Pharmacological Reports 69 (2017) 199–205

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original article

Acute behavioral effects of co-administration of mephedrone and MDMA in mice

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ARTICLE INFO

Article history:
Received 22 July 2016
Received in revised form 7 October 2016
Accepted 7 October 2016
Available online 11 October 2016

Keywords:
Mephedrone
MDMA
Memory
Anxiety
Depression
Mice

ABSTRACT

Background: Abuse of more than one psychoactive drug is becoming a global problem. Our experiments were designed to examine the effects of a concomitant administration of 3,4-methylenedioxy-methamphetamine (MDMA) and mephedrone on depression- and anxiety-like behaviors and cognitive processes in Swiss mice.
Methods: In order to investigate the drug interactions the forced swimming test (FST) – an animal model of depression, the passive avoidance (PA) test – a memory and learning paradigm, as well as the elevated plus maze (EPM) test – test for anxiety level were used.
Results: The results revealed that a concomitant administration of non-effective doses of mephedrone (1 mg/kg) and MDMA (1 mg/kg) exerted marked antidepressive effects in the FST. Also a co-administration of mephedrone (2.5 mg/kg) and MDMA (1 mg/kg) displayed a pro-cognitive action in the PA paradigm. Furthermore, even though mephedrone and MDMA can, in general, exert some anxiogenic effects in mice, the concomitant administration of nonactive doses of both drugs (0.05 and 0.1 mg/kg, respectively) in the EPM test, did not show any synergistic effect in our study.
Conclusions: The effects of mephedrone and MDMA combination on mammalian organisms were attempted to be evaluated in our study and the results are described in the present report. These results may help explain the reasons for and consequences of a concomitant administration of psychoactive substances with regards to the central nervous system, while being possibly useful in the treatment of polydrug intoxication.

Introduction

Simultaneous consumption of several psychoactive drugs is a problem among young recreational drug users [1]. Like other recreational drugs of abuse, mephedrone [(RS)-2-methylamino-1-(4-methylphenyl) propan-1-one] and MDMA, [3,4-methylene-dioxy-methamphetamine] are often used in combination. These drugs show a close structural and mechanistic convergence (see Fig. 1) which can contribute to life-threatening interactions. Mephedrone is a semi-synthetic derivative of cathinone [2]. β-ketoamphetamines (e.g., cathinone and mephedrone) exhibit a significant structural similarity to the neurotransmitters, noradrenaline (NA), adrenaline and dopamine (DA), as well as to exogenous substances that stimulate the central nervous system (CNS), such as amphetamine, methamphetamine and MDMA. Only a few behavioral and biochemical experimental studies have investigated the effects of mephedrone in laboratory animals. The results indicate that mephedrone enhances the extracellular levels of DA and serotonin (5-HT) in the nucleus accumbens and can thus be self-administered to produce locomotor activation [3–6]. *In vitro* release studies indicate that mephedrone is a nonselective substrate for transporters of plasma membrane monoamine, such as NA (NAT), DA (DAT) and 5-HT (SERT) [3,7]. MDMA, similar to mephedrone, is able to elevate DA and NA levels but in smaller amounts than 5-HT level. MDMA can also delay their metabolism by inhibition of monoamine oxidase (MAO) and bind to distinct 5-HT and NA receptors [8–10]. The above-mentioned mechanisms contribute to the general increase of extracellular monoamine level in the CNS after both drugs are taken.

Furthermore, mephedrone users describe the effects of the drug as more MDMA-than cocaine-like, including positive psychotomimetic effects, e.g., euphoria, elevated mood stimulation, as well as negative, e.g., anxiety and panic [11].

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http://dx.doi.org/10.1016/j.pharep.2016.10.006
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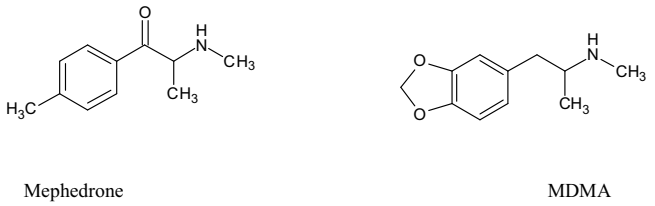

Mephedrone MDMA

Fig. 1. Chemical structure of mephedrone [(RS)-2-methylamino-1-(4-methylphenyl) propan-1-one] and MDMA [3,4-methylenedioxy-methamphetamine].

Considering these findings, it is most likely that an acute mephedrone and MDMA co-administration may contribute to behavioral changes in both humans and animals. Much research has focused on evaluating the long lasting neurotoxic effects of mephedrone, using a binge like regime in animal models [12–15]. However, to the best of our knowledge, there is no preclinical information relating to the acute, concomitant use of mephedrone with MDMA, even though the problem seems to be significant. For this reason, we chose three behavioral procedures, i.e., the passive avoidance (PA) test, the forced swimming test (FST) and the elevated plus maze (EPM) test to assess the acute effects on memory, depression- and anxiety-like behaviors, respectively, after co-administration of these drugs. The results from our experiments may explain, whether acute exposures to the two amphetamines have any short-lasting behavioral consequences.

Materials and methods

Animals

Male Swiss mice weighing 20–25 g were used in the experiments. The animals were maintained under standard laboratory conditions (12 h light/dark cycle, room temperature 21 ± 1 °C, cage dimension of 26 × 20 × 14 cm, 8 animals per cage) with free access to tap water and laboratory chow (Agropol, Poland) and adapted to laboratory conditions for, at least, one week. Each experimental group consisted of 8–10 animals. All the experiments were conducted, according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and to the European Community Council Directive for the Care and Use of Laboratory Animals of 22 September 2010 (2010/63/EU), and were approved by the local ethics committee. Each mouse was used in one procedure only.

Drugs

The following compounds were tested: MDMA (3,4-methylenedioxy-methamphetamine hydrochloride; 0.1, 0.5, 1, 2.5, 5, 10 and 20 mg/kg; Tocris, UK) and mephedrone ((RS)-2-methylamino-1-(4-methylphenyl) propan-1-one; 0.05, 1, 2.5 and 5 mg/kg; Toronto Research Chemicals Inc., Canada). The drugs were dissolved in saline solution (0.9% NaCl), and intraperitoneally (*ip*) administered at a volume of 10 ml/kg. Fresh drug solutions were prepared on each day of experimentation. Control groups received saline injections in the same volumes and *via* the same route of administration.

The doses of mephedrone and MDMA were based on literature data [16–19] and our preliminary studies.

The FST procedure

According to Porsolt et al. [20], mice were individually placed in glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water at 23–25 °C for 6 min. The total duration of immobility was recorded after 2 min of familiarization to the environmental conditions. Immobility was recognized when a mouse stopped struggling and continued only slight movements in order to keep its head above water level.

The animals were divided into following groups: saline + saline, saline + mephedrone (1, 2.5, 5 mg/kg, *ip*), MDMA (1, 2.5, 5, 10 mg/kg, *ip*) + saline, or mephedrone (1 mg/kg, *ip*) co-administered with MDMA (1 mg/kg, *ip*). The test was conducted in 15 min after drug administration.

The PA procedure

The apparatus and PA procedure was fully described in our previous article [21]. On the first day of experiment (pre-test), mice were individually placed in a light compartment and allowed to explore the light box for 30 s. After that time period, a guillotine door was raised and, when the mice entered a dark compartment, the guillotine door was closed again and an electric foot-shock (0.2 mA) of 2 s duration was delivered. The latency time to enter the dark compartment was recorded (TL1). In a subsequent trial (retention) 24 h later, the same mouse was again placed individually in the light compartment of the PA apparatus and the time taken to reenter the dark compartment was recorded (TL2). If the animal did not enter the dark compartment within 300 s, the test was stopped and TL2 was recorded as 300 s.

The experimental procedure involved examination of memory consolidation (the animals received injections of the substance after pre-test) [22,23].

The animals were allocated into the following drug groups: mephedrone (2.5 mg/kg, *ip*) + saline, MDMA (1, 2.5, 5, 10 mg/kg, *ip*) + saline, or mephedrone (2.5 mg/kg, *ip*) co-administered with MDMA (1 mg/kg, *ip*). The drugs were administered immediately after pre-test (memory consolidation) and the mice were re-tested 24 h later.

The EPM procedure

The apparatus, made of dark Plexiglas, was cross-shaped and consisted of a central platform (5 × 5 cm), with two open arms (30 × 5 cm) opposite to each other and two equal-sized enclosed (30 × 5 × 15 cm) arms opposite to each other. The maze was elevated to the height of 50 cm above the floor and illuminated by dim light.

The procedure was based on our recently published data [21,24]. The procedure was similar to the method of Lister [25].

The animals were divided into the following groups: saline + saline, MDMA (0.1, 0.5, 1, 2.5, 5, 10 and 20 mg/kg, *ip*) + saline, saline + mephedrone (0.05 mg/kg, *ip*), or mephedrone (0.05 mg/kg, *ip*) co-administered with MDMA (0.1 mg/kg, *ip*) The test was conducted in 15 min after drug administration.

Locomotor activity

Photoresistor actimeters (circular cages, two light beams, diameter of 25 cm, were used to measure the locomotor activity of experimental mice. The animals were individually placed in an actimeter for 40 min. The number of episodes of light beam crossing by the mice was recorded as their locomotor activity after 15 min. In order to measure the locomotor effects of mephedrone (1 mg/kg, *ip*), or MDMA (1 mg/kg, *ip*), the animals, naive for any drug treatment, were injected with the drug and immediately placed in the activity chamber. Their locomotor activity, i.e., the number of photocell beam breaks was automatically recorded.

Statistical analysis

For each experiment, a two-way analysis of variance (ANOVA) with repeated measures was used for comparisons between groups pretreated with saline and MDMA or mephedrone, followed by the *post hoc* Tukey's test for multiple comparisons. All the data are shown as means (±SEM). The confidence limit of $p < 0.05$ was considered statistically significant.

Regarding the memory related behaviors, changes in PA performance were expressed as differences between retention and training latencies and taken as an index of latency (IL). IL was calculated for each animal and reported as the following ratio:

$$IL = TL2 - L1/TL1$$

TL1 – the time period to enter the dark compartment during training

TL2 – the time period to reenter the dark compartment during retention [26].

Results

Effects of single mephedrone injection on depression-like behaviors in the FST

Fig. 2 illustrates mephedrone effects on depression-like behaviors in the FST (one-way ANOVA: $F(3,28) = 33.04$, $p < 0.0001$). The *post hoc* Tukey's analysis showed significant changes after mephedrone administration (doses of 2.5 and 5 mg/kg) in the immobility time, as compared with the saline-treated mice ($p < 0.05$ and $p < 0.001$, respectively) (Fig. 2).

Effects of single MDMA injection on depression-like behaviors in the FST

Fig. 3 illustrates MDMA effects on depression-like behaviors in the FST (one-way ANOVA: $F(4,41) = 78.30$, $p < 0.0001$). The *post hoc* Tukey's analysis showed significant changes after MDMA administration (doses of 2.5, 5 and 10 mg/kg) in the immobility time, as compared with the saline-treated mice ($p < 0.001$) (Fig. 3).

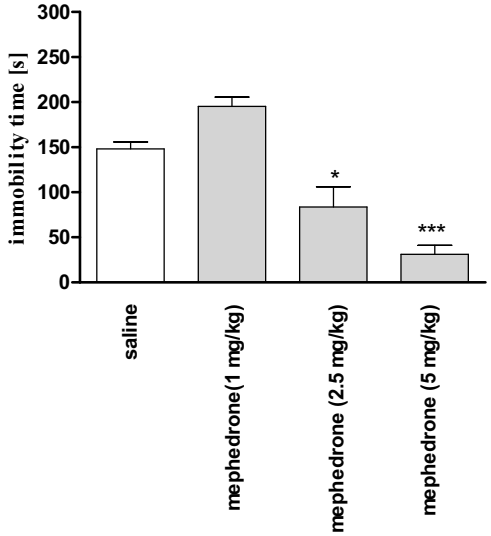

Fig. 2. Effects of an acute mephedrone (1, 25 and 5 mg/kg; *ip*) or saline injection on the total duration of immobility in the FST in mice Mephedrone or saline were administered 15 min before the test; n = 8–10; the means ± SEM; $^{*}p < 005$; $^{***}p < 0001$ *vs.* saline control group; Tukey's test.

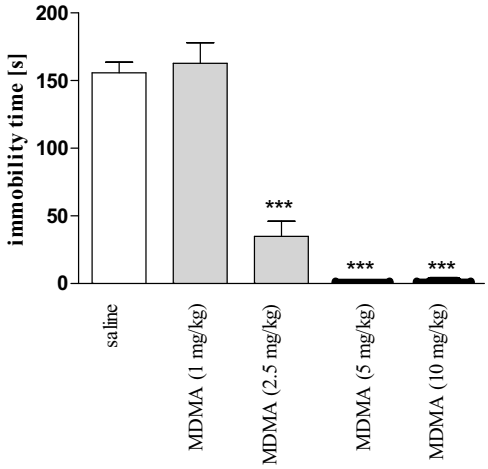

Fig. 3. Effects of an acute MDMA (1, 25, 5 and 10 mg/kg; *ip*) or saline injection on the total duration of immobility in the FST in mice MDMA or saline were administered 15 min before the test; n = 8–10; the means ± SEM; $^{***}p < 0001$ *vs.* saline control group; Tukey's test.

Effects of the co-administration of mephedrone and MDMA on depression-like behaviors in the FST

Two-way ANOVA revealed significant interactions between mephedrone and MDMA [$F(1,33) = 10.35$, $p = 0.01$], both without pretreatment [$F(1,33) = 1.83$, $p = 0.19$] and after treatment [$F(1,33) = 3.37$, $p = 0.08$] (Fig. 4). The co-administration of nonactive doses of mephedrone (1 mg/kg) and MDMA (1 mg/kg) decreased immobility time in the FST *vs.* the MDMA- or mephedrone-treated mice ($p < 0.05$ and $p < 0.01$, respectively, see the *post hoc* Tukey's test) (Fig. 4).

Effects of single MDMA injection on memory-related processes in the PA test

One-way ANOVA revealed that, at the consolidation trial, the acute *ip* administration of MDMA (1, 2.5, 5 and 10 mg/kg) significantly changed IL values [$F(4,41) = 7.25$; $p = 0.0002$]. The

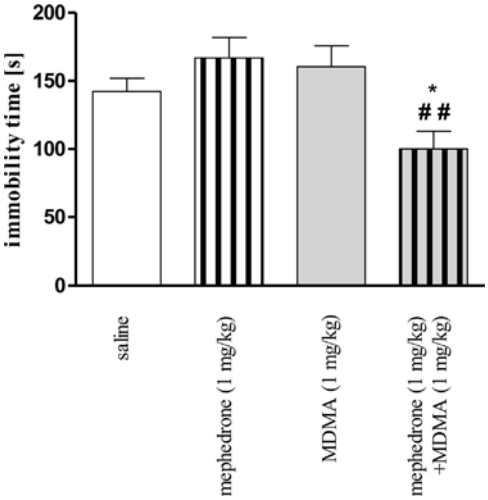

Fig. 4. The effect of co-administration of mephedrone (1 mg/kg, *ip*) and MDMA (1 mg/kg, *ip*) on the total duration of immobility in the FST in mice Data represent the means ± SEM; n = 8–10; $^{*}p < 005$ *vs.* MDMA-treated control group; $^{\#\#}p < 001$ *vs.* mephedrone–treated control group; Tukey's test.

post hoc Tukey's test showed that MDMA, at the doses of 2.5 and 5 mg/kg, significantly increased IL, as compared to the saline-treated mice, indicating that MDMA, at the used doses, improved memory and learning processes ($p < 0.01$) (Fig. 5).

For mephedrone-induced acute cognitive effects, measured in the PA task – see our recent paper of Budzynska et al. [16]. The nonactive doses of mephedrone and MDMA in the PA test were chosen to study interactions in the cognitive processes.

Effects of the co-administration of mephedrone and MDMA on memory-related behaviors in the PA test

The co-administration of nonactive doses of mephedrone (2.5 mg/kg, *ip*) and MDMA (1 mg/kg, *ip*) affected memory consolidation during the retention trial in the PA task [two-way ANOVA: pre-treatment ($F(1,30) = 10.22$, $p = 0.003$), treatment ($F(1,30) = 4.14$, $p = 0.005$) and interactions ($F(1,30) = 4.01$, $p = 0.005$)], and significantly improved memory and learning processes *vs.* the MDMA- or mephedrone-treated mice ($p < 0.01$ and $p < 0.05$, respectively, see the *post hoc* Tukey's test) (Fig. 6).

Effects of single MDMA injection on anxiety-related processes in the EPM test

As shown in Fig. 7, MDMA, administered acutely in 15 min prior to the EPM test, exerted a significant effect on the percentage of the time spent on the open arms [one-way ANOVA: $F(7,66) = 9.49$, $p < 0.0001$], as well as on the percentage of open arm entries [one-way ANOVA: $F(7,66) = 13.84$, $p < 0.0001$]. The *post hoc* Tukey's analysis showed that MDMA, at a large range of doses (0.5–20 mg/kg), significantly decreased the percentage of the time spent on the open arms ($p < 0.01$ for 0.5 and 20 mg/kg; $p < 0.001$ for 1, 2.5, 5 and 10 mg/kg) (Fig. 7A). Moreover, MDMA significantly decreased the percentage of open arms entries at the doses of 0.5–20 mg/kg ($p < 0.001$) (Fig. 7B) *vs.* saline-treated mice, indicating an anxiogenic effect. The dose of 0.1 mg/kg of MDMA did not cause any effect in the EPM paradigm, thus it was chosen for the subsequent experiments.

The effect of MDMA on the enclosed arms entries, regarded as a measure of locomotor activity, is shown in Table 1 (one-way ANOVA: $F(8,78) = 3.28$, $p = 0.003$). The *post hoc* Tukey's test showed that MDMA significantly increased locomotor activity when

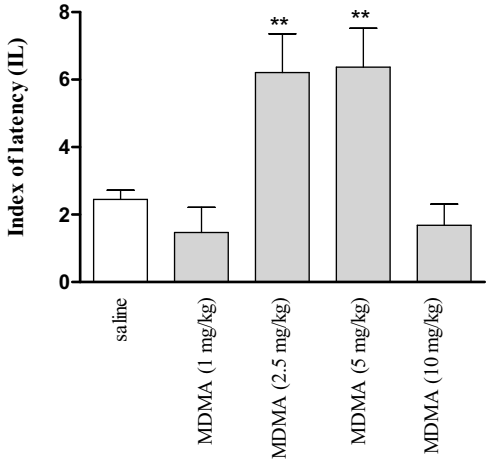

Fig. 5. Effects of an acute MDMA (1, 25, 5 and 10 mg/kg; *ip*) or saline injection on the memory consolidation trial using the PA test in mice MDMA or saline were administered immediately after the pre-test; n = 8–10; the means ± SEM; $^{**}p < 001$; *vs.* saline control group; Tukey's test.

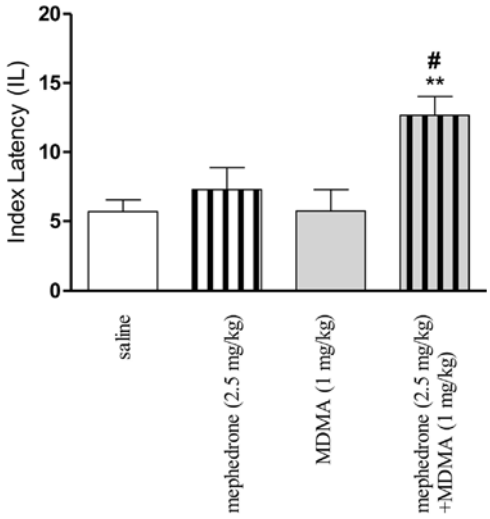

Fig. 6. Effects of co-administration of mephedrone (25 mg/kg, *ip*) and MDMA (1 mg/kg, *ip*) on memory consolidation trial using the PA test in mice Appropriate groups of mice received compounds immediately after the pre-est Data represent the means ± SEM and are expressed as latency index (IL); n = 8–10; $^{**}p < 001$ *vs.* MDMA–treated control group; $^{\#}p < 005$ *vs.* mephedrone-treated control group; Tukey's test.

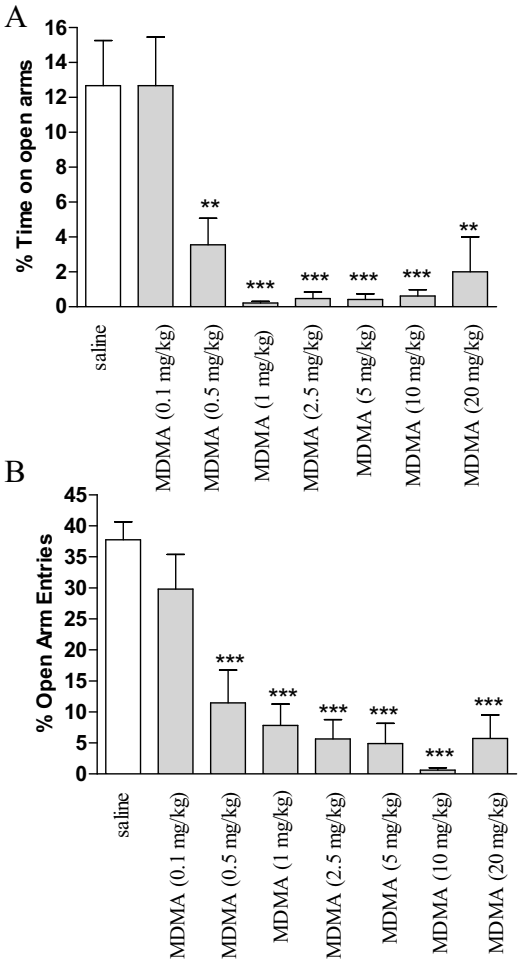

Fig. 7. Effects of an acute MDMA (01, 05, 1, 25, 5, 10 and 20 mg/kg; *ip*) or saline injection on percentage time spent in open arms (A) and percentage open arms entries (B) in the EPM test in mice. Drugs were administered 15 min prior the EPM test; n = 8–10; $^{**}p < 001$; $^{***}p < 0001$, *vs.* saline control group, Tukey's test.

Table 1
Mean (±SEM) enclosed arm entries in the EPM test in mice. MDMA (0.1, 0.5, 1, 2.5 5, 10 and 20 mg/kg) or saline were administered 15 min prior the EPM test; n = 8–10; $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* saline control group, Tukey's test.

treatment	saline	MDMA 0.1 mg/kg	MDMA 0.5 mg/kg	MDMA 1 mg/kg	MDMA 2.5 mg/kg	MDMA 5 mg/kg	MDMA 10 mg/kg	MDMA 20 mg/kg	F, *p*
enclosed arm entries	13.13 ±0.97	12.67 ±1.52	8.83 ±1.99	9.25 ±1.39	9.33 ±1.11	20.00 ±2.57**	18.14 ±3.60*	10.18 ±4.00	F(7,54) = 3.86 $p = 0.02$

injected at the doses of 10 mg/kg ($p < 0.05$) and 5 mg/kg ($p < 0.01$), as compared with the saline-treated mice, while MDMA (0.1, 0.5, 1, 2.5 and 20 mg/kg), given acutely, did not provoke any changes in the number of enclosed arm entries in the EPM test, thus causing no changes in the locomotor activity of the animals.

See our recent paper for effects of acute mephedrone administration on anxiety-like behaviors in the EPM [16]. The nonactive doses of mephedrone and MDMA in the EPM paradigm test were chosen to study interactions in anxiety-related processes.

Effects of the co-administration of mephedrone and MDMA on anxiety-related processes in the EPM test

As shown in Table 2, mephedrone (0.05 mg/kg), as well as MDMA (0.1 mg/kg), administered either separately or in combination, did not exert any effect on the percentage of time spent on the open arms, the percentage of open arm entries or on closed arm entries. Thus no synergistic effect was revealed between both drugs in that paradigm.

Effects of mephedrone and MDMA on locomotor activity

Neither mephedrone (1 mg/kg) nor MDMA (1 mg/kg), administered separately or in combination, influenced the locomotor activity of experimental mice in 15 min after administration (mean photocell beam-break: saline 155.4 ± 25.40; mephedrone 152.0 ± 24.52; MDMA 170.8 ± 33.19; mephedrone + MDMA 198.0 ± 22.71; two-way ANOVA pretreatment $F(1,31) = 0.08$, $p = 0.7751$, treatment $F(1,31) = 86.73$, $p = 0.0673$, interactions $F(1,31) = 0.75$, $p = 0.3939$.

Discussion

In spite of the general increase in the use of mephedrone and MDMA as "club drugs", there are currently no scientific publications on behavioral interactions between these drugs. We showed for the first time that a concomitant administration of MDMA and mephedrone could lead to antidepressive effects. Those experiments also revealed that MDMA and mephedrone, injected concomitantly, improved the consolidation of memory processes. Moreover, both drugs exerted strong anxiogenic effects at a wide range of doses. However, the simultaneous administration of nonactive doses of both drugs in the EPM test did not show any synergistic effect.

The primary goal of the present study was to determine whether MDMA and mephedrone co-administration would influence depression-like behaviors in the FST in male mice. This test was designed by Porsolt et al. as the primary screening test for antidepressants [20] and it is still one of the best paradigms for the assessment of depressive behaviors in laboratory animals. While decreased immobility time periods were reported in rodents during acute MDMA administration [27], to our knowledge, this is the first study, where increased immobility time periods were examined in the FST, following mephedrone or MDMA/mephedrone combination.

The monoamine hypothesis of depression implies that reduced levels of 5-HT, NA and/or DA in the CNS are at the base of the disease. These assumptions are confirmed by the effectiveness of antidepressant drugs that increase the levels of these neurotransmitters in the brain and are effective in relieving the symptoms of depression [28]. The results obtained in the present study follow this hypothesis.

Table 2
Mean (±SEM) percentage time spent in open arms, percentage open arms entries and enclosed arm entries in the EPM test in mice. Mephedrone (MEPH, 0.05 mg/kg), MDMA (0.1 mg/kg) or saline were administered 15 min prior the EPM test; n = 8–10.

treatment	saline + saline	MDMA 0.1 mg/kg + saline	saline + MEPH 0.05 mg/kg	MDMA 0.1 mg/kg + MEPH 0.05 mg/kg	F, *p*
percentage time spent in open arms	11.13 ±2.37	10.77 ±2.67	7.97 ±1.16	8.73 ±3.7	pretreatment $F(3,31) = 1.66$; $p = 0.21$ treatment $F(3,31) = 0.13$; $p = 0.72$ interactions $F(3,31) = 0.25$; $p = 0.60$
percentage open arms entries	22.58 ±3.94	15.11 ±2.79	23.15 ±4.21	26.10 ±4.32	pretreatment $F(3,31) = 2.25$; $p = 0.14$ treatment $F(3,31) = 0.34$; $p = 0.56$ interactions $F(3,31) = 0.25$; $p = 0.18$
enclosed arm entries	13.20 ±1.02	12.67 ±1.52	9.20 ±1.43	9.87 ±0.99	pretreatment $F(3,31) = 4.73$; $p = 0.03$ treatment $F(3,31) = 0.04$; $p = 0.87$ interactions $F(3,31) = 0.03$; $p = 0.85$

Both humans and animals, up to an hour after MDMA administration, express an initial increase 5-HT levels, followed by a significant decline, which is suppressed within 3 days [29]. As mentioned above, mephedrone also increases the levels of DA, 5-HT and NA in the CNS. However, several studies established contradictory data, concerning the neurotoxic effect of mephedrone, e.g., Angoa-Pérez and coworkers [12–14] showed that mephedrone did not cause any damage of the long lasting hippocampal 5-HT and DA nerve endings in mice or did not enhance the effect of MDMA on SERT or tryptophan hydroxylase 2, whereas, Martínez-Clemente et al. [15] observed a loss of dopaminergic and serotoninergic neurons. However, decreased immobility time periods in mice were observed in our study during a short period after drug administration. It is therefore certain that the antidepressive-like activity of MDMA, mephedrone and MDMA/mephedrone combination may result already from an initial increased level of 5-HT. Indeed, further research relating to the long-term effects of a single administration of both drugs may provide interesting results.

The reported study also addressed the effects of MDMA and mephedrone on consolidation of memory processes, measured in the PA test in mice. This test is a fear-motivated paradigm, useful for the assessment of drug effects on cognitive processes, as well as for evaluation of the mechanisms, involved in memory and learning in laboratory animals [30]. Psychostimulants, such as amphetamines, are widely used for cognitive enhancement by healthy young people [31]. In accordance, it was found that amphetamine improved memory consolidation in rats [32,33]. However, other experimental evidence shows that a chronic intake of these drugs may result in neurodegeneration and memory deficits [34,35]. In the present study, the animals, which received non-effective doses of mephedrone and MDMA, showed improved memory retention. Our study corresponds to the findings of Strupp et al. [36], showing that injection of d-amphetamine after training improves the consolidation phase of memory. It is worth mentioning that the post-training administration of the drugs immediately after training induced memory consolidation improvement 24 h later. It cannot be excluded that measurements, made on the following days, may show memory impairment resulting from neurotoxic profile of MDMA and mephedrone [15].

This finding seems to emphasize that the acute effects of both drugs involve multiple neurotransmitters, which may influence memory performance. The effects, observed in the reported study, could have been mediated by actions of the dopaminergic systems [37]. A behavioral study has suggested that DA is one of the major neurotransmitters in the late memory consolidation [38]. An acute administration of amphetamine and its derivatives directly stimulates dopaminergic neurotransmission immediately after administration, and DA improves memory performance *via* reward pathways [39]. Additionally, DA has been implicated in hippocampal acetylcholine (Ach) release [40]. Thus, an acute administration of low doses of MDMA and mephedrone may cause transient stimulation of dopaminergic transmission and induce short lasting memory improvement, as observed in the present experiments.

The subsequent step of the study was an evaluation of the effects of concomitant administration of MDMA and mephedrone on anxiety-like behaviors in the EPM test in mice. The EPM paradigm is based on the natural aversion of rodents to elevated, open spaces. Therefore, rodents spend naturally more time in the enclosed, arms of the maze [25]. Furthermore, the pathogenesis of anxiety is associated with changes in the release of various neurotransmitters and involves many brain structures. It is well established that a neuronal system, such as cholinergic, adrenergic, dopaminergic, GABA-ergic, serotoninergic and glutamatergic [41], as well as neuropeptides, e.g., the adrenocorticotropic hormone, cholecystokinin, neuropeptide Y and the corticotrophin-releasing hormone play roles in the anxiety processes [42].

Several preclinical and clinical studies showed that amphetamine-like drugs increased neuropsychiatric symptoms, like anxiety in animal models, as well as in humans [43–46]. However, it was observed that depression- and anxiety- related behaviors were not present after mephedrone administration (30 mg/kg twice daily for 4 days) [47]. Recent findings showed biphasic, anxiolytic and anxiogenic MDMA actions, however, the mechanism of these effects remains still unknown [48,49]. An important finding of that study was such that MDMA and mephedrone, when administered separately, showed a strong anxiogenic effect in the EPM test. Of particular interest is the increased anxiety after low doses of MDMA and mephedrone in comparison to depressive and memory processes. Considering the close structural and mechanistic similarities between mephedrone and MDMA, it was surprising to find that the co-administration of both drugs did not cause any increase in the number of entries into the open arms or the time, spent in the open arm of the maze. Thus, no synergistic action was detected.

As it has already been mentioned, both drugs are active releasers of 5-HT, DA and NA, and all these transmitter systems are related to anxiety-like behaviors [41]. Thus, a question arises whether the absence of effects resulted from a biphasic activity of MDMA or whether these three neurotransmitters play some role or interact with each other in the mediation of anxiogenic-like effects of MDMA, co-administered with mephedrone.

In summary, it was revealed that mice, given acute MDMA/mephedrone combinations of inactive doses, had shown several significant behavioral changes, including an antidepressive-like activity and improvement in the consolidation of memory without any influence on anxiety–like behaviors in mice. The results may help explain why psychoactive substances are abused and why polydrug administration is still a social problem. It emphasizes the importance to assess further behavioral and biochemical effects of the coadministration of novel psychoactive drugs.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This study was supported by grant no 2013/11/B/NZ7/04837 from the National Science Centre, Poland.

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