Evaluation of antidepressant-related assays sensitive to clinical relevant doses of R-/S-ketamine.

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Introduction

The non-competitive NMDA antagonist R-/S-ketamine has been shown to elicit a rapid and long-lasting antidepressant effects in treatment resistant depressed subjects and in preclinical models. However, discrepancies have been observed between the plasmatic concentration of clinically effective infusion and the doses routinely used in rodent limiting the translatability between these species. Indeed, both Zarate et al. (2012) and Zhao et al. (2012) estimated a maximal plasmatic concentration (Cmax) of 200 ng/ml after iv infusion of the sub-anesthetic dose of 0.5 mg/kg for 40 min in human whereas we estimate a Cmax of 2000-10000 ng/ml after injection of the routinely used 10-30 mg/kg in rodents. This observation raise the possibility that the antidepressant-like effects of R-/S-ketamine observed in preclinical models could be mediated by off-target effects such as engagement of opioids, dopamine or nicotinic receptors. In the present study, we attempt to evaluate the sensitivity of different animal models/behavioral paradigms to detect antidepressant-like effects of clinical relevant doses of R-/S-ketamine such as tail suspension, forced swim test and novelty suppressed feeding test in mice and rats. Both rapid and long-lasting effects of R-/S-ketamine were investigated testing the animals 30 min as well as 24h after drugs administration.

Rapid and long-lasting Antidepressant-like effects of R-/S-ketamine in the tail-suspension test (TST) assay.

Protocol: Male CD-1 (20-25 g the day of the test) were tested three times in the TST. During the first test (Pretest), animals were injected with saline and exposed the tail suspension in order to ensure that the animals were assigned into balanced groups based on equivalent immobility in the test. 24h after, the mice were injected either with saline, R-/S-ketamine (3, 10, 30mg/kg; s.c) or imipramine prior to a 6-min tail suspension test. Twenty-four hours later, mice were injected with saline, 30 min prior the tail-suspension session. An automated TST device (Med Associates, St Albans, VT) was used to measure the duration of behavioral immobility (Crowley et al., 2004).

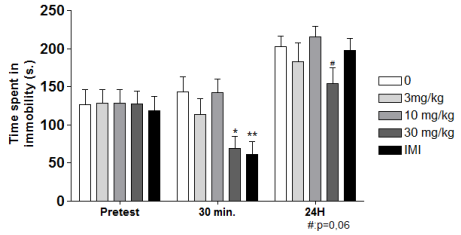

Figure 1. R-/S-ketamine at 30 mg/kg decreased significantly the time spent in immobility in the tail suspension 30min after administration. Imipramine at 30 mg/kg induced similar antidepressant effects in the model. 24h post administration, R-/S-ketamine at 30 mg/kg but not imipramine decreased the time spent in immobility. N=13-14/groups. * p<0.05;** p<0.01 vs saline.

- In the TST, Koike et al. (2011) observed antidepressant effect of R-/S-ketamine 30 min, 24 and 72h post injection at 30 mg/kg (i.p.) in CD-1 strain. Here, R-/S-ketamine **exhibits antidepressant-like effect in the tail-suspension at 30 mg/kg, s.c., 30 min and 24h post administration in CD-1 strain.**

Anxiolytic effects of R-/S-ketamine in novelty suppressed feeding (NSF) test.

Protocol: The NSF test was carried out during a 5-min period as described previously. In brief, the testing apparatus consisted of a plastic box (50 × 50 × 20 cm), the floor of which was covered with approximately 2 cm of wooden bedding. Twenty-four hours before behavioral testing, all the food was removed from the home cage. At the time of testing, a single pellet of food (regular chow) was placed on a white paper platform positioned in the center of the box. C57bl6j were placed in a corner of the box, and a stopwatch was immediately started. The latency to eat (defined as the mouse sitting on its haunches and biting the pellet with the use of forepaws) was timed. R-/S-ketamine or chlordiazepoxyde were injected 30 min prior the test.

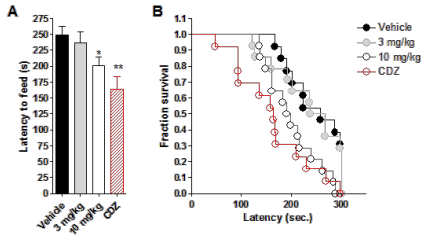

Figure 2. (A) Both R-/S-ketamine (10 mg/kg) and chlordiazepoxide (2, 5 mg/kg) decreased the latency to feed. **(B)** Kaplan–Meier curves for the animals receiving R-/S-ketamine and chlordiazepoxide: and cumulative survival percentage of animals that have not eaten). N=13-14 * p<0.05;** p<0.01 vs saline.

- In the NSF, Koike et al. (2013) observed Anxiolytic effects of R-/S-ketamine 30 min, 24 H at 30 mg/kg (i.p.) in C57/Bl6j strain. **Here, R-/S-ketamine decreased significantly the latency to eat at 10 mg/kg (s.c.).**

Pharmacokinetic and selectivity of R-/S-ketamine

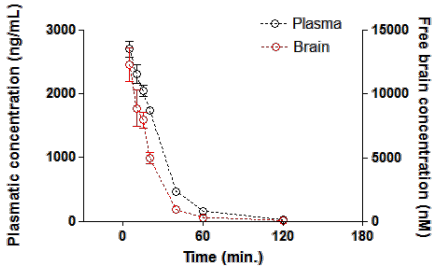

Figure 3. Time profile of plasma and brain concentration of R-/S-ketamine (10mg/kg, s.c.) after administration in C57BL6j. Data are presented as means ± SEM.

- In humans, studies estimated the plasmatic concentration around 200 ng/ml (Cmax) after the clinically effective infusion of R-/S-ketamine (0.5 mg/kg). In C57Bl6j, we observed a Cmax of **2700 ng/ml in the plasma and brain concentration 12312 nM after an injection of 10 mg/kg of R-/S-ketamine (s.c.).**

Binding Assay	% control (10uM)	IC50 (nM)	KI (nM)	nHill
PCP	91	630	580	1.1
M4 (h)	50	59000	26000	1.2
mu (h) (MOP) (agonist site)	42	21000	7538	1
kappa (KOP)	31	36000	24000	1
NK1 (h)	28			
M1 (h)	27			
CRF1 (h)	25			
5-HT transporter (h)	24			
5-HT2B (h) (agonist site/DOI)	24			
Ca2+ channel (L- diltiazem site) (benzothiazepines)	23			
sigma (non-selective)	21			
DA transporter (h)	20			
GABAB	20			
beta 2 (h)	19			
5-HT2A (h) (agonist site)	18			

Figure 4. CEREP selectivity screen undertaken to determine the general pharmacological activity of R-/S-ketamine..

- R-/S-ketamine exhibits some activities at Mu and Kappa opioid receptors suggesting that "antidepressant-like" effects using the preclinical of 10 and 30 mg/kg might be mediated by off-target engagement.

Rapid antidepressant-like effects of R-/S-ketamine in wistar-kyoto (WKY)

Protocol: Wistar Kyoto rats have been suggest as genetic model of depression: Insensitive to acute SSRI in the FST (López-Rubalcava et al 2000), Altered HPA axis, High Anxiety level. Males wistar-kyoto rats were injected 25 minutes prior exposure to locomotor apparatus then tested in a single session of FST (5 min). The time spent in immobility was measured by Two different blind observers.

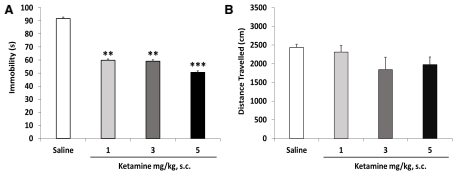

Figure 5. R-/S-ketamine at 1, 3 and 5 mg/kg elicit a rapid antidepressant like effect in the FST **(A)** without affecting locomotor activity **(B)** in Wistar Kyoto rats. N=10/groups in FST and 4/groups in LMA. * p<0.01;*** p<0.001 vs saline.

- **Thirty minutes after administration, R-/S-ketamine elicited a robust antidepressant-like** at 1, 3 and 5 mg/kg without influencing locomotor activity.

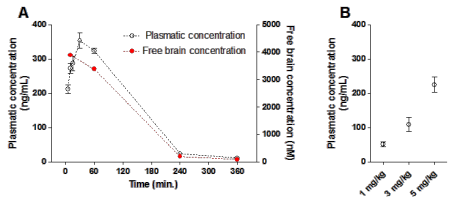

Figure 6. (A) Time profile of plasma and brain concentration of R-/S-ketamine (5mg/kg, sc) after R-/S-ketamine administration in Wistar Kyoto rats. **(B)** Plasma concentration 36 minutes R-/S-ketamine administration in Wistar Kyoto rats exposed to Forced Swim Test.

- Maximum plasma and brain concentrations of R-/S-ketamine (Figure 1A) were achieved 30 min (353 ng/ml) after s.c. administration. Thirty minutes after administration, plasmatic concentration were **51 ng/ml, 108 ng/ml and 225 ng/ml** (1 mg/kg, 3 mg/kg and 5 mg/kg, respectively).

Long-lasting antidepressant of R-/S-ketamine in wistar-kyoto rats.

Protocol: Males Wistar Kyoto rats were injected 24 hours prior exposure to locomotor apparatus then tested in a single session of FST (5 min) The time spent in immobility was measured by Two different blind observers.

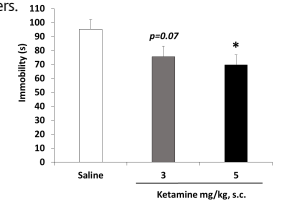

Figure 7. R-/S-ketamine at 1, 3 elicit a rapid antidepressant like effect in the FST 24h after administration in wistar-kyoto rats. N=9/groups in FST and p<0.05 vs saline.

- Twenty-four hours after administration, R-/S-ketamine elicited an antidepressant-like at 3 mg/kg.

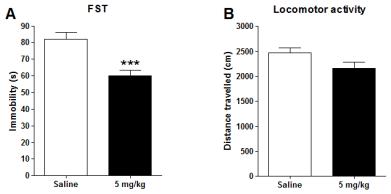

Figure 8. R-/S-ketamine at 5 mg/kg elicited a robust long-lasting antidepressant like effect in the FST (A) without affecting locomotor activity in Wistar kyoto rats(B). N=20/groups in FST and 8/groups in LMA. * *** p<0.001 vs saline.

- Twenty-four hours after administration, R-/S-ketamine elicited a Robust antidepressant-like effect at 5 mg/kg. Moreover, increasing the number of animals/group to 20 increased significantly the power (Power with alpha= 0.050: 0.978)

Conclusions

We identify the Wistar Kyoto rats as one of the most optimal model since we observed both fast and prolonged effect of ketamine at clinical relevant doses. Consequently, we will consider this model as the most appropriate entry-point in order to assess antidepressant properties of novel agents acting on NMDA.

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