KETAMINE INDUCED STRUCTURAL PLASTICITY IN HUMAN IPSC-DERIVED DOPAMINERGIC NEURONS

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Ketamine is a non competitive NMDA receptor antagonist and dissociative anaesthetic that is also used as a "street drug", being addictive. Recently, interest was rekindled in ketamine with reports of sustained antidepressant effects in preclinical and clinical studies (Iadarola et al. 2015). While its mechanism of action is only partially understood, ketamine induced mTOR-dependent structural plasticity in neurons of prefrontal cortex at doses displaying antidepressant effects (Li et al. 2010). Since NMDA receptors control dopamine (DA) transmission, and ketamine increases DA release in telencephalic areas, structural plasticity in DA neurons may be implicated in the antidepressant and other effects of ketamine.

In previous works, we showed that addictive substances that induce DA release such as amphetamine, cocaine and nicotine promoted structural plasticity in primary cultures of mouse mesencephalic dopaminergic neurons via activation of D3 receptor and Akt-mTOR pathway (Collo et al. 2012: Collo et al. 2013).

Here we studied the effects of ketamine in human dopaminergic neurons differentiated from inducible pluripotent stem cells (iPSCs) (Kriks et al. 2012). Human iPSCs were generated from healthy donors and differentiated into dopaminergic neurons that were cultured for 60-80 days to induce maturation. At this stages dopaminergic neurons expressed differentiation markers, including tyrosine hydroxylase (TH), dopamine transporter (DAT), D3R, NMDA subunit NR2B and AMPA subunits GluR1 and GluR2. They formed a network with GABAergic and glutamatergic neurons.

Ketamine dose-dependently increased dendritic arborisation and soma size of dopaminergic neurons as measured using computer-assisted morphometry. Similar effects were observed using the NMDA antagonist Ro 25-6981. These effects were inhibited by pre-treatment with the AMPA antagonists NBQX and GYKI 52466. The intracellular pathways involved in this structural plasticity were studied by western blot and immunofluorescence. Ketamine increased phosphorylation of p-70S6 kinase, located downstream of mTOR, an effect blocked by LY294002, a PI3 kinase inhibitor, and rapamycin, a mTORC-1 complex inhibitor. In previous works we had shown that amphetamine, cocaine and nicotine promoted structural plasticity of dopaminergic neurons by indirect DA-mediated activation of dopamine D3 receptors (D3R) (Collo et al. 2012: Collo et al. 2013). By analogy, the effects of ketamine, including mTOR recruitment, were blocked by the selective D3 receptor antagonists SB277011-A and S33084.

These results show that ketamine promotes structural plasticity in DA neurons via a complex pattern of actions involving AMPA receptor activation and engagement of D3R-Akt-mTOR pathways. This long-term structural plasticity may constitute a substrate for prolonged antidepressant effects and/or the induction of dependence.

Collo et al. (2012). J Neurochem. 120, 765-778.

Collo et al. (2013). Mol Pharmacol. 83, 1176-1189.

Iadarola et al. (2015). Ther Adv Chronic Dis. 6,97-114.

Kriks et al. (2011). Nature 480, 547-551.

Li et al. (2010). Science 329, 959-964.