

Ketamine, not TPS potentiates glutamate-induced Ca neurotoxicity

There is growing concern about anaesthesia effects on the developing brain because commonly used anaesthetics can cause neurodegeneration and long-term neurocognitive deficits. In the developing brain, GABA-R agonists and NMDA-R antagonists cause neurotoxicity and apoptosis. Comparing their effects on the developing nervous system helps derive optimal dose and duration of administration. In this study, we used calcium imaging and morphological examinations *in vitro* to determine whether prolonged exposure to thiopental sodium (TPS), a GABA agonist, or ketamine, a NMDA antagonist, during perinatal stages would result in dose-related potentiation of exogenous glutamate-induced intracellular calcium elevation and neurotoxicity after intravenous anaesthetics are discontinued.

We examined the degree of L-glutamate-induced intracellular calcium ($[Ca^{2+}]_i$) elevation and neurotoxicity in primary cultured neurons exposed to anaesthetics. Primary cortical neurons from E17 Wistar rats were preincubated with various concentrations of ketamine or TPS for the first 72 h of culturing. Two weeks later, the neurons were exposed to L-glutamate. The extent of glutamate toxicity was evaluated using Ca^{2+} -imaging and morphological experiments.

Preincubation with high concentration (100 mM) of ketamine but not with other concentrations of ketamine (? 10 mM) and TPS (100 mM) for the first 72 h in culture significantly enhanced L-glutamate-induced $[Ca^{2+}]_i$ elevation 2 weeks later. Morphology experiments showed that vulnerability to L-glutamate-mediated neurotoxicity was only altered in neurons preincubated with 100 mM ketamine. Vulnerability to L-glutamate-mediated neurotoxicity was unchanged in neurons preincubated with TPS at any concentration.

Ketamine resulted in an upregulation of NMDA receptor NR1 subunit protein, accompanied by enhanced apoptosis. This upregulation leads to accumulation of cytosolic calcium levels, making neurons more vulnerable to subsequent excitotoxicity via glutaminergic neurotransmission even after ketamine is eliminated. Our results support these data by showing a similar calcium dysregulation that lasted for 10 days after ketamine removal. In rats, the vulnerable period to ketamine exposure is PD1–14, shortly after neurons have differentiated and migrated to their destination. During this period, a brain growth spurt and rapid synaptogenesis occur. When an NMDA-R antagonist is administered during this critical period, NMDA-R upregulation in response to continuous blockade of the receptors causes neural cell death. Even after ketamine washout, activation of these upregulated NMDA-Rs in the immature brain leads to a toxic accumulation of intracellular calcium under normal physiological conditions. These upregulated NMDA-Rs cause calcium dysregulation, increased oxidative stress, and activation of NF- κ B signalling, thereby increasing neural susceptibility to hypoxic or ischemic events.

In contrast, preincubation with higher concentrations of TPS showed no enhancement of either

glutamate-induced $[Ca^{2+}]_i$ elevation or neurotoxic effects on 13–14 DIV. This may be attributed to the differences between the anaesthetics.

Although preincubation with high concentration of ketamine showed enhancement of L-glutamate-induced $[Ca^{2+}]_i$ elevation 2 weeks later, long-term exposure to TPS or ketamine at clinical doses during developmental periods may not result in a dose-related potentiation of exogenous glutamate-induced neurotoxicity, once the intravenous anaesthetics are discontinued.

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[Only extra-high dose of ketamine affects l-glutamate-induced intracellular \$Ca\(2+\)\$ elevation and neurotoxicity.](#)

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