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Review

Present and future of metabolic and metabolomics studies focused on classical psychedelics in humans

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ABSTRACT

Psychedelics are classical hallucinogen drugs that induce a marked altered state of consciousness. In recent years, there has been renewed attention to the possible use of classical psychedelics for the treatment of certain mental health disorders. However, further investigation to better understand their biological effects in humans, their mechanism of action, and their metabolism in humans is needed when considering the development of future novel therapeutic approaches. Both metabolic and metabolomics studies may help for these purposes. On one hand, metabolic studies aim to determine the main metabolites of the drug. On the other hand, the application of metabolomics in human psychedelics studies can help to further understand the biological processes underlying the psychedelic state and the mechanisms of action underlying their therapeutic potential. This review presents the state of the art of metabolic and metabolomic studies after lysergic acid diethylamide (LSD), mescaline, N,N-dimethyltryptamine (DMT) and β-carboline alkaloids (ayahuasca brew), 5-methoxy-DMT and psilocybin administrations in humans. We first describe the characteristics of the published research. Afterward, we reviewed the main results obtained by both metabolic and metabolomics (if available) studies in classical psychedelics and we found out that metabolic and metabolomics studies in psychedelics progress at two different speeds. Thus, whereas the main metabolites for classical psychedelics have been robustly established, the main metabolic alterations induced by psychedelics need to be explored. The integration of metabolomics and pharmacokinetics for investigating the molecular interaction between psychedelics and multiple targets may open new avenues in understanding the therapeutic role of psychedelics.

1. Introduction

Classical psychedelics are drugs that induce an altered state of consciousness, characterized by dose-dependent distortions in perception and thought, and amplification of emotional state [1,2]. Classical psychedelics can be divided into indoleamine and phenylalkylamine derivatives. Some indoleamines, such as *N*,*N*-dimethyltryptamine (DMT), 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT), lysergic acid diethylamide (LSD), and psilocybin are used for recreational, ritualistic and therapeutic purposes [3–5]. Phenylalkylamines, such as mescaline (3,4,5-trimethoxy-β-phenylethylamine) and 2,5-dimethoxy-4-iodoamphetamine (DOI), share the pharmacological mechanism of action. Hence, both produce similar subjective effects, even though they display different chemical structure [6].

Many psychedelic compounds can be found in plants, animals, and fungi. For example, ayahuasca is a psychedelic brew originally from

Abbreviations: 2-MTHBC, 2-methyl-1,2,3,4-tetrahydro-b-carboline; 4-HIAA, 4-hydroxyindole-3-acetic acid; 4-HTP, 4-hydroxytryptophol; 5-OH-DMT, 5-hydroxy-N,N-dimethyltryptamine; 5-MeO-DMT, 5-methoxy-N,N-dimethyltryptamine; CD, coulometric detection; DMT, *N*,*N*-dimethyltryptamine; ECD, electrochemical detection; FD, fluorescence detection; LC, liquid chromatography; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; IAA, indoleacetic acid; LSD, lysergic acid diethylamide; MS, mass spectrometry; ND, not declared; NMR, nuclear magnetic resonance; THH, tetrahydroharmine.

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South America that is traditionally made with the bark of *Banisteriopsis caapi* and the leaves of *Psychotria viridis*. These plant species contain a synergistic combination of β-carboline alkaloids (harmine, tetrahydroharmine, and harmaline) and DMT. Other tryptamines, such as 5-MeO-DMT and 5-hydroxy-DMT (5-OH-DMT, also known as bufotenine), are alkaloids secreted by the parotid glands of *Bufo alvarius* (*Incilius alvarius*) found in the Sonora region [7]. Psilocybin is found in several species of psychedelic mushrooms (*Psilocybe*) [8]. Mescaline can be found in the Peyote cactus (*Lophophora williamsii*), whereas 2,5-dimethoxy-4-iodoamphetamine (DOI) was synthesized by Alexander Shulgin [9].

Indigenous communities have been using some of these substances for millennia. For example, Miller and colleagues identified chemical remnants of 5-hydroxy-DMT, DMT, harmine, and cocaine as organic residues from a ritual bundle in Bolivia, radiocarbon dated to approximately 1000 C.E. [10]. Nevertheless, there is controversy regarding the antiquity of alleged pre-Columbian ritual use, such as that of *Bufo alvarius* [11].

Historically, research with psychedelic substances started last century with the synthesis of LSD by Albert Hofmann in 1938 [12]. After almost three decades of flourishing investigation, research on psychedelics was mainly conducted in very restricted approaches [4,13]. Psychedelics were used as an adjunct for psychotherapy during the 50 s and early 60 s [14,15]. After several seminal studies [16] [17] [18] with varying degrees of success, scheduling, and stigma drastically reduced the interest in psychedelics [19].

After several decades of ostracism, recently interest has been resurgent regarding the therapeutic potential of these substances in treating an array of different mental health disorders, including depression, anxiety, substance use disorders, obsessive-compulsive disorder, post-traumatic stress disorder, [20–35] The use of psychedelics has also been suggested as a promising tool for a better understanding of the physiopathology of mental illness [21,36]. Additionally, there is a renewed focus on the potential neuroplasticity, promoting qualities of psychedelic substances [22]. Thus, the effect of some psychedelics in enhancing cognitive function has been [37,38].

Despite all this evidence pointing towards a potential therapeutic use of psychedelics in human health, understanding of most biological processes mediating enhancement remains limited [39]. Regarding the mechanisms of action, there is evidence that both phenylalkylamine (mescaline, DOI) and indoleamines (DMT, 5-MeO-DMT, LSD) are 5-HT_2 receptor agonists (5-HT_{2A}, 5-HT_{2B}, and 5-$HT_{2\ C}$ receptors), being the phenylalkylamine psychedelics more selective for these receptors than indoleamines [6,40–42]. Among the current knowledge, the stimulation of $5HT_{2A}$ enhances presynaptic glutamatergic transmission in the prefrontal cortex and affects sensory perception, behavioral regulation, and mood [40]. Interactions with other receptor sites may affect the psychopharmacological and behavioral effects of indoleamine drugs [43]. In agreement with this mechanism of action, some psychedelics have been reported to modulate in a dose-dependent way the concentration of serotonin and its metabolites in the brain [44]. Furthermore, psychedelics combined with monoamine oxidase (MAO) inhibitors reduce their metabolism and prolong their effects [45]. For example, in the case of ayahuasca, this effect is produced by the β-carboline compounds naturally present in ayahuasca brew that act by inhibiting the MAO activity, preventing the metabolism of DMT by this enzyme [46] and leading to neural interactions [47,48].

Investigating these and other currently unknown mechanisms of action in therapeutic use can not only help establish treatment guidelines but can also provide evidence of the possible risks associated with the use of these substances. The use of psychedelics in the therapeutic field raises several questions. Despite the described benefits in mental illness, intervention with psychedelics may cause overwhelming distress, and this could lead to unpredictable behaviors [49] in susceptible individuals or when the dose has not been individually adjusted. Reoccurring drug-like experiences, or flashbacks, after the administration of some psychedelics, such as LSD, are rare phenomena that may course without any clinically relevant problem in controlled studies with healthy participants [50]. However, the persisting and distressing manifestation of these experiences may lead to hallucinogen-persisting perception disorder [51]. Related risks are prolonged psychoses triggered by these compounds, especially in individuals with a personal or family history of psychotic disorders or other psychiatric disorders [52].

Cardiac, gastrointestinal, neurological, and clinical alterations have also been reported [53,54]. Therefore, given the growing interest in the use of psychedelics in the treatment of psychiatric illnesses, understanding their mechanisms of action is crucial to establishing their benefit/risk ratio [55].

Performing both metabolic and metabolomics studies is crucial to highlight both the consequences of exposure to psychedelics and simultaneously consider environmental and phenotyping factors on users [56]. On the one hand, metabolic studies aim to establish the main metabolic pathways involved in the biotransformation of the drug in the body. These studies are required to characterize a drug properly, obtain suitable biomarkers for drug consumption, and elucidate differences in the susceptibility of the individuals. The identification of the drug metabolites, their activity, and their concentrations are necessary for investigating the physiological mechanisms and risk. In most cases, metabolic transformations may be the most direct pathway for drug inactivation. Thus, the evaluation of the metabolites and their association with the drug effects is a suitable tool to identify one of the common reasons for the different susceptibility i.e., the presence of polymorphisms in metabolic enzymes that are key. In other cases, metabolites may be even more active than the parent drug. In these cases, the evaluation of the metabolite concentrations may be more adequate to establish safe doses than the evaluation of the parent drug. For most psychedelic drugs, metabolic, pharmacokinetic, and pharmacodynamic studies have been conducted, and plasma and urine concentrations of the parent drug and major metabolites have been estimated.

On the other hand, metabolomics studies aim to determine the specific changes in the metabolome, the complete set of metabolites in the body, produced by the drug administration. Metabolomics allows for the detection of alterations in systems-level metabolites within biological pathways, thereby providing insights into the mechanisms that underlie various physiological conditions and pathologies [56,57]. Therefore, metabolomics provides a thorough understanding of the metabolome and biochemical processes associated with systems biology [58]. One of its possible uses is the ability to evaluate the molecular mechanisms associated with the physiological condition and to monitor the response to such condition through the impact on the endogenous metabolome. The use of metabolomics has allowed the elucidation of different physiological mechanisms of these events [59]. Successful metabolomics applications in drugs relevant to psychiatry such as ketamine [60], medical cannabis [61], or MDMA [62–64] have been shown to contribute to the therapeutic field. These studies provided new insights into the mechanisms of action of the drugs associated with the therapeutic benefits of the use by correlating alterations in the metabolome with clinical outputs such as the severity of depression or behavioral symptoms commonly observed in subjects with autism spectrum disorder. These studies also reveal the mechanisms of action potentially associated with the deleterious side effects of the drug.

However, very few reviews on metabolic and metabolomic studies of psychedelics in humans can be found in the literature. Some of them are focused either on one specific psychedelic, such as LSD [65] or mescaline [66] or on one specific analytical technique, such as NMR [67]. To our knowledge, there is a lack of systematic reviews focused on both metabolic and metabolomics studies without restriction in analytical techniques and studying a wide range of classical psychedelics.

In this systematic review, we aim to report evidence on the research ongoing on human metabolic and metabolomics studies of classical psychedelics. Before performing metabolic and metabolomics studies,

several analytical considerations such as the biological matrix (plasma/serum, urine), the analytical technique (liquid or gas chromatography-mass spectrometry [LC-MS, GC-MS] and NMR), and the analytical strategy (targeted or untargeted) should be properly selected to obtain reliable and interpretable results from these studies. Thus, we first describe the characteristics of the published research regarding drug, strategy, demographics, biological matrix, and analytical considerations such as sample treatment and instrumentation. Afterward, we review the main results obtained by both metabolic and metabolomics studies in classical psychedelics stratified by drugs.

2. Material and methods

Literature query and article selection were performed using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) procedure [68], which is the most common method used for systematic reviews. The search sentence was constructed using the following search terms *TITLE-ABS ((psychedelics OR psilocybin OR (lysergic acid diethylamide) OR dimethyltryptamine OR (5-methoxy-N, N-dimethyltryptamine) OR (2,5-dimethoxy-4-iodoamphetamine) OR mescaline OR peyote) AND (urine OR plasma OR serum OR blood OR excretion OR biofluid*) AND (human* OR men OR women OR patient* OR volunteer*) AND (metabolite* OR biokinetics OR biotransformation OR volatile OR volatiles OR (metabolite profiling) OR (metabolite analysis) OR (metabolic profiling) OR (metabolic fingerprinting) OR (metabolic characterization) OR metabolome OR metabolomics OR metabolomic OR metabonomics OR metabonomic OR lipidome OR lipidomics OR lipidomic))*. The end date of search on the repositories of PubMed (https://pubmed.ncbi.nlm.nih.gov/) and SCOPUS (https://www.scopus.com/) was January 2023. The review was limited to papers in English language, and no restrictions were applied to the publication date. The research papers applying metabolomics approaches were selected by one or more skilled researchers from the list of retrieved references in the procedure outlined in Fig. 1. Data obtained on the query were merged into an Excel file, including authors, year, title, and abstracts. Duplicated research papers were removed after reading the title and abstract. The exclusion criteria included publications calculating the peripheral concentration of the drug after administration at a known dose, publications including drugs other than classical psychedelics, in vitro studies, animal studies, reviews, book chapters, conference papers, and case reports.

3. Results and discussion

The results of the literature search are summarized in Fig. 1. The query results in a total of 136 reports from Scopus (66) and PubMed (73), plus 8 additional works that were detected by checking cites from the resulting works. After the removal of duplicates, a total of 118 articles were selected after screening based on title and abstract. Among them, 81 references were removed due to exclusion criteria. The full text of the remaining 37 papers was downloaded and reviewed.

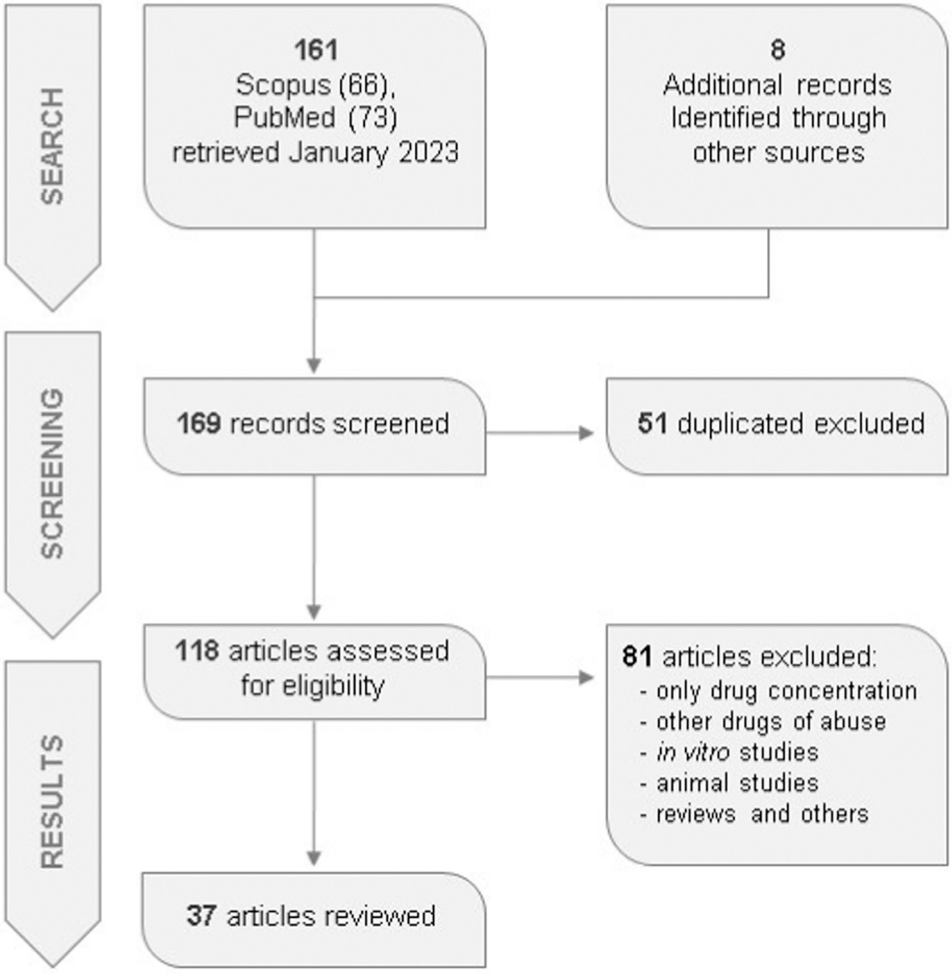

Fig. 1. Algorithm of the literature search for reviewed articles.

3.1. Characteristics of the studies

3.1.1. Drug

For the 37 studies meeting the criteria for the systematic review, we classified the references based on the studied drug. Results are shown in Table 1. LSD has been the most studied psychedelic (14 papers), followed by psilocybin, ayahuasca, and mescaline (9, 6, and 5 papers, respectively). Less information is available for DMT alone (2 publications). Remarkably, only one reference included the study of several psychedelics (e.g., psilocin, 5-OH-DMT, and LSD).

3.1.2. Types of study

Three types can be distinguished in the literature: (i) targeted detection of drug metabolites as indicative of use, (ii) pharmacokinetic study of the drug/metabolites, and (iii) metabolomics studies aiming to establish mechanisms of action.

Overall, most of the papers were focused on either the detection of drug use or pharmacokinetics. Only a few examples were found regarding metabolomics studies.

Regarding the targeted detection of known drugs/metabolites as indicative of use, several studies aimed to detect either the same psychedelic compound or its major metabolite in a given biological matrix as markers of drug consumption. The determination of the parent drug and 1–2 metabolites is common in this type of study (Table 1). The detection of several metabolites provides additional confirmation of the use of the drug. Additionally, these metabolites may increase the time in which the use can be detected. Studies aiming to evaluate the pharmacokinetics of the drug also determined the drug and several of its metabolites. The inclusion of metabolites in this type of study may help in clarifying the role of drug-metabolizing enzymes in the pharmacokinetics of the drug.

Remarkably, all the studies related to the detection of drug metabolites are focused on the main metabolites previously reported in other species. We found a lack of studies involving the comprehensive study of psychedelic metabolism in humans. These studies would be able to elucidate the structure of unknown human metabolites. This limitation leads to only a partial understanding of the human metabolism of psychedelics. We also found that metabolomics studies that focused on the effect of psychedelics on the human metabolome were very scarce [69–72]. Consequently, in most cases, valuable information on the physiological processes behind the drug's therapeutic effects, and/or side effects, cannot be extracted.

3.1.3. Demographics

It is worth mentioning that humans are greatly diverse, and several human studies have shown that the pharmacological response to a specific drug is multi-factorial, involving individual factors such as genetics and epigenetics, fasting condition, lifestyle, age, ethnic, body composition, and sex [73–76]. This diversity may substantially alter the metabolism of the psychedelic drug, and its pharmacokinetics, contributing to differences in susceptibility.

Regarding drug metabolism, it has been reported that the CYP2D6 genetic polymorphism may introduce considerable variability in the response to multiple psychedelics [45,77–79]. The alteration of psychedelics' metabolism produced by CYP2D6 genetic polymorphisms is one of the main sources of this variability. In this review, only one human-based study investigating the genetic influence (CYP2D6) on the metabolism of these substances was found [77].

The endogenous metabolome is also strongly impacted not only by inter- and intraindividual variations such as age, fasting status, and sex [80,81] but also by other factors such as circadian rhythm, seasonal differences, menstrual cycle, stress, and gut microbiota [82,83]. Therefore, these factors should be considered before extracting conclusions about the potential effects of psychedelics in the metabolome. Ideally, a rigorous study design needs to take into consideration specific research objectives, appropriate sample sizes, and participant characteristics, allowing for effective control and analysis of the factors of interest.

In this review, we found that almost half of the studies were carried out only in men. Only 20 studies out of 37 included women as well. However, in most of these studies, the number of women and men was not balanced, and only 8 papers included a similar number of men and women. In some cases, no sex information was given. Evaluating the sex differential impact of psychedelics on the metabolome is not only important to establish potential sex bias in the metabolism but also because sex differences have been described in the serotonergic mechanisms in the behavioral effects of psychedelics [84,85]. This raises the possibility that the metabolism of psychedelics might be different in men and women, and future studies should consider this likelihood.

The influence of fasting status on metabolite levels necessitates standardized protocols. Standardizing the fasting duration or considering fasting status as a covariate in statistical analyses helps mitigate the confounding effects of recent food intake. In this context, several studies restrict the food intake before the intake of the drug [86–88].

3.1.4. Biological matrix and sample preparation

Most metabolic studies focused on the determination of major drug metabolites are mainly performed on blood (plasma/serum) and urine. However, there is a wide variety of many other biological specimens e.g., saliva, seminal fluids, amniotic fluid, cerebrospinal fluid, synovial fluid, digestive fluids, and lung aspirates, among others [89] that can be used in metabolomics studies to assess the effect that the drug has on the endogenous metabolome and the drug metabolism.

In this review, we found that, except for one publication that included post-mortem vitreous humor [90], only blood-based (plasma/serum) and urine specimens were used for metabolic/metabolomics studies of psychedelics (Table 1). This exclusivity is probably promoted by the ease of sample collection and the large number of samples available for these matrices.

The sample preparation primarily aims to selectively extract the analytes from the biological matrices, removing at the same time the matrix components that may hinder the analysis depending on the analytical technique [91,92]. Additionally, sample preparation may also preconcentrate the metabolite increasing the sensitivity of the targeted compound by MS-based methods. Some of the reviewed studies used a solid phase extraction (SPE) step for that purpose [93–95].

Sample preparation may also be modulated by the characteristics of the selected biological matrix. In this respect, urine reflects the human metabolism at the end of the absorption, distribution, metabolism, and excretion course of the drug [92,96] whereas plasma/serum samples provide a global "snapshot" of the circulation substances reflecting internal and external perturbations of the organism [97]. Therefore, urine displays a higher number of conjugated metabolites (glucuronides, sulfates) than plasma/serum. For this reason, sample preparation may include a hydrolysis step to release the unconjugated metabolite. Several of the reviewed studies utilized enzymatic hydrolysis treatment of the samples in both urine and plasma/serum. This enzymatic hydrolysis increased the levels of several drug metabolites suggesting an important role of conjugation (both with glucuronide and sulfate) in psychedelic metabolism [98–100].

3.1.5. Instrumentation

LC-MS, GC-MS, and NMR are the most widely used analytical techniques in both metabolic and metabolomics studies. Although NMR spectroscopy offers ease of sample preparation, high reproducibility, and is an intrinsically quantitative technique, no NMR-based metabolomics studies were found in this literature review. [101]MS techniques are more sensitive with lower limits of detection typically being some orders of magnitude and LC-MS/MS is routinely used for high-throughput pharmacokinetic screening [102]. As can be seen in Table 1, most of the reviewed studies are based on LC-MS/MS, while few studies described the use of GC/MS-based techniques. Other techniques

Table 1
Overview of metabolic and metabolomic studies on psychedelics in humans.

Author	Reference	Drug	Metabolites	Gender	Analytical method	Biosample
McIlhenny et al., 2010	[100]	Ayahuasca	DMT, DMT-N-oxide, THH, harmine, harmaline, harmalol, harmol, 2MTHBC, 5-hydroxy-DMT	3 males	HPLC-MS/MS	urine
Riba et al., 2003	[105]	Ayahuasca	Plasma: DMT, harmaline, THH, harmalol, harmol; Urine: normetanephrine	15 males, 3 females	Plasma: HPLC-FD Urine: HPLC-CD	plasma urine
McIlhenny et al., 2012	[124]	Ayahuasca	DMT, DMT-N-oxide, THH, tetrahydroharmol, harmine, harmol, harmaline, harmalol, 2-MTHBC, IAA	3 males	HPLC-MS/MS	plasma
Riba et al., 2012	[127]	Ayahuasca	DMT, DMT-N-oxide, IAA, 2MTHBC, N-methyltryptamine	10 males	HPLC-MS/MS	urine
Yritia et al., 2002	[104]	Ayahuasca	DMT, harmine, harmaline, THH, harmol, harmalol	4 males	HPLC-FD	plasma
Madrid-Gambin et al., 2022	[130]	Ayahuasca	Tyrosine, DHEA, OEA, POEA, AEA, 5HIAA, serotonin, 2-OG, DGLEA, 20α-DHE, 20β-DHE, glutarate, α-hydroxybutyrate, leucine, 2-LG, cortisone, LEA, β-hydroxybutyrate, acetylcarnitine, carnitine, 2-AG, glutamate, glutamine, octanoic, glyoxylate, isoleucine, MAG/DAG 18:1, DEA	14 males and 9 females	LC-MS/MS	plasma
Eckernäs et al., 2022	[143]	DMT	DMT, DMT N-oxide, IAA	7 males, 6 females	LC-MS/MS	plasma
Riba et al., 2015	[123]	DMT	DMT, DMT-N-oxide, IAA	3 males, 3 females	HPLC-MS/MS	urine
Nelson et al., 1992	[110]	LSD	LSD, iso-LSD, and N-demethyl-LSD	ND	GC/MS/MS	Blood and urine
Favretto et al., 2007	[90]	LSD	Blood: LSD, nor-LSD; Urine: LSD, iso-LSD, nor-LSD, 2-oxo-3-hydroxy-LSD; Vitreous humor: LSD, nor-LSD	ND	LC-ESI-MS/MS	blood urine Vitreous humour
Dolder et al., 2018	[113]	LSD	LSD, nor-LSD, lysergic acid mono ethylamide, lysergic acid ethyl-2-hydroxyethylamide, 2-oxo-LSD, 13/14-hydroxy-LSD	12 males, 12 females	LC-MS	plasma
Holze et al., 2019	[71]	LSD	LSD, nor-LSD, 2-oxo-3-hydroxy-LSD	13 males, 14 females	UHPLC–MS/MS	plasma
Vizeli et al., 2021	[77]	LSD	LSD, 2-oxo-3-hydroxy-LSD	40 males, 41 females	UHPLC–MS/MS	plasma
Steuer et al., 2017	[112]	LSD	LSD, iso-LSD, nor-LSD, 2-oxo-3-hydroxy-LSD, hydroxy-LSD, hydroxy-LSD glucuronide	8 males and 8 females	MFLC-MS/MS	plasma
Reuschel et al., 1999	[95]	LSD	LSD, 2-oxo-3-hydroxy-LSD	Kinetics: 1 male, 1 female; n = 49 for screening (ND)	GC-MS/MS	urine
Poch et al., 1999	[93]	LSD	LSD, 2-oxo-3-hydroxy-LSD	ND	GC-MS: LSD, iso-LSD; LC-MS/MS: nor-LSD, 2-oxo-3-hydroxy-LSD	urine
Klette et al., 2002	[108]	LSD	LSD, 2-oxo-3-hydroxy-LSD, nor-LSD, iso-LSD	ND	LC-MS	urine
Burnley et al., 2003	[109]	LSD	2-oxo-3-hydroxy-LSD	19 males, 6 females	GC-MS	urine
Horn et al., 2003	[94]	LSD	2-oxo-3-hydroxy-LSD	ND	LC-MS	urine
Lim et al., 1988	[111]	LSD	LSD, 2-oxo-3-hydroxy-LSD	1 male	GC-MS	urine
Masuda et al., 1960	[144]	LSD	p-hydroxyphenyl-acetic acid, feruloylglycine, vanilloylglycine, vanillic acid	21 males and 2 females	paper chromatography	urine
Dolder et al., 2016	[69]	LSD	LSD, 2-oxo-3-hydroxy-LSD	8 males, 8 females	LC-MS/MS	plasma
Thomann et al., 2022	[120]	Mescaline	Mescaline, 3,4,5-trimethoxyphenylacetic acid, N-acetyl mescaline	ND	LC-MS/MS	plasma
Demisch et al., 1979	[121]	Mescaline	Prolactin, growth hormone	4 males and 1 female	radio-immune assay	serum
Demisch et al., 1978	[145]	Mescaline	3,4,5-trimethoxybenzoic acid	ND	GC-MS	urine
Charalampous et al., 1966	[115]	Mescaline	Urine: mescaline, 3,4,5-trimethoxyphenylacetie acid, N-acetyl-β-(3,4-dimethoxy-5-hydroxyphenyl)-ethylamine, N-acetylmescaline, O-demethylated phenylacetic acid Plasma: mescaline, 3,4,5-trimethoxyphenylacetic acid, N-acetylmescaline, N-acetyl-β-(3,4-dimethoxy-5-hydroxyphenyl)-ethylamine Cerebrospinal fluid: mescaline, 3,4,5-trimethoxyphenyl-acetic acid, N-acetylmescaline, N-acetyl-β-(3,4-dimethoxy-5-hydroxyphenyl)-ethylamine	12 males	paper chromatography	urine, plasma and cerebrospinal fluid
Richter et al., 1938	[106]	Mescaline	mescaline	ND	colorimetry	urine
Madsen et al., 2021	[146]	Psilocybin	psilocin (unconjugated)	9 males and 6 females	UPLC-MS/MS	plasma
Bambauer et al., 2021	[140]	Psilocybe sp.	Psilocin-O-glucuronide	ND	HILIC-HRMS/MS	urine
Kolaczynska et al., 2021	[137]	Psilocybin	Psilocin, psilocin glucuronide, 4-HIAA	ND	LC-MS/MS	plasma
Hasler et al., 2002	[103]	Psilocybin	Psilocin, psilocin glucuronide	4 males and 4 females	HPLC-ECD	urine
Hasler et al., 1997	[70]	Psilocybin	Psilocin, 4-HIAA	8 males and1 female	HPLC-ECD	plasma

(*continued on next page*)

Table 1 (*continued*)

Author	Reference	Drug	Metabolites	Gender	Analytical method	Biosample
Madsen et al., 2019	[135]	Psilocybin	Psilocin	5 males and 3 females	UHPLC–MS/MS	plasma
Brown et al., 2017	[72]	Psilocybin	Psilocybin	10 males and2 females	LC-MS	plasma and urine
Martin et al., 2013	[114]	Psilocybin	Urine Psilocybin: psilocin, bufotenine	1 male	LC-MS/MS	urine
		LSD	Urine LSD: LSD, iso-LSD, nor-LSD, 2-oxo-3-hydroxy-LSD, bufotenine;	1 male and 3 females		urine
			Serum LSD: LSD, iso-LSD, 2-oxo-3-hydroxy-LSD			serum

Abbreviations: 4-HIAA, 4-hydroxyindole-3-acetic acid; CD, coulometric detection; DMT, N,N-dimethyltryptamine; ECD, electrochemical detection; FD, fluorescence detection; HPLC, high-performance liquid chromatography; HRMS, high resolution mass spectrometry; IAA, indoleacetic acid; LSD, lysergic acid diethylamide; MS, mass spectrometry; ND, not declared; THH, tetrahydroharmine.

such as HPLC with electrochemical [70,103], fluorescence [104,105] and colorimetry [106] detection have been also used for the quantification of major metabolites of the drug.

3.1.6. *Analytical strategy*

In the field of psychedelics, metabolic studies are usually performed by targeted approaches, thus an analytical method specifically developed for the determination of targeted compounds is applied. Although untargeted approaches based on the open detection of all potential metabolites may provide additional information such as the occurrence of unexpected metabolites, this type of strategy is very uncommon in metabolic studies of psychedelics.

In contrast, in the case of metabolomics studies, both targeted and untargeted approaches are common. The targeted approach or metabolic profiling refers to the analysis, generally quantitative, of a part of the metabolome that has already been previously predefined. The analysis is focused on several predefined metabolic pathways potentially altered by the administration of the drug (such as the serotonin metabolic pathway). Untargeted approaches or metabolic fingerprinting refers to the analysis of the metabolome in a broad sense and that includes

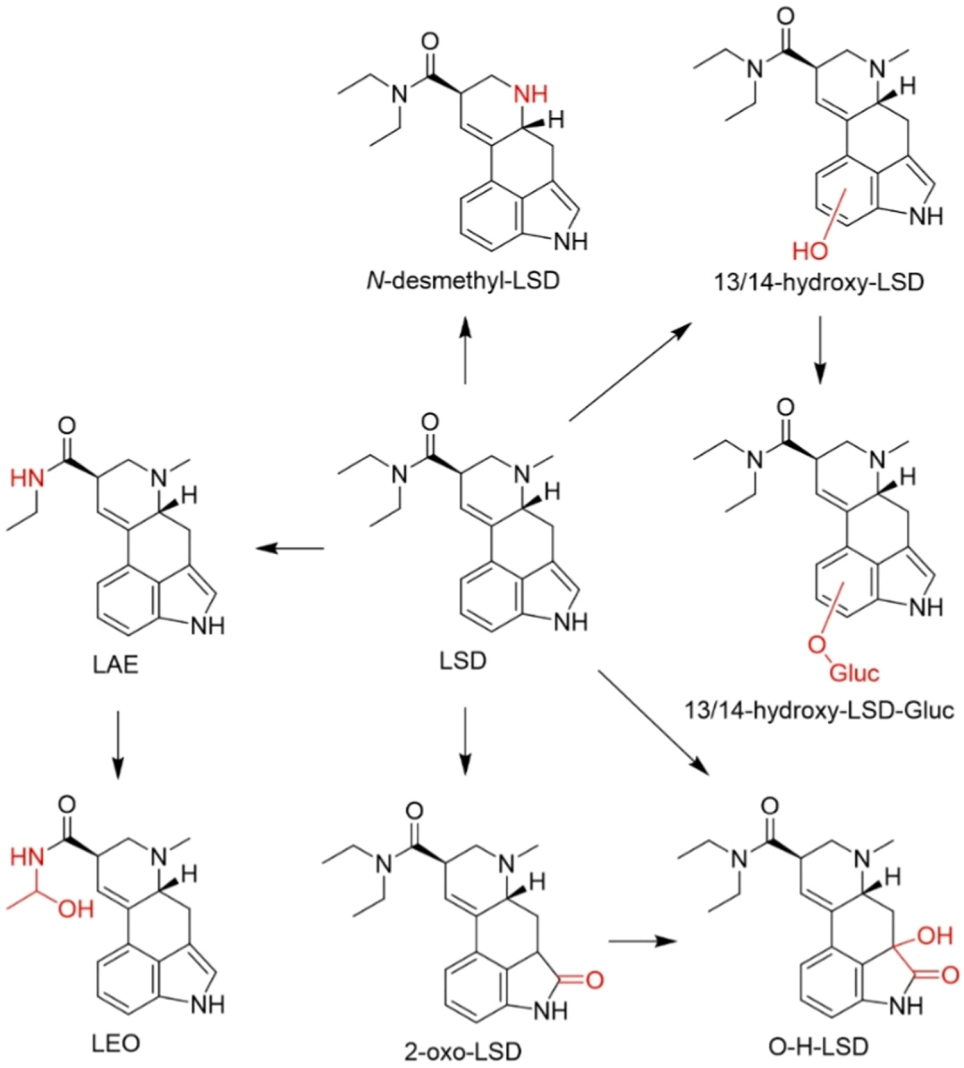

Fig. 2. The metabolism of LSD and its main metabolites in humans. Abbreviations: gluc, glucuronide; LAE, lysergic acid ethylamide; LEO, lysergic acid ethyl-2-hydroxyethylamide; LSD, lysergic acid diethylamide; O-H-LSD, 2-oxo-3-hydroxy lysergic acid.

the largest number of metabolites without prior knowledge. Therefore, it also includes those poorly characterized or unknown metabolites. The untargeted approach gives us a complete qualitative view of the metabolome, intending to detect the maximum number of metabolites possible associated with drug exposure [107].

All the studies considered in this review used targeted approaches for the determination of known compounds. In the case of metabolic studies, the psychedelic itself and/or its major metabolites are normally targeted. The few metabolomics studies published determined predefined parts of the metabolome including metabolic pathways theoretically altered by the administration of the drug.

3.2. Drug metabolites

3.2.1. LSD

Most references found on LSD deal with either drug concentration or pharmacokinetic profile. Due to legal restrictions on the administration of LSD, there is limited literature on human metabolism. Based on this information, Fig. 2 shows the metabolic pathway of LSD. LSD is mainly metabolized in 2-oxo-3-hydroxy lysergic acid (O-H-LSD) by different CYP enzymes. These metabolites are commonly detected using MS-based techniques [94,95,108–110]. Other main metabolites such as *N*-desmethyl-LSD [93,111], iso-LSD, hydroxyl-LSD, and its glucuronide [112] have been also reported. Finally, some minor metabolites such as lysergic acid ethylamide (LAE), lysergic acid ethyl-2-hydroxyethylamide (LEO), 2-oxo-LSD, and trioxylated-LSD have been also reported [113]. Although the 13/14-hydroxy-LSD standard was tentatively detected during the development of the LC-MS/MS method, the metabolite was only sporadically detected in human plasma samples after LSD administration. Remarkably, in a paper investigating a small number of drug metabolites, the authors detected small amounts of 5-OH-DMT in the urine of a couple of LSD users after recreational use [114]. Conversely, the authors cannot guarantee that these volunteers did not consume drugs other than LSD.

The pharmacokinetics of LSD and/or its main metabolite O-H-LSD have been extensively investigated in both plasma and urine (Table 2). After oral administration, LSD and O-H-LSD showed maximal urinary concentrations at 1.5 h and 4 h, respectively, [69]. The authors also reported a substantial decrease in LSD during the first 12 h, while the O-H-LSD decrease occurred between 16 and 24 h after administration. Regarding the rest of the metabolites, a high fluctuation of *N*-desmethyl-LSD in plasma and delayed elimination of LSD metabolites, compared with LSD have been reported [112]. This fluctuation of *N*-desmethyl-LSD in plasma was confirmed in a more recent publication [113]. In the study, the metabolite could only be detected in 2 out of 24 plasma samples of healthy subjects after LSD intake.

Despite being LSD the most investigated psychedelic, we did not find any metabolomics study investigating the effects of LSD administration

Table 2
Summary of the main pharmacokinetic parameters published for classical psychedelics. Cmax: maximum concentration, Tmax: time taken to reach the maximum concentration AUC_{∞}: AUC from time zero to infinity, $t_{1/2}$: half-life time, CL/F: Clearance, Vz/F: apparent volume of distribution.

	Dose	Cmax	Tmax	AUC_{∞}	$t_{1/2}$	CL/F	Vz/F	Reference
	(μg/Kg)	**(ng/ml)**	**(h)**	**(ng/ml·h^{-1})**	**(h)**	**(l/h)**	**(l)**	
Ayahuasca								
DMT	600	12.14	1.5	21.55	1.07	2281	3510	[105]
DMT	850	17.44	1.5	38.33	1.06	1813	2506	[105]
Harmaline	70	2.48	1.5	8.13	2.01	746	2041	[105]
Harmaline	90	4.32	2.0	14.87	1.95	597	1439	[105]
THH	820	23.06	2.5	172.07	4.78	560	3070	[105]
THH	1160	39.40	3	351.89	4.68	365	2073	[105]
Harmol	1000*	10.95	1.5	28.33	1.64			[105]
Harmol	1400*	17.57	2	52.27	1.49			[105]
Harmalol	70*	6.74	2.5	206.93	30.33			[105]
Harmalol	90*	9.59	2.75	333.54	48.64			[105]
Mescaline								
Mescaline	300[a]	895	2.09	6800	4.2			[120]
Mescaline	500[a]	3500	2		6			[115]
TMPAA	300*[a]	794	2.36	6067	4.51			[120]
NAM	300*[a]	24.7	2.42	128.8	2.72			[120]
LSD								
LSD	1–1.8	1.7	1.7	13	3.6	7.5	39	[71]
LSD	2.04–3.85	3.5	1.5	22	4.2	140		[112]
LSD	2.04–3.85	4.3	1.5	28	3.6			[69]
LSD (*functional CYP2D6*)	1.1–2.1	2.2	1.7	15	4.1			[77]
LSD (*non-functional CYP2D6*)	0.9–2.1	2.5	1.8	27	7.5			[77]
Oxo-OH-LSD	1–1.8*	0.11	5.0	1.7	5.2	51	490	[71]
Oxo-OH-LSD	2.04–3.85*	0.11	4.0	1.6	9.0			[112]
Oxo-OH-LSD	2.04–3.85*	0.4	4	3.8				[69]
Oxo-OH-LSD (*functional CYP2D6*)	1.1–2.1*	0.11	4.5	1.6	9.3			[77]
Oxo-OH-LSD (*non-functional CYP2D6*)	0.9–2.1*	0.13	6.2	3.1	16			[77]
Iso-LSD	2.04–3.85*	0.70	2.3	16	12			[112]
Nor-LSD	2.04–3.85*	0.01	4.6	0.28	11			[112]
Psilocybin								
Psilocin	224*	8.2	1.75	32.7	2.71			[70]
Psilocin	300*	16	2.03	140	2.98	164		[72]
Psilocin	450*	26	2.03	213	2.98	164		[72]
Psilocin	600*	37.6	2.05	267	2.98	164		[72]
Psilocin	200*	10.81	3.71	33	2.24			[86]
Psilocin unconjugated	70*	20	2	83	1.8			[147]
Psilocin Glucuronide	70*	82	4	712	4.7			[147]
Psilocin total	70*	96	3	798	4.3			[147]
4-HIAA	224*	150	1.88	514.3	1.61			[70]
4-HIAA	70*	105	2	347	1.6			[147]

* Dose of the precursor,
[a] Dose reported in mg of substance. Abbreviations:

on the metabolome.

3.2.2. Mescaline

Early reports already demonstrated the importance of metabolism in mescaline excretion [106]. The metabolism for mescaline based on the reviewed work is depicted in Fig. 3. A major part of the detoxification of mescaline comprises oxidative deamination to 3,4,5-trimethoxyphenylacetaldehyde, which is then oxidized to produce the inactive 3,4,5-trimethoxyphenylacetic acid (TMPAA) or reduced to produce the inactive 3,4,5-trimethoxyphenylethanol [115–118]. There is still debate over which enzyme deaminates mescaline into its aldehyde derivative. Both diamine oxidases (DAO) and MAO may be involved in this reaction. As a result of its metabolism, TMPAA is converted into 3,4-dihydroxy-5-methoxyphenylacetic acid or 3,4,5-trimethoxybenzoic acid. TMPAA demethylation is further combined with glutamine by glutamine N-acyltransferase, to form 3,4-dihydroxy-5-methoxyphenacetylglutamine [119]. Other minor metabolites such as *N*-Acetylmescaline (*N*-[2-(3,4,5-trimethoxyphenyl)ethyl]acetamide), *N*-acetyl-β-(3,4-dimethoxy-5- hydroxyphenyl)-ethylamine (NAHM) and 4-desmethylmescaline (3,5-dimethoxy-4-hydroxyphenethylamine) have been also reported [115] [120].

The main pharmacokinetics parameters reported for mescaline are summarized in Table 2. Charalampous and colleagues showed by administration of the radioactive compound that an average of 87% of the dose was excreted within the first 24 h, and 92% during the first 48 h [115]. The authors showed that after an oral administration, 13.2% of the dose was excreted as 3,4,5-trimethoxyphenylacetic acid (TMPAA). Accordingly, 87% of TMPAA was excreted within the first 24 h and 96% within 48 h. *N*-Acetylmescaline (N-[2-(3,4,5-trimethoxyphenyl)ethyl]acetamide) and *N*-acetyl-β-(3,4-dimethoxy-5- hydroxyphenyl)-ethylamine (NAHM) were also detected in much lower amounts [115]. The maximum plasmatic concentration of mescaline is found two hours after administration. Similar Tmax are obtained for mescaline's metabolites TMPAA and NAM. Current analytical methods did not reach the required sensitivity for obtaining reliable pharmacokinetic data for minor metabolites such as 4-desmethyl mescaline [120].

Although the administration of mescaline has been reported to alter some critical biological processes such as the secretion of both prolactin and growth hormone [121], we could not find any metabolomics study showing the effects of mescaline administration on the endogenous metabolome.

3.2.3. Ayahuasca (DMT and β-carboline alkaloids)

The main metabolic pathways of both DMT and β-carboline alkaloids (harmine, tetrahydroharmine, and harmaline) have been deeply studied. On the one hand, DMT is mainly metabolized through oxidative processes, as illustrated in Fig. 4. The reported urinary metabolites for DMT after ayahuasca consumption include the DMT-*N*-oxide (major) [100], *N*-methyltryptamine (minor) [122], and its subsequent transformation by MAO to indole-3-acetic acid [123]. In the case of plasma samples, enzymatic hydrolysis did not exhibit any substantial rise in any of the major metabolites, indicating that DMT metabolites are in their free form in circulation [124]. The presence of small amounts of 5-OH-DMT in urine has been also reported after ayahuasca consumption, increasing its concentration after glucuronidase–sulfatase hydrolysis [100]. This finding remains unusual since 5-OH-DMT is an alkaloid naturally found in certain species of plants, mushrooms, and *Bufo alvarius* [125] but not in common ayahuasca sources (the bark of *Banisteriopsis caapi* and the leaves of *Psychotria viridis*). Two potential reasons may be behind this result. First, it is known that the preparation of the ayahuasca brew and its ingredients can vary considerably among regions [46]. 5-OH-DMT may come from the metabolism of small amounts of 5-MeO-DMT present in some ayahuasca brews [100]. Second, the indole-hydroxylation of DMT for producing 5-OH-DMT by the effect of L-tryptophan-5-hydroxylase might also occur.

Regarding pharmacokinetics, the main pharmacokinetic parameters obtained after ayahuasca consumption are summarized in Table 2.

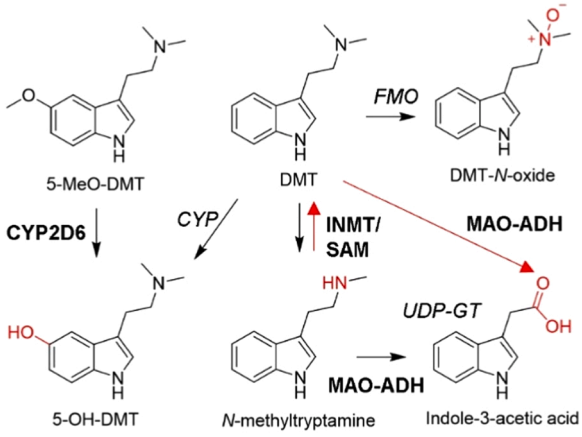

Fig. 4. The metabolism of DMT, 5-MeO-DMT and their main metabolites in humans. Bold enzymes are those which are reported in the literature and the rest are hypothesized regarding to the existing data. Abbreviations: 5-OH-DMT, 5-hydroxy-N,N-dimethyltryptamine; 5-MeO-DMT, 5-methoxy-N,N-dimethyltryptamine; DMT, N,N-dimethyltryptamine.

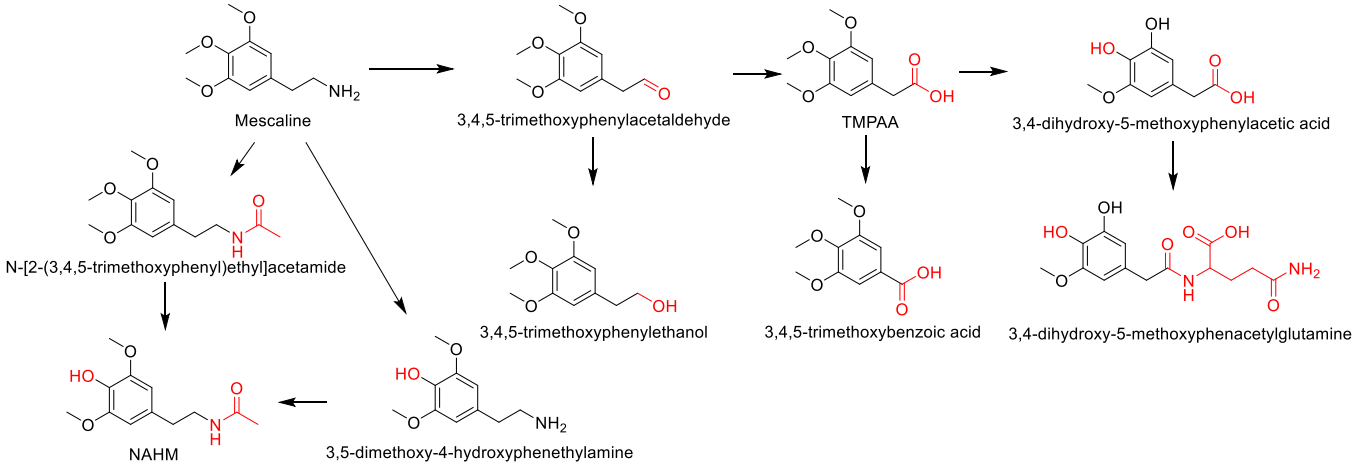

Fig. 3. The metabolism of mescaline and its main metabolites in humans. Abbreviations: TMPAA, 3,4,5-trimethoxyphenylacetic acid; NAHM, N-acetyl-β-(3,4-dimethoxy-5-hydroxyphenyl) ethylamine.

Although the intravenous injection of DMT produces a maximum DMT level in human plasma after 2 min but negligible after 30 min [126], the maximum DMT concentration after ayahuasca consumption is obtained after 1.5 h [105]. MAO inhibition seems to be behind these differences. Since MAO is a key enzyme in DMT metabolism (Fig. 4), the administration of a MAO inhibitor considerably affects the metabolism and pharmacokinetics of DMT [123]. That is the case of ayahuasca brew in which the β-carboline alkaloids are reversible inhibitors of MAO-A. Due to the presence of these inhibitors, the excretion of DMT metabolites is more commonly found after ayahuasca consumption than after oral administration of pure DMT [100,122]. The maximum urinary concentration of excreted DMT-*N*-oxide occurs after 4–8 h of consumption [100], while this metabolite is also the major circulating metabolite in plasma [124]. Riba and colleagues later corroborated the excretion of unmetabolized DMT, DMT-*N*-oxide, indole-3-acetic acid, and *N*-methyltryptamine, with the highest concentrations after the first 4 h post-consumption [127]. However, despite MAO inhibition indole-3-acetic acid continued to be one of the most abundant metabolites.

The major β-carboline alkaloids present in ayahuasca are harmine and harmaline [128,129] (Fig. 5). After ayahuasca consumption, free harmol, tetrahydroharmine, and harmalol were found in urine and plasma at considerable concentrations being tetrahydroharmine the major compound excreted after 8–24 h post-administration [100,104]. These metabolites are produced through the *O*-demethylation of harmine and harmaline (Fig. 5) [128,129]. Riba and colleagues also reported harmol, harmalol, and 2-MTHBC as a metabolite from β-carboline alkaloids [127], as well as tetrahydroharmol as an *O*-demethylated product of tetrahydroharmine. The studies reporting metabolites from β-carboline alkaloids coincide with the fact that the treatment with β-glucuronidase/sulfatase enzymes increased the levels of the metabolites, suggesting that conjugation with either glucuronides or sulfates plays a role in β-carboline metabolism. These conjugates may be suitable biomarkers of consumption since they are the longest detectable metabolites appearing between 16 and 24 h post-consumption [127]. The main pharmacokinetics parameters for β-carboline alkaloids are summarized in Table 2.

Despite the potential importance of metabolomics studies in psychedelics (see Introduction section), Ayahuasca is the only drug for which we found studies related to its effect on the metabolome. A seminal work by Riba et al. studied for the first time the effect of ayahuasca consumption on the main metabolites for monoamine neurotransmitters, including vanillylmandelic acid, homovanillic acid, and 5-hydroxyindole-3-acetic acid (5-HIAA), metanephrine, and normetanephrine. The authors detected an increase of normetanephrine in pooled urine (0–24 h) after ayahuasca consumption. However, other deaminated metabolites, such as vanillylmandelic acid, homovanillic acid, and 5-hydroxy-3-indoleacetic acid, remained unaltered [105]. This study revealed a potential mechanism of action of ayahuasca and directed the first step toward a more complex metabolomics approach. Similarly, Strassman et al. reported an increase in some metabolites such as cortisol after intravenous DMT injection [126]. A recent metabolomics analysis in humans performed by our group revealed alterations of several plasmatic large-neutral amino acids (LNAA) and circulating N-acyl-ethanolamine and 2-acyl-glycerol endocannabinoids after ayahuasca consumption [130]. Interestingly, LNAAs were further associated with the subjective psychedelic state. For example, circulating tryptophan was found to be positively correlated with "ego-dissolution" items of the Altered States of Consciousness Rating Scale. These findings support the hypotheses that ayahuasca consumption increases the amount of circulating aromatic amino acids (e.g., tryptophan) which are the precursors for the later synthesis of neurotransmitters (e.g., serotonin), which may then trigger the associated subjective effects.

3.2.4. *5-MeO-DMT*

Unlike DMT, 5-MeO-DMT has been less investigated and human metabolic studies relative to 5-MeO-DMT were not found using this search strategy on this date. To date, only a clinical trial of 5-MeO-DMT in humans has been published with vaporized dosing up to 18 mg [28] but it did not provide data either on circulating concentrations of the drug or on the nature and levels of its metabolites. Therefore, our knowledge of 5-MeO-DMT metabolism is very limited. It has been suggested that 5-MeO-DMT is deaminated by MAO-A to 5-methoxyindoleacetic acid (5-MIAA) and O-demethylated by CYP2D6 to 5-OH-DMT (Fig. 4) [7,131]. The implication of CYP2D6 into 5-MeO-DMT metabolism could suggest differences in susceptibilities due to CYP2D6 polymorphisms although this fact remains to be explored.

Regarding the effects of the administration of 5-MeO-DMT in the endogenous metabolome, 5-MeO-DMT has been reported to alter the levels of salivary cortisol [132]. However, comprehensive metabolomics studies aiming to study the effects of 5-MeO-DMT in the metabolome are not found in the literature.

3.2.5. *Psilocybin (4-phosphoryloxy-N,N-dimethyltryptamine)*

Psilocybin, or 4-phosphoryloxy-*N*,*N*-dimethyltryptamine, is another indole alkaloid similar to the neurotransmitter serotonin [133]. After consumption, psilocybin is rapidly metabolized to psilocin (4-OH-DMT) by intestinal alkaline phosphates and undetermined esterases [134], as illustrated in Fig. 6. This metabolite is another example of a bioactive metabolite since it has been reported to be responsible for psychoactive effects by stimulation of $5HT_{2A}$ receptors [135]. Psilocin is then metabolized by UDP-glucuronosyltransferases to psilocin-*O*-glucuronide, which is the major metabolite found in human fluids [136]. Psilocin may be also deaminated and oxidized by liver aldehyde

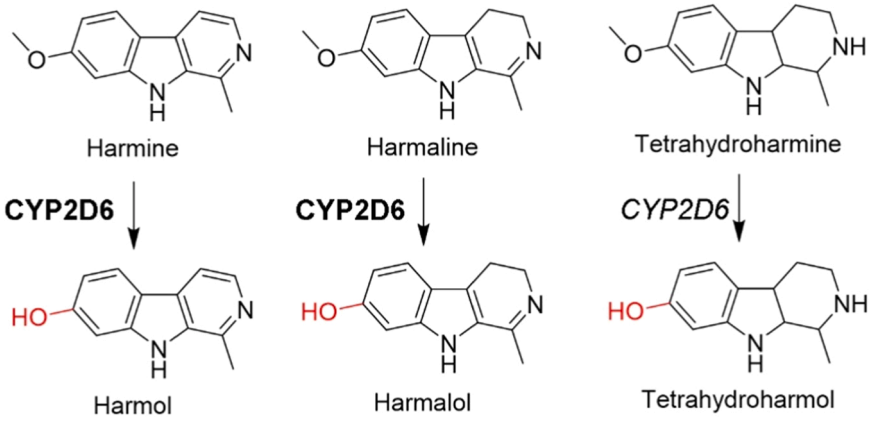

Fig. 5. The metabolism of major β-carboline and their main metabolites in humans. Bold enzymes are those which are reported in the literature and the rest are hypothesized regarding to the existing data.

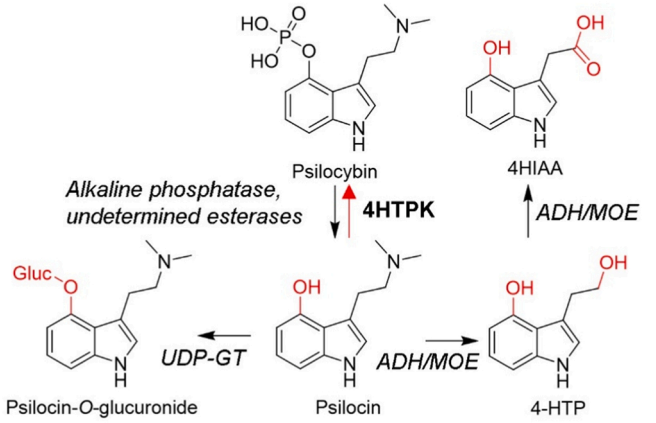

Fig. 6. The metabolism of psilocybin and their main metabolites in humans. Bold enzymes are those which are reported in the literature and the rest are hypothesized regarding to the existing data. Abbreviations: 4-HIAA, 4-hydroxyindole-3-acetic acid; 4-HTP, 4-hydroxytryptophol; gluc, glucuronide.

dehydrogenase and MAO to 4-hydroxytryptophol (4-HTP) and 4-hydroxyindole-3-acetic acid (4HIAA), respectively [8,70].

The main pharmacokinetics parameters for psilocybin and its metabolites are shown in Table 2**.** Psilocybin has not been detected in either plasma or urine [72]. In contrast, psilocin and 4HIAA have been quantified in plasma, whereas psilocin and psilocin-*O*-glucuronide are detectable in urine. The study of the pharmacokinetic profiles of psilocin demonstrated maximum plasma concentrations of 1.5–2 h after oral administration and subsequent elimination with a half-life between 2 and 3 h [70,72,137]. Kamata et al., quantified psilocin and psilocin-O-glucuronide in serum [138] and urine [139] after the ingestion of 9 g of dried psilocybe mushrooms, using both LC-MS and LC-MS/MS. The authors detected 71.0 ng/ml of total psilocin and 13.3 ng/ml of free psilocin in serum 5 h after ingestion. They were able to detect psilocin in serum up to 52 h after ingestion. Conversely, they detected 3.55 μg/ml of conjugated psilocin in urine only after the enzymatic hydrolysis, and psilocin was not detected without hydrolysis. Similar to some results already discussed for LSD [114], the presence of a small urinary amount of 5-OH-DMT after enzymatic cleavage with β-glucuronidase has been reported after psilocybe mushroom consumption [114]. The urinary presence of 5-OH-DMT-*O*-glucuronide after psilocybe mushrooms consumption was later confirmed by Bambauer and colleagues [140].

Similar to other psychedelics, although the effect of psilocybin administration on the endogenous metabolome has been evaluated for some specific pathways such as the hypothalamic–pituitary–adrenal axis [141,142], there is a lack of metabolomics studies devoted to determining the comprehensive effect of this drug in the metabolome.

4. Final remarks and future perspective

This review concludes that the studies on metabolism and metabolomics research in classical psychedelic drugs progress at different speeds. Whereas contrasted information about psychedelics metabolism - such as the establishment of main metabolites, enzymes involved, and pharmacokinetics and pharmacodynamics data - have been reported, metabolomics studies on psychedelics are just starting to take their first steps.

Although the published information about the metabolism of psychedelic substances is profuse, it is based on the determination of previously known metabolites. In contrast with other drugs, there is a lack of studies aiming to elucidate the structure of unknown metabolites of psychedelics in humans. All this may result in a partial understanding of psychedelic drug metabolism in humans. Thus, it is feasible that some psychedelic drug metabolites, even active ones, remain unnoticed by the current research on the topic. Due to the importance of the determination of metabolites to understand variability in the subjective effects and activity of the drug (in case active metabolites are formed), it would be advisable that such studies be performed.

We anticipate an increase in the number of metabolomics studies related to psychedelics administration in the next years. These metabolomics studies would open new avenues in the study of psychedelics by providing useful information about unanswered questions in the field. Thus, the expected results from future metabolomics studies would help in (i) establishing the mechanisms of action behind both the beneficial and the deleterious effects of psychedelics, (ii) predicting the response and the side effects of the drug in a specific subject and (iii) monitoring the evolution of the treatment with psychedelics. With the discoveries obtained by metabolomics studies, new hypotheses could be generated about the mechanisms of action and, therefore, consider both new therapeutic targets in psychiatric diseases and discover the mediating agents in the recently described therapeutic treatment. In order to obtain the maximum of these benefits from the metabolomics studies, it is required to perform controlled studies of administration in which the most important factors altering the metabolome (sex, age, ethnicity, BMI, time of sample collection, comorbidities) are properly considered from the moment of the study design.

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CRediT authorship contribution statement

Francisco Madrid-Gambin: Formal analysis, Writing – original draft preparation. **David Fabregat-Safont:** Writing – Reviewing. **Alex Gomez-Gomez:** Writing – Reviewing. **Eulàlia Olestid:** Writing – review & editing. **Natasha L. Mason:** Writing – Reviewing. **Johannes G. Ramaekers:** Writing – Review & Validation. **Oscar J. Pozo:** Conceptualization and Supervision.

Declaration of Competing Interest

none.

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