Understanding the pharmacokinetics of synthetic cathinones: Evaluation of the blood–brain barrier permeability of 13 related compounds in rats

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Funding information
Universitat Jaume I de Castelló, Grant/Award Number: UJI-B2016-45; Generalitat Valenciana, Grant/Award Number: PROMETEO/2016/076; Ministerio de Economía y Competitividad-FEDER, Grant/Award Number: BFU2016-77691-C2-1-P; Ministerio de Educación, Cultura y Deporte, Grant/Award Number: FPU15/02033; University Jaume I, Grant/Award Number: UJI-B2018-19

Abstract
Synthetic cathinones are the second most commonly seized new psychoactive substance family in Europe. These compounds have been related to several intoxication cases, including fatalities. Although the pharmacological effects, metabolism, and pharmacokinetics of cathinones have been studied, there is little information about the permeability of these compounds through the blood–brain barrier (BBB). This is an important parameter to understand the behavior and potency of cathinones. In this work, 13 selected cathinones have been analyzed in telencephalon tissue from Sprague–Dawley rats intraperitoneally dosed at 3 mg/kg. Our results revealed a direct relationship between compound polarity and BBB permeability, with higher permeability for the more polar cathinones. The chemical moieties present in the cathinone had an important impact on the BBB permeability, with lengthening of the α-alkyl chain or functionalization of the aromatic ring with alkyl moieties resulting in lower concentration in telencephalon tissue. Our data suggest that transport of cathinones is a carrier-mediated process, similar to cocaine transport across the BBB.

KEYWORDS
blood–brain barrier, new psychoactive substances, pharmacokinetics, pharmacology, synthetic cathinones, toxicological analysis

1 | INTRODUCTION

The consumption of synthetic cathinones represents an important public health problem, according to the most recent report from the United Nations Office on Drugs and Crime, which illustrates that this new psychoactive substance (NPS) family is one of the most commonly seized worldwide, together with synthetic cannabinoids. Most of the cathinone seizures are powder, together with pills and similar products. These compounds have also been found as adulterants in “classical” illegal drugs such as cocaine, illustrating that their prevalence of consumption could be underestimated. In addition to the data obtained by seizure analysis, the public health problem related to cathinones is also illustrated by numerous intoxication cases related to these substances, and even some fatalities. The synthetic cathinones prevalence can also be illustrated by analytical data obtained from wastewater analysis, illustrating that these compounds are being consumed worldwide.

It is almost impossible to ban all the cathinone derivatives existing nowadays due to the continuous change in structure of new
compounds appearing on the market. Besides, new compounds that could replace banned ones surface in mere weeks, in a similar way to what occurs with synthetic cannabinoids. To face this public health problem, the scientific community must be able to provide information about novel compounds, their chemical, pharmacological, and toxicological properties. Thus, a notable number of papers have been published, as illustrated by the reviews available in literature about the metabolism of these substances, the associated pharmacological behavior, toxicology, and even their neurotoxicity.

An important pharmacological issue to highlight is how cathinones affect endogenous compounds, producing a psychoactive effect. Several studies have demonstrated that cathinones act as nonselective monoamine uptake inhibitors, increasing the levels of dopamine and serotonin, producing effects similar to cocaine. Thus, the potency of cathinones and other NPS may be studied using in vitro approaches, in a similar way to synthetic cannabinoids. Although in vitro studies provide valuable information about the intrinsic potency of a compound, the in vivo effect must be determined by the extent to which a compound reaches its site of action. One of the key barriers in this context is the blood–brain barrier (BBB), modulating the exchange of compounds between the brain and the blood. The BBB is a complex system that presents different “entry routes” that can be used by drugs or hormones, such as passive diffusion (usually used by nonpolar compounds such as steroids) and carrier-mediated influx (used by some psychoactive substances such as cocaine), whereby a specific transporter helps the compound to cross the BBB and reach the brain. To complement the in vitro data and better understand the pharmacokinetics (and in vivo potency) of cathinones, it is therefore essential to generate accurate data on the BBB permeability of these compounds.

This work is the first to quantify an extensive series of cathinones in brain samples from rats intraperitoneally injected with these compounds, with the objective of relating the permeability through the BBB with their structure. To this aim, we have developed and validated advanced analytical methodology based on ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) for the determination of 13 cathinones (Figure 1) in Sprague–Dawley rats’ telencephalon tissue. UHPLC coupled to mass spectrometry (MS) plays an essential role in cathinone analysis, using both high-resolution MS (HRMS) and low-resolution MS/MS. Thus, most studies about the identification of novel cathinones and the elucidation of their metabolites have utilized UHPLC-HRMS, taking profit of the full-spectrum acquisition and high mass accuracy provided by this technique. UHPLC-MS/MS is the preferred technique for the accurate and sensitive determination of a predetermined list of compounds and has often been used for studying the pharmacokinetics and pharmacodynamics of cathinones.

As shown in Figure 1, taking pentedrone as a template, the cathinones investigated in this study differ in the amine functionalization, aromatic ring substitutions, and/or length of the alkyl chain of the alpha carbon atom. The studied compounds were thoroughly selected in order to cover most of the possible combinations of moiety changes usually appearing in cathinones. Using a human in vitro BBB permeability model, Simmler et al. readily assessed the transendothelial transport of a series of cathinones, whereas the permeability of three cathinones with different alkyl chain length (methylone, butylone, and pentylnone) was evaluated.
by Grecco et al., using Sprague-Dawley rats.\textsuperscript{41} Our work further elaborates on this by testing in vivo an extensive set of well-chosen cathinones with different changes in the moieties of the molecule.

2. MATERIALS AND METHODS

2.1. Reagents and chemicals

Research chemicals containing cathinones were provided by Energy Research chemicals containing cathinones were provided by Energy Control (ABD Foundation, Barcelona, Spain). All the compounds were characterized and purity tested by UHPLC-HRMS and nuclear magnetic resonance, following the same procedures already reported in literature.\textsuperscript{42,43} Cathinone stock solutions were prepared at approximately 1 mg/ml in methanol (0.01-mg accuracy). HPLC-grade water was obtained by purifying demineralized water using a Milli-Q system from Millipore (Bedford, MA, USA). HPLC-grade methanol, HPLC-grade acetonitrile, HPLC-grade ethanol, formic acid, and acetone were purchased from Scharlau (Scharlab, Barcelona, Spain). Physiological saline solution was purchased from Laboratorios ERN (Barcelona, Spain).

2.2. Animal testing

For animal experiments, dose and brain dissection time were selected based on the information available in literature. In this work, a realistic 3 mg/kg dose\textsuperscript{40} was preferred over the very large doses (around 20 mg/kg) used in other pharmacokinetic studies.\textsuperscript{39,41} This is relevant because extremely high doses could affect the metabolome, metabolic routes, or active transport—among other parameters—that could be involved in the pharmacology and pharmacokinetics of these compounds. It should be noted that, depending on the compound, the doses reported by users are vastly different. For example, in the case of pentedrone, the compound used as reference in this work, 30 to 60 mg has been described as a typical dose when using intravenous route (roughly 0.5 to 1.0 mg/kg).\textsuperscript{44} However, for the aim of this work, it seemed reasonable to use similar doses for all the tested compounds, in order to facilitate the interpretation of the results and avoid the influence of different cathinone concentrations on the data obtained.

Peters and colleagues\textsuperscript{40} quantified three cathinones in rat brains by LC-MS/MS, dosing the rats at 3 mg/kg for methylene and mephedrone and at 1 mg/kg for MDPV. Pharmacokinetic data obtained revealed that the highest concentration was achieved 20 min after administration. Also another study\textsuperscript{18} studying the variation of monoamine transporters in brain tissue from rats injected at 1, 3, and 10 mg/kg reported the highest concentration in brain tissue 20 min after injection. Based on these studies, 3 mg/kg was selected as the dosage to be administered to the rats for all the compounds, and the brain was dissected 20 min post injection.

Thus, 30 female Sprague-Dawley rats (8 weeks of age), weighing between 280 and 320 g, were purchased from Janvier Labs (Le Genest-Saint-Isle, France). Animals were housed in groups of three animals in polypropylene plastic cages under controlled temperature (24 ± 2°C) and lighting conditions (12 h:12 h; lights ON at 8 am), with ad libitum access to food and water. Before drug administration, animals were handled and habituated to the experimental room for 1 week. Experiments were conducted in accordance with the standard ethical guidelines (European Communities Directive 86/600-EEC) and approved by the Valencian Region government ethical committee (Generalitat Valenciana, Direcció General d’Agricultura, Ganaderia i Pesca, ref. 2019/VSC/PEA/0048).

Two rats were dosed per compound, and the brains were pooled in order to avoid possible animal differences. In total, 26 rats received intraperitoneal injections of the 13 different cathinones at a dose of 3 mg/kg in 300 μl of physiological saline solution containing 5% ethanol—the latter for increasing the solubility of the synthetic cathinones. Four additional animals were injected with the same volume of the vehicle and used to obtain blank brain tissue samples, to be used to prepare quality control samples and matrix-matched calibration curves. After 20 min, the rats were anesthetized with CO₂ and decapitated immediately. The brain was dissected (avoiding blood that could contaminate it), and the telencephalon (both cerebral hemispheres) was isolated, quickly frozen in liquid nitrogen and stored at −23°C until analysis.

2.3. Sample treatment

Brain tissue samples were homogenized and crushed with dry ice (Praxair, Valencia, Spain) using an electric grinder, followed by a −23°C overnight storage for CO₂ evaporation. After that, approximately 250 mg were accurately weighed (±0.1 mg) in 1.5-ml polypropylene tubes, and 750 μl of acetonitrile containing 1% of formic acid were added. Samples were extracted for 30 min under agitation using a vortex (Velp Scientifica, Usmate Velate, Italy) at 1200 rpm. After keeping extracts for 30 min at −23°C, samples were centrifuged at 12 000 rpm for 10 min. Finally, the supernatant was diluted with ultrapure water for UHPLC-MS/MS analysis: in the case of samples used for method validation, the supernatant was tenfold diluted, whereas for the brain samples obtained after administration of cathinones, supernatant was 1000-fold diluted.

The sample treatment procedure was adapted from literature,\textsuperscript{19} with the only difference being the homogenization procedure. In the present study, homogenization was performed using an electric grinder and dry ice, followed by extraction with acetonitrile and 1% formic acid, a freezing step as clean-up and dilution of the supernatant with HPLC-grade water. Sample weight, extraction volumes, and dilutions were designed according to information available in literature,\textsuperscript{40} with some modifications to improve method sensitivity. For a detailed description on analytical methodology validation and the results obtained, see the Supporting Information.
2.4 | Instrumentation

Samples were analyzed using an Acquity UPLC™ H-Class liquid chromatography system (Waters Corp, Mildford, MA, USA) coupled to a Xevo TQ-S mass spectrometer (Waters Corp, Manchester, UK) equipped with a triple quadrupole mass analyzer, using a Z-Spray electrospray interface (ESI). Further information about the UHPLC-MS/MS instrument, the conditions employed, and its optimization can be found in the Supporting Information.

3 | RESULTS

Cathinone concentrations found in the brain displayed wide differences, from 762 ng/g brain tissue for N,N-dimethylpentylone to 10 596 ng/g for N-ethyl-pentylone, the concentrations for most of the remaining compounds ranging between 1000 and 4000 ng/g. In all cases, the concentrations were well above the analytical performance, in terms of sensitivity and limits of quantification of our methodology. In addition, reliability of the analytical methodology was supported by analysis of quality control samples in duplicate, spiked at 1 and 10 ng/g, included in the sample batch. Recoveries between 70% and 120% were obtained, confirming the correct quantification of the cathinones in telencephalon tissue.

The concentrations found in telencephalon samples for all the compounds are shown in Tables 1–3. The differences observed in the cathinone levels suggest that the BBB permeability of these compounds is structure dependent, being associated with their polarity, as discussed further.

4 | DISCUSSION

4.1 | Cathinone penetration through the BBB

The main objective of our study was to evaluate the relationship between the structure of cathinones and their BBB permeability, in order to get better acquainted with the pharmacological behavior of these substances.

Pronounced concentration differences were observed in the telencephalon for different cathinones, ranging from 762 ng/g (N,N-dimethylpentylone) to 10 596 ng/g (N-ethyl-pentylone). This difference is surprising given the very high structural similarity of these two compounds, which only differ in the amine functionalization (dimethyl vs. ethyl).

Table 1 shows the concentrations found in telencephalon tissue for cathinones that differ by the functionalization of the amine moiety (N). In the two groups (those without aromatic ring substitution and those with a 3,4-methylenedioxy substituent), cathinones with an N-methyl (pentedrone and pentylone) moiety were found at a higher concentration than those with a pyrrolidine ring (α-PVP and MDPV). Regarding the compounds with an N-ethyl group, N-ethyl-pentylone had lower permeability than the N-methyl analog whereas N-ethyl-pentylone had a higher permeability than the N-methyl analog. It is also remarkable that the cathinone with a N,N-dimethyl group (N,N-dimethylpentylone) seemed to have the lowest permeability of the BBB.

N-ethyl-pentylone was, by far, the cathinone with the highest concentration in telencephalon tissue. Also known as ephylone or bk-EBDP, this substance is a recently reported cathinone that has been involved in numerous recent intoxication cases including 151 deaths between 2014 and 2018, which raises high concerns regarding the toxicity of this compound. The high N-ethyl-pentylone concentration found in telencephalon tissue is in line with a recent study about the pharmacokinetic behavior of this cathinone, which also suggested a high BBB permeability, which could explain its elevated toxicity.

Another common modification seen in cathinone analogs is altering the length of the alkyl chain. In this study, we evaluated three pairs of cathinones that only differed from each other in the length of the alkyl chain. (Table 2): buphedrone and pentedrone, N-ethyl-pentylone and N-ethyl-hexedrone, and butylone and pentylone. In all three cases, lengthening the alkyl chain led to a reduction of the BBB permeability, as shown in Table 2. These results are in concordance with data reported in a similar study, where the permeability of methylenone, butylone, and pentylone through the BBB was evaluated. The reported concentrations in cerebrospinal fluid were around 13 mg/L for butylone and 7 mg/L for pentylone after dosing Sprague–Dawley rats at 20 mg/kg. These results are coherent with those of the present study, where around 6000 and 3700 ng/g butylone and pentylone, respectively, were found in telencephalon for rats dosed at 3 mg/kg. Based on these data, the increment of the nonpolarity of the cathinones due to the increase of the alkyl chains produces a reduction of the BBB permeability. Strangely, the most potent cathinone analogs in terms of dose reported by consumers are those that have a three-carbon alkyl chain: MDPV, pentylone, α-PVP, pentedrone, and so forth, with the dose being higher if the length is shortened or increased further in most cases. This could indicate that the mechanisms of toxicity of these compounds are not directly linked to their BBB permeability. As can be seen in the case studies for bk-EBDP intoxications, users frequently report a long duration of action for this compound, which is not so common for other cathinones. Perhaps the duration of effects indicates that bk-EBDP lingers in the body for an unusually long amount of time, and some of the toxicity may stem from this phenomenon.

The last typical change in the cathinone structure is functionalization of the aromatic ring. As can be observed in Table 3, the functionalizations studied were the addition of a 3,4-methylenedioxy moiety, a methyl group, a 3,4-dimethoxy group, and the addition of an halogen atom (in this case, a fluorine). Three of the four cathinone couples with/without a 3,4-methylenedioxy moiety (buphedrone and butylone, pentedrone and pentylone, and α-PVP and MDPV) presented a reduction of the permeability through the BBB when this moiety was added to the molecule (Table 3), and it could also be related to the increment of the nonpolarity of the compound by this modification. The remarkably low brain tissue concentration (860 ng/g) for...
MDPV is in line with previously reported concentrations for MDPV in rat brain, quantified around 260 ng/g at 30 min when dosing a rat at 1 mg/kg. Only for the couple N-ethyl-pentedrone and N-ethyl-pentylone, the cathinone with the 3,4-methylenedioxy moiety presented a higher concentration in telencephalon tissue. Similar to the results obtained when analyzing the N-functionalization (Table 1), N-ethyl-pentylone produced an unexpected result when compared with the other cathinones. The higher telencephalon concentration of 4-fluoropentedrone, when compared with pentedrone, suggests that the presence of a halogen atom may increase BBB permeability, potentially due to an increment of the compound's polarity. The reason for the increment of the permeability observed when adding a
methyl group (N-ethyl-pentedrone vs. N-ethyl-4-methylpentedrone) or a 3,4-dimethoxy group (α-PVP vs. 3,4-dimethoxy-α-PVP) is unclear. It is possible that the presence of these terminal methyl groups allows for easier passing through the BBB. In order to confirm this, more cathinones with these aromatic ring changes should be evaluated.

The concentration differences discussed above, when changing the N-functionalization, alkyl chain length, and aromatic ring substitution, point at a positive correlation between polarity and BBB permeability, as also suggested by others. However, based on an in vitro model using TY09 conditionally immortalized human brain capillary endothelial cells, Simmler and colleagues suggested the opposite: these authors reported that a decrease in polarity of cathinones produces an increment of the permeability of the BBB, with nonpolar cathinones presenting a particularly high transendothelial permeability. Although these in vitro data apparently contradict our findings and those of the previous literature, the use of live animals instead of a cell culture is a closer representation of the real pharmacokinetic behavior of these compounds in a process as complex as BBB permeability.

In fact, the BBB is not only composed of endothelial cells but also includes associated cell elements such as astrocyte endfeet, pericytes, and microglia. There are several important routes of transport across the BBB, passive diffusion and the carrier-mediated influx being the most common ones related to psychoactive substances. The coexistence of both influx processes for cocaine through the BBB has been reported in literature using an in vivo model with Swiss mice; here,
TABLE 3  Concentration levels found in rat telencephalon tissue for cathinones differing on the aromatic ring substitutions (rats dosed at 3 mg/kg)

| Compound                  | Aromatic ring substitution | Brain tissue conc. (ng/g) | Ratio found/dosed (μg/mg) | Structure |
|---------------------------|-----------------------------|---------------------------|---------------------------|-----------|
| Buphedrone                |                             | 6067                      | 1.69                      | ![Structure](image1) |
| Butylone                  |                             | 4659                      | 1.29                      | ![Structure](image2) |
| Pentedrone                |                             | 3718                      | 1.03                      | ![Structure](image3) |
| Pentylone                 |                             | 3113                      | 0.86                      | ![Structure](image4) |
| 4-Fluoropentedrone        |                             | 4806                      | 1.34                      | ![Structure](image5) |
| N-ethyl-pentedrone        |                             | 1432                      | 0.40                      | ![Structure](image6) |
| N-ethyl-pentylone         |                             | 10 596                    | 2.94                      | ![Structure](image7) |
| N-ethyl-4-methylpentedrone|                             | 2541                      | 0.71                      | ![Structure](image8) |

(Continues)
the carrier-mediated influx rate was 3.4 times greater than its passive diffusion rate.\textsuperscript{26} The same publication indicates that MDPV is also a substrate for the cocaine transporter. Based on this evidence and in line with the data presented in this work as well as in literature,\textsuperscript{41} it can be deduced that the penetration of cathinones through the BBB is a carrier-mediated process. An exhaustive study about the solute carrier transporters involved in cathinone transport should be performed in order to confirm this hypothesis.

In addition to compound polarity and moieties present in the distinct structures, different physicochemical properties of the compounds such as logP and logD, topological polar surface area, number of rotatable bonds, fraction of sp3 carbons, heavy atom count, among other parameters, can be also be related to BBB permeability. For the compounds studied in this work, topological polar surface area values (obtained from PubChem) were evaluated for 11 compounds. No relationship between this parameter and BBB permeability was found. No additional parameters were found in compound databases; consequently, those should be determined experimentally or theoretically to evaluate their contribution to BBB permeability.

In summary, the relationship between cathinone structure and their ability to cross the BBB was studied in this work. To this aim, telencephalon tissues from Sprague-Dawley rats dosed at 3 mg/kg with 13 different cathinones were analyzed by a validated UHPLC-MS/MS procedure. The results obtained showed that permeability of cathinones is related to their polarity, with better crossing of the BBB with increasing polarity. These findings are in accordance with previously published data using rats\textsuperscript{41} but differ from those obtained using in vitro experiments,\textsuperscript{17} demonstrating the importance of not solely relying on in vitro data. Less polar N functionalizations, such as the presence of a pyrrolidine ring, reduced the cathinone transport through the BBB. In a similar way, cathinones with longer alkyl chain were less able to cross the BBB. Nonpolar aromatic ring substitutions such as 3,4-methylenedioxy reduced BBB permeability, whereas the presence of a fluorine atom increased BBB transport. All these data, together with information available in literature from similar studies\textsuperscript{41} and the BBB transport\textsuperscript{26} suggest that cathinones cross the BBB through a carrier-mediated process. Additionally, this study shows that studying the pharmacology and pharmacokinetics of cathinones, and NPS as a whole, is crucial for a better understanding of the in vivo potency of these compounds, complementing other studies such as dopamine and serotonin uptake inhibition. Our future work will be focused on the study of additional cathinones that will appear on the continuously evolving NPS market, in order to support the role of carrier-mediated processes in the BBB passage of cathinones.

**ACKNOWLEDGEMENTS**

D. Fabregat-Safont, J.V. Sancho, F. Hernández, and M. Ibáñez acknowledge financial support from the University Jaume I (UJI-B2018-19). D. Fabregat-Safont acknowledges Ministerio de Educación, Cultura y Deporte in Spain for his predoctoral grant (FPU15/02033). F. Martínez-García and M. Barneo-Muñoz acknowledge financial support from Ministerio de Economía y Competitividad-FEDER (BFU2016-77691-C2-1-P), the Generalitat Valenciana (PROMETEO/2016/076), and the Universitat Jaume I de Castelló (UJI-B2016-45). X. Carbón and M. Ventura acknowledge the

**TABLE 3** (Continued)

| Compound          | Aromatic ring substitution | Brain tissue conc. (ng/g) | Ratio found/dosed (μg/mg) | Structure |
|-------------------|----------------------------|---------------------------|---------------------------|-----------|
| α-PVP             |                            | 3054                      | 0.85                      |           |
| MDPV              |                            | 863                       | 0.24                      |           |
| 3,4-Dimethoxy-α-PVP |                           | 2083                      | 0.58                      |           |
grants from Subdirecció General de Drogodependències, Departament de Salut, Generalitat de Catalunya, and Plan Nacional sobre Drogas. The authors also acknowledge Esther Fuentes-Ferragud for helping during method development.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHORS CONTRIBUTION

DF-S, JVS, and MI conceived the work. MB-M and FM-G performed animal experiment and brain dissection. DF-S and MI performed sample treatment, instrumental analysis, and data process. DF-S, JVS, MB-M, FM-G, XC, MV, and MI interpreted and discussed the results. FH and FM-G contributed with new reagents and analytical tools. DF-S and MI wrote the first draft of the manuscript. JVS, MB-M, FM-G, XC, MV, CS, and FH provided useful comments and feedback for the manuscript.

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How to cite this article: Fabregat-Safont D, Barneo-Muñoz M, Carbón X, et al. Understanding the pharmacokinetics of synthetic cathinones: Evaluation of the blood–brain barrier permeability of 13 related compounds in rats. Addiction Biology. 2021;26:e12979. https://doi.org/10.1111/adb.12979