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Relationship between ketamine-induced developmental neurotoxicity and NMDA receptor-mediated calcium influx in neural stem cell-derived neurons

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ABSTRACT

Ketamine, a noncompetitive NMDA receptor antagonist, is used as a general anesthetic and recent data suggest that general anesthetics can cause neuronal damage when exposure occurs during early brain development. To elucidate the underlying mechanisms associated with ketamine-induced neurotoxicity, stem cell-derived models, such as rodent neural stem cells harvested from rat fetuses and/or neural stem cells derived from human induced pluripotent stem cells (iPSC) can be utilized. Prolonged exposure of rodent neural stem cells to clinically-relevant concentrations of ketamine resulted in elevated NMDA receptor levels as indicated by NR1subunit over-expression in neurons. This was associated with enhanced damage in neurons. In contrast, the viability and proliferation rate of undifferentiated neural stem cells were not significantly affected after ketamine exposure. Calcium imaging data indicated that 50 μM NMDA did not cause a significant influx of calcium in typical undifferentiated neural stem cells; however, it did produce an immediate elevation of intracellular free Ca^{2+} $[Ca^{2+}]_i$ in differentiated neurons derived from the same neural stem cells.

This paper reviews the literature on this subject and previous findings suggest that prolonged exposure of developing neurons to ketamine produces an increase in NMDA receptor expression (compensatory up-regulation) which allows for a higher/toxic influx of calcium into neurons once ketamine is removed from the system, leading to neuronal cell death likely due to elevated reactive oxygen species generation. The absence of functional NMDA receptors in cultured neural stem cells likely explains why clinically-relevant concentrations of ketamine did not affect undifferentiated neural stem cell viability.

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1. Introduction

Ketamine, a noncompetitive NMDA receptor antagonist, is used primarily in adult, pediatric (Deasy and Babl, 2010) and veterinary (Muir, 2010) medicine. It is also highly abused for its hallucinogenic and out-of-body producing experiences (Reynaud-Maurupt et al., 2007) and has proven useful in the study of psychiatric disorders (Bubenikova-Valesova et al., 2008; Machado-Vieira et al., 2009; Krystal et al., 2010). Clinically, ketamine is used to provide sedation, analgesia, and general anesthesia to children during painful procedures (Kohrs and Durieux, 1998). At clinically-relevant concentrations, ketamine acts to block the NMDA receptor, a glutamate receptor subtype which is involved in a variety of processes including: development and differentiation of the nervous system; learning and memory; and synaptic plasticity (Collingridge et al., 1983; Meldrum and Garthwaite, 1990; D'Souza et al., 1993; Muller et al., 1996). NMDA receptors, along with other glutamate receptors, are abundantly expressed in the developing brain and are excitatory on neurons that play key roles in many physiological and pathological processes. Preclinical studies using rodent and nonhuman primate models have shown that ketamine can induce accelerated programmed nerve cell death (apoptosis) when administered in high doses and/or for prolonged exposure periods (Larsen et al., 1998; Lahti et al., 2001; Scallet et al., 2004; Maxwell et al., 2006; Slikker et al., 2007; Shi et al., 2010).

Noncompetitive antagonism of NMDA receptors is thought to be the mechanism by which ketamine produces its primary therapeutic effect. It has been reported that NMDA receptor

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NR1 expression in ketamine-treated rat pup brain tissues is significantly higher than in controls (Slikker et al., 2007; Zou et al., 2009b; Shi et al., 2010). It has been postulated that this up-regulation of the NMDA receptor is responsible for – or at least contributes to – ketamine-induced neurotoxicity, because it allows for a potentially toxic accumulation of intracellular calcium once ketamine is washed out of the system.

Neural stem cells are a subclass of precursor cells that have several specific characteristics that include: 1) self-renewal – the capability of making additional copies of itself by division; 2) multipotency – the capability of making daughter cells, including committed progenitors, neurons, astrocytes and oligodendrocytes; and 3) the capability of generating all or part of neural tissue. Stem cell models, in the context of toxicological insult, might provide important structures around which information can be arrayed in levels of a biological platform. Thus, stem cell-based approaches to developmental toxicology may be configured to predict the functional outcomes of stimuli using integral *in vitro* models that allow for the directional and quantitative descriptions of responses at the cellular and molecular levels. Stimuli eliciting such responses include environmental alterations and exposures to drugs or other chemicals and infectious agents. Specifically, the utilization of stem cell models in exploring issues relevant to pediatric general anesthetic-induced brain damage has the potential to advance our understanding of brain-related biological processes, including those critical for normal processes such as neural plasticity, and calcium flux, as well as those associated with the expression of toxicity (Keirstead et al., 2005; Li et al., 2005; Lamba et al., 2006; Kang et al., 2007).

This review focuses on how the availability of stem cell-derived models, especially embryonic neural stem cells, along with their capacity for proliferation and differentiation, can provide valuable systems for examining the developmental effects of ketamine-induced general anesthesia *in vitro*. This review also addresses the issue of how changes in the expression of functional neuronal transmitter receptors, as monitored using advanced intracellular calcium imaging approaches, can effect neurotoxicity induced by ketamine and how these bridging models may decrease the uncertainty associated with the process of extrapolating pre-clinical data to the clinical situation.

2. NMDA-type glutamate receptor expression and ketamine-induced neuronal damage

It is known that the most frequently used general anesthetics have either NMDA receptor blocking or GABA receptor enhancing properties. Accumulated data indicate that ketamine-induced general anesthesia can cause neuronal damage in several major brain regions in animal models, including rodents and nonhuman primates, during certain periods of development, particularly the brain growth spurt (Ikonomidou et al., 1999; Scallet et al., 2004; Slikker et al., 2007; Zou et al., 2009a). In nonhuman primates such exposures have been shown to result in significant disruption in cognitive function later in life (Paule et al., 2011). Glutamatergic transmission is mediated by receptor families that are classified as ionotropic (iGluRs) or metabotropic (mGluRs) receptors. iGluRs are ligand-gated ion channels that are sub-classified into the following groups based upon their ligand binding properties: *N*-methyl-D-aspartate (NMDA); alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA); and kainate (KA). NMDA receptors are heteromeric complexes composed of obligatory NR1 subunits as well as subunits from the NR2 subfamily (NR2A, NR2B, NR2C, NR2D) or the NR3 subfamily (NR3A or NR3B) (Premkumar and Auerbach, 1997; Laube et al., 1998; Furukawa et al., 2005; Ulbrich and Isacoff, 2007). Various combinations of subunits generate a large number of different NMDA receptors with differing pharmacological and biological properties. NMDA receptors are excitatory on neurons that play key roles in many physiological and pathological processes and are well-known mediators of neuronal cell death in numerous neuropathological conditions (Kari et al., 1978; Bubenikova-Valesova et al., 2008; Wakschlag et al., 2010; Medeiros et al., 2011).

Data from previous reports (Wang et al., 2006; Slikker et al., 2007) suggest that the enhanced apoptotic cell death associated with ketamine exposure is not directly associated with the *in situ* blood and brain ketamine levels at the time of cell death, but likely reflect some indirect or compensatory mechanisms. It has been hypothesized (Wang et al., 2005, 2006; Slikker et al., 2007) that continuous blockade of NMDA receptors by ketamine causes a compensatory up-regulation of NMDA receptors and that this up-regulation makes neurons bearing these receptors more vulnerable, after ketamine washout, to the excitotoxic effects of endogenous glutamate.

Several lines of evidence have demonstrated that prolonged ketamine exposure affects specific NMDA receptor subunit expression, alters receptor function and elevates ROS generation, all of which are thought to contribute to subsequent neuronal damage. Previous studies (Wang et al., 2005, 2006; Liu et al., 2013) have provided direct evidence at the protein level that prolonged ketamine exposure causes a compensatory up-regulation of the NMDA receptor NR1 subunit, and quantitative densitometry revealed that ketamine administration produced a significant increase (about two-fold) in protein expression ratio of NR1 protein to β-actin (Liu et al., 2013). In addition to ketamine, the administration of other noncompetitive NMDA receptor antagonists such as phencyclidine (PCP) and MK-801 to rats during a critical period of development also results in neurotoxicity/neurodegeneration in several major brain areas (Ikonomidou et al., 1999; Scallet et al., 2004). Previous *in vivo* data demonstrated that the gene expression of the NMDA receptor subunit gene, *Grin1* (*NR1*), was significantly up-regulated in ketamine-exposed rat pups as detected using *in situ* hybridization and microarray techniques (Shi et al., 2010). To determine whether the administration of antisense oligonucleotide (ODNs) targeted to specific NMDA receptor subunits could block the ketamine-induced up-regulation of subunit mRNA, an 18-mer antisense oligodeoxynucleotide corresponding to nucleotide 4–21 of the NR1 subunit mRNA – which directly follows the translation initiation codon – was used (Wang et al., 2006). It was demonstrated that ketamine-induced neurodegeneration could be effectively blocked by the co-administration of NR1 antisense oligonucleotides in cultured rat and monkey neurons (Wang et al., 2005, 2006), providing additional evidence supporting this hypothesis. Also, previous work has demonstrated altered expression levels of the NMDA receptor NR2 family, such as NR2A and NR2C (Shi et al., 2010), after repeated ketamine exposure.

Importantly, previous studies (Wang et al., 2005, 2006) have demonstrated that, after ketamine washout, application of the AMPA/kainite receptor antagonist, CNQX, had only a small (negligible) effect as compared to the strong protective effect obtained with the selective NMDA receptor antagonist, d-APV. Similarly, nifidipine, an antagonist of L-type voltage-sensitive calcium channels, did not produce a significant protective effect.

Taken together, previous and current mRNA, protein and biochemical data demonstrate that extended exposure of the developing mammal to NMDA receptor antagonists (e.g., ketamine) perturbs the endogenous NMDA receptor system and up-regulates the expression of NMDA receptors, especially the essential NR1 subtype. These results strongly suggest that enhanced neurodegeneration after prolonged ketamine exposure is associated the up-regulation of NMDA receptors.