDMA in rats, suggested that the antinociceptive properties of MDMA may contribute to the popularity of this compound as a recreational drug. In addition, caffeine is used as an adjuvant analgesic for various types of pain such as headache, dental and postoperative pain, etc.

The primary aim of the present paper was to investigate the relationship between MDMA-induced neurotoxicity and the potential $D_2$ and 5-$HT_2$ receptor density regulation and, secondly, we sought to evaluate the effects of this relationship in MDMA plus caffeine-treated animals in order to better understand the possible consequences of a chronic MDMA consumption pattern in tandem with caffeine. An additional goal of the present study was to determine and compare the analgesic efficacy of MDMA and caffeine in mice, using different animal models of acute (thermal or chemical stimuli) or chronic (formalin) pain. The possible interaction (potentiation) between these compounds at the antinociceptive level, and their interaction with the dopaminergic or serotonergic system were also studied.

## 2. Results

### 2.1. *Antinociceptive effect*

#### 2.1.1. *Effects of MDMA and caffeine in the writhing test*

MDMA elicited a dose-dependent analgesic response as demonstrated by a significant inhibition of abdominal contractions in mice receiving acetic acid with respect to control-treated (saline + acetic acid) animals. Caffeine showed a slight antinociceptive effect (about 20%) only at the highest dose assayed (10 mg/kg) (Fig. 1).

When associated, caffeine (10 mg/kg) plus MDMA (1 mg/kg), had a cumulative antinociceptive effect. The number of abdominal stretching movements was significantly reduced compared with animals treated with MDMA or caffeine alone (see Fig. 1). At the lowest dose (1 mg/kg), caffeine did not modify the MDMA effect (number of abdominal stretching movements: 36.75 ± 1.32, $n = 6$ CAF + MDMA vs. 36.25 ± 1.03, $n = 8$ MDMA).

When given alone at a dose up to 2 mg/kg, methysergide, a nonselective 5-HT receptor antagonist, had no effect on abdominal stretching movements and failed to modify the effect of MDMA (1 mg/kg) or caffeine (10 mg/kg).

When given at doses of 0.1 and 0.5 mg/kg haloperidol, a nonselective DA receptor antagonist, reduced stretching abdominal movements: 44.55 ± 5.73, $n = 6$ ($P < 0.05$, vs. saline) and 30.05 ± 3.96, $n = 7$ ($P < 0.01$), respectively. However, at this dose range locomotor impairment was evident. A subsequent dose of 0.05 mg/kg haloperidol, which did not interfere with measurements, was chosen. At this dose, the compound modified neither the effect of MDMA (1 mg/kg) nor of caffeine (10 mg/kg) (Fig. 1).

#### 2.1.2. *Effects of MDMA and caffeine in the hot-plate test*

In this test, MDMA and caffeine induced a significant increase in the pain threshold. Table 1 shows the latency time, measured before and 20 min after drug administration.

The association of caffeine (10 mg/kg but not 1 mg/kg) plus MDMA (1 mg/kg) elicited an antinociceptive response similar to that obtained with MDMA or caffeine alone. Under our conditions, methysergide (2 mg/kg) did not modify the basal pain threshold. Furthermore, methysergide pretreatment significantly antagonized the analgesic response induced by MDMA (5 mg/kg) but not by caffeine (5 mg/kg). Haloperidol at a