P.783 PHARMACODYNAMICS OF S-KETAMINE AND KETAMINE RACEMATE IN RAT PREFRONTAL CORTEX AND HIPPOCAPMUS AFTER DRY POWDER INHALATION, INTRATRACHEAL AND INTRAVENOUS ADINISMTRATION

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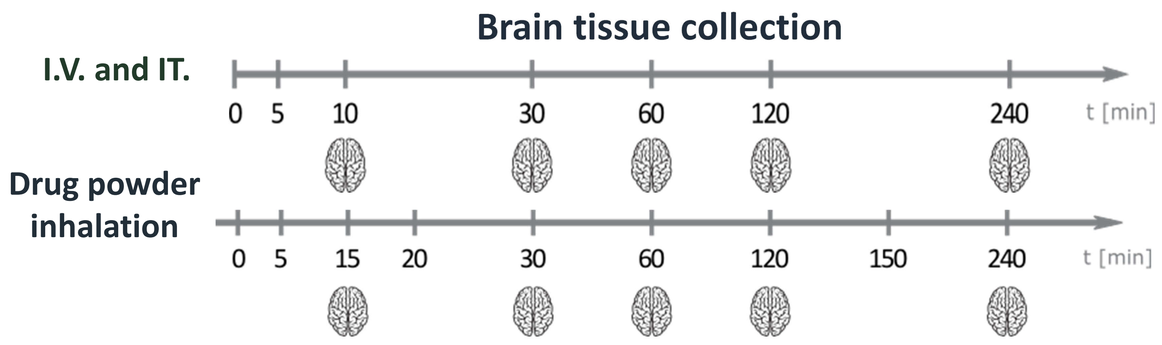
INTRODUCTION

The antidepressant mechanisms of ketamine (NMDA receptor antagonist) are still controversial, not completely understood and appear to be driven by multiple processes [1]. It was demonstrated that a single, subanesthetic doses of ketamine produces a rapid (within hours) antidepressant response that may last for up to 15 days [2]. There are several publications showing pharmacodynamic and antidepressive effects of ketamine in rodents involving mTOR signaling pathway, inhibiton of eEF2kinase and de-suppression of BDNF leading to a rapid increase in synaptic protein synthesis [3],[4],[5]. Therefore, this study was performed to determine, compare and extend published evidence of pharmacodynamic effect, for **S-ketamine** and **ketamine racemate**, in the rat prefrontal cortex and hippocampus after different routes of administration **including dry powder inhalation that may provide a novel solution for ketamine delivery.**

MATERIALS AND METHODS

Animals

The studies were performed using male Wistar rats (n=3) treated with either vehicle, S-ketamine (10 mg/kg) or ketamine racemate (20 mg/kg) that were administered either via intravenous (I.V.), intratracheal (I.T.) or dry powder inhalation (INH.) route. Upon completion of blood sampling, the animals were euthanized (isoflurane followed by exsanguination) at the different time points and the brain tissue was collected:



PK study

Brain homogenate samples were extracted with 150ul of acetonitrile spiked with internal standard (Ketamine-d4). Extracts were analyzed by a LC-MS/MS method.

Preparation of synaptic protein extraction

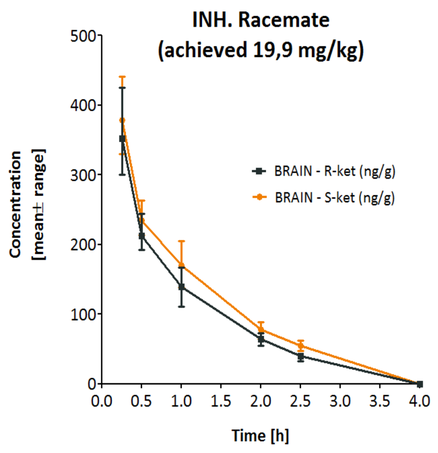
Synaptoneurosomes were purified from rat prefrontal cortex (PFC) and hippocampus (HP) with Syn-PER Reagent (Thermo Fisher Scientific) acc. to manufacturer's protocol.

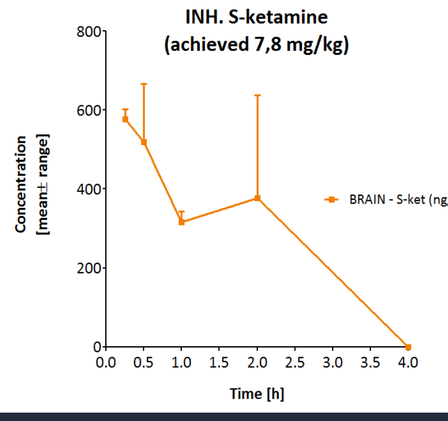
Western Blotting and Elisa methods

Synaptoneurosome suspensions were analyzed by Western Blotting analysis using antibodies (Cell Signaling, Merck) against: phospho-Akt (Ser473), phospho-ERK 1/2 (Thr202/Tyr204), phospho-4E-BP1 (Thr37/46), phospho-P70S6K (Ser235/236), phospho-eEF2 (Thr56), phospho-mTOR (Ser2448), pGSK3β (Ser9), with corresponding total proteins and synaptic protein expression (PDE-95, Synapsin-1, ARC,Glur). Stain-Free technology (BioRad) was exploited as normalization prodedure. Every sample was analyzed in two independent runs. pAKT (RayBio) and the p-mTOR ELISA assays (Abcam) were performed according to the manufacturer's protocol.

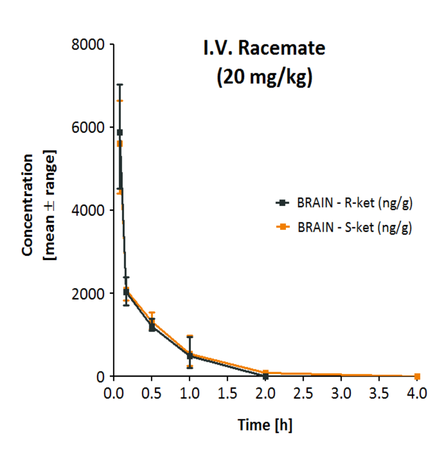
PHARMACOKINETICS

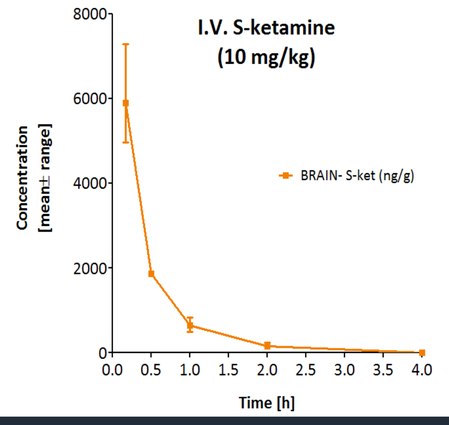
DRUG POWDER INHALATION



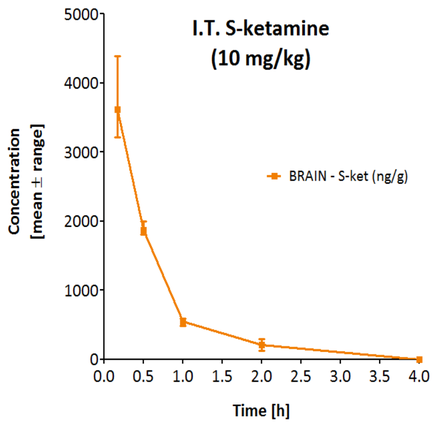


INTRAVENOUS





INTRATRACHEAL

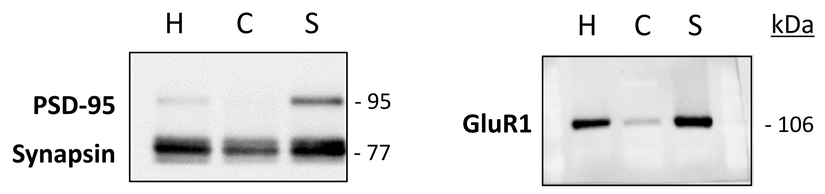


SUMMARY OF BIOAVAIBILITY OF S-KETAMINE

Administration	Cmax (ng/ml)	AUC (ng*h/ml)	F
INH. Ketamine Rac. (19,9 mg/kg)	1578	1383	~85%
INH. S-ketamine (7,8 mg/kg)	828	867	~85%
IT. S-ketamine (10mg/kg)	5750	1259	~96%

PHARMACODYNAMICS

ENRICHMENT OF SYNAPTIC PROTEIN

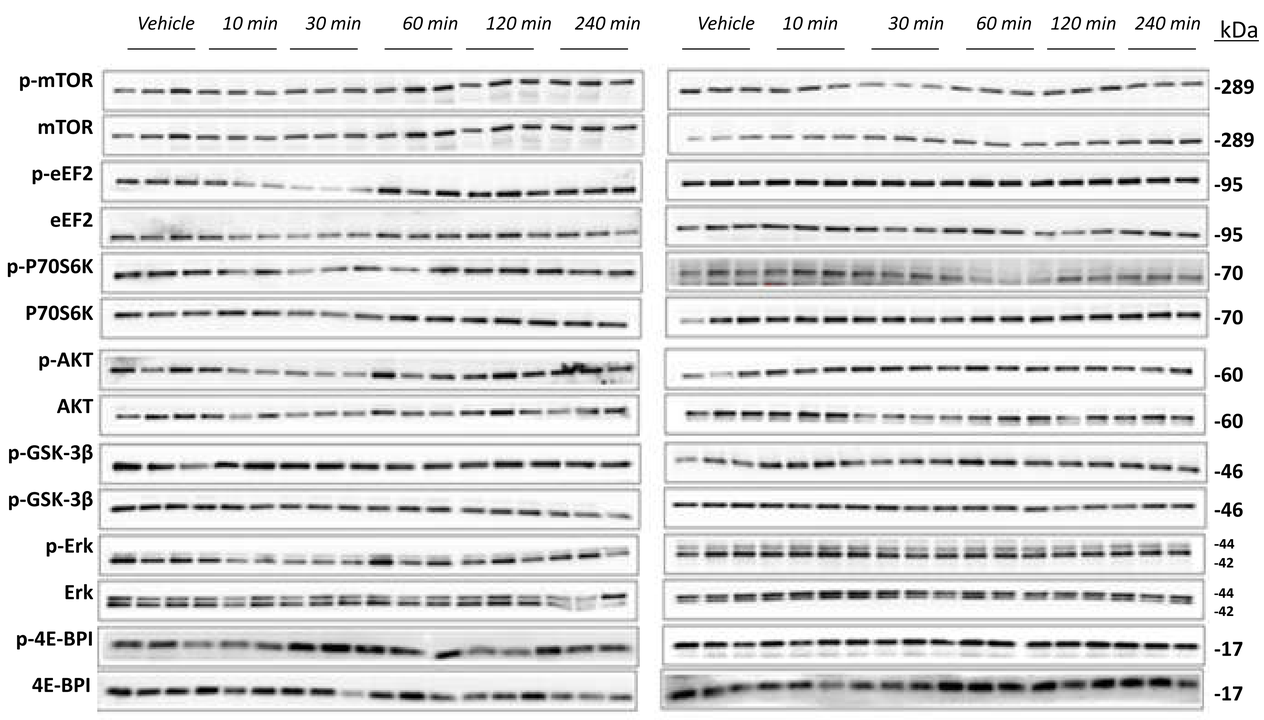


Total protein from rat brain tissue in **homogenate (H) and cytosol (C) fraction**, and **synaptosome suspension (S**) was analyzed by WB. The pre- and post-synaptic protein markers including synapsin, post-synaptic density protein 95 (PSD95) and AMPA receptors (GluR1) were evaluated as purity controls after synaptoneurosomes preparation.

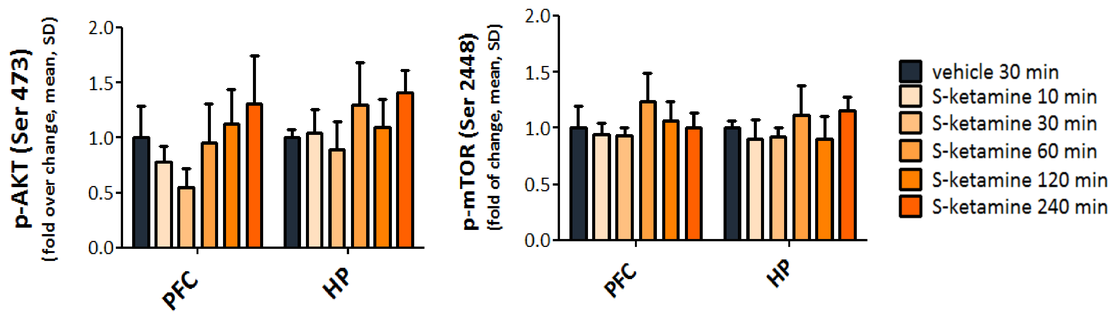
EFFECT OF S-KETAMINE ON INTRACELLULAR SIGNALING PATHWAYS

WB

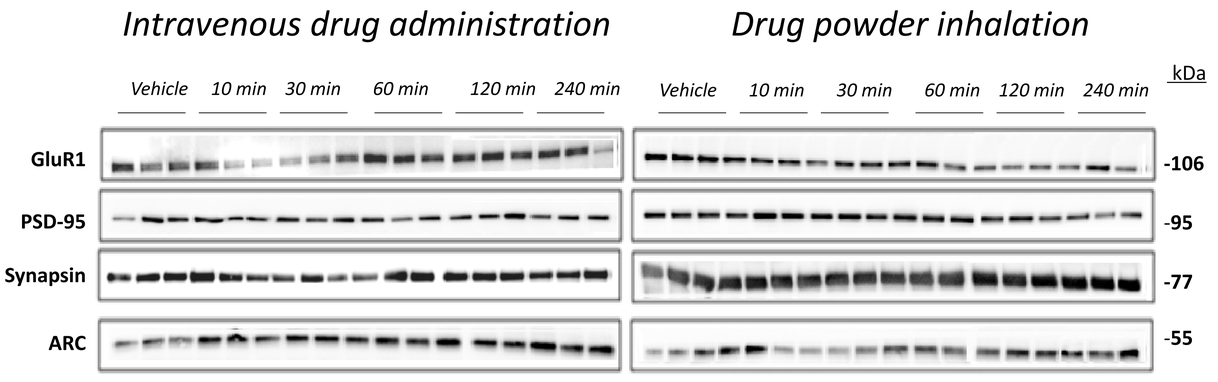
Protein phosphorylation in synaptoneurosomes of PFC



Phosphorylation of mTOR and AKT in synaptoneurosomes of PFC and HP - *ELISA*



Synaptic proteins



CONSLUSION

The present study evaluated pharmacodynamic effects of S-ketamine and ketamine racemate via i.v., i.t. or dry powder inhalation administration route. Though **ketamine was high bioavailable (85% for inhalation and 96% for i.t. route) and highly abundant in the brain tissue, no changes in phosphorylation of mTOR, 4E-BP1, p70S6K, AKT, ERK 1/2, eEF2, GSK3β and no changes in expression of Synapsin 1, PSD95, GluR1 and Arc in PFC and HP at any studied time point for both S-Ketamine and ketamine racemate were observed.**

REFERENCES

[1] Zanos, P., Gould, T.D.,2018. Mechanisms of ketamine action as an antidepressant. Mol Psychiatry 23(4), 801-811.
[2] Zarate, C. A., Singh, J. B., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A., et al., 2006. A randomized trial of an N-methyl-D-aspartate antagonist in treatment resistant major depression. Archives of general psychiatry, 63(8), 856-864.
[3] Li, N., Lee, B., Liu, R.J., Banasr, M., Dwyer, J.M et al., 2010. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science, 329 (5994).
[4] Autry, A.E., Adachi, M., Nosyreva, E., Na, E.S., Los, M.F, et al., 2011. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature, 475(7354), 91-95.
[5] Zanos, P., Moaddel, R., Morris, P.J., Georgiou, P., Fischell, J., Elmer, G.I, et al., 2016. NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature, 533(7604), 481-6.