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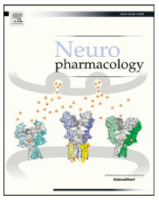

# CYP 450 enzymes influence *(R,S)*-ketamine brain delivery and its antidepressant activity

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ABSTRACT

Esketamine, the *S*-stereoisomer of (*R,S*)-ketamine was recently approved by drug agencies (FDA, EMA), as an antidepressant drug with a new mechanism of action. *(R,S)*-ketamine is a *N*-methyl-D-aspartate receptor (NMDA-R) antagonist putatively acting on GABAergic inhibitory synapses to increase excitatory synaptic glutamatergic neurotransmission. Unlike monoamine-based antidepressants, *(R,S)*-ketamine exhibits rapid and persistent antidepressant activity at subanesthetic doses in preclinical rodent models and in treatment-resistant depressed patients. Its major brain metabolite, (2*R*,6*R*)-hydroxynorketamine (HNK) is formed following *(R,S)*-ketamine metabolism by various cytochrome P450 enzymes (CYP) mainly activated in the liver depending on routes of administration [e.g., intravenous (largely used for a better bioavailability), intranasal spray, intracerebral, subcutaneous, intramuscular or oral]. Experimental or clinical studies suggest that (2*R*,6*R*)-HNK could be an antidepressant drug candidate. However, questions still remain regarding its molecular and cellular targets in the brain and its role in *(R,S)*-ketamine's fast-acting antidepressant effects. The purpose of the present review is: 1) to review *(R,S)*-ketamine pharmacokinetic properties in humans and rodents and its metabolism by CYP enzymes to form norketamine and HNK metabolites; 2) to provide a summary of preclinical strategies challenging the role of these metabolites by modifying *(R,S)*-ketamine metabolism, e.g., by administering a pre-treatment CYP inducers or inhibitors; 3) to analyze the influence of sex and age on CYP expression and *(R,S)*-ketamine metabolism. Importantly, this review describes *(R,S)*-ketamine pharmacodynamics and pharmacokinetics to alert clinicians about possible drug-drug interactions during a concomitant administration of *(R,S)*-ketamine and CYP inducers/inhibitors that could enhance or blunt, respectively, *(R,S)*-ketamine's therapeutic antidepressant efficacy in patients.

## 1. Introduction

The glutamate hypothesis of depression implicates alterations to glutamate and γ-aminobutyric acid (GABA)-related synaptic function (Sanacora et al., 2012). Unlike conventional antidepressant drugs, some modulators of the glutamatergic system such as (*R,S*)-ketamine (2-(2-chlorophenyl)-(1-methylamino-cyclohexanone) (heretofore referred to as ketamine), are non-competitive antagonists of the *N*-methyl-D-aspartate receptor (NMDA-R). Administration of a single, sub-anesthetic dose of *(R,S)*-ketamine evokes rapid and sustained (from 24 h to 1 week) antidepressant effects in patients with treatment-resistant depression (TRD) (Berman et al., 2000; Zarate et al., 2006).

*(R,S)*-ketamine is a racemic mixture of (*S*)-ketamine (or esketamine) and (*R*)-ketamine (or arketamine). Ketamine is rapidly and stereoselectively metabolized into several metabolites (Table 1). For example, (*S*)-ketamine is metabolized to (*S*)-norketamine and (*2S,6S*)-hydroxynorketamine (*2S,6S*)–HNK. Recent studies suggest that active ketamine metabolites, e.g., (*2R,6R*)-HNK, may mediate the antidepressant effects of ketamine via an AMPAR- or NMDAR-dependent mechanism (Suzuki et al., 2017; Zanos et al., 2016).

*(R,S)*-ketamine has been first described in 1965 and used as an anesthetic and analgesic veterinary drug since 1970 (Chen and Chen, 2010; Craven, 2007; Hijazi and Boulieu, 2002). *(R,S)*-ketamine has multiple properties and indications, i.e., in anesthesia, treatment of pain, and has sympathomimetic effects. Recently, several clinical trials have emphasized the rapid and sustained antidepressant action of (*R,*

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**List of abbreviations**

| *Abbreviations* | *Definition* |
|---|---|
| 1-ABT | 1-Aminobenzotriazole |
| 5-HT | serotonin |
| ADME | absorption, distribution, metabolism and excretion |
| AMPA | α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid |
| AUC | area under the curve |
| BBB | brain blood barrier |
| BDNF | brain-derived neurotrophic factor |
| CSDS | chronic social defeat stress |
| CNS | central nervous system |
| CSF | cerebrospinal fluid |
| CYP | cytochromes P450 enzymes |
| DHNK | dehydronorketamine |
| DRN | dorsal raphe nucleus |
| eEF2 | eukaryotic elongation factor-2 kinase |
| EEG | electroencephalographic |
| EMA | European Medical Agency |
| FDA | Food Drug Administration |
| fMRI | functional magnetic resonance imaging |
| FST | forced swim test |
| GABA | γ-aminobutyric acid |
| HK | hydroxyketamine |
| HNKs | Hydroxynorketamine metabolites |
| $IC_{50}$ | the half maximal inhibitory concentration |
| i.m. | intramuscular |
| i.n. | intranasal |
| i.p. | intraperitoneal |
| i.v. | intravenous |
| icv | intracerebroventricular |
| KET | *(R,S)*-ketamine or ketamine |
| MDD | major depressive disorder |
| MDE | major depressive episode |
| mPFC | medial prefrontal cortex |
| MRI | magnetic resonance imaging |
| mTOR | mammalian target of rapamycin |
| NK | norketamine |
| NMDA | N-methyl-D-aspartate |
| NMDA-R | *N*-methyl-D-aspartate receptor |
| p.o. | oral administration |
| P-gp | P-glycoprotein |
| PSD95 | postsynaptic density protein 95 |
| PV | parvalbumine |
| s.c. | subcutaneous |
| s.l. | sublingual |
| sEPSCs | small excitatory post-synaptic currents |
| SNPs | single nucleotide polymorphisms |
| SNRI | serotonergic noradrenergic reuptake inhibitors |
| s.p. | suppository |
| SSRI | selective serotonin reuptake inhibitors |
| SST | somatostatin |
| TRD | treatment-resistant depression |
| TrkB | tropomyosin receptor kinase B |

*S*)-ketamine after administration of a single subanesthetic intravenous (i.v.) injection (0.5 mg/kg) perfused for 40 min for treatment-resistant depression (TRD) (see the meta-analyses (Caddy et al., 2015; Marcantoni et al., 2020; Wilkinson et al., 2018; Xu et al., 2016):). This antidepressant effect is maintained after at least 1 week (and lasts up to 2 weeks) in TRD patients, i.e., patients resistant to conventional antidepressant drug treatments (Zarate et al., 2006). Multiple randomized clinical trials confirmed this new pharmacological property [see meta-analysis reviews (Caddy et al., 2014; Fond et al., 2014; Newport et al., 2015; Xu et al., 2016):]. In addition, the FDA approved (*S*)-ketamine nasal spray (esketamine, SPRAVATO®; Janssen Pharmaceuticals) as a new antidepressant in conjunction with an oral antidepressant drug for TRD in adults.

There are several hypotheses for ketamine's mechanism of action. One main hypothesis is the disinhibition hypothesis of pyramidal cells *via* a decreased output of fast-spiking GABAergic interneurons in the

**Table 1**
CYP450 enzymes involved in the formation of twelve metabolites of (*R,S*)-ketamine.

| Drug/Metabolite | Cytochrome P450 (CYP) enzymes | NMDA receptor affinity (μm) | Antidepressant-like effect | References |
|---|---|---|---|---|
| (*R,S*)-Ketamine | | 0.53 | Yes | (Pham et al., 2018; Wei et al., 2020; Yang et al., 2017) |
| (*R*)-Ketamine | | 1.4 | Yes | (Yamaguchi et al., 2018; Yang et al., 2015) |
| (*S*)-Ketamine | | 0.3 | Yes | (Molero et al., 2018) |
| (*R,S*)-Norketamine | CYP2B6, CYP3A, CYP2A6, CYP2C8, CYP2D6, CYP2C9 | | Yes | (Sałat et al., 2015) |
| (*R*)-Norketamine | | 13 | | |
| (*S*)-Norketamine | | 1.7 | Yes | (Yang et al., 2018a; Yokoyama et al., 2020) |
| (*R,S*)-Dehydronorketamine | CYP2B6, CYP2A6, CYP2C8 | | | |
| (*2S,6S; 2R,6R*)-hydroxynorketamine | CYP2B6, CYP2A6, CYP3A, CYP2C19, CYP2C8 | >10 | Yes, No | (Yamaguchi et al., 2018; Zanos et al., 2016) |
| (*2S,6R; 2R,6S*)-hydroxynorketamine | | | | |
| (*2S,5S; 2R,5R*)-hydroxynorketamine | | | | |
| (*2S,4S; 2R,4R*)-hydroxynorketamine | | | | |
| (*2S,4R; 2R,4S*)-hydroxynorketamine | | | | |
| (*2S,5R; 2R,5S*)-hydroxynorketamine | | | | |
| (*2S,6S; 2R,6R*)-hydroxyketamine | CYP2A6, CYP2C19, CYP3A | | | |
| (*2S,6R; 2R,6S*)-hydroxyketamine | | | | |

mPFC and hippocampus has been proposed as a key mechanism that triggers *(R,S)*-ketamine's antidepressant response. A low subanesthetic dose of *(R,S)*-ketamine blocks NMDA-Rs located on GABA interneurons in the mPFC, resulting in decreased inhibitory inputs and increased small excitatory post-synaptic currents (sEPSCs) on layer V neurons (Gerhard et al., 2020) or increased glutamate release in the mPFC (Moghaddam et al., 1997). This hypothesis was investigated by several groups, e.g. (Widman and McMahon, 2018),). However, it is important to note that different *in vitro/in vivo* protocols have been used in each separate study. Widman and McMahon (2018) performed *in vitro* electrophysiology to study neuronal excitability immediately after ketamine application *in vitro* on dorsal hippocampus slices from 'naïve' rats. They found that ketamine reduced inhibitory input onto pyramidal cells and increased synaptically driven pyramidal cell excitability measured at the single-cell and population levels. These authors used a seconds-to-minutes time frame consistent with the rapid effects occurring *in vivo* when i.v. ketamine reaches brain. Thus, they captured the immediate effects of ketamine on neuronal excitability. These *in vitro* results illustrate the 'induction' phase of the response to ketamine.

By contrast, in BALB/cJ mice, a preclinical rodent model of chronic stress, a sustained antidepressant-like response of a single ketamine dose occurred 24 h post-administration i.e., when ketamine and its main metabolites are no longer present in the body (Pham et al., 2018). Ketamine mechanism of action evokes a TrkB-dependent mechanism via an activation of brain-derived neurotrophic factor (BDNF), causing plastic changes in the central nervous system within 24 h.

This time corresponds to the sustained antidepressant activity of ketamine in TRD patients (Price et al., 2009; Wilkinson and Sanacora, 2016) as well as to its sustained neuronal and behavioral effects in rodents (Chowdhury et al., 2017; Li et al., 2010). Under these experimental conditions, ketamine increased GABA release in the mPFC at 24h (Pham et al., 2018, 2020). The mechanism involved in the delayed increase in GABA release in the mPFC is currently unknown. An intra-cortical perfusion of muscimol, a $GABA_A$-R agonist did not change ketamine-induced increase in GABA release in the mPFC, but blocked the sustained antidepressant like activity of ketamine at t24h (Pham et al., 2020). GABA release could involve (*2R,6R*)-HNK, known to mediate, at least in part, the sustained antidepressant effects of ketamine (Zanos et al., 2016). A pretreatment with CYP inhibitors and inducers before a systemic ketamine administration could help to test this hypothesis.

In addition, anxiety frequently coexists with depression and adding benzodiazepines to antidepressant is a common practice to treat patients with MDD. Question has recently been asked whether such a co-administration may attenuate ketamine fast antidepressant effects (Andrashko et al., 2020; Irwin and VandenBerg, 2021).

These results are in line with those of Perrine et al. (2014) showing that chronic stress yielding a depressed phenotype decreased brain tissue GABA levels, and a subanesthetic dose of (*R,S*)-ketamine normalized these changes (Perrine et al., 2014). Furthermore, major depression is associated with low plasma and cerebrospinal fluid GABA concentrations in depressed patients (Sanacora et al., 2002). Thus, this disinhibition hypothesis needs to be further investigated and validated by comparing the balance excitation-inhibition in the mPFC *versus* the hippocampus. One study that attempted to elucidate how the two regions connect used optogenetic activation of the ventral hippocampus - mPFC circuit (Carreno et al., 2016).

These *in vivo* results presumably reflect the 'expression' phase of the response to ketamine. Recently, the time course of different phases of ketamine-induced neural plasticity and behavioral effects were recapitulated as reported by previous studies in mice and humans (Wu et al., 2021). However, the full complement of molecular and cellular factors involved in ketamine-induced synaptogenesis remains to be elucidated from 2 to 48 h post-treatment.

Beyond NMDA-R antagonism, it has been shown that ketamine induces a cascade of postsynaptic intracellular events activated by *(R,S)*-ketamine increases phosphorylation of mammalian target of rapamycin mTOR (Duman et al., 2019; Miller et al., 2014; Yang et al., 2018b) and the expression of synaptic proteins (eukaryotic elongation factor-2 (eEF2) kinase, brain-derived neurotrophic factor (BDNF), synapsin 1, PSD95) involved in synaptogenesis in mPFC pyramidal neurons usually 24 h after a single *(R,S)*-ketamine dose (Pham and Gardier, 2019). In addition, several concerns were raised in clinal trials about esketamine efficacy (Turner, 2019). Cellular and molecular signatures governing ketamine's antidepressant effects need to be investigated further.

## 2. (*R,S*)-ketamine and its (*R*)- and (*S*)-enantiomers

(*R,S*)-ketamine undergoes rapid and extensive metabolism by cytochrome P450 (CYP) enzymes in the liver and intestine (Kharasch and Labroo, 1992; Rao et al., 2016). (*R,S*)-ketamine is first metabolized to (*R,S*)-norketamine, which is then further metabolized into 12 different (*R,S*)-hydroxynorketamine (HNK) metabolites in humans and in rodents (Can et al., 2016; Moaddel et al., 2015; Noppers et al., 2011) (Table 1). Two main enantiomers of (*R,S*)-ketamine are ()-ketamine (or arketamine) and (*S*)-ketamine (or esketamine). (*R,S*)-ketamine is a racemic mixture composed of equal amounts of (*S*)-ketamine and (*R*)-ketamine, but these enantiomers are not equipotent at NMDARs as shown in a [$^3$H] MK-801 binding study to the NMDAR *in vitro* in rat brain. Thus, it makes a straight dose-dose comparison difficult (Zanos et al., 2016). (*R*)-ketamine has lower affinity for the NMDAR compared to (*S*)-ketamine, but (*R*)-ketamine has greater potency and longer-lasting antidepressant-like actions in animal models of depression (Hashimoto, 2020).

Hashimoto's group suggested that (*R*)-ketamine has a greater potency and longer-lasting antidepressant effects than (*S*)-ketamine in rodent models of depression (10 mg/kg, i.p (Hashimoto, 2016; Zhang et al., 2014). A systemic administration of (*R*)-ketamine exhibited more potent antidepressant effects than (*S*)-ketamine in a rodent model of neonatal dexamethasone exposure, while (*R*)-ketamine was devoid of side effects of ketamine and (*S*)-ketamine (Zhang et al., 2014). In the CSDS model, the order of potency of antidepressant effects after a single intranasal administration was (*R*)-ketamine > (*R,S*)-ketamine > (*S*)-ketamine (Chang et al., 2019). The authors also found that administration of (*R*)-ketamine would be a safer antidepressant than (*R,S*)-ketamine and (*S*)-ketamine (Chang et al., 2019; Yang et al., 2015). Hashimoto's group also showed that metabolites of (*R*)-ketamine such as (*2R,6R*)-HNK, are devoid of antidepressant effects (Yamaguchi et al., 2018).

In contrast, Ago's group supports the benefits of (*S*)-ketamine metabolites. They compared the effects of (*R*)-norketamine (*R*)-NK, (*S*)-NK, (*2R,6R*)-HNK, and (*2S,6S*)–HNK in a mouse model of depression (Yokoyama et al., 2020). A single administration of (*S*)-NK and (*2S,6S*)–HNK at one dose 20 mg/kg reduced the enhanced immobility in the forced swim test (FST) at 30 min after injection in chronic CORT-treated mice, while R enantiomers did not. These results suggest that (*S*)-ketamine metabolites have potent acute and sustained antidepressant effects in rodents.

Interestingly, in another rodent model, chronic social defeat stress (CSDS), Yao et al. (2018) highlighted differences between (*R*)- and (*S*)-ketamine (Yang et al., 2018a). They found that the two drugs remodel prefrontal and hippocampal neurons *via* a mechanism involving downstream activation of AMPA receptors. However, mTOR and ERK signaling pathways play a role in the antidepressant effects of (*R*)- and (*S*)-ketamine, respectively. Differences between these enantiomers may also rely on brain regions and circuits. In addition, Ago and Hashimoto tried to explain these differences in a joint publication (Abdallah, 2020; Ago et al., 2019; Andrade, 2017a,b). In sum, they found that (*R*)-ketamine strongly activates the prefrontal serotonergic system through an AMPA receptor-independent mechanism, while (*S*)-ketamine-induced serotonin and dopamine release was AMPA receptor-dependent. These findings provide a neurochemical basis for the underlying pharmacological differences between ketamine enantiomers and their

metabolites.

In humans, the S(+)-enantiomer has been postulated to be a four times more potent anesthetic and analgesic than the R(−)-enantiomer and approximately two times more effective than the racemic mixture of ketamine (Peltoniemi et al., 2016). However, to our knowledge, there are no direct comparisons between (*S*)-ketamine and either (*R*)-ketamine or racemic ketamine in depressed patients; therefore, it is not possible to make an informed choice when considering ketamine enantiomers and the racemate for treatment-resistant depression (Andrade, 2017a). However, despite the lack of strong data, an esketamine nasal spray SPRAVATO® was approved by the US FDA and European EMA drug agencies in 2019 and is currently being administered to patients (FDA News Release, 2019; Mahase, 2019).

Though CYPs are known to metabolize ketamine in the liver and intestine, it is unknown whether they also play a substantial role in drug metabolism in the brain. To the best of our knowledge, there are no evidence that ketamine is metabolized in the brain. However, we cannot ignore this possibility. The role of these enzymes in drug metabolism in the brain needs to be investigated in a cell type- and region-specific manner (Toselli et al., 2016). Rat whole brain CYP tissue content is 1–4% of hepatic levels (Toselli et al., 2016). Mammalian brain neural and glial cells exhibited high constitutive and inducible expression of CYPs in rodents (Hart et al., 2009; Naud et al., 2016) and in humans (Tripathi et al., 2017). *(R,S)*-ketamine is one of the substrates of CYP2B6, an enzyme located in the brain (Miksys and Tyndale, 2013). The expression and function of CYP isoforms in drug metabolism in rodent brains are consistent with clinical data obtained in humans (Meyer et al., 2007). The role of CYPs in ketamine's metabolism could be confirmed *via* a pre-treatment with CYP inhibitors (Yamaguchi et al., 2018) or inducers in animal models of anxiety/depression. The intra-nasal route of administration (i.n.) of ketamine is another way to test this hypothesis because it allows a direct access of drugs to the brain (Zanger and Schwab, 2013). Yet, it is too early to conclude whether CYPs act in the brain to metabolize ketamine.

## 3. Ketamine and its active metabolites

### 3.1. Role of (R,S)-ketamine and (2R,6R)-HNK in mediating antidepressant-like actions

Questions are currently being investigated regarding the pharmacological properties and clinical efficacy of (*R,S*)-ketamine metabolites. Of these 12 different HNKs, (2*S,6S*;2*R,6R*)-HNK are the most abundant circulating HNK metabolites detected in the plasma in humans (Moaddel et al., 2015; Zarate et al., 2013; Zhao et al., 2012). Plasma and brain tissues were also measured following an intravenous administration of (*R,S*)-ketamine in rodents (Pham et al., 2018; Zanos et al., 2016). However, drug metabolites formed by the liver by CYP enzymes may cross the BBB, and further confound the estimation of brain contribution (Miksys and Tyndale, 2013).

Our understanding of the antidepressant effects of (*R,S*)-ketamine and the role of (2*R,6R*)-HNK needs to be clarified. In particular, questions still remain regarding the sharing of roles between ketamine and HNKs: is it the combination of (*R,S*)-ketamine and (2*R,6R*)-HNK that produces the antidepressant effect or only one of the two compounds, either (*R,S*)-ketamine or HNK alone? It is also unclear whether HNK's antidepressant action is better, worse than, or equal to that of the racemic mixture (*R,S*)-ketamine.

### 3.2. Contribution of (2R,6R)-HNK to ketamine's antidepressant effects

There are two main pre-clinical strategies to study whether (*2R,6R*)-HNK mediates the antidepressant effects of ketamine or participates to its effects: the use of deuterated ketamine or a pre-treatment with a CYP inducer or inhibitor. To directly determine whether (2*R,6R*)-HNK is required for (*R,S*)-ketamine antidepressant actions, Zanos et al. (2016) deuterated (*R,S*)-ketamine or (*R*)-ketamine (Zanos et al., 2016). Such a change alters the pharmacological properties of (*R,S*)-ketamine without changing its binding affinity to NMDARs or to brain (*R,S*)-ketamine levels; however, it prevents its metabolism to (2*R,6R*)-HNK (Gant, 2014). As a consequence, deuterated ketamine failed to induce antidepressant-like effects in behavioral tests predicting antidepressant responses 24h after administration. These data suggest a role of (2*R,6R*)-HNK in the sustained antidepressant efficacy of the parent drug (Zanos et al., 2016, 2019). The same strategy was successfully described for deuterated (d6)-dextromethorphan-induced antidepressant-like effects in mice (Nguyen et al., 2017).

A pre-treatment with a CYP inducer or inhibitor has not yet been investigated in preclinical studies with the racemic (*R,S*)-ketamine. However, a cocktail of CYP inhibitors was administered 1 h before an acute dose of R-ketamine (3, 10 and 30 mg/kg) (Yamaguchi et al., 2018). They found that metabolism to *(2R,6R)*-HNK is not necessary for the antidepressant effects of (*R*)-ketamine and that unmetabolized (*R*)-ketamine itself may be responsible for its antidepressant actions. Benefits of such a strategy are developed below in paragraph 3.

### 3.3. Antidepressant effects of (2R,6R)-HNK itself, i.e., when given alone

To test whether (*2R,6R*)-HNK displays antidepressant effects independently of ketamine, a comparison between ketamine *versus* (*2R,6R*)-HNK should be done. In order to study these questions, preclinical experiments have been performed following systemic or intra-mPFC drug infusion to compare ketamine or (2*R,6R*)-HNK neurochemical and behavioral effects (Zanos et al., 2016; Pham et al., 2018). Working on hippocampal slices, Suzuki et al. (2017) reported that 10 μM (2R, 6R)-HNK blocked synaptic NMDARs in a similar manner to its parent compound. Unfortunately, a systemic administration of (*2R,6R*)-HNK is often realized at the same dose than the parent drug, e.g., 5 and 10 mg/kg, i.p., which equates to the maximum concentration of brain exposure (10.69 μM in mice) and also resulted in significant antidepressant actions (Suzuki et al., 2017; Zanos et al., 2016). However, dose-dependent concentrations of the metabolite in the brain relevant to its antidepressant effects need to be further investigated.

Zanos et al. (2016) initially suggested that (2*R,6R*)-HNK is necessary and sufficient to produce (*R,S*)-ketamine's full antidepressant-like effects in mice (Zanos et al., 2016). HNKs exhibit a low affinity to NMDAR's, which has brought up questions about its contribution to (*R,S*)-ketamine-induced antidepressant effect (Zanos et al., 2016). (2*R,6R*)-HNK does not block NMDAR's like ketamine does (Zanos et al., 2016), but it also requires BDNF signaling to exert its activity as ketamine. In addition, the $IC_{50}$ as determined by measuring whole-cell currents in Xenopus oocytes expressing distinct NMDAR subtypes showed that (*2S,6S*)–HNK inhibited NMDAR subtypes to a greater degree than its isomeric counterpart (*2R,6R*)-HNK but a lower degree than ketamine (Lumsden et al., 2019). However, these data do not mean that (2*R,6R*)-HNK plays no role in the antidepressant actions of ketamine or has an antidepressant activity of its own. For instance, direct effects of HNKs have been found on excitatory synaptic transmission, independent of NMDAR inhibition were observed using extracellular slice electrophysiology in CA1 hippocampus in naïve rats (Riggs et al., 2020).

To address whether (2*R,6R*)-HNK plays a role in ketamine's antidepressant activity, we recently showed that a systemic or intra-cortical administration of (*R,S*)-ketamine and (*2R,6R*)-HNK displayed a similar "sustained" antidepressant-like activity (i.e., at 24 h post-injection) in the forced swim test (FST), but do note that this rodent test is not considered to reflect behavioral despair, helplessness or depression. This effect was associated with enhanced glutamate and GABA release by pyramidal neurons and interneurons, respectively, in the mPFC in hyperanxious BALB/cJ mice (Dulawa et al., 2004; Pham et al., 2018).

These initial findings demonstrating antidepressant-relevant biological activity of (2*R,6R*)-HNK have been widely replicated and expanded, including Rasenick's (Wray et al., 2019), Gardier's (Pham

et al., 2017), Chergui's (Yao et al., 2018), Collo's (Cavalleri et al., 2018; Collo and Merlo Pich, 2018), Chou's (Chou et al., 2018; Ye et al., 2019), Castren's (Fred et al., 2019) and Denny's (Chen et al., 2020). Fukumoto et al. (2019) also studied the antidepressant effects of systemic administration of (*2R,6R*)-HNK (3, 10 and 30 mg/kg) in mice (Fukumoto et al., 2019). A bilateral infusion of (*2R,6R*)-HNK (10 ng per side) in the mPFC also induced a rapid effect in behavioral tests in naïve mice. Interestingly, the antidepressant actions of (2*R*,6*R*)-HNK were blocked in mice with a knock-in of the BDNF Val66Met allele, which blocks the processing and activity-dependent release of BDNF. Thus, an activity-dependent BDNF release and downstream TrkB and mTOR signaling increased synaptic function in the mPFC, a cascade of events required for the rapid and long-lasting antidepressant effects of (2*R*, 6*R*)-HNK.

However, (2*R*,6*R*)-HNK's antidepressant-like activity and its contribution to the actions of (*R,S*)-ketamine is still a matter of debate (Abdallah, 2020; Chaki and Yamaguchi, 2018; Collingridge et al., 2017; Farmer et al., 2020; Riggs et al., 2020; Zanos et al., 2017). In particular, for Hashimoto's group, (2*R*,6*R*)-HNK does not exert rapid and sustained antidepressant-like effects in different mouse preclinical tests of antidepressant effectiveness (Yamaguchi et al., 2018; Yang et al., 2017). Yamaguchi et al. (2018) also reported that metabolism to (2*R*,6*R*)-HNK is not necessary for the antidepressant effects of the I-ketamine enantiomer.

## 4. Routes of ketamine administration in humans and rodents

Regarding its antidepressant activity, i.v. is the main route of *(R,S)*-ketamine administration in humans. (*R,S*)-ketamine can be administered through multiple routes, such as i.v., intraperitoneal (i.p.),

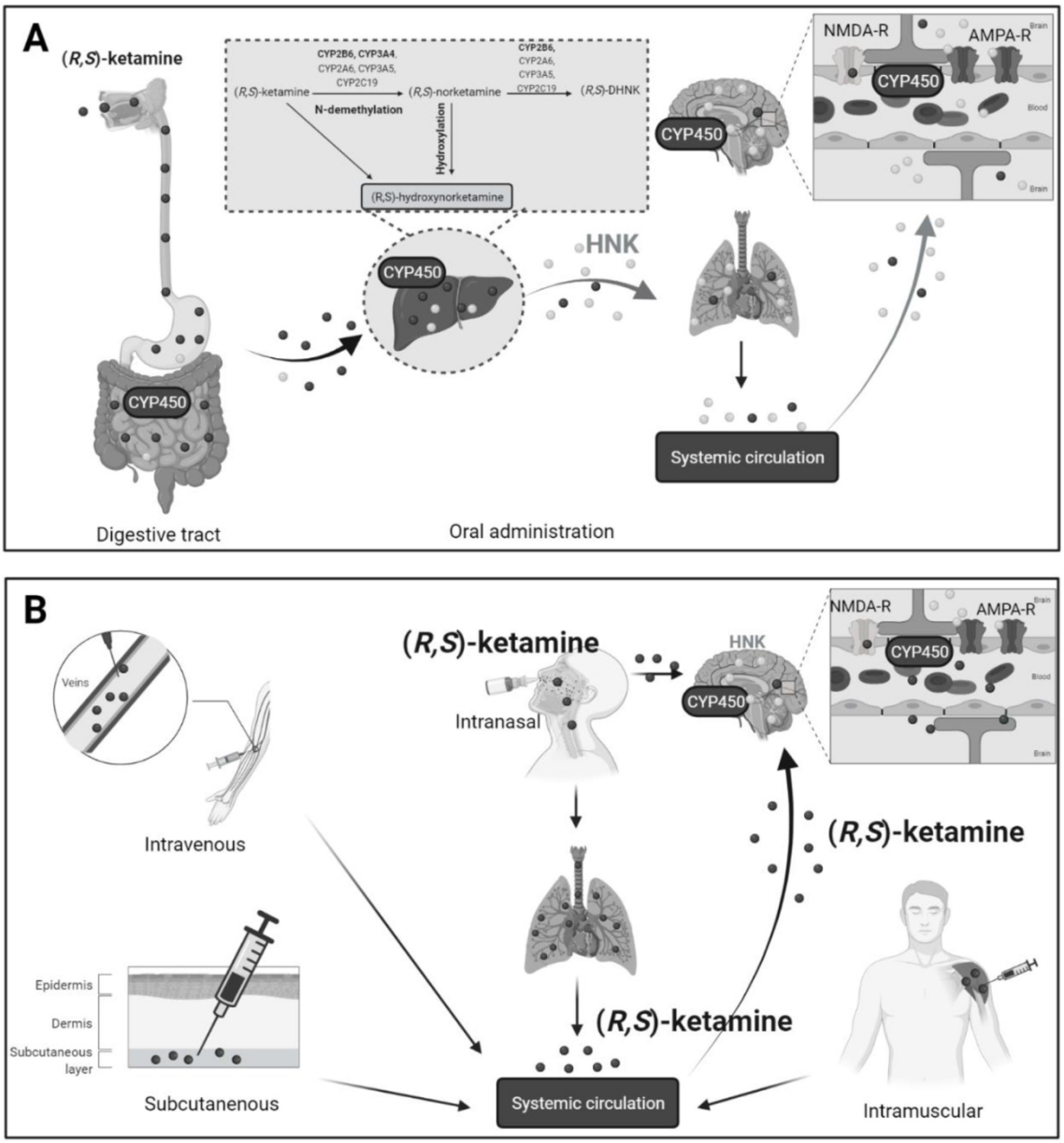


**Fig. 1.** Role of cytochrome P450 enzymes in the liver and the brain.
**A**) After oral administration, (*R,S*)-ketamine is metabolized by CYP enzymes expressed in the intestine and the liver, leading to norketamine, and then HNKs formation. (*R,S*)-ketamine and its metabolites then cross the blood-brain barrier and bind to their respective molecular target to exert antidepressant effects.
**B**) After parental administration (intravenous, subcutaneous, or intramuscular) and intranasal administration, (*R,S*)-ketamine distributes directly in the systemic circulation and reaches the brain (target organ). In this case, higher *(R,S)*-ketamine levels are metabolized by brain CYP enzymes to norketamine and then HNK.

intramuscular (i.m.), subcutaneous (s.c.), oral (p.o.), intranasal (i.n.), epidural, and intrarectal (Andrade, 2017b; Malinovsky et al., 1996). Lipophilic drugs have a bioavailability of almost 100%, but that of polar molecules is estimated at about 10% (Panek et al., 2020). *(R,S)*-ketamine is very liposoluble easily crosses the blood-brain-barrier (BBB).

The role of brain CYPs in modulating the effects of drugs has been difficult owing to the challenge of distinguishing the effects of hepatic and brain metabolite synthesis (Ferguson and Tyndale, 2011). However, as"a "highly speculative possibility", brain CYP could have a substantial impact on local drug metabolism and the resulting drug activity in the brain, e.g., on HNK synthesis and the rapid antidepressant effect of *(R,S)*-ketamine. Such an impact also depends on the route of the drug administration. The different stages of the d'ug's fate in the body as indicated in pharmacokinetic studies include absorption of the molecule, distribution in the body, metabolism and excretion (ADME). The absorption step (bioavailability) can be incomplete after an oral administration where the drug must first pass through the intestinal wall, then the portal circulation to the liver before reaching the systemic circulation for distribution. The absorption step can be shorter, direct and complete after an i.v. administration.

After an oral administration (bioavailability 10–20%) at a subanesthetic dose, a substantial *(R,S)*-ketamine metabolism by CYP enzymes occurs in the intestine and the liver, which generates metabolites such as HNK. The metabolites and some parent drug can then enter the brain from the periphery and bind to their molecular targets (i.e., NMDA-R or AMPA-R) to exert antidepressant effects (Fig. 1A). Orally, *(R,S)*-ketamine concentration arriving at its site of action in the brain is lower (due to a low bioavailability) compared to the intravenous-intranasal routes. The oral bioavailability of (*2R,6R*)-HNK was around 40% in rodents (Highland et al., 2018). Other routes of administration have been used for ketamine (Fig. 1B).

By contrast, an i.v. bolus or i.n. administration of *(R,S)*-ketamine allows its direct distribution to the brain, its target organ, more quickly, and at higher concentration compared to the oral route since the bioavailability is 100%. The i.v. infusion is the route of drug administration used as a reference for pharmacokinetic analyses. CYP inhibitors and antibodies can modify this process, thus altering the response of centrally acting drugs such as *(R,S)*-ketamine or propofol (Khokhar and Tyndale, 2011).

In humans, intravenous (i.v.) administration is the most common route that quickly achieves maximum plasma concentrations (Clements et al., 1982; Weber et al., 2004; Zanos et al., 2018). In TRD patients, $C_{max}$ plasma of *(R,S)*-ketamine is 185 ng/ml or 0.78 μM following a 40-min infusion at 0.5 mg/kg (Zanos et al., 2018; Zarate et al., 2012). In rats, after an i.v. administration at 40 mg/kg, $C_{max}$ plasma of *(R,S)*-ketamine reaches 34.52 ± 2.93 μM (Zarate et al., 2012).

Intraperitoneal (i.p.) injection is a classic route of administration in preclinical studies. However, drug bioavailability may be decreased because of the extensive first-pass metabolism in the liver and drug's metabolites are formed by the catalysis of CYP450 enzymes. In mice, $C_{max}$ plasma of *(R,S)*-ketamine and HNKs are 2.36 μM and 2.81 μM, respectively, following an i.p. injection at 10 mg/kg (Zanos et al., 2016). Unfortunately, mass spectrometry cannot discriminate between the 12 isoforms of HNKs.

Intramuscular (i.m.) injection is used in emergency situations in non-cooperative people, neonates and infants (Zanos et al., 2018). *(R,S)*-ketamine intramuscular administration generates maximum plasma concentrations rapidly post-treatment, with a relatively high bioavailability (Grant et al., 1981; Peltoniemi et al., 2016). In healthy volunteers, after an i.m. administration of 2–4 mg/kg, *(R,S)*-ketamine is quickly absorbed, peak plasma concentrations reached within 5–30 min post-dose and bioavailability is roughly 93% (Clements et al., 1982; Grant et al., 1981).

Oral bioavailability of *(R,S)*-ketamine is poor because of extensive first-pass metabolism. Oral *(R,S)*-ketamine administration is uncommon owing to unpredictable gastrointestinal absorption and low bioavailability (Ritter et al., 2020). Indeed, oral bioavailability is only 8–24% due to extensive first-pass hepatic metabolism and $C_{max}$ of *(R,S)*-ketamine achieves within 20–120 min (Chong et al., 2009; Clements et al., 1982; Fanta et al., 2015; Grant et al., 1981; Kharasch and Labroo, 1992; Peltoniemi et al., 2016; Yanagihara et al., 2003). Following oral intake, plasma concentrations of *(R,S)*-ketamine metabolites are significantly higher compared to those of the parent drug (Fanta et al., 2015).

Subcutaneous (s.c.) route is one of promising methods for *(R,S)*-ketamine administration in depression (Loo et al., 2016). *(R,S)*-ketamine s.c. injection has a greater bioavailability due to avoiding first-pass hepatic metabolism, so ketamine metabolites were not formed. In a pilot trial, it has been demonstrated that s.c. *(R,S)*-ketamine was also well-tolerated and plasma concentrations were comparable to i.v. *(R,S)*-ketamine (Loo et al., 2016).

Intranasal (i.n.) route is also a promising administration for *(R,S)*-ketamine treatment in depression. Intranasal administration has a few potential advantages for antidepressant drugs. Appropriate drug concentrations delivered intranasally are quickly transported across the nasal capillary network and delivered to the systemic circulation, thereby avoiding the absorption-limiting effects of first-pass metabolism (Panek et al., 2020). Therapeutic drug concentrations are rapidly attained in the cerebrospinal fluid (CSF), making intranasal administration an efficient mode of delivery into the brain. In addition, the nasal route of antidepressant delivery is noninvasive, improves drug bioavailability compared to the oral route, as well as helps to avoid limitations with crossing the blood-brain barrier, gastrointestinal absorption, and first-pass hepatic metabolism. Although early studies of intranasal *(R,S)*-ketamine are promising in the treatment of chronic pain and suicidality, information regarding its use in the treatment of central nervous system (CNS) disorders is currently very limited. For example, a first phase 3 study in patients with MDD suggests esketamine nasal spray may fulfill the unmet need for a rapid-acting antidepressant at 4-h and 24-h time points in patients undergoing an acute crisis of suicidal ideation (Ionescu et al., 2021). Furthermore, a clinically relevant, favorable improvement in depressive symptoms was also observed with esketamine nasal spray combined with an ineffective antidepressant in patients with TRD. These clinically meaningful benefits were measured 24 h after the first dose (Popova et al., 2019). These benefits of esketamine nasal spray observed in clinical trials must be confirmed in a daily practice, i.e., in the"real life".

Nasal administration produces rapid maximal plasma *(R,S)*-ketamine concentrations with relatively high bioavailability (Peltoniemi et al., 2016). *(R,S)*-ketamine i.n. administration has a greater bioavailability (45–50% in children) than that after oral intake (20%) and sublingual (30%) (Malinovsky et al., 1996). Thus, the fraction of a 28-mg intranasal dose of esketamine absorbed through the nasal cavity was 54% in healthy volunteers and patients with TRD (Perez-Ruixo et al., 2021). The remaining 46% was swallowed and underwent a first-pass intestinal metabolism and 18.6% of the swallowed dose reached the systemic circulation. The absolute bioavailability of 56 and 84 mg of intranasal esketamine was similar, around 50% (Perez-Ruixo et al., 2021).

Intranasal (i.n.) administration is a non-invasive method of drug delivery, which provides both direct and indirect pathways for the transmission of drugs to the central nervous system (CNS). Nasal anatomy and physiology suggest that drugs may bypass the BBB to allow direct access to the CNS to obtain therapeutic effects (Lochhead and Thorne, 2012). Intranasal delivery of *(R,S)*-ketamine and esketamine via its transport from the nasal epithelium to the CNS is a useful strategy to treat major depressive episode (MDE) or TRD patients (Canuso et al., 2018; Daly et al., 2018; Lapidus et al., 2014). Increasing evidence suggests that perineural and perivascular spaces of the olfactory and trigeminal nerves are involved in drug delivery and distribution into the brain (Lochhead and Davis, 2019). However, there is also an indirect passage suggesting that *(R,S)*-ketamine could return from the nasal

epithelium to the peripheral organs (Quintana et al., 2016). Because of less invasive, rapid absorption and low hepatic first-pass effect, i.n. administration is considered to an alternative i.v. injection of *(R,S)*-ketamine in depression treatment (Malinovsky et al., 1996).

## 5. Importance of brain CYP enzymes in drugs' metabolism

### 5.1. Brain CYP enzymes' expression in humans and rodents

Cytochromes P450 enzymes (CYP) are omnipresent enzymes engaged in the metabolism of endogenous or exogenous substrates, especially medications (Zanger and Schwab, 2013). This metabolism occurs primarily in the liver, but it can also take place in extrahepatic organs, including the brain (Miksys and Tyndale, 2013; Naud et al., 2016). CYP isoforms can reduce, increase, or alter the effect of many drugs acting in the brain such as antidepressant drugs (Toselli et al., 2016; Zanger and Schwab, 2013). At the cellular level, these CYP isoforms in the brain are found in the endoplasmic reticulum, mitochondria and cell surface in both the neurons and glial cells. Changes in brain CYP enzyme expression patterns and activity, which carry out drug demethylation and hydroxylation, can affect therapeutic drug responses (Desta et al., 2012; Navarro-Mabarak et al., 2018). Brain CYP tissue content is less than 4% of hepatic levels in rodents (Toselli et al., 2016).

It is now recognized that brain CYP enzymes play an important role in drug metabolism (Toselli et al., 2016). Based on gene expression patterns, CYP genes have been categorized into different families by cluster analysis (Choudhary et al., 2005; Hines, 2007). However, among the CYP enzymes identified in humans and rodents, those involved in drug metabolism are not well known (Nelson et al., 2004). Furthermore, because of the lack of selective antibodies against most of the CYP proteins, quantification using CYP mRNA array is often used to describe gene expression patterns (Hart et al., 2009). Therapeutic agents are not the only parameters modifying CYP gene expression profiles; they also depend on age, gender (Watzka et al., 1999), genetic polymorphism and environmental (diet), or epigenetic factors. Expression patterns of drug-metabolizing CYP genes can also differ by mouse strains, e.g., the liver in C57BL/6 versus BALB/cJ mice: Hart et al., 2009). To our knowledge, equivalent data in the brain of rodents are scarce.

CYP enzymes have been found in the brain of several species, including rodents, pigs and monkeys as well as humans (Ferguson and Tyndale, 2011). Total CYP levels in the brain are significantly lower than those in the liver (about 0.5–2% CYP levels in the liver) (Hedlund et al., 2001). Therefore, CYP-regulated drug metabolism in the brain is unlikely to affect systemic metabolite levels considerably (Hedlund et al., 2001). Indeed, brain CYPs are clustered near drug targets in specific brain regions and cell types and may substantially affect local drug metabolism and their therapeutic properties (Ferguson and Tyndale, 2011). For example, CYP2B6 in the frontal cortex of the human brain is highly expressed in astrocytes surrounding blood vessels in layer I, whereas CYP2D6 is found predominantly in pyramidal neurons in layers III-V (Ferguson and Tyndale, 2011; Meyer et al., 2007). However, CYP levels in the brain are low compared to those in the liver: whether it achieves therapeutic relevance for centrally acting drugs is still unclear. This information is scarce regarding drugs used in psychiatry (e.g., *(R,S)*-ketamine) and neurology.

Forty-one CYP enzymes have been found in the human brain (Dutheil et al., 2008), which are consistently distributed among different brain regions and cell types. Sex-related differences in brain CYP enzymes have also been identified (Dutheil et al., 2008). Accordingly, brain CYP expression was higher in male compared to female rats, but sex differences in the CYP expression were not observed in human brains (Roselli and Resko, 1997; Stoffel-Wagner et al., 1999). The majority of brain CYPs metabolize substrates with similar affinities and selectivity to their hepatic counterparts (Miksys and Tyndale, 2013). However, some CYP enzymes are specific to the brain or more highly expressed in the brain than other tissues, such as CYP2D4, CYP2D18, and some isoforms of the CYP3A family in rats (Miksys and Tyndale, 2013). CYP1A1, CYP2B, CYP2E1, and CYP3A are found mainly in neurons while others are present in both neurons and glial cells, such as CYP2B and CYP2D (Miksys and Tyndale, 2013). CYP2B6 RNA and protein were detected in the human brain.

*(R,S)*-ketamine is one of the substrates of CYP2B6 in the brain (Miksys and Tyndale, 2013). Brain CYP2B6 is higher in smokers and alcoholics, while liver CYP2B6 is unaffected by smoking (Miksys and Tyndale, 2013). In mice, about 3% CYPs were detected in the brain (Renaud et al., 2011), including CYP2 and CYP4 families (Renaud et al., 2011).

Overall, the role of CYP enzymes in the brain may be of considerable importance in understanding the antidepressant-like activity of *(R,S)*-ketamine and that of its major brain metabolite, *(R,S)*-HNKs, in rodents and humans.

### 5.2. Drug-drug interactions and ketamine

Since CYP belongs to a set of important hepatic drug-metabolizing enzymes, drug-drug interactions can occur by enzyme induction and inhibition. Here, we include a list of substrates, inhibitors and inducers of CYP450 (Table 2). Some CYPs such as CYP2B6 and CYP2C9 are more particularly involved in *(R,S)*-ketamine metabolism in humans (Peltoniemi et al., 2011). Even though CYP activities exhibit dissimilarity among different species, the activity of CYP1A1, CYP1A2, CYP2E1, and CYP3A in mouse and rat hepatic microsomes is similar to that of human microsomes (Bogaards et al., 2000; Hart et al., 2009). Thirteen protein-coding genes for CYPs expressed in the liver have been identified in mice (Hart et al., 2009). An *in vitro* comparison of *(R,S)*-ketamine metabolism on rat liver fractions versus humans shows that the formation of norketamine is mainly carried out by CYP3A in the two mammalian species, with however a greater contribution of the isoenzyme CYP2C in rats (Santamaria et al., 2014).

There are few studies on CYP inhibitors-ketamine interaction in rodents. In 1975, White and colleagues reported that halothane, a substrate of many CYPs (3A4, 2B6, 2A6, 2C9, 2E1), increased $t_{1/2}$ of (*R,S*)-ketamine in both plasma and brain in male Sprague-Dawley rats (White et al., 1975). In the same year, Lo and Cumming demonstrated that secobarbital (a CYP2B6 inhibitor) and diazepam (a substrate of CYP2C19 and CYP3A4) also climbed $t_{1/2}$ of (*R,S*)-ketamine in isolated rat livers (Lo and Cumming, 1975). More than 20 years later, it was reported that cyclophosphamide (CYP2B6 substrate) increased the duration of anesthesia caused by *(R,S)*-ketamine in male BALB/c mice (Rojavin et al., 1996). As expected, prior treatment with a cocktail of CYP inhibitors, including ticlopidine (a CYP2B6 inhibitor) and 1-ABT (a multiple CYP inhibitor), decreased *(R,S)*-ketamine metabolism and the subsequent formation of (*2R,6R*)-HNK in male C57 mice (Yamaguchi et al., 2018). The CYP2B6 enzyme is the major isoform primarily catalyzing *(R,S)*-ketamine N-demethylation to *(R,S)*-norketamine *in vitro* in human liver microsomes (Hijazi and Boulieu, 2002; Li et al., 2013). CYP2B6, one of the most polymorphic CYP genes in humans with over 100 described single nucleotide polymorphisms (SNPs). CYP2B6 plays a role in the metabolism of 2%–10% of clinically prescribed drugs in humans (Hedrich et al., 2016). The most common functionally deficient allele is CYP2B6*6 [Q172H, K262R], which occurs at frequencies of 15 to over 60% in different populations. Another important variant, CYP2B6*18 [I328T], occurs predominantly in Africans (4–12%) and does not express functional protein (Zanger and Klein, 2013). Thus, significant inter-individual variability in the expression and function of the human CYP2B6 gene variants exists and can result in altered clinical outcomes in patients receiving treatment with CYP2B6-substrate drugs. However, few clinical studies focused on *(R,S)*-ketamine. One of them found that the allelic variant CYP2B6*6 polymorphism did not affect *(R,S)*-ketamine metabolism in healthy volunteers (Rao et al., 2016). The work is in progress since a large number of uncharacterized variants are currently emerging from different ethnicities in the course of the Human

**Table 2**
Examples of clinical substrates, inhibitors and inducers of CYP450-mediated metabolism (FDA, 2019).

| CYP450 | Substrates | | Inhibitors | | | Inducers | | |
|---|---|---|---|---|---|---|---|---|
| | Sensitive substrates | Moderate sensitive substrates | Strong inhibitors | Moderate inhibitors | Weak inhibitors | Strong inducers | Moderate inducers | Weak inducers |
| **1A2** | caffeine, alosetron, melatonin, duloxetine, ramelteon, tasimelteon, tizanidine | clozapine, ramosetron, pirfenidone, theophylline | enoxacin, ciprofloxacin, fluvoxamine | mexiletine, methoxsalen, oral contraceptives | allopurinol, acyclovir, cimetidine, piperine, peginterferon alpha-2a, zileuton | – | phenytoin, smoking, rifampin, ritonavir, teriflunomide | – |
| **2B6** | bupropion | efavirenz | – | – | tenofovir, clopidogrel, ticlopidine, voriconazole | carbamazepine | efavirenz, rifampin | nevirapine, ritonavir |
| **2C8** | repaglinide | pioglitazone, montelukast, rosiglitazone | gemfibrozil | clopidogrel, teriflunomide, deferasirox | trimethoprim | – | rifampin | – |
| **2C9** | celecoxib | phenytoin, tolbutamide, glimepiride, warfarin | – | fluconazole, miconazole, amiodarone, piperine | diosmin, fluvastatin, disulfiram, fluvoxamine, voriconazole | – | rifampin, enzalutamide | carbamazepine, apalutamide, ritonavir, aprepitant, |
| **2C19** | omeprazole, S-mephenytoin | lansoprazole, rabeprazole, diazepam, voriconazole | fluconazole, fluvoxamine, fluoxetine, ticlopidine | felbamate | omeprazole, voriconazole | rifampin | efavirenz, apalutamide, enzalutamide, phenytoin | ritonavir |
| **2D6** | desipramine, dextromethorphan, atomoxetine, eliglustat, nebivolol, perphenazine, tolterodine, nortriptyline, R-venlafaxine | imipramine, propafenone, propranolol, metoprolol, tramadol, trimipramine, encainide, S-venlafaxine | bupropion, paroxetine, quinidine, fluoxetine, terbinafine | cimetidine, duloxetine, fluvoxamine, cinacalcet, mirabegron | amiodarone, celecoxib, abiraterone, cimetidine, clobazam, desvenlafaxine, escitalopram, cobicistat, labetalol, lorcaserin, ritonavir, sertraline, vemurafenib | – | – | – |
| **3A4** | alfentanil, simvastatin, avanafil, buspirone, tacrolimus, conivaptan, darifenacin, darunavir, ebastine, everolimus, ibrutinib, lomitapide, lovastatin, midazolam, naloxegol, nisoldipine, saquinavir, budesonide, dasatinib, dronedarone, eletriptan, eplerenone, felodipine, indinavir, lurasidone, maraviroc, quetiapine, sildenafil, ticagrelor, tolvaptan sirolimus, tipranavir, triazolam, vardenafil | alprazolam, aprepitant, eliglustat, pimozide, atorvastatin, rilpivirine, rivaroxaban, colchicine, tadalafil | boceprevir, itraconazole, ketoconazole, cobicistat, danoprevir and ritonavir, elvitegravir and ritonavir, indinavir and ritonavir, lopinavir and ritonavir, paritaprevir and ritonavir and (ombitasvir and/or dasabuvir), posaconazole, ritonavir, saquinavir, ritonavir, telaprevir, tipranavir and ritonavir, clarithromycin, idelalisib, nefazodone, nelfinavir, telithromycin, troleandomycin, voriconazole, grapefruit juice | ciprofloxacin, aprepitant, conivaptan, crizotinib, diltiazem, dronedarone, erythromycin, fluconazole, fluvoxamine, imatinib, tofisopam, cyclosporine, verapamil | cilostazol, cimetidine, clotrimazole, istradefylline, ivacaftor, lomitapide, ranitidine, ranolazine, fosaprepitant, chlorzoxazone, ticagrelor | phenytoin, apalutamide, carbamazepine, mitotane, rifampin, enzalutamide, St. John's wort | phenobarbital etravirine, bosentan, efavirenz, primidone | modafinil, armodafinil, rufinamide |

Genomes Project.

Thus, drug-drug interactions may be of concern if *(R,S)*-ketamine is prescribed with either a CYP inducer or a CYP inhibitor. CYP inducers (e.g., rifampicin, phenytoine and carbamazepine) induce a gradual increase in synthesis of microsomal liver CYP enzymes. In Daly et al. JAMA Psychiatry 2019, it is indicated that subjects may not take a known potent inducer of hepatic CYP3A activity within 2 weeks of the first administration of intranasal study medication until at least 24 h after the last intranasal dose of study medication. Examples: efavirenz, nevirapine, barbiturates, carbamazepine, glucocorticoids, modafinil, oxcarbazepine, phenobarbital, phenytoin, rifabutin, rifampin, and St. John's wort. Therefore, these drugs were prohibited during the phase 3 clinical trials of esketamine (SPRAVATO®) (Daly et al., 2019).

Conversely, CYP inhibitors (e.g., ketoconazole and fluconazole) inhibit synthesis of microsomal hepatic CYP3A4/5 enzymes, thus could lead to an increase in plasma levels of the parent drug (*(R,S)*-ketamine), together with a decrease in HNK levels. Such an interaction of a CYP inhibitor could increase plasma levels of the parent drug (*(R,S)*-ketamine), while decreasing those of its metabolites (HNK). This is well documented hypothesis for drug-drug interactions (e.g., triazole drugs – fentanyl interaction (Saari et al., 2008; Ziesenitz et al., 2015):). Interactions between ketamine and CYP inducers are shown in Tables 4–6.

If this parent drug has inactive metabolites, the efficacy of this drug should increase (e.g., clarithromycin, a strong inhibitor of CYP3A4 and oral esketamine in healthy volunteers (Hagelberg et al., 2010): or the CYP3A4/5 inhibitor ketoconazole and the SNRI milnacipran (Chen et al., 2015).

We are currently facing **two hypotheses**: 1) If HNK metabolites are active, a CYP inhibitor should *decrease (R,S)*-ketamine efficacy; 2) by contrast, if metabolites are inactive, a CYP inhibitor should *increase (R,S)*-ketamine efficacy in behavioral tests (Yamaguchi et al., 2018). In both cases, we should measure a decrease in plasma metabolite *(R,S)*-norketamine and HNKs levels (Peltoniemi et al., 2011). However, a possibility remains regarding *(R,S)*-ketamine and HNKs: both the parent drug and its metabolite could produce an antidepressant effect.

## 6. Pharmacokinetics of ketamine and its metabolites

### 6.1. Pharmacokinetics in humans

In humans, the pharmacokinetic profiles for two isomers (*S*)- aI(*R*)- of ketamine did not differ significantly from the racemic mixture *(R,S)*-ketamine. Both racemic *(R,S)*-ketamine and its isomers have high clearance and a moderately large volume of distribution (White et al., 1985). After systemic administration, *(R,S)*-ketamine can rapidly be distributed into the brain and other tissues (Peltoniemi et al., 2016). The elimination half-life ($t_{1/2}$) of *(R,S)*-ketamine is short (from 7 min to 4 h) (Domino, 2010) and its binding to plasma protein is quite low (10–30%) (Dayton et al., 1983; Hijazi et al., 2003). Three days after the administration of a single dose of *(R,S)*-ketamine (0.5 mg/kg by intravenous route, for 40 min), it was eliminated in the urine: 2.3% in unchanged form, 1.6% in *(R,S)*-norketamine, 16.2% in dehydronorketamine and 80% in the hydroxylated conjugated derivatives of ketamine (Domino, 2010; Zarate et al., 2012). These metabolites are then glucuronidated and eliminated through the urine and bile (Desta et al., 2012; Noppers et al., 2011). Some parameters of the pharmacokinetic profiI of (*R*)-ketamine, (*S*)-ketamine et racemic *(R,S)*-ketamine are shown in Table 3.

Most drugs can be metabolized in the liver leading to one or more metabolites, generally less active than the parent drug (inactivation). However, depending on the route of administration, *(R,S)*-ketamine undergoes metabolism in the liver (p.o. or i.p. administration) or bypasses the liver (i.v., s.c. and i.n. administration).

### 6.2. Pharmacokinetics in rodents

In rodents, (*R,S*)-ketamine has a short elimination half-life ($t_{1/2} = 30$ min in mice) (Maxwell et al., 2006). Similarly to humans, (*R,S*)-ketamine is rapidly metabolized to (*R,S*)-norketamine and (*R,S*)-HNKs by many different CYP450 after administration in rodents. *(R,S)*-ketamine undergoes oxidative metabolism, mainly to *(R,S)*-norketamine by CYP3A and CYP2B6 enzymes in humans (Peltoniemi et al., 2016). In rat hepatic microsomes, norketamine, HNKs and hydroxyketamine present at 80%, 15% and 5%, respectively (Kharasch and Labroo, 1992; Noppers et al., 2011). Metabolites norketamine, HNKs and DHNK are detected in plasma 10–15 min after a 10 mg/kg dose of *(R,S)*-ketamine given intraperitoneally (i.p.) in rats (Can et al., 2016; Moaddel et al., 2015). The brain-to-plasma ratio for HNK metabolites is approximately 1:1 in rodents (see (Highland et al., 2021) for a review). Plasma and brain levels of *(R,S)*-ketamine metabolites (2*R*,6*R*)-HNK were five times higher than that of the parent drug at 30 min after an i.p. administration (Pham et al., 2018). However, neither *(R,S)*-ketamine nor HNK levels were detected in these samples at 24 h post-treatment in mice, when a positive correlation between cortical neurotransmitter release and antidepressant-like effects of *(R,S)*-ketamine was observed (Pham et al., 2017, 2018, 2020). Although electroencephalographic (EEG) slow waves' studies and neuroimaging techniques such as functional fMRI and proton magnetic resonance spectroscopy (1)H-MRS have identified a characteristic signature of *(R,S)*-ketamine in the living human brain, its brain and CSF levels have not been measured directly in humans.

Similarly to *(R,S)*-ketamine, its main metabolites norketamine and (2*R*,6*R*)-HNK, display antidepressant-like effects in some rodent studies (Pham et al., 2018; Yang et al., 2018a; Zanos et al., 2016). Following an i.v. administration of *(R,S)*-ketamine at 10 mg/kg, $C_{max}$ of norketamine reached 26 ng/mL after 20 min, and its AUC was 11.2 ng h/mL (Chong et al., 2009). After *(R,S)*-ketamine p.o. administration at 25 mg/kg, $C_{max}$, $t_{max}$ and AUC of norketamine were 86 ng/mL, 90 min and 12.7 ng h/mL, respectively (Chong et al., 2009).

In plasma, the maximum drug concentrations were 561 ng/ml (2.3 μM) for *(R,S)*-ketamine and 1,098 ng/ml (4.9 μM) for norketamine at t10 min of i.p. administration, and 674 ng/ml (2.8 μM) for HNKs at t30 min. In the brain of C57BL/6 mice (Zanos et al., 2016), the concentrations at t10 min are: 1,162 ng/g *(R,S)*-ketamine of fresh tissue (4.9 μM/kg of tissue), 451 ng/g norketamine of fresh tissue (2.0 μM/kg of tissue) and 498 ng HNKs of fresh tissue (2.1 μM/kg of tissue).

## 7. Factors affecting CYP expression and drug metabolism

### 7.1. Gender differences: male versus female (sex-dependent CYP expression)

Only a few studies have investigated sex differences in the CYP expression in the liver and the brain. Sex-based differences in hepatic CYP activity were found in both animals and humans, which may be an important cause of individual differences in drug responses (Waxman and Holloway, 2009). Gender differences in human hepatic P450-catalyzed drug metabolism are less significant than in rats (Zhang et al., 2011). In humans, more than 1,300 genes whose mRNA expression was greatly influenced by sex, of which 75% displayed higher expression in females (Zhang et al., 2011). Many clinical studies showed that women metabolize drugs more rapidly than men (Zanger and Schwab, 2013). Indeed, CYP3A4 protein level in liver tissue of women is about 2-fold higher than that of men (Lamba et al., 2010; Schmidt et al., 2001; Wolbold et al., 2003; Yang et al., 2010). Accordingly, some CYP3A4 substrates such as cyclosporine (Kahan et al., 1986), erythromycin (Austin et al., 1980), and nifedipine (Krecic-Shepard et al., 2000), demonstrate higher clearance rates in female. Similarly, there is also evidence for higher CYP2B6 and CYP2A6 activity in women (Lamba et al., 2010; Sinues et al., 2008). In contrast, the expression of CYP1A2, CYP2E1 and CYP2D6 in men is higher than that in women. As a result, men display more rapid clearance of CYP1A2 substrates (e.g., caffeine, olanzapine and clozapine), CYP2E1 substrates (e.g., chlorzoxazone) and CYP2D6 substrate propranolol, metoprolol, dextromethorphan,

**Table 3**
Pharmacokinetic profile of (*R*)-ketamine, (*S*)-ketamine and the racemic mixture (*R,S*)-ketamine.

| Drug, route, dose | Isomer | T1/2 (min) | Tmax (min) | Cmax (ng/mL) | Cl (ml $kg^{-1}min^{-1}$) | Vd ($L.kg^{-1}$) | AUC0-∞ (h·ng/mL) | Patient population | Ref |
|---|---|---|---|---|---|---|---|---|---|
| **(*R,S*)-KET, i.m., 0.5 mg/kg** | (*R,S*)-KET | 155 ± 12 | 22 ± 4 | 240 ± 50 | – | – | – | Healthy volunteers | Grant et al. (1981) |
| | (*R,S*)–NK | – | 77 ± 14 | 90 ± 10 | – | – | – | Healthy volunteers | Grant et al. (1981) |
| **(*R,S*)-KET, p.o., 0.5 mg/kg** | (*R,S*)-KET | – | 30 ± 5 | 45 ± 10 | – | – | – | Healthy volunteers | Grant et al. (1981) |
| | (*R,S*)–NK | – | 60 ± 13 | 200 ± 44 | – | – | – | Healthy volunteers | Grant et al. (1981) |
| **(*R,S*)- KET, i.v., 275 ± 25 mg** | (*R,S*)-KET | 132 ± 32 | | | 16.1 ± 4.6 | 2.9 ± 0.5 | | Healthy volunteers | White et al. (1985) |
| **(*S*)-KET, i. v., 140 ± 21 mg** | (*S*)-KET | 158 ± 45 | | | 21.3 ± 1.6 | 4.7 ± 1.1 | | Healthy volunteers | White et al. (1985) |
| **(*R*)-KET, i. v., 429 ± 37 mg** | (*R*)-KET | 155 ± 42 | | | 17.4 ± 2.5 | 3.9 ± 1.3 | | Healthy volunteers | White et al. (1985) |
| **(*R,S*)-KET, i.v., 20 mg** | (*R*)-KET | 126.8 ± 47.7 | – | – | 18.3 ± 3.3 | 2.5 ± 0.6 | 138.0 ± 15.6 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*S*)-KET | 117.3 ± 47.9 | – | – | 20 ± 1.7 | 2.5 ± 0.8 | 126.3 ± 15.1 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*R*)-NK | – | 23.3 ± 5.8 | 38.1 ± 6.1 | – | – | 173.8 ± 26.5 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*S*)-NK | – | 23.3 ± 5.8 | 34.9 ± 6.4 | – | – | 149.8 ± 9.5 | Healthy volunteers | Yanagihara et al. (2003) |
| **(*R,S*)-KET, p.o., 50 mg** | (*R*)-KET | – | 33.3 ± 15.3 | 42.6 ± 13.3 | – | – | 72.3 ± 22.4 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*S*)-KET | – | 30.0 ± 30.0 | 40.4 ± 14.2 | – | – | 64.9 ± 27.4 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*R*)-NK | – | 50.0 ± 17.3 | 188.1 ± 4.7 | – | – | 533.8 ± 47.1 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*S*)-NK | – | 50.0 ± 17.3 | 172.0 ± 13.7 | – | – | 494.9 ± 73.0 | Healthy volunteers | Yanagihara et al. (2003) |
| **(*R,S*)-KET, s.l., 50 mg** | (*R*)-KET | – | 40.0 ± 20.0 | 61.6 ± 21.3 | – | – | 108.8 ± 17.3 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*S*)-KET | – | 36.7 ± 20.8 | 56.9 ± 22.8 | – | – | 110.5 ± 18.4 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*R*)-NK | – | 113.3 ± 70.2 | 123.4 ± 10.7 | – | – | 462.9 ± 65.2 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*S*)-NK | – | 93.3 ± 75.7 | 109.7 ± 16.6 | – | – | 408.7 ± 35.1 | Healthy volunteers | Yanagihara et al. (2003) |
| **(*R,S*)-KET, s.p., 50 mg** | (*R*)-KET | – | 23.3 ± 5.7 | 42.8 ± 31.1 | – | – | 102.4 ± 76.7 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*S*)-KET | – | 23.3 ± 5.7 | 38.8 ± 30.2 | – | – | 87.9 ± 78.9 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*R*)-NK | – | 33.3 ± 15.3 | 85.3 ± 35.1 | – | – | 322.6 ± 106.5 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*S*)-NK | – | 33.3 ± 15.3 | 78.7 ± 30.9 | – | – | 282.6 ± 74.2 | Healthy volunteers | Yanagihara et al. (2003) |
| **(*R,S*)-KET, i.n., 25 mg** | (*R*)-KET | – | 22.5 ± 9.6 | 29.4 ± 16.5 | – | – | 76.8 ± 27.9 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*S*)-KET | – | 17.5 ± 5.0 | 29.3 ± 13.8 | – | – | 72.7 ± 17.5 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*R*)-NK | – | 120.0 ± 52.0 | 33.1 ± 25.3 | – | – | 115.9 ± 17.6 | Healthy volunteers | Yanagihara et al. (2003) |
| | (*S*)-NK | – | 100.0 ± 17.3 | 29.4 ± 21.3 | – | – | 100.7 ± 39.6 | Healthy volunteers | Yanagihara et al. (2003) |
| **(*R,S*)-KET, i.v., 10 mg** | (*R,S*)-KET | 312 (204–384) | – | 202 (123–344) | 15 (11.7–15) | 5 (4–6) | 13.3 (11–16)[a] | Patients with neuropathic pain | Chong et al. (2009) |
| | (*R,S*)–NK | 330 (258–474) | 19.8 (19.8–27.6) | 26 (20–48) | – | – | 11.2 (9.4–14) | Patients with neuropathic pain | Chong et al. (2009) |
| **(*R,S*)-KET, p.o., 25 mg** | (*R,S*)-KET | 336 (216–426) | 120 (72–150) | 21 (12–35) | 50 (50–83) | 24.5 (19–26) | 2.5 (2.1–3.7) | Patients with neuropathic pain | Chong et al. (2009) |
| | (*R,S*)–NK | 234 (186–348) | 90 (54–138) | 86 (69–107) | – | – | 12.7 (8.6–16) | Patients with neuropathic pain | Chong et al. (2009) |
| **(*R,S*)-KET, s.l., 25 mg** | (*R,S*)-KET | 306 (246–492) | 30 (18–48) | 30 (24–32) | 66.7 (16.7–70.83) | 19.7 (9.9–26.4) | 4.2 (2.6–6.5) | Patients with neuropathic pain | Chong et al. (2009) |
| | (*R,S*)–NK | 384 (306–426) | 108 (90–120) | 74 (41–85) | – | – | 8.8 (6.7–12.9) | Patients with neuropathic pain | Chong et al. (2009) |
| **(*R,S*)-KET, i.n., 0.2 mg/kg** | (*R,S*)-KET | – | 10 ± 6.3 | 27.7 ± 5.9 | – | – | – | Patients with neuropathic pain | Huge et al. (2010) |
| | (*R,S*)–NK | – | 81 ± 59 | 18.3 ± 14.9 | – | – | – | Patients with neuropathic pain | Huge et al. (2010) |

(*continued on next page*)

**Table 3** (*continued*)

| Drug, route, dose | Isomer | T1/2 (min) | Tmax (min) | Cmax (ng/mL) | Cl (ml $kg^{-1}min^{-1}$) | Vd ($L.kg^{-1}$) | AUC0-∞ (h·ng/mL) | Patient population | Ref |
|---|---|---|---|---|---|---|---|---|---|
| **(*R,S*)-KET, i.n., 0.4 mg/kg** | (*R,S*)-KET | – | 14 ± 5 | 34.3 ± 22.2 | – | – | – | Patients with neuropathic pain | Huge et al. (2010) |
| | (*R,S*)–NK | – | 75.0 ± 39.7 | 34.3 ± 5.5 | – | – | – | Patients with neuropathic pain | Huge et al. (2010) |
| **(*S*)-KET, i.v., 0.1 mg/kg** | (*S*)-KET | 354 ± 66 | 12 (12–18) | 32.5 ± 7.5 | 84 ± 21[c] | 427 ± 118[b] | 81 ± 15 | Healthy volunteers | Peltoniemi et al. (2012a) |
| | (*S*)-NK | 468 ± 114 | 60 (18–180) | 14.2 ± 4.5 | – | – | 139 ± 39.9 | Healthy volunteers | Peltoniemi et al. (2012a) |
| **(*S*)-KET, p.o., 0.3 mg/kg** | (*S*)-KET | 360 ± 96 | 42 (18–60) | 12.4 ± 5.9 | – | – | 27.2 ± 12.5 | Healthy volunteers | Peltoniemi et al. (2012a) |
| | (*S*)-NK | 342 ± 53.4 | 60 (18–180) | 77.5 ± 21.2 | – | – | 385 ± 76.8 | Healthy volunteers | Peltoniemi et al. (2012a) |
| **(*S*)-KET, p.o., 0.2 mg/kg** | (*S*)-KET | 252 ± 144 | 54 (18–90) | 5.3 ± 2.7 | – | – | 13.6 ± 6.4 | Healthy volunteers | Hagelberg et al. (2010) |
| | (*S*)-NK | 312 ± 108 | 78 (18–120) | 38.4 ± 7.9 | – | – | 186 ± 49 | Healthy volunteers | Hagelberg et al. (2010) |
| **(*S*)-KET, p.o., 0.3 mg/kg** | (*S*)-KET | 390 ± 180 | 48 (18–90) | 16.2 ± 5.78 | – | – | 32.9 ± 13.4 | Healthy volunteers | Peltoniemi et al. (2012c) |
| | (*S*)-NK | 420 ± 156 | 60 (18–60) | 50.1 ± 11.0 | – | – | 232 ± 77 | Healthy volunteers | Peltoniemi et al. (2012c) |
| **(*S*)-KET, p.o., 0.2 mg/kg** | (*S*)-KET | 294 ± 84 | 42 (18–60) | 8.3 ± 4.2 | – | – | 17.4 ± 9.0 | Healthy volunteers | Peltoniemi et al. (2012b) |
| | (*S*)-NK | – | 60 (18–90) | 50.0 ± 16.4 | – | – | 256.6 ± 61 | Healthy volunteers | Peltoniemi et al. (2012b) |
| **(*S*)-KET, p.o., 0.2 mg/kg** | (*S*)-KET | 438 ± 156 | 42 (18–120) | 10.6 ± 6.0 | – | – | 28.8 ± 19.6 | Healthy volunteers | Peltoniemi et al. (2011) |
| | (*S*)-NK | – | 60 (18–120) | 56.9 ± 22.3 | – | – | 345 ± 112 | Healthy volunteers | Peltoniemi et al. (2011) |
| **(*R,S*)-KET, i.v, 0.5 mg/kg** | (*R,S*)-KET | – | – | 177.23 ± 53.8 | – | – | 975.4 | Patients with bipolar depression | (Zanos et al., 2018; Zarate et al., 2012) |
| | (*2R,6R*)-HNK | – | – | 37.59 ± 14.23 | – | – | 1366 | Patients with bipolar depression | (Zanos et al., 2018; Zarate et al., 2012) |
| | (*R,S*)-KET | – | – | 204.13 ± 101.46 | – | – | 873.5 | Patients with MDD | (Zanos et al., 2018; Zarate et al., 2012) |
| | (*2R,6R*)-HNK | – | – | 23.19 ± 11.88 | – | – | 1038 | Patients with MDD | (Zanos et al., 2018; Zarate et al., 2012) |
| **(R,*S*)-KET, i.v, 10 mg** | (*R,S*)-KET | 270 (150–420) | – | 128.1 (81.1–226.7) | 39.2 (33.4–59.8)[c] | 237 (126–385)[b] | 255 (167.2–299.4) | Healthy volunteers | Rolan et al. (2014) |
| **(R,*S*)-KET, s.l., 25 mg** | (R,*S*)-KET | 204 (108–330) | 45 (15–60) | 71.1 (50.0–128.3) | – | – | 184.6 (161.6–211.3) | Healthy volunteers | Rolan et al. (2014) |
| **(*S*)-KET, i.v, 0.5 mg/kg** | (*S*)-KET | 287.50 ± 110.20 | 1.17 (0.28–1.25) | 2277.60 ± 2697.60 | 18.10 ± 3.20 | 7.39 ± 2.70 | 475.20 ± 86.50 | Chinese patients received conventional gastroscopy | Wang et al. (2019a) |
| | (*S*)-NK | 519.00 ± 117.00 | 20.17 (10.15–20.22) | 129.70 ± 28.9 | 10.10 ± 3.30 | 7.59 ± 2.40 | 871.00 ± 262.30 | Chinese patients received conventional gastroscopy | Wang et al. (2019a) |
| **(*R,S*)- KET, i.v., 1 mg/kg** | (*S*)-KET | 283.20 ± 117.80 | 1.15 (0.23–1.28) | 1976.80 ± 1518.70 | 18.40 ± 3.40 | 7.29 ± 2.54 | 469.00 ± 99.50 | Chinese patients received conventional gastroscopy | Wang et al. (2019a) |
| | (*R*)-KET | 343.40 ± 112.70 | 1.15 (0.23–1.28) | 2064.40 ± 1573.90 | 15.80 ± 3.10 | 7.61 ± 2.15 | 547.30 ± 121.60 | Chinese patients received conventional gastroscopy | Wang et al. (2019a) |
| | (*S*)-NK | 475.10 ± 65.90 | 10.19 (10.12–20.20) | 136.00 ± 29.10 | 10.60 ± 3.00 | 7.81 ± 2.17 | 782.80 ± 158.20 | Chinese patients received conventional gastroscopy | Wang et al. (2019a) |
| | (*R*)-NK | 453.80 ± 64.40 | 20.13 (10.15–30.0) | 149.40 ± 32.60 | 9.50 ± 2.80 | 6.64 ± 1.795 | 882.40 ± 217.00 | Chinese patients received conventional gastroscopy | Wang et al. (2019a) |

KET: ketamine.
NK: norketamine.
i.v.: intravenous.
i.n.: intranasal.
s.l.: sublingual.

s.p.: suppository.
Values represent mean ± SD or mean/median (range).
MDD: major depressive disorder.
[a] Dose-normalized AUC from baseline to 8 h, expressed in μg.h/L/mg.
[b] L.
[c] L/h.

**Table 4**
Effect of CYP inhibitors on the pharmacokinetics of (*R,S*)-ketamine and its isomers in humans.

| CYP inhibitors, dose, route | Isomers of ketamine, dose, route | Affected CYPs | Type and object of study, n | Consequences | References |
|---|---|---|---|---|---|
| Diazepam, 0.15 mg/kg, i.m. | Ketamine, 10 mg/kg, i.m., 30 min after diazepam | Substrate of CYP2C19, CYP3A | patients 2–9 years old | ↑$t_{1/2}$ of ketamine | Lo and Cumming (1975) |
| Secobarbital, 2 mg/kg, i.m. | Ketamine, 10 mg/kg, i.m., 30 min after secobarbital | CYP2B | patients 2–9 years old | ↑$t_{1/2}$ of ketamine | Lo and Cumming (1975) |
| Medetomidine | (*R,S*)-ketamine, (*R*)-ketamine, (*S*)-ketamine | CYP3A4, CYP2C9 | human liver microsomes | Inhibit N-demethylation of ketamine and its isomers | Kharasch et al. (1992) |
| Orphenadrine, 500 μM | (*R*)-ketamine, 5 μM | CYP2B6 | human liver microsomes | ↓67% N-demethylase activity | Yanagihara et al. (2001) |
| Orphenadrine, 500 mM | (*S*)-ketamine, 5 μM | CYP2B6 | human liver microsomes | ↓64% N-demethylase activity | Yanagihara et al. (2001) |
| Sulfaphenazole, 100 mM | (*R*)-ketamine, 5 μM | CYP2C9, CYP2B6 | human liver microsomes | ↓62% N-demethylase activity | Yanagihara et al. (2001) |
| Sulfaphenazole, 100 mM | (*S*)-ketamine, 5 μM | CYP2C9, CYP2B6 | human liver microsomes | ↓57% N-demethylase activity | Yanagihara et al. (2001) |
| Ketoconazole, 2 μM | Ketamine, 0.05 mM | CYP3A4 | human liver microsomes | ↓ 40% ketamine N-demethylation activity | Hijazi and Boulieu (2002) |
| Ketoconazole, 10 μM | Ketamine, 0.05 mM | CYP3A4 | human liver microsomes | ↓ 65% ketamine N-demethylation activity | Hijazi and Boulieu (2002) |
| Orphenadrine, 100 μM | Ketamine, 0.05 mM | CYP2B6 | human liver microsomes | ↓ 20% ketamine N-demethylation activity | Hijazi and Boulieu (2002) |
| Orphenadrine, 500 μM | Ketamine, 0.05 mM | CYP2B6 | human liver microsomes | ↓ 60% ketamine N-demethylation activity | Hijazi and Boulieu (2002) |
| Clarithromycin, 500 mg twice daily for 4 days, p.o. | (*S*)-ketamine, 0.2 mg/kg, 1h after clarithromycin on day 4, p.o. | CYP3A | a randomized controlled cross-over study, 2 phases, 10 healthy people | ↑ 3.6 times of Cmax of ketamine.<br>↑ 2.6 times of AUC0–∞ of ketamine<br>↑ 35% Cmax of norketamine<br>↓tmax of norketamine from 1.3h to 0.6h<br>↓ 54% $AUC_{norket}/AUC_{ket}$ | Hagelberg et al. (2010) |
| 1-aminobenzotriazole | (*R,S*)-ketamine | general CYP inhibitor | human liver microsomes | Block ketamine N-demethylation activity in a concentration-dependent manner | Mossner et al. (2011) |
| Ketoconazole, 2 μM | (*R,S*)-ketamine | CYP3A4 | human liver microsomes | ↓ 52% ketamine N-demethylation activity | Mossner et al. (2011) |
| Sulfaphenazole, 10 μM | (*R,S*)-ketamine | CYP2C9 | human liver microsomes | ↓ 32% norketamine formation | Mossner et al. (2011) |
| Tranylcypromine | (*R,S*)-ketamine | CYP2A6 | human liver microsomes | ↓ norketamine formation | Mossner et al. (2011) |
| Nootkatone, 2 μM | (*R,S*)-ketamine | CYP2C19 | human liver microsomes | ↓ 35% norketamine formation | Mossner et al. (2011) |
| Clopidogrel, 2 μM | (*R,S*)-ketamine | CYP2B6 | human liver microsomes | ↓ minor extent of ketamine N-demethylation activity | Mossner et al. (2011) |
| Itraconazole, 200 mg once daily for 6 days, p.o. | (*S*)-ketamine, 0.2 mg/kg, 1h after itraconazole on day 6, p.o. | CYP3A4 | a randomized, blinded, crossover study, 11 healthy volunteers | No change in the $AUC_{0-\infty}$, Cmax and $t_{1/2}$ of ketamine.<br>↓ $AUC_{norket}/AUC_{ket}$ | Peltoniemi et al. (2011) |
| Ticlopidine, 250 mg twice daily for 6 days, p.o. | (*S*)-ketamine, 0.2 mg/kg, 1h after ticlopidine on day 6, p.o. | CYP2B6 | a randomized, blinded, crossover study, 11 healthy volunteers | ↑ 2.1 times of $AUC_{0-\infty}$ of ketamine<br>↓ $AUC_{norket}/AUC_{ket}$ | Peltoniemi et al. (2011) |
| Grapefruit juice, 200 ml, three times a day for 5 days, p.o. | (*S*)-ketamine, 0.2 mg/kg on day 5, p.o. | CYP3A | a randomized, open-label crossover study design with two phases, 12 healthy volunteers | ↑ 3.0 times of $AUC_{0-\infty}$ of ketamine<br>↑ 2.1 times of $C_{max}$ of ketamine<br>↑ 24% $t_{1/2}$ of ketamine<br>↓ 57% $AUC_{norket}/AUC_{ket}$ | Peltoniemi et al. (2012b) |
| Halogen | (*R,S*)-ketamine | CYP2B6 substrate | recombinant human proteins | ↓ systemic clerance | Wang et al. (2019b) |

desipramine, and mirtazapine) (Franconi et al., 2007; Schwartz, 2007; Waxman and Holloway, 2009).

In rodents, sex-based differences of CYP expression is common in laboratory animals, including mice and rats, were regulated by differences in growth hormone, thyroid hormone, sex hormones and other chemicals between female and male animals (Kato and Yamazoe, 1992; Waxman and Holloway, 2009; Zanger and Schwab, 2013). More than 1,000 genes whose expression is sex-dependent have been identified in mice and rats (Waxman and Holloway, 2009). In mice, hepatic expressions of Cyp3a16, Cyp3a41a/b, 3a44, and Cyp3a59 in females are

**Table 5**
Effect of CYP inducers on the pharmacokinetics of (*R,S*)-ketamine in humans.

| CYP inducers, dose, route | Isomers of ketamine, dose, route | Affected CYPs | Type and object of study, n | Consequences | References |
|---|---|---|---|---|---|
| Barbiturate, chronic | (*R,S*)-ketamine, 1.1–1.3 mg/kg/h, continuous infusion, at least 3 days | CYP3A4, CYP2C9, CYP2B6 | 27 patients for long-term analgosedation | ↓ steady-state plasma levels of ketamine | (Hijazi and Boulieu, 2002; Koppel et al., 1990) |
| Rifampicin, 600 mg once daily for 6 days, p.o. | (*S*)-ketamine, 0.1 mg/kg on day 6, i.v.; 0.3 mg/kg on day 6, p.o. | CYP3A4 | a 4-session paired cross-over study design, 12 healthy volunteers | ↓ 14% $AUC_{0-\infty}$ of IV (*S*)-ketamine<br>↓ 86% $AUC_{0-\infty}$ of oral (S)-ketamine<br>↓ 81% $C_{max}$ plasma of oral (S)-ketamine<br>↓ 66% $AUC_{norket}/AUC_{ket}$ after IV (*S*)-ketamine<br>↑ 147% $AUC_{norket}/AUC_{ket}$ after oral (*S*)-ketamine | (Peltoniemi et al., 2012a) |
| St John's wort, 300 mg twice daily for 14 days, p.o. | (*S*)-ketamine, 0.3 mg/kg on day 14, 1h after St John's wort, p.o. | CYP3A4 | a randomized cross-over study, two phases, 12 healthy subjects | ↓ 58% $AUC_{0-\infty}$ of (*S*)-ketamine<br>↓ 66%$C_{max}$ plasma of (*S*)-ketamine<br>↓ 18% $C_{max}$ plasma of norketamine<br>↓ 23% $AUC_{0-\infty}$ of norketamine | (Peltoniemi et al., 2012c) |

**Table 6**
Effect of CYP inhibitors and inducers on the pharmacokinetics of (*R,S*)-ketamine and its isomers in rodents.

| CYP inhibitors, dose, route | Isomers of ketamine, dose, route | Affected CYPs | Type and object of study, n | Consequences | References |
|---|---|---|---|---|---|
| Halothane | (*R,S*)-ketamine, 5, 10, 20 or 50 mg/kg, i.m. | Substrate of CYP 3A4, 2B6, 2A6, 2C9, 2E1 | 259 male Sprague-Dawley rats, 300–350 g | ↑ $t_{1/2}$ of ketamine | White et al. (1975) |
| Secobarbital, 2 mg | (*R,S*)-ketamine, 4 mg | CYP2B6 inhibitor | Isolated rat livers | ↑ $t_{1/2}$ of ketamine | Lo and Cumming (1975) |
| Diazepam, 0.15 mg | (*R,S*)-ketamine, 4 mg | Substrate of CYP2C19 and CYP3A4 | Isolated rat livers | ↑ $t_{1/2}$ of ketamine | Lo and Cumming (1975) |
| Cyclophosphamide, 100 mg/kg, i.p. | (*R,S*)-ketamine, 80 mg/kg | CYP2B6 substrate | Male BALB/c mice | ↑ $t_{1/2}$ of ketamine (↑ the duration of ketamine anesthesia) | Rojavin et al. (1996) |
| Ticlopidine+1-ABT | (*R*)-ketamine | 1-ABT: multiple CYP inhibitor<br>Ticlopidine: CYP2B6 inhibitor | Male C57BL/6 mice (naïve and LPS-model) | ↑ Cmax and ↑ $AUC_{0-3h}$ of ketamine.<br>↑ $AUC_{0-3h}$ but no change $C_{max}$ of norketamine.<br>The formation of (*2R,6R*)-HNK was blocked | Yamaguchi et al. (2018) |
| Phenobarbital, 70 mg/kg, i.p | (*R,S*)-ketamine | CYP2B6 inducer | Microsomes of male Sprague-Dawley rats | ↑ 4 times of ketamine metabolites | Woolf and Adams (1987) |

higher compared with males. By contrast, CYP3A4 expression in male rat liver, is shown to be 5–10 fold higher than that in female rat liver (Bogaards et al., 2000). Moreover, adult male mice displayed higher of CYP2C18 and CYP2C19 mRNA levels in the liver and kidney compared to female mice (Löfgren et al., 2009). However, female mice have higher hepatic expressions of Cyp2b9 (30%), 2b13 (30%), CYP2b10 (75%), CYP2c44 (35%), Cyp2c69 (8000%) than male mice (Renaud et al., 2011).

As indicated above, *(R,S)*-ketamine was metabolized by CYP3A4, CYP2C9, and CYP2B6 in the liver. With respect to (*S*)-ketamine, a sex-related difference in pharmacokinetic has been documented (Sigtermans et al., 2009). Accordingly, women have 20% higher elimination clearance of (*S*)-ketamine and (*S*)-norketamine than men, leading to higher plasma concentrations of both compounds in men compared with women. These differences may be related to sex differences in drug plasma protein binding, liver perfusion, and/or activity of CYP3A4 (Sigtermans et al., 2009).

### 7.2. Age-dependent CYP expression

In humans, age is one of the most important factors affecting drug metabolism capacity, especially at the extremes of life, where drug metabolism capacity seems to be significantly lowered. In infants, poor drug metabolism is caused by immaturity of several enzyme systems including CYP450 (Stevens, 2006; Stevens et al., 2008; Zanger and Schwab, 2013). The ability to clear drugs is significantly decreased in the elderly population, especially for drugs with a narrow therapeutic range, such as antipsychotics, antidepressants, anticoagulants, and beta-blockers (Zanger and Schwab, 2013).

Investigations on the influence of aging on CYP activity in humans have shown conflicting results (Wauthier et al., 2007). The impact of age on enzyme expression in humans has been observed in several CYPs, such as CYP2C19, CYP2D6 and CYP3A4 (Ishizawa et al., 2005; Stevens, 2006; Stevens et al., 2008; Wauthier et al., 2007). Indeed, it was observed that a significant reduction of 32% in total CYP450 content of liver biopsy samples and a decline of 29% of the antipyrine clearance in elderly people (>70 years old) compared to young people (Sotaniemi et al., 1997). In another study, total cytochrome P450, CYP2E1 and CYP3A contents decreased with increasing age, while some isoforms (CYP1A2 and CYP2C) unchanged (George et al., 1995). According to Yang et al. (2010), there were positive correlations between age and CYP activities, especially CYP2C9, and weak interaction between impact of age and sex on CYP1A2, CYP2A6, CYP2B6, CYP2C8, and CYP3A4 (Yang et al., 2010). By contrast, some studies have not observed the influence of age on CYP450 activity (Gorski et al., 2003; Parkinson et al., 2004; Shimada et al., 1994; Simon et al., 2001).

In rodents, several studies have also identified an age-related decrease in the clearance of drugs undergoing biotransformation by hepatic microsomal monooxygenases (Belpaire et al., 1990; Hammerlein et al., 1998; Watkins et al., 1989; Wauthier et al., 2007). In rats, significant reductions in Cyp3a2 and Cyp2c11 protein levels with age have been indicated (Mori et al., 2007). Similarly, Cyp2e1 activity is reduced by 37%; CYP2c11 and CYP3a2 protein levels are declined by 85% and 65%, respectively in senescent rats (Wauthier et al., 2007). A decrease in drug metabolism has also been reported. Indeed, it has been indicated that the *in vitro* metabolism of phenacetin, chlorzoxazone, triazolam, and midazolam in senescent rats decreased by 60%, 37%, 50%, and 55%, respectively (Dhir and Shapiro, 2003; Wauthier et al., 2004, 2006a, 2006b; Zanger and Schwab, 2013).

## 8. Conclusion

Currently approved antidepressant drugs take weeks to exert their full therapeutic benefits. Over the past decade, preclinical and clinical *(R,S)*-ketamine findings have generated a lot of excitement about the promise of a rapid and sustained antidepressant drug treatment in TRD. The targets by which *(R,S)*-ketamine produce glutamate bursts that trigger the fast (at 30 min) or sustained (at t24h post-dose) antidepressant-like activity must also be questioned (Fuchikami et al., 2015). By modulating the plasticity of cortico-mesolimbic synapses, *(R,S)*-ketamine can alleviate the symptoms of depression, but also induces adverse effects such as psychotic-like episodes in a dose-dependent manner (Muller et al., 2016). To date, psychotomimetic symptoms associated with its therapeutic effects limit (*R,S*)-ketamine prescription in depressed patients.

Several cytochrome enzymes have been found to be involved in (*R,S*)-ketamine metabolism in the digestive tract, the liver and the brain, but there is no strong evidence of individual polymorphisms manifesting in clinical outcomes (Saba et al., 2017).

Indeed, *(R,S)*-ketamine metabolites were originally thought to be inactive. The study of its mechanism of action is complicated by the fact that *(R,S)*-ketamine is a racemic miIre of (*R*)- and (*S*)-ketamine. Preclinical studies demonstrated differences in affinity for NMDA-R *in vitro* and in the potency/efficiency of the antidepressant effect between these two isomers. In addition, norketamine and HNK metabolites would have a potent antidepressant-like activity (Sałat et al., 2015; Zanos et al., 2016).

These last years, most preclinical studies have focused on (2*R,*6*R;*2*S,*6*S*)–HNK, which is the major HNK metabolite in the brain, but its molecular targets have not been clearly identified. Questions still remain about the cellular pathway underlying its antidepressant activity in a brain region-specific manner. Hypotheses regarding the role of ketamine's metabolites are as follows: first, it is thus possible that the combination of the two molecules, *(R,S)*-ketamine (as NMDA-R antagonist) and (2*R,*6*R*)-HNK (by increasing AMPA receptor signaling), produces antidepressant effects and AMPA-R agonist, respectively). Thus, this metabolite could contribute to antidepressant effects. Second, (2*R,*6*R*)-HNK could be seen as an antidepressant drug independently from its parent drug. It could have less adverse effects such as psychotomimetic-like behaviors and abuse potential as suggested in animal tests (Chaki and Yamaguchi, 2018; Zanos et al., 2018). The leading hypothesis is not yet determined. Thus, the possible use of this metabolite in the treatment of depression needs further preclinical and clinical investigations.

Experimental protocols using a pre-treatment with a CYP enzyme inducer/inhibitor may help to resolve the issue regarding the contribution of (2*R,*6*R*)-HNK to *(R,S)*-ketamine's antidepressant-like activity. Studying *(R,S)*-ketamine metabolism and CYP expression will aid to determine *in vivo* effects of these compounds, such as on the balance between glutamate/GABA neurotransmission associated with their antidepressant-like activity in animal models of anxiety or depression. Characterize the role of liver and brain CYP enzymes in *(R,S)*-ketamine metabolism may help to confirm the antidepressant potential of its metabolites in TRD. The role of these metabolites in *(R,S)*-ketamine efficacy also depends on which route of administration is used.

Overall, this review emphasizes pharmacodynamic and pharmacokinetic properties of *(R,S)*-ketamine. Its metabolism is greatly influenced by CYP enzyme expression in various biological tissues (intestine, liver, brain), the route of drug administration, the gender, and the age of rodents and patients. We urge clinicians to be informed about possible drug-drug interactions during a concomitant administration of CYP inhibitors or inducers and *(R,S)*-ketamine and esketamine that could limit or enhance, respectively, their therapeutic antidepressant efficacy in patients.

Thus, information collected in preclinical studies must be translated to the clinic. However, we must remember that differences in CYP-dependent metabolism between rodents and humans have been described (Hart et al., 2009). Thus, a translational approach, from bench to bedside, is another challenge for future studies dedicated to the research on fast antidepressant drugs acting on the brain glutamate/GABA neurotransmission. These steps are critical for developing more efficient antidepressant drugs to address a major public health concern.

## Declaration of competing interest

None for this work.

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