Research article

The effects of methamphetamine and buprenorphine, and their interaction on anxiety-like behavior and locomotion in male rats

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HIGHLIGHTS

- The effects of Methamphetamine (Meth) on anxiety are controversial.
- Buprenorphine (Bup) is used to treat anxiety-related behaviors.
- The administration of Meth and Bup alone was anxiolytic in rats.
- The coadministration of Bup and Meth was anxiogenic.
- These effects might occur via dopamine, catecholamine, or glutamate pathways.

ABSTRACT

Methamphetamine (Meth) abuse and dependence are major global problems. Most of previous studies showed that Meth is anxiogenic. While buprenorphine (Bup) is used to treat anxiety-related behaviors, the effects of Meth in combination with Bup on anxiety-like behavior are unclear. In this study, we examined the effects of these drugs on anxiety-like behavior with the elevated plus maze (EPM) and open field (OF) tests, which are widely used to assess anxiety-like behavior in small rodents.

Forty male Wistar rats were divided into four groups: sham, Meth, Bup, and Bup + Meth. The groups were administered their assigned treatments for 7 days. The time spent in the open arms, and number of total entries into the arms (total activity) in the EPM were recorded. In addition, locomotor activity and number of entrances into the center area in the OF were recorded.

The 7-day administration of Meth or Bup increased open arm exploration in the EPM. In contrast, the combined administration of Bup and Meth had the opposite effects. In addition, Meth and Bup had no effects on total and locomotor activity. Furthermore, the rats in the Meth and Bup groups spent more time in the center of the OF, while the group given both Bup and Meth spent less time in the center of the OF. The administration of Meth and Bup alone was anxiolytic in rats, whereas the coadministration of Bup and Meth was anxiogenic.

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1. Introduction

Methamphetamine (Meth), which is one of the most harmful and addictive drugs, is widely abused worldwide [1]. It is currently listed as the second most abused illicit substance in the world [2]. The popularity of this drug worldwide seems to be due to its easy production, low cost [3], and powerful psychostimulant properties [1,4–7]. Meth has serious effects on the behavior of both humans and laboratory animals [8]. It induces aggressive behavior and changes social interactions [3]. Anxiety, depression, insomnia, and psychosis are among the most commonly reported symptoms associated with Meth dependence, and individuals presenting to the emergency department due to Meth intoxication may be agitated, violent, or suicidal [9].

Buprenorphine (Bup) is a semisynthetic drug [10] that has partial agonist effects on the µ receptor, antagonistic effects on the δ and κ opioid receptors, and limited euphoric effects [5,11]. It is currently used to treat opiate addiction and chronic pain [12]. Bup,
which is a highly lipophilic derivative of the morphine alkaloid thebaine, has a rapid onset and long duration of action in rodents [14]. Bup has a more favorable clinical profile than morphine and its related agonists [15], because it has limited physical dependence and less abuse potential [16].

Several studies have investigated the interactions of psychostimulants and opioid receptor agonists [5]. Psychosocial interventions, such as the matrix method, are a major treatment component of Meth dependency [17]. This mode of treatment was originally designed as an integrated neurobehavioral treatment that was based on several research-based techniques and that targeted clients’ behavioral, emotional, cognitive, and relationship issues [18]. Adding Bup to the matrix method significantly reduced Meth cravings [17], Bup has been shown to attenuate Meth-induced self-injurious behavior in mice [5]. In addition, notable changes have been observed in Meth-induced behaviors in a prenatally Bup-exposed animals [19].

Anxiety consists of a painful state of helplessness and vague sense of apprehension [20,21]. These feelings, with the accompanying organic and physiological disturbances, or, more precisely, secretory and motor discharges, can overwhelm the subject and represent an unbearable state and incomparable experience in the most severe cases [20]. Anxiety-related behaviors are caused by increases in dopaminergic transmission and plasma catecholamine concentrations and the potentiation of glutamatergic responses [22,23].

Most studies have demonstrated anxiogenic effects of Meth and other psychostimulants [8,24,25]. Meth administration in mice results in the expression of anxiety-related behavior in the elevated plus maze (EPM) test [24]. Conversely, some animal studies have shown that Meth injections decrease anxiety in EPM tests [8]. Bup has shown promise in the treatment of anxiety-related and other adverse behaviors and possibly in the patient population refractory to conventional anxiolytics [15,26]. Thus, the anxiolytic-like properties of Bup may have clinical significance. This study was designed to investigate the effects of Bup and Meth on anxiety-like behavior in male rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats, weighing 250–300 g, were used in the present study. The rats were randomly divided into four groups (n = 10) at the beginning of the study. They were housed on a 12-h light schedule (lights on at 07:00 AM) in a temperature-controlled (22 ± 2°C) colony room. They were allowed ad libitum access to standard rat chow and water. All procedures of research and animal care were approved by the Veterinary Ethics Committee of the Hamadan University of Medical Sciences (VECHUMS) and were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985).

2.2. Drugs

Methamphetamine hydrochloride was obtained from the Presidency Drug Control Headquarters (Tehran, Iran). It was dissolved in 0.9% saline [27] and used at a dose of 2 mg/kg body weight [4,6,19,24]. Bup (Faran Shimi Pharmaceutical Co., Tehran, Iran) was dissolved in 0.9% saline [16] and used at a dose of 5 mg/kg body weight [28].

2.3. Groups

In this experiment, 40 male rats were categorized into the following four groups. The Sham group was intragastrically (IG) administered saline by gavage once a day for 7 days. The Meth group was intraperitoneally administered Meth at 2 mg/kg once a day [4,19,29] for 7 days [24,29,30]. The Bup group was administered Bup IG at 5 mg/kg [28] once a day for 7 days [31]. Finally, the Bup + Meth group was administered Bup (once a day for 7 days) and Meth (once a day for 7 days). On the seventh day, Meth and Bup were administered to the groups 30 [8] and 60 [32] min, respectively, before the test. Experimental timeline is shown in Fig. 1.

2.4. Elevated plus-maze test

Anxiolytic activity of substances was measured using the EPM test. This test has been widely validated to measure anxiety in rodents [33]. On the day of the experiment, rats were moved to the testing room, where they remained in their home cage for a 60-min acclimation period [3]. Briefly, for rats, the apparatus consisted of two open arms (50 × 10 × 1 cm each), two enclosed arms (50 × 10 × 50 cm each), and a central platform (10 × 10 cm), arranged in such a way that the two arms of each type were opposite to each other. The maze was elevated 50 cm above the floor [34,35]. In the EPM, the rats were placed in the center of the maze facing the open arms. The rats were allowed to explore the maze, and their behaviors were monitored for 10 min by a digital camera that was located above the maze. The time spent in the open arms, and number of entries into the open arms were calculated [27,36,37]. All of the animals were tested in the EPM 30 min after the Meth injections [4] or 60 min after Bup administration.
2.5. Locomotor activity in the open field (OF)

Locomotor activity was measured in an OF, which was made of white acrylic with a 50 × 50 cm surface area and 38-cm-high walls. The field was lit by low ambient room lights [38]. The times that the animals spent in the central and peripheral zones were recorded by an overhead video camera and analyzed by video track software. The central zone was square-shaped and 25 cm from each wall. The rats were placed in the middle of the OF and allowed to explore for 10 min [39]. The total distance traveled (locomotor activity), number of entries into the center, and times in the periphery and center of the apparatus were measured [40]. In the OF, decreased anxiety-like behavior was indicated by increased time in the center of the OF [41].

2.6. Statistical analysis

The data from the EPM and OF tests were analyzed with a one-way analysis of variance (ANOVA) and Tukey’s post hoc test in a computerized analysis. In all cases differences were considered significant if \( p < 0.05 \). Results were expressed as Mean ± SEM (standard error of the mean).

3. Results

3.1. Elevated plus maze test

3.1.1. Effects on open arm entries

The rats in the Bup group (8 ± 0.7) showed a significant increase \( (p < 0.05) \) in the number of entries into the open arms compared with the number of entries of the Sham group (1.57 ± 0.36). Meth administration (8.17 ± 3.06) significantly increased \( (p < 0.05) \) the number of entries into the open arms compared with those of the Sham group. The coadministration of Bup and Meth (0.29 ± 0.18) had a different effect on the number of entries into the open arms. The coadministration resulted in a significant decrease in the number of entries into the open arms compared with those of the Meth \( (p < 0.05) \) and Bup \( (p < 0.01) \) groups. The coadministration of these two drugs did not significantly differ compared with the Sham group \( (p > 0.05) \) (Fig. 2A).

3.1.2. Effects on the time spent in the open arms

The rats in the Bup (80.25 ± 18.78 s) group showed a significant increase \( (p < 0.05) \) in the time spent in the open arms compared with the Sham group (9.40 ± 3.44 s). The Meth group spent significantly more time \( (p < 0.05) \) in the open arms (83.57 ± 22.7 s) compared with the Sham group. The Bup + Meth group (0.43 ± 0.29 s) exhibited a significant decrease in the time spent in the open arms compared with the Meth group \( (p < 0.01) \) and Bup group \( (p < 0.05) \) (Fig. 2B).

3.1.3. Effects on the number of entries into the arms (total activity)

The number of entries into the arms (total activity) did not differ among any of the groups (Meth, 19.2 ± 3.01; Bup, 20.88 ± 2.43; Meth + Bup, 24.33 ± 3.84; and Sham, 17.17 ± 3.26; \( p > 0.05 \) for all) (Fig. 2C).

3.2. Open field

Locomotor activity (distance traveled) did not differ among any of the groups (Meth, 13.28 ± 1.49; Bup, 17.62 ± 1.16; Meth + Bup, 17.19 ± 1.15; and Sham, 17.70 ± 2.26; \( p > 0.05 \) for all) (Fig. 3A).

3.2.1. Effects on the number of entries into the center area

The rats in the Bup (18.28 ± 1.83) group showed significant increases \( (p < 0.05) \) in the number of entries into the center area compared with the Sham group \( (10.85 ± 1.38) \). Meth administration significantly increased \( (p < 0.05) \) the number of entries into the central area in that group \( (17.4 ± 2.01) \) compared with the Sham group. The rats who were coadministered Bup and Meth exhibited a significant decrease in the number of entries into the center area \( (6.8 ± 0.58) \) compared with the Meth \( (p < 0.01) \) and Bup \( (p < 0.001) \) groups (Fig. 3B).

3.2.2. Effects on the time spent in the center area

Compared with the sham group \( (82.42 ± 3.02 s) \), the rats in the Bup \( (173.4 ± 7.91 s) \) and Meth \( (176.57 ± 4.76 s) \) groups exhibited significant increases in the time spent in the center area \( (p < 0.001) \) for both. The coadministration of Bup and Meth resulted in a significant decrease in the time spent in the center area \( (62 ± 5.2 s) \) compared with the Meth and Bup groups \( (p < 0.001) \) for both and no difference compared with the Sham group \( (p > 0.05) \) (Fig. 3C).

4. Discussion

Our results demonstrated that the administration of either Meth or Bup in the doses used in this study produced anxiolytic effects in rats. However, the coadministration of Bup and Meth produced anxiogenic effects as this group of rats exhibited a significant decrease in open arm exploration in the EPM, which indicated the expression of anxiety-related behavior [25]. These findings in our study were different from most of previous studies. They are unique. In addition, the results of this study showed that none of the drugs had any significant effects on the number of total entries into the arms (total activity) in the EPM test or locomotor activity in the OF test. Also, the administration of either Meth or Bup in the doses used in this study produced anxiolytic effects in the rats in the OF test. Similar to the EPM test, the coadministration of Bup and Meth produced anxiogenic effects in the rats in the OF test.

In accordance with our results, animal studies have shown that Meth injections decrease anxiety in EPM tests [8]. In contrast with our results, the anxiogenic effects of Meth resulted in decreased visits to the open arms in the EPM [25]. Similarly, other studies have found that Meth decreased social contacts in male rats in tests of social interactions. Decreased social interactions indicate anxiogenic behavior [42]. Psychostimulants have been reported to induce aggressive behavior and anxiogenic effects and change social interactions [3].

Some experiments have found anxiogenic effects of Bup administration [43]. The anxiogenic-like actions of Bup have been hypothesized to result from its antagonistic properties at the \( \delta \) receptor and lack of agonist activity on \( \kappa \) receptors [43]. In contrast, rat behavioral studies have demonstrated that the \( \kappa \)-opioid receptor agonist U-50 488H has anxiolytic-like effects [11,15]. Accordingly, Bup induces robust, consistent, and reproducible antidepressant-like and anxiolytic-like effects [12]. Another study showed that Bup produces antidepressant and anxiolytic-like responses in mice. The anxiolytic effects of Bup were evident as early as 24 h after acute treatment, which suggests that Bup may have benefits during the early phase of treatment in patients dealing with anxiety [44].

In order to describe this result, it is suggested that the coadministration of Meth and Bup might increase anxiety through increases in the activity of dopamine, or glutamate pathways. Increasing evidence suggests the involvement of the dorsal striatum (DS) in drug addiction. The DS is a major target for Meth [6]. Numerous observations indicate that anxious states might be related to increased dopaminergic transmission [45] and linked...
Fig. 2. The effects of methamphetamine (Meth, 2 mg/kg, intraperitoneal), buprenorphine (Bup, 5 mg/kg; intragastric), and their coadministration (Bup + Meth) on the number of entrance into open arms (A), time spent in open arms (B), and number of entrance into arms (total activity) (C) in elevated plus maze (EPM) test. *: P < 0.05, Meth and Bup in comparison to sham; $: P < 0.05$, $\$: P < 0.01, Bup + Meth in comparison to Bup; #: P < 0.05, $$: P < 0.01$ Bup + Meth in comparison to Meth.

to increased dopaminergic transmission involving both the D1 and D2 dopamine receptors [22]. Meth increases the extracellular concentrations of dopamine in some brain regions, in part, by reversing the dopamine transporter and facilitating cytoplasmic dopamine release as well as releasing vesicular stores of dopamine [7]. In the DS, Meth administration results in a massive release of dopamine from presynaptic terminals into the synaptic cleft [6]. Meth acts as a pseudotransmitter and binds to the dopamine transporter on dopaminergic nerve terminals, which then promotes dopamine release and inhibits dopamine reuptake, thus increas-
Fig. 3. The effects of methamphetamine (Meth, 2 mg/kg, intraperitoneal), buprenorphine (Bup, 5 mg/kg; intragastric), and their coadministration (Bup + Meth) on the locomotor activity (traveled distance [meters]) (A), number of entrances into the center area (B), and time spent in center area (C) in the open field test. *: P < 0.05, **: P < 0.001, Meth and Bup in comparison to Sham; $$$: P < 0.001, Bup + Meth in comparison to Bup; $$$$: P < 0.001, Bup + Meth in comparison to Meth.

Meth increases the release of dopamine in the nucleus accumbens (NAc) [46]. On the other hand, the repeated administration of Meth leads to attenuation of the inhibition of dopamine release from the NAc via presynaptic dynorphin-sensitive receptors [48]. Neurochemical or molecular changes within the NAc have been associated with changes in anxiety states [49]. Like this, Bup enhances dopamine basal levels and release in the NAc and progressively increases extracellular dopamine [50]. Previous studies have shown that this partial μ agonist stimulates dopamine release through the mesolimbic system, much like other μ receptor agonists [51]. Accordingly, partial agonists of μ-opioid receptors, such as Bup, have been reported to increase the extracellular concentrations of dopamine in the NAc and striatum when it is administered systemically or into the VTA or substantia nigra (SN) [52].

In addition, recent studies have shown that opioid receptor agonists modify the pharmacodynamic effects of Meth on the dopaminergic system [6,50]. Prenatal exposure to Bup has greater effects on Meth-induced behaviors and the dopaminergic system compared with other opioids [19].
Meth cravings by Bup might be explained by the similar mechan-isms of Meth in the NAc, which act through the cocaine and amphetamine-regulated transcript, which is a peptide that acts in regions, including the NAc, related to the brain’s reward system [17].

The glutamate system has received much attention in anxiety research because both preclinical animal studies and human drug trials have provided good evidence of the efficacy of glutamatergic drugs in the treatment of anxiety [53]. The potentiation of glutamate responses promotes potent depolarization, which leads to the expression of anxiety-like behaviors [23]. Evidence suggests that Meth enhances glutamate release in several brain regions, such as the cerebral cortex, striatum, and hippocampus [29]. In addition, chronic Bup treatment increases the basal levels of glutamate in the NAc in drug-naïve and cocaine-pre-exposed rats [54]. In contrast, chronic exposure to Bup might reduce glutamate function as chronic treatment with Bup has been shown to decrease glutamatergic activity in the NAc and striatum [50].

In conclusion, although Meth and Bup both increase the extracellular concentrations of dopamine in the NAc, the results of this study showed that the administration of Meth or Bup alone produced anxiolytic effects in rats. In contrast, the coadministration of Bup and Meth produced anxiogenic effects. Future studies should examine the effects on anxiety-like behavior of different doses, other routes of administration, and other treatment durations of Bup and Meth.

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References


