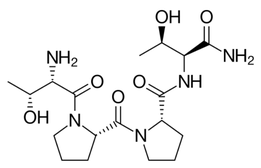
Rapastinel antidepressant-like activity is independent of increased efflux of dopamine, 5-HT and glutamate as observed for S-(+)-ketamine in the rat mPFC

J Kehr[1,2]*, T Yoshitake[2] , F-H Wang[1], S Yoshitake[1], S Schmidt[1], P Banerjee[3], J Donello[3]

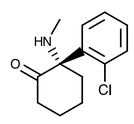
[1]Pronexus Analytical AB, Gringstuvägen 44, 167 33 Bromma, Sweden
[2]Department of Physiology and Pharmacology, Karolinska Institutet, Nanna Svartz väg 2, 171 77 Stockholm, Sweden
[3]Allergan, Inc., Jersey City, New Jersey, USA

Introduction

Rapastinel (Glyx-13) is a novel NMDA receptor modulator with glycine-like partial agonist properties. Rapastinel is in late-stage clinical development as an adjunct therapy for major depressive disorder. Ketamine, a noncompetitive NMDA receptor channel blocker, is known to produce rapid and sustained antidepressant effect in treatment-resistant patients with major depression. However, ketamine, a schedule II controlled substance, is abused frequently and is known to produce addictive and psychotomimetic effects in animals and humans. Both ketamine and rapastinel produce antidepressant-like effects in rodent models of depression but rapastinel does not exhibit ketamine-like CNS adverse effects. For example, rapastinel does not produce ketamine-like sedation and rewarding effects or disrupt sensorimotor gating in rodents (Burgdorf et al, 2013). In the present study, we evaluated acute effects of rapastinel and S-ketamine on extracellular levels of dopamine (DA), 5-HT, glutamate Glu, glycine (Gly) and GABA in rat medial prefrontal cortex (mPFC) using intracerebral microdialysis. The onset and the duration of antidepressant-like effects of rapastinel and ketamine were evaluated in the rat forced swim test (FST).



Rapastinel (Glyx-13)



S-ketamine

Conclusions

Rapastinel produced rapid and long-lasting antidepressant-like effect in the rodent FST model. Rapastinel administered at the doses which were effective in the FST had no influence upon the basal extracellular levels of DA, 5-HT, Glu, GABA and Gly in the rat mPFC. In contrast, the rapid increases in brain DA, 5-HT and Glu levels induced by S-ketamine observed in this study and reported elsewhere for ketamine may play a significant role in ketamine high abuse potential and psychotomimetic-like effects. These data demonstrate a distinct difference in the neurochemical basis for rapastinel's antidepressant-like activity and favourable CNS adverse effects compared to ketamine.

Materials and Methods

Drugs

Rapastinel (Glyx-13) was kindly provided by Allergan(USA), S-(+)-ketamine HCl and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.).

Experimental procedures

The microdialysis and FST experiments were carried out on awake male Sprague Dawley rats following the microdialysis protocol described elsewhere (Kehr, 1999; Kehr et al., 2001) and in separate cohorts of rats, the FST protocol as described by Porsolt et al. (1979) with some minor modifications.

The stereotaxic surgery was performed under aseptic conditions under isoflurane anaesthesia. Briefly, the guide cannulae were surgically implanted into the mPFC using the standard stereotaxic coordinates: AP +3.2 mm, L +0.5 mm, V -0.5 mm; from bregma and the dural surface, according to the stereotaxic atlas of Paxinos and Watson, 2007. Following 5-7 days of recovery, the microdialysis probe (Eicom A-I: 0.22 mm o.d., 3 mm membrane length with cut-off 50 kDa) was inserted into the guide cannula of the awake rat the probe was perfused at a constant flow-rate of 1 µl/min with aCSF solution (148 mM NaCl, 4 mM KCl, 0.8 mM $MgCl_2$, 1.4 $CaCl_2$, 1.2 mM Na_2HPO_4, 0.3 mM NaH_2PO_4, pH 7.2). After the 2-3 h stabilization period, the microdialysis samples were collected in 20-min intervals. The first three samples were used for estimation of basal levels of DA, 5-HT, Glu, GABA and Gly. Thereafter, rapastinel (3, 10 or 30 mg/kg s.c) or S-ketamine (20 mg/kg i.p.) or vehicle were injected s.c. to separate groups of rats and the fractions were collected for additional 240 min. After finalizing the experiment, the animals were sacrificed by overdose of isoflurane and dislocation of the neck. The brains were removed and examined for correct placement of the probe (the probe track) in the rat brain.

All experiments were performed in accordance with general recommendations of Swedish animal protection legislation following the directives of the 'Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Council of the European Communities (86/809/EEC).

The concentrations of DA, 5-HT, Glu, GABA and Gly, as well as the concentrations of rapastinel in the dialysates were measured by using HPLC and LC-MS/MS methods.

Results

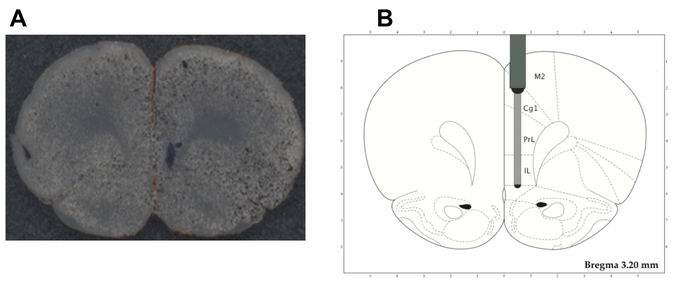


Fig. 1.
Representative photomicrograph of a dialysis probe placed in the mPFC of a rat (panel A); panel B shows a schematic illustration of a dialysis probe in the mPFC area (Cg1- cingulate cortex; PrL - perilimbic cortex ; IL - infralimbic cortex, adapted from Paxinos and Watson, 1997).

References

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Results

The antidepressant-like effects of single dose of Rapastinel last longer than those of S-ketamine

Both rapastinel (3-30 mg/kg) and ketamine (3-30 mg/kg) decreased immobility time in the rat FST when tested at 1-hr post-dose. This antidepressant-like effect of both drugs persisted at day 2 and at day 7. In the dialysis assay, ketamine significantly elevated extracellular levels of dopamine and glutamate in the mPFC within the first 60 min of dosing. By contrast, rapastinel did not increase dopamine or glutamate levels in mPFC at any of the doses tested.

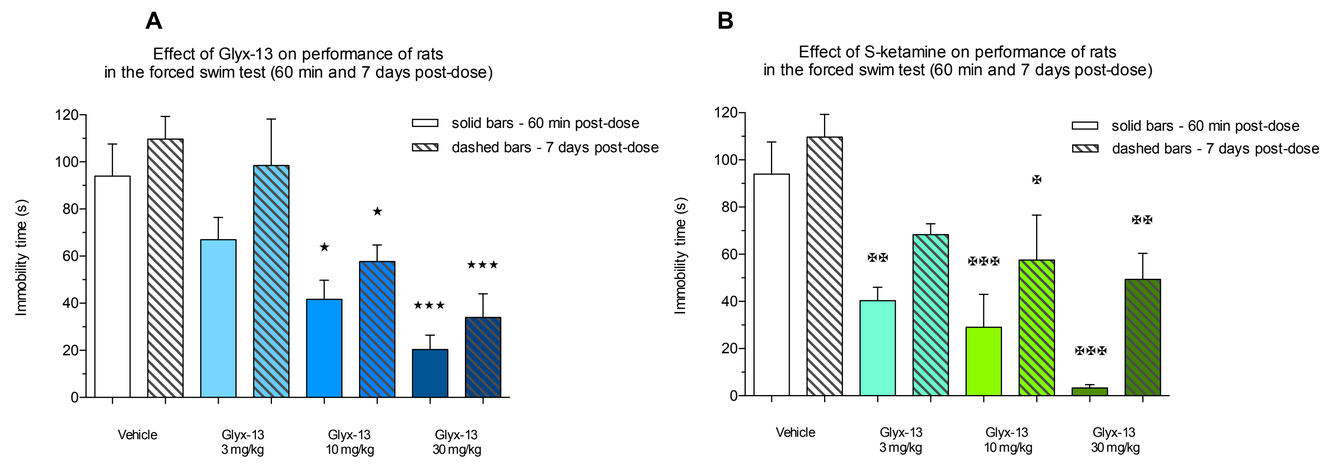

Fig. 2.
Both **A**) rapastinel (Glyx-13) and **B**) S-ketamine dose-dependently produced sustained antidepressant-like effects in the rat FST model after a single dose; the antidepressant effects of rapastinel and S-ketamine were measured at 60 min and 7 days post-dose. Rapastinel: ★★★ P < 0.001; ★ P < 0.05; S-ketamine: ✠ ✠ ✠ P < 0.001; ✠ ✠ P < 0.01; ✠ P < 0.05, mean ± SEM, n = 6.

Rapastinel is present in the brain microdialysates, the Cmax levels are observed already in 20-40 min samples

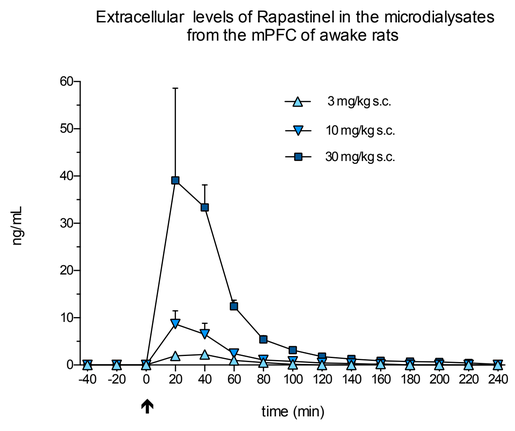

Fig. 3.
Kinetics of rapastinel in the extracellular space (ECS) of rat medial prefrontal cortex (mPFC) after a single SC dose of rapastinel (3 or 10 or 30 mg/kg). ECS levels of rapastinel increased within 20 min postdose and dissipated rapidly (within 60-80 min postdose). Note: the antidepressant effects of rapastinel was measured at 60 min and 7 days postdose. The ECS-Cmax of rapastinel at 10 or 30 mg/kg is ~10 ng/mL (25 nM) or ~40 ng/mL (98 nM); in the calcium imaging studies, NMDAR activation by rapastinel is observed at 30-300 nM, suggesting that the antidepressant effect of rapastinel is mediated by NMDAR activation. Rapastinel in the dialysates was measured using a HPLC-MS/MS method.

Rapastinel does not increase the levels of DA, 5-HT and Glu in the rat mPFC, in contrast to S-ketamine

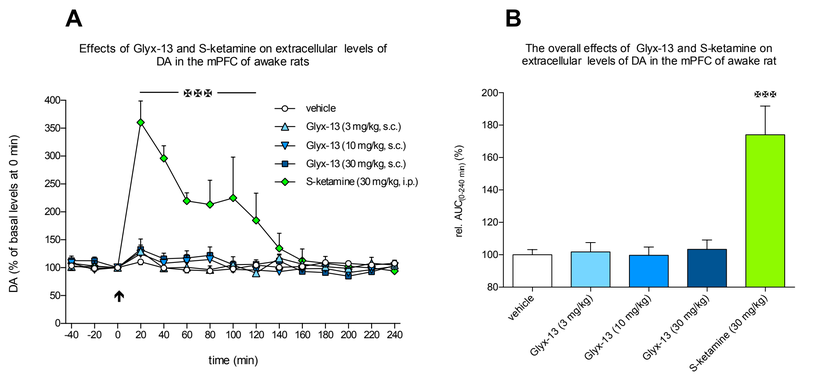

Fig. 4.
S-ketamine (30 mg/kg) significantly increased DA levels in rat mPFC, evaluated as **A**) percentage increase over baseline and **B**) relative $AUC_{(0\text{-}240\ min)}$; ✠ ✠ ✠ P < 0.001.
Rapastinel (3, 10 or 30 mg/kg) did not increase the DA levels in mPFC.

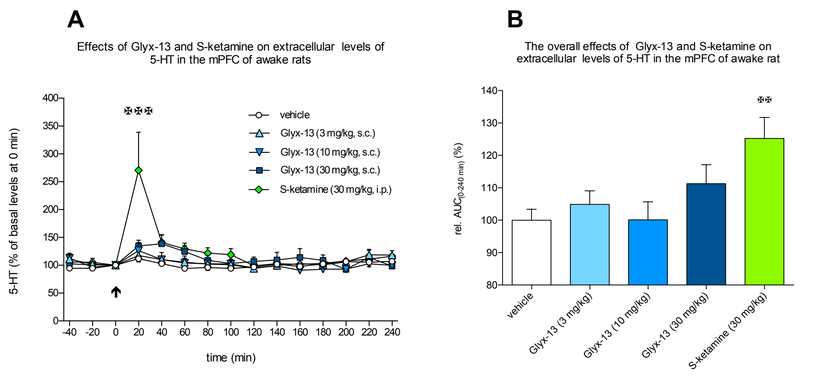

Fig. 5.
S-ketamine (30 mg/kg) significantly increased 5-HT levels in rat mPFC, evaluated as **A**) percentage increase over baseline and **B**) relative $AUC_{(0\text{-}240\ min)}$; ✠ ✠ ✠ P < 0.001; ✠ ✠ P < 0.01.
Rapastinel (3, 10 or 30 mg/kg) had no effect on the 5-HT levels in mPFC.

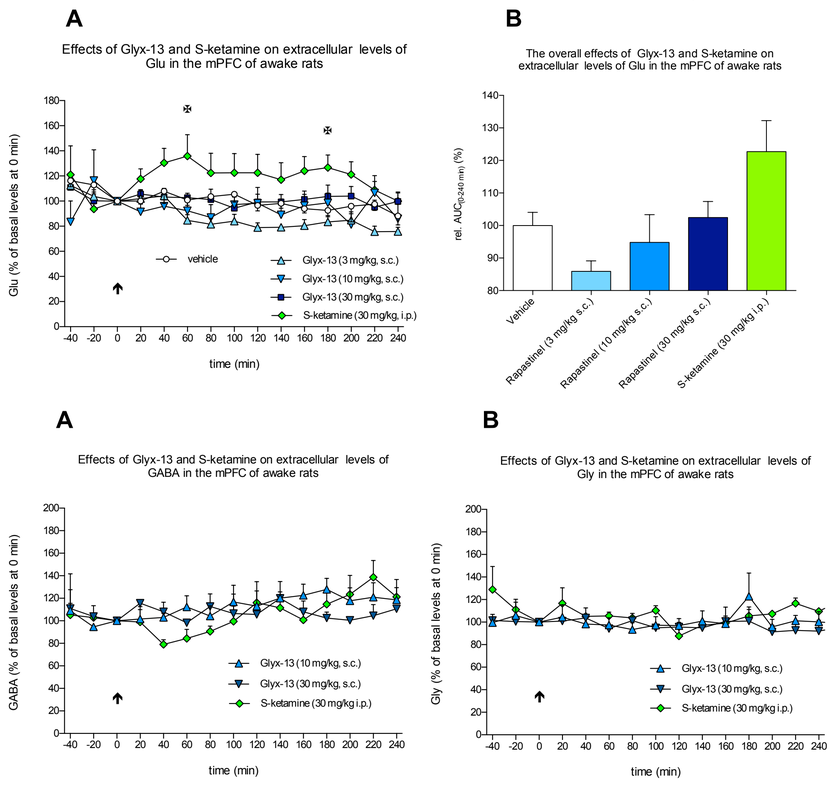

Fig. 6.
S-ketamine (30 mg/kg) significantly increased Glu levels in rat mPFC, evaluated as **A**) percentage increase over baseline; ✠ P < 0.05, but showed only a trend when calculated as **B**) relative $AUC_{(0\text{-}240\ min)}$. Rapastinel (3, 10 or 30 mg/kg) had no effect on the Glu levels in mPFC.

Fig. 7.
Neither S-ketamine (30 mg/kg i.p.) nor rapastinel (10 or 30 mg/kg s.c.) altered the extracellular levels of **A**) GABA and **B**) Gly in the rat mPFC.

Disclosure

This study was funded by Allergan (Irvine, CA). All authors met ICMJE authorship criteria. Neither honoraria nor payments were made for authorship. Financial arrangements of the authors with companies whose products may be related to the present report are listed below, as declared by the authors. P. Banerjee and J. Donello are full-time employees of Allergan. T. Yoshitake is a visiting scientist at Karolinska Institutet, J. Kehr, F-H. Wang, S. Yoshitake and S. Schmidt are full-time employees of Pronexus Analytical AB.