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Neurotoxicology of Synthetic Cathinone Analogs

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Abstract

The present review briefly explores the neurotoxic properties of methcathinone, mephedrone, methylone, and methylenedioxypropylvalerone (MDPV), four synthetic cathinones most commonly found in “bath salts.” Cathinones are β -keto analogs of the commonly abused amphetamines and display pharmacological effects resembling cocaine and amphetamines, but despite their commonalities in chemical structures, synthetic cathinones possess distinct neuropharmacological profiles and produce unique effects. Among the similarities of synthetic cathinones with their non-keto analogs are their targeting of monoamine systems, the release of neurotransmitters, and their stimulant properties. Most of the literature on synthetic cathinones has focused on describing their properties as psychostimulants, their behavioral effects on locomotion, memory, and potential for abuse, whereas descriptions of their neurotoxic properties are not abundant. The biochemical gauges of neurotoxicity induced by non-keto analogs are well studied in humans and experimental animals and include their ability to induce neuroinflammation, oxidative stress, excitotoxicity, temperature alterations as well as dysregulation of neurotransmitter systems and induce changes in monoamine transporters and receptors. These neurotoxicity gauges will serve as parameters to discuss the effects of the four previously mentioned synthetic cathinones alone or in combination with either another cathinone or with some of their non-keto analogs. Bath salts are not a defined combination of drugs and may consist of one synthetic cathinone compound or combinations of more cathinones. Furthermore, this review also presents some of the mechanisms that are thought to underlie this toxicity. A better understanding of the cellular and molecular mechanisms involved in the synthetic cathinones-induced neurotoxicity should contribute to generate modern therapeutic approaches to prevent or attenuate the adverse consequences of use of these drugs in humans.

Keywords

MDPV; Mephedrone; Methcathinone; Methylone; Neurotoxicity; Synthetic cathinones

1 Neuroinflammation

One of the most relevant issues related to non-ketoamphetamines-induced neurotoxicity is that they can trigger inflammatory processes in those brain areas that exhibit terminal

degeneration [1]. Studies have demonstrated that glial activation participates in the events that induce neuronal damage, since chronic neuroinflammation elevates the levels of glia-derived cytokines that exert neurotoxic effects on vulnerable neurons [2]. Microglia and astrocytes are the primary modulators of inflammation in the CNS and have been associated with the toxicity induced by administration of methamphetamine [3], amphetamine and parachloroamphetamine [4], and 3,4-methylenedioxy-methamphetamine (MDMA) [5]. Studies of the effects of β -ketoamphetamines on neuroinflammation are summarized in Table 1.

To the best of our knowledge, no reports have been made on the neuroinflammatory effects of methcathinone.

With regard to mephedrone, *in vivo* studies reported that there were no signs of striatal [17] or cortical [25] astroglial activation after administration of mephedrone in a binge paradigm. Similarly, no signs of microglial activation were observed in the striatum at 2 or 7 days after administration of mephedrone [17]. Lopez-Arnau and colleagues measured [3 H]PK11195-specific binding to investigate the microglial activation after neuronal injury in rats killed 24 h post-treatment with mephedrone. PK11195 is an isoquinoline carboxamide that purportedly binds to microglia in conditions of brain injury. In these animals, no increase in the density of [3 H]PK11195 binding sites was detected, indicating a lack of microglial activation [20]. However, Martinez-Clemente reported an increase in reactive astrocytes in the dentate gyrus of the hippocampus of mice treated with mephedrone at 7 days after a binge of 3 doses of 25 mg/kg for 2 days [25].

There were no signs of striatal, hippocampal, or cortical microgliosis in methylone-treated rats using a regimen of 4 doses of 20 mg/kg every 3 h [39]. However, a significant increase of reactive astrocytes was reported in the frontal cortex of methylone-treated rats. By contrast, no significant differences were found in the expression of the astroglial marker GFAP in striatum or any subregion of the hippocampus (dentate gyrus, CA1 or CA3) [39]. MDPV was not found to elicit GFAP increases when administered in a binge regimen of 4 doses of 30 mg/kg every 2 h in mice [19].

None of the β -ketoamphetamines mephedrone, methylone, or MDPV in combination with each other resulted in changes in the levels of GFAP in the striatum of mice [19]. However, methylone was able to enhance by approximately 50% the expression of GFAP induced by administration of methamphetamine [19]. MDPV, on the contrary, prevented the striatal increases in GFAP observed after administration of methamphetamine and MDMA [19].

2 Thermoregulation

A common adverse effect of non-ketoamphetamines such as methamphetamine [52], amphetamine, and MDMA [53] is an increase in body temperature. This hyperthermia is dependent on the frequency of exposure, dosing, age, ambient temperature, and route of exposure [52]. MDMA and methamphetamine produce hyperthermia following acute and repeated exposure at ambient temperature and elevated ambient temperature [14, 54–58]. Hyperthermia is an important factor known to exacerbate the deleterious effects of

amphetamine-type drugs. Studies of the effects of β -ketoamphetamines on thermoregulation are summarized in Table 1.

A single dose of 10 mg/kg of methcathinone caused a sustained increase in rectal temperature that was not accompanied by any significant concomitant change in tail temperature in individually housed rats [7]. Similarly, an acute intoxication with a methcathinone infusion (5 mg/kg/min; 100 mg/mL) caused hyperthermia in rats [6]. By contrast, acute exposure to mephedrone produces hypothermia in rats [7, 59]. This hypothermic response at ambient and elevated temperatures is rat-strain specific, with the reduction in body temperature detected in Wistar but not Sprague-Dawley rats [27]. Alpha-1 adrenoreceptor and dopamine D1 receptor blockade seem to enhance the hypothermic response induced by mephedrone [7]. On the other hand, when mephedrone is administered repeatedly in a binge paradigm, it produces hyperthermia in both mice [17, 19, 25] and rats [24]. These studies indicate that mephedrone differs from MDMA and methamphetamine in its thermoregulatory effects despite their neuropharmacological similarity. Lopez-Arnau and colleagues showed that the effect of mephedrone changes with the dose using a 2-day binge paradigm of 3 doses of 25 mg/kg a day every 2 h [20]. On day 1, after receiving the first dose of mephedrone, the treated animals showed a significant transient reduction in body temperature; after the second dose, temperature increased over the saline values but this hyperthermic response was significant only after the third dose. On day 2, no significant hyperthermic response was evidenced. Consistent with that report, other studies have suggested that the mephedrone-induced hypothermia is attenuated with repeated dosing and that this response can be attenuated by 6-hydroxydopamine or abolished by 5,7-dihydroxytryptamine [26]. Taken together, mephedrone produces hypothermia following acute exposure, while producing hyperthermia following binge models of dosing [60].

Studies of self-administration of mephedrone in rats evidenced that its thermoregulatory effects also differed between rat strains, with the Sprague-Dawley rats being most sensitive. While in Sprague-Dawley rats the administration of mephedrone produced a dose-dependently decreased body temperature, in Wistar rats, the response was biphasic, starting with a decrease during the first 15 min, followed by an increase during the next 25–30 min [61]. Another study with these two strains of rats monitored the effects of the subcutaneous administration of mephedrone under conditions of low (23°C) and high (27°C) ambient temperature. A reliable reduction of body temperature was produced by mephedrone in Wistar rats at low and high temperatures with only minimal effect in Sprague-Dawley rats. Furthermore, hypothermia produced by serotonin (5-HT) 1A/7 receptor agonists was similar in each strain [27].

Similar to mephedrone, methylone produces hyperthermia following binge dosing [60]. In mice, 4 injections of methylone (20 mg/kg) every 3 h produced a robust hyperthermic response that reached a peak between 25 and 35 min after each drug administration. This effect of methylone increased significantly with the dose, so that the last dose induced a greater increase in body temperature than the first one [38]. In rats, methylone treatment (3 injections at 3 and 10 mg/kg every 2 h) caused significant hyperthermia from 2 h through 6 h post-injection [24]. The thermoregulatory effects caused by a single administration of methylone did not differ from the outcomes observed after repeated administration of the

drug. Piao and colleagues evaluated the acute effects of methylone using 5-HT transporter (SERT) and dopamine (DA) transporter (DAT) knockout (KO) mice and observed a slight diminution in the hyperthermic effects of methylone in DAT KO mice, whereas a slight enhancement of these effects was seen in SERT KO mice. Administration of selective D1 and D2 receptor antagonists reduced methylone-induced hyperthermia, but these drugs also had hypothermic effects of their own in saline-treated mice, which complicates interpretation of the findings [41].

MDPV exhibits a few important differences in altering body temperature by comparison to other β -ketoamphetamines. Acute exposure to MDPV produces hyperthermia [47] at elevated temperatures but not at normal ambient temperatures, which contrasts with what is observed for MDMA and methamphetamine [60]. MDPV has been shown to elevate body temperature in some cases of human medical emergency and fatal overdose deaths [62]. In rats, treatment with 1.0, 5.6, and 10.0 mg/kg of MDPV elicited a significant hypothermia when compared with the vehicle condition [44]. The effects were dose dependent and lasted up to 3 h after dosing [44]. However, an unexpected lack of dose dependence was characterized when MDPV was administered in the 20°C ambient environment. At that cool ambient temperature, MDPV doses from 1 to 30 mg/kg each induced a rise in core temperature of approximately 1.5°C, which was not different from that observed following saline administration, and the time course of this effect was also similar across all tested doses [46]. Besides affecting body temperature, MDPV has been found to induce brain hyperthermia through an increase in peripheral vasoconstriction [40]. Furthermore, an age-dependent effect of MDPV on thermoregulation was documented in rats. While adolescent rats increased their body temperature following MDPV administration, adults showed a decrease in temperature [48].

The β -ketoamphetamines mephedrone, methylone, and MDPV differentially affect the temperature effects of their non- β -ketoamphetamine counterparts [19]. In vivo studies demonstrated that the mephedrone-induced hyperthermia is not enhanced by concomitant administration of methamphetamine [23]. When given in two-drug combinations, mephedrone, methylone and MDPV caused significant increases in body temperature [19]. When methylone or MDPV are given with mephedrone, the initial hypothermic effect of mephedrone was retained and slightly exaggerated. Combined treatment with MDPV and methylone results in a steady 1–2°C increase in core body temperature that becomes evident within 15 min of treatment and persists for at least 8–9 h [19]. Neither MDPV nor methylone attenuated the hyperthermic effects of methamphetamine in mice [19].

3 Neurotransmitters

Alterations in monoaminergic systems are one of the hallmarks of the most studied non-keto-amphetamines. Methamphetamine is perhaps best known for its toxic effects on DA nerve terminals of the striatum [63, 64]. MDMA has also been shown to cause long-term deficits in DA and 5-HT nerve ending in both laboratory animals and humans [65–67]. Even amphetamine has been linked to nerve terminal damage [66]. Amphetamine neurotoxicity manifests as long-term depletion of DA and 5-HT, inhibition of their biosynthetic enzymes tyrosine hydroxylase (TH) and tryptophan hydroxylase 2 (TPH-2), inactivation of DAT and

SERT, reduction in function of the vesicle monoamine transporter (VMAT), degeneration of fine, unmyelinated axons, and apoptosis [3]. Reductions in DA, TH, and DAT have been documented in the postmortem striatum of chronic methamphetamine users [68]. Studies of the effects of β -ketoamphetamines on neurotransmitter systems are summarized in Table 1.

Methcathinone is a potent releaser of DA but not 5-HT, similar to amphetamine and methamphetamine [15]. Methcathinone has previously been shown to release radiolabeled DA [16] and 5-HT from rat brain preparations with similar DA versus 5-HT selectivity to amphetamine and methamphetamine, but with two- to threefold lower potency [15]. Multiple administrations of methcathinone caused persistent deficits in monoaminergic systems [11], reflected by decreases in DA and 5-HT uptake capacity, tissue content and associated TH and TPH-2 activities in frontal cortex, hippocampus, and neostriatum after four doses of 30 mg/kg in rats [8, 9]. However, the effects of this drug seemed to be more accentuated in rats compared to mice. While in mice methcathinone produced long lasting depletions of striatal DA, in rats it caused significant depletions of both DA and 5-HT [11]. A single high-dose administration of methcathinone increased striatal DA release, as measured by microdialysis in conscious rats [8]. Methcathinone was also found to increase the metabolites homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) levels in striatum [7].

Mephedrone alone did not cause persistent reductions in the levels of DA, 5-HT, or TPH-2 [18, 19, 22, 28, 69] aside from a small decrease in the DA metabolite HVA in the mouse striatum [28]. Mephedrone is considered a more potent releaser of DA than MDMA [15, 70]. An in vivo microdialysis study in rats showed that mephedrone produced a rapid and pronounced increase in DA levels in the nucleus accumbens that was comparable with amphetamine and greater than MDMA, which only elevates DA levels moderately [71]. While both mephedrone and MDMA also produced strong increases in extracellular 5-HT, amphetamine had only a moderate effect on 5-HT levels [71]. Self-administration of mephedrone was shown to decrease the levels of striatal 5-HIAA in rats [21]. Mephedrone administered in a binge of 3 doses of 10 mg/kg every 2 h showed that the extracellular increase in striatal DA seen after the first mephedrone injection was similar in magnitude and time course to those following the second and third injections. However, the extracellular overflow of striatal 5-HT was more variable but was enhanced when second and third injections were given when compared with the first response [26]. Other microdialysis studies in the rat nucleus accumbens showed that mephedrone elevated extracellular DA and 5-HT levels, with relatively higher effects on 5-HT levels [24, 27], similar to MDMA and unlike methamphetamine, which preferentially increases DA [24]. Thus, mephedrone shares some of the DA-releasing properties of amphetamine and methamphetamine and the 5-HT-releasing property of MDMA [15]. Repeated administration of mephedrone in rats showed no significant effect on tissue concentrations of DA, 5-HT, or their metabolites in the striatum or frontal cortex and hippocampus 7 days post-administration although the concentration of DOPAC was significantly increased in this region following mephedrone [29]. The same study further evaluated the acute effects of mephedrone in comparison with MDMA, and reported reductions in hippocampal 5-HT and 5-HIAA 2 h after a single injection of MDMA but not following acute mephedrone [29]. The expression of TH, a biochemical marker of neuronal integrity in DA neurons, was found

to be decreased in frontal cortex but not in the striatum after a binge regimen of 25 mg/kg of mephedrone [20]. The overall effects of mephedrone do not involve long-lasting depletions of DA but they seem to affect 5-HT. Repeated administration of mephedrone in rats caused persistent decreases in hippocampal 5-HT levels but no changes were observed in striatal DA after 7 days of treatment [70]. Another study reported decreases in TH and TPH-2 after a binge of mephedrone for 2 consecutive days [25]. The fact that mephedrone has DA-releasing capability resembling methamphetamine and yet does not cause DA deficits is of significant interest for studying the differential mechanisms of damage induced by stimulants.

Methylone administration does not result in damage to DA nerve endings in mice [19]. Binge administration of methylone to single-housed rats (3 or 10 mg/kg, 3 doses) has no long-lasting effects on brain tissue monoamines [24] but produced significant elevations on extracellular levels of DA and 5-HT [24, 34, 42]. There seem to be species differences in the sensitivity to long-term neurochemical effects of methylone [28]. The effects of drug treatments on mouse brain monoamine levels in the frontal cortex, striatum, and hippocampus indicate that methylone did not cause any significant changes in neurotransmitter levels. However, in the rat brain methylone had a profound impact on 5-HT levels, causing a decrease in 5-HT levels in the frontal cortex, striatum, and hippocampus comparable to that induced by amphetamine. Additionally, 5-HIAA levels were reduced in the striatum and hippocampus [28].

In vivo microdialysis studies indicate that MDPV increases extracellular concentrations of DA in the nucleus accumbens in a dose-related manner similar to cocaine. However, MDPV was tenfold more potent than cocaine in its ability to increase extracellular dopamine [49]. This robust stimulation of DA transmission by MDPV predicts serious potential for abuse [49]. Despite the high potency to block the DAT, MDPV did not produce DA efflux. Thus, this cathinone is thought to be a pure transporter uptake inhibitor [15].

Studies in mice indicate that combined treatment with mephedrone and methamphetamine or MDMA did not change the status of 5-HT nerve endings to an extent that was different from either drug alone [18]. Methamphetamine and MDMA alone caused mild reductions in 5-HT but did not change SERT and TPH2 levels [23]. While mephedrone did not produce changes in the 5-HT system, it enhanced the DA and TH depletions induced by methamphetamine, amphetamine, and MDMA in striatum [23].

In mice, none of the β -ketoamphetamines mephedrone, methylone, or MDPV in combination with each other resulted in changes in striatal DA or TH, but mephedrone and methylone potentiated the depletions of DA and TH induced by administration of methamphetamine [19, 23]. On the other hand, MDPV was able to prevent the striatal decreases in DA and TH observed after administering methamphetamine, MDMA, and MPTP [19]. Consistent with this study, in vitro data showed that MDPV blocked methamphetamine-induced DA release with high potency reflecting its elevated efficiency as an uptake inhibitor. The finding suggests that the more potently a drug antagonizes the DA release produced by methamphetamine, the more potently it also blocks DA uptake [51].

4 Biochemical Mechanisms: Oxidative Stress and Cytotoxicity

Evidence indicates that reactive oxygen species (ROS) are responsible for amphetamine-related damage but neither the manner by which these drugs cause oxidative stress nor the cellular source of the reactant species is known [4, 68]. Oxidative stress is believed to be a prominent factor in methamphetamine-induced cellular toxicity [72]. By increasing DA release, amphetamines increase the available DA for oxidation and its metabolism into ROS [73]. Methamphetamine's ability to flood the intracellular medium with DA is thought to be the first step in a cascade that leads to mitochondrial dysfunction, enhanced excitatory neurotransmission, increases in oxidative stress, nerve ending damage, and apoptosis [74]. Similar to the other amphetamines, metabolism of MDMA also results in the formation of ROS, which ultimately induce long-term neurotoxic effects [2]. However, none of the β -ketoamphetamines (methcathinone, mephedrone, methylone, and MDPV) showed cytotoxicity at the highest concentrations tested in functional assays [36]. Studies of the effects of β -ketoamphetamines on oxidative stress and cytotoxicity are summarized in Table 1.

Methcathinone is manufactured by a clandestine process that involves potassium permanganate oxidation of ephedrine and pseudoephedrine contained in readily available pharmaceuticals. Intravenous injections of such methcathinone preparations expose users to a high manganese load because the resultant methcathinone is not purified [75]. Although studies of methcathinone abusers have described movement disorders similar to Parkinson's disease attributed to the manganese toxicity, the syndrome lacks typical features of this condition such as resting tremor and gait initiation failure [75]. The accumulation of manganese can lead to the development of encephalopathy and might trigger secondary pathogenic mechanisms, such as mitochondrial dysfunction and oxidative stress [76].

Animal studies showed that mephedrone induced an increase in the expression of the antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase in the hippocampus, striatum, and frontal cortex in rats. Along with these enzyme protein increases, treatment with mephedrone caused a significant increase in the levels of lipid peroxidation in the frontal cortex [20]. In mice, a single injection of mephedrone at 2.5 mg/kg caused both an increase in lipid peroxidation levels and a decrease of catalase activity in the hippocampus and prefrontal cortex [37] whereas at a dose of 25 mg/kg, mephedrone induced a significant increase of glutathione peroxidase in striatum [30].

The exposure of mouse cultured cortical cells to various concentrations of mephedrone for 24 h or 48 h caused a concentration-dependent decrease in metabolically active cells. The calculated LD50 value for mephedrone after 24 h of incubation was significantly higher to that obtained after 48 h of drug exposure [25]. In addition, neuroblastoma cells exposed to mephedrone showed an increase in cytotoxic damage only at high concentrations [31]. Cell culture studies documented the cytotoxicity of methylone and methamphetamine in CHO cells stably expressing the rat transporters DAT, NET, and SERT. Methylone was not cytotoxic to any cell line except that expressing the SERT [43], indicating higher specificity for the 5-HT system.

The cytotoxic effects of MDPV have only been evaluated in a developing brain mouse model, where a single administration of the drug caused a prominent increase in the number of apoptotic cells in the piriform cortex, retrosplenial area, hippocampus CA1, and nucleus accumbens, without an overall change in the density of cells. The neurons of the nucleus accumbens appeared to be especially sensitive to MDPV as they showed an increase of apoptotic cells in the core and shell regions of the accumbens. However, this effect of MDPV was not observed in the brain of adult mice [45]. While methylone alone was reported to be cytotoxic in cell lines expressing the rat SERT, the combination of methamphetamine with methylone caused a significant increase in the toxicity in the cells stably expressing the rat monoamine transporters DAT, NET, and SERT but not in the control CHO cells [43].

5 Neurotransmitter Transporters and Receptors

The non-keto-amphetamines including amphetamine, methamphetamine, and MDMA cause neurotoxic effects to monoaminergic systems in part through alterations in DA and 5-HT transporters and receptors [66]. Methamphetamine causes acute increases in both DA and 5-HT release that result from the direct and indirect actions on the DAT and SERT. Amphetamines can disrupt the vesicle proton gradient to cause an increase in cytoplasmic DA and 5-HT from vesicular compartments by altering the function of the vesicular monoamine transporter-2 (VMAT-2). Both methamphetamine and MDMA also increase 5-HT release through similar transporter mediated mechanisms, though MDMA has a preferential affinity for SERT over DAT and consequently more pronounced effects on the 5-HT system [74]. Studies of the effects of β -ketoamphetamines on neurotransmitter transporters and receptors are summarized in Table 1.

In animals, repeated administration of methcathinone was shown to reduce the content of DA and 5-HT, the number of transporter sites, as well as the activity of TPH-2 and TH [9]. In humans, persistent reductions of DAT density have been reported using PET in methcathinone users and are suggestive of loss of DAT or loss of DA terminals [10, 77]. Methcathinone exhibited a monoamine transporter inhibition profile that was very similar to that of the non-keto analogs amphetamine and methamphetamine, with high inhibitory potencies at the DAT and low potencies at the SERT [13, 15, 78]. It is believed that the deficits in DAT and SERT produced by methcathinone may reflect potential long-term damage to DA and 5-HT neurons. Nevertheless, to become evident, these neural deficits require massive, multiple doses of methcathinone over several days. Such doses are 10 to 100 times higher than behaviorally active doses [12]. Deficits in DA function induced by methcathinone were prevented by pretreatment with dopamine D1 or D2 receptor antagonists, whereas 5-HT changes were prevented with a depletion of striatal DA by lesioning with 6-hydroxydopamine [8]. Apparently the serotonergic neurotoxicity of methcathinone is promoted by the presence of the *N*-methyl group on the drug molecule as it was earlier reported that no long-term changes in 5-HT levels were observed with repeated high doses of the *des*methyl parent compound, cathinone [12]. In a cell study, methcathinone was less efficacious in releasing preloaded radiolabeled neurotransmitter via VMAT-2 than methamphetamine [33]. Methcathinone also exhibited low μ M potency at 5-HT_{2A} receptor binding [15].

Mephedrone administration alone did not cause persistent reductions in the levels of SERT [18]. Uptake inhibition studies using rat synaptosomes found that mephedrone potently inhibits DAT and SERT [35, 70], and the drug is a substrate for DAT, SERT, and the norepinephrine transporter (NET) [24]. Similar to methcathinone, mephedrone can bind to 5-HT_{2A} receptors and stimulation of these receptors has also been shown to enhance DA release potentially increasing abuse liability [79]. Mephedrone and methcathinone also exhibited affinity for α 1A adrenoceptors, which have been implicated in stimulant-induced vasoconstriction, hyperthermia, and euphoria [15] and methcathinone has been found to be a low potency partial agonist at the 5-HT_{1A} receptors [33].

In mice, repeated administration of mephedrone induced a significant loss in DA and 5-HT reuptake sites in striatum, hippocampus, and frontal cortex [25]. In addition, mephedrone decreased the number of D2 receptors in striatum and the number of 5-HT_{2A} receptors in frontal cortex and hippocampus of treated mice [25]. In adolescent mice, mephedrone elicited an increase in D3 receptors in the striatum [30]. In rats, a binge of mephedrone induced a significant loss in DAT in frontal cortex and a decrease in the density of SERT in striatum, cortex, and hippocampus. This effect was accompanied by decreased TPH-2 expression in all the three brain areas and a moderate decrease in the concentration of D2 receptors in the striatum [20]. The effects of mephedrone on the human monoamine transporters were studied using cell lines stably expressing the human NET, DAT, and SERT. These data indicate that mephedrone and MDMA were equally potent in inhibiting noradrenaline uptake at NET. Compared to their NET inhibition potency, both drugs were weaker uptake inhibitors at DAT and SERT, with mephedrone being more potent than MDMA at DAT and less potent than MDMA at SERT. Nonetheless, mephedrone and MDMA differed most in their inhibition of DA uptake by synaptic vesicles isolated from human striatum, with MDMA being tenfold more potent than mephedrone, and their ability to release DA from human VMAT expressing cells [80]. In general, the in vitro releasing capabilities of mephedrone resemble those of MDMA. With regard to selectivity ratios, mephedrone displayed NET/DAT ratios and DAT/SERT ratios close to unity, similar to MDMA [24]. Interestingly, a recent report suggests that the para ring-substitution of the methyl group in mephedrone left-shifted the SERT inhibition curves over the DAT inhibition curves (DAT:SERT inhibition ratios <1), resulting in monoamine transporter inhibition profiles that were more similar to MDMA and less similar to the parent compound methcathinone [36]. Similarly, in vitro and in vivo studies have shown that methcathinone para ring-substituted analogs increase monoamine release via SERT relative to DAT, and that this shift in selectivity markedly reduces the abuse-related effects of the drugs as assessed by intracranial self-stimulation [81, 82].

Methylone was reported to act somewhat more potently in inhibiting DAT than SERT at the human transporter [15], but equally potent for DAT and SERT inhibition in rat synaptosomes [83]. Methylone was a substrate for NET and DAT, with slightly lower potency at SERT, displaying a selectivity profile similar to mephedrone but about half as potent. In general, the in vitro releasing capabilities of methylone resembled those of MDMA. With regard to selectivity ratios, methylone displayed NET/DAT ratios and DAT/SERT ratios close to unity, similar to MDMA [24]. In cells expressing the VMAT-2, methylone elicited less than 35% of methamphetamine maximal efficacy to stimulate release of neurotransmitter via the

VMAT-2 [33]. Similar to mephedrone, methylone is also a low potency partial agonist at the 5-HT_{1A} receptors, and an antagonist with very low potency at the 5-HT_{2A} receptor [33]. While methamphetamine and MDMA are likely to be substrates for VMAT-2, methcathinone and methylone are not. Therefore, the behavioral effects of methcathinone and methylone arise largely from the drugs' effects at the plasma membrane transporters, not VMAT-2. In summary, due to the large decrease in potency at VMAT-2, methcathinone and methylone are highly selective for the plasma membrane catecholamine transporters and moderately selective for SERT. As a result of its greater potency at the SERT, methylone is somewhat less discriminating than methcathinone at the plasma membrane [13].

MDPV exhibited very high affinity for the DAT and NET in the low nanomolar range (<10 nM) in vitro, consistent with its high potency as a DAT and NET inhibitor [15, 33, 49]. MDPV exhibited the most potent DAT inhibition [15], being at least tenfold more potent than cocaine and methamphetamine [15]. In contrast, MDPV is a weak inhibitor of the SERT, resulting in high DAT selectivity, with DAT/SERT inhibition ratios >100. MDPV is also one of the most potent NET inhibitors [15]. Studies using fast-scan cyclic voltammetry in mouse striatal slices indicate that MDPV is more potent than cocaine at inhibiting DA clearance [49]. In contrast to mephedrone, MDPV is a very potent NET and DAT inhibitor with very low 5-HT activity, reflected by high DAT:SERT inhibition ratios. The 3,4-methylenedioxy ring substitution that is shared by MDMA and MDPV would be predicted to increase serotonergic activity compared with the non-substituted compound methamphetamine. However, in the case of MDPV, the SERT inhibition potency is very low despite the presence of this substitution [36]. In this regard, data have shown that the carbonyl and the extended alpha alkyl groups in MDPV have greater contributions to this drug's affinity for DAT than the methylenedioxy group [84]. In addition, in vitro findings revealed that the presence of a pyrrolidine ring in any cathinone-like compound such as MDPV confers potent blocking properties at DAT and NET [85].

An examination of methylone's ability to influence the reverse transport of substrates through DAT, NET, and SERT was done in comparison with methamphetamine, since unlike cocaine, methamphetamine induces the release of monoamines via a reversal of transport. Similar to methamphetamine, methylone elicited the release of radiolabeled DA, NE, and 5-HT from CHO cells expressing the rat DAT, NET, and SERT. In addition, the combination of methylone with methamphetamine did not cause a further increase in the release of substrates [43]. None of the β -ketoamphetamines mephedrone, methylone, or MDPV in combination with each other resulted in changes in striatal DAT [19]. In combination with methamphetamine, mephedrone and methylone enhanced the reductions in DAT observed in mouse striatum [19, 23]. In contrast, administration of MDPV prevented the depletions in DAT observed after methamphetamine, amphetamine, MDMA, and MPTP [19].

6 Transendothelial Blood–Brain Barrier Dysfunction

The rate at which drugs reach the brain parenchyma depends not only on their route of administration but also on their ability to cross the cerebral endothelium, also called the blood–brain barrier (BBB), which constitutes the main brain interface modulating the exchange of compounds between the brain and blood [50]. Alterations in BBB function are

likely involved in drug abuse neurotoxicity [1, 86]. Both MDMA and METH have been shown to produce disruption of the BBB as reflected by IgG extravasation and Evans Blue leakage [5, 87]. In fact, it was previously shown that METH compromises BBB function and its capacity to protect the brain against infection by the human immunodeficiency virus [88]. Studies of the effects of β -ketoamphetamines on BBB dysfunction are summarized in Table 1.

Methcathinone exhibited a brain permeability ratio ≈ 3 , indicating high permeability. However, the apical to basolateral transport of methcathinone was not consistent with active transport by one of the blood-to-brain influx carriers [15]. No studies on the compromise of the BBB by methcathinone have been reported to date. The permeability ratio for mephedrone was >10 , suggesting very high BBB permeability [15], and confirming that mephedrone readily enters the brain [15, 70]. Although highly permeable into the brain, mephedrone administration has not been linked to any BBB dysfunction. It is well recognized that compounds with a brain/plasma concentration ratio greater than 1 freely cross the blood–brain barrier and the obtained brain/plasma ratio for methylone of 1.42 demonstrates access to central nervous system [89]. As a reference, methamphetamine, amphetamine, and MDMA brain/plasma ratios are >3 [15].

Similar to mephedrone, the permeability ratio for MDPV was >10 , suggesting a very high permeability [15]. The apical to basolateral transport of MDPV was significantly greater than basolateral to apical transport, consistent with active transport by one of the blood-to-brain influx carriers [15]. MDPV is a monoamine uptake inhibitor that is more lipophilic and potent than other cathinone derivatives. The high lipophilicity of this substance is caused by the pyrrolidine ring and the tertiary amino group creating a less polar molecule more able to cross the blood–brain barrier [90]. No combinations of the β -ketoamphetamines mephedrone, methylone, or MDPV with each other or any other amphetamine compound have been evaluated on BBB dysfunction to date.

7 Mechanisms of Action

Drugs that target monoamine transporters can be classified generally as either substrates, such as methamphetamine, or blockers, such as cocaine [83]. Both types of compounds elicit profound psychostimulant effects that render them liable for recreational abuse [91]. Substrates or blockers increase monoamine neurotransmitter concentrations in the synaptic cleft but this action can be the result of at least two distinct mechanisms [13]. One mechanism is through drug inhibition of plasma membrane transporter-mediated uptake of released neurotransmitters (i.e., for transporter blockers). The inhibition is believed to arise from competition by drugs for substrate binding sites in the monoamine uptake transporters, thereby reducing the effectiveness with which DA, 5-HT, and NE are cleared from the synapse following release. Typical DAT blockers are expected to fully inhibit DA uptake and to fully inhibit binding of another blocker, as well as release of substrate by reverse transport [92]. A second mechanism is through the drug-evoked release of the monoamine neurotransmitters, apparently by transporter-mediated exchange (i.e., for transporter substrates). The drug-evoked neurotransmitter release arises from two intracellular compartments. Methamphetamine and MDMA induce the release of newly synthesized,

vesicular transporters and the degrading enzymes monoamine oxidase (MAO) and catechol-*O*-methyl transferase [91], both of which may contribute to their toxic properties. Amphetamine interactions with these three targets are the core tenet of the so-called weak base hypothesis [96]. Amphetamines enter nerve terminals via plasmalemmal transporters and disrupt vesicular storage as weak bases by dissipating the proton gradient across the membrane [96]. A reduction of the vesicular pH gradient promotes the reverse transport of DA into the cytosol. DA is then released into the synaptic space via reverse transport through the DAT. This flooding of the cytoplasm and synaptic space with the oxidatively labile DA is thought to be a critical first step in the neurotoxic cascade of the amphetamines [73]. These conditions of elevated concentrations of cytosolic monoamines could be further aggravated by inhibition of MAO [91]. Unlike amphetamines, mephedrone and methylone have little if any affinity for VMAT-2 [33]. Therefore, their lack of neurotoxicity could derive from an inability to promote the release of DA from storage vesicles into the cytoplasm.

If not toxic on their own, how can mephedrone and methylone increase the neurotoxicity of methamphetamine, MDMA, and amphetamine? We hypothesize that the enhancement of neurotoxicity elicited by the combination of methamphetamine plus either mephedrone or methylone could be explained by a reversal of greater numbers of DAT molecules than caused by either drug alone, resulting in heightened DA efflux into the cytoplasm (i.e., via methamphetamine actions on the VMAT) and synapse (i.e., via combined methamphetamine plus mephedrone actions on the DAT). This possibility is supported by the observation that amphetamine-induced DA release is greater when originating from both synaptic vesicles and cytoplasmic stores than from cytoplasmic stores only [97]. In addition, a possible inhibition of MAO could be speculated for mephedrone and methylone as it has been shown that MAO inhibitors increase significantly the DA depletion induced by methamphetamine [95]. However, a well-established mechanism to explain why some β -ketoamphetamines such as mephedrone and methylone are not neurotoxic on their own but are capable of potentiating the damage induced by amphetamines remains to be elucidated.

8 Conclusion

As β -keto analogs of amphetamines, synthetic cathinones may be expected to have amphetamine-like effects because of their structural similarity. However, β -ketoamphetamines are a diverse class of chemical compounds with differential neurotoxic properties on monoaminergic neurons. Some of the benchmarks used to gauge the neurotoxicity induced by amphetamines include inflammation, disruption of monoaminergic neurotransmitters, their transporters and receptors, alterations in thermoregulation, oxidative stress, and cytotoxicity. Compared to the effects induced by amphetamines on these parameters, the effects described for β -ketoamphetamines seem to be more moderate. Administration of synthetic cathinones is not consistently associated with long-term depletions in the levels of DA and 5-HT or with inhibition of these neurotransmitters biosynthetic enzymes. While hyperthermia has been established as one of the hallmark effects of amphetamines, synthetic cathinones elicit more complex responses that involve hypothermia and oscillations between hyper and hypothermia. Neuroinflammation markers such as microglial activation have not been documented after administration of synthetic cathinones and reports of increases in GFAP have been sparse. The evaluation of the effects

of these cathinones on oxidative stress and cytotoxicity are limited and mostly circumscribed to in vitro studies, where concentrations are very high. Nonetheless, some studies in animals have described increases in lipid peroxidation and in the expression of antioxidant enzymes after mephedrone. Deficits in DAT and SERT were only observed after multiple doses that are several times higher than behaviorally active doses or with exacerbation of other factors such as high ambient temperature. Although only a few studies have reported the neurotoxic effects of β -ketoamphetamines alone or in combination with other drugs of the same group or with amphetamines, the overall outcome appears to be associated with their interaction with the vesicular and plasma transporters. The fact that mephedrone and methylone cause little or no toxicity themselves on the one hand, while being capable of enhancing amphetamines toxicity on the other hand, remains a provocative and open question that requires additional research. The role of these synthetic cathinones as weak bases to collapse the vesicular pH gradient necessary for monoamine storage, their capacity to increase the releasable pool of cytosolic monoamines, their potential to inhibit monoamine degrading enzymes, their ability to increase monoamine oxidation and metabolism into ROS, as well as their additive effects in recruiting DAT molecules along with amphetamines to enhance the DA efflux into the synapse, constitute some of the possible manners in which these β -ketoamphetamines may heighten the neurotoxicity induced by amphetamines.

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Table 1 Summary of studies that have investigated the neurotoxic effects of β -ketoamphetamines in vivo and in vitro

	Inflammation	Thermoregulation	Neurotransmitter depletion	Oxidative stress	Neurotransmitter transporters and receptors	Transendothelial dysfunction	Combinations
Methcathinone	n/a	[6, 7]	[8–11]	n/a	[8, 9, 12–15]	[15]	[16]
Mephedrone	[17–22]	[17–20, 23–27]	[7, 17–25, 28, 29]	[20, 30, 31]	[15, 24, 26, 30, 32–36]	[15]	[18, 19, 23, 30, 32, 37]
Methylone	[19, 38, 39]	[19, 24, 38–41]	[19, 24, 28, 38, 39]	n/a	[13, 15, 24, 33, 34, 42, 43]	[15]	[19, 43]
MDPV	[19]	[19, 40, 44–48]	[19, 45]	n/a	[15, 32, 33, 36, 49]	[15, 50]	[19, 32, 46, 51]