Neurotoxicology and Teratology 54 (2016) 52–60

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Distinct effects of ketamine and acetyl L-carnitine on the dopamine system in zebrafish

Bonnie L. Robinson, Melanie Dumas, Elvis Cuevas, Qiang Gu, Merle G. Paule, Syed F. Ali, Jyotshna Kanungo *

Division of Neurotoxicology, National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR 72079, USA

ARTICLE INFO

Article history:
Received 12 June 2015
Received in revised form 28 January 2016
Accepted 15 February 2016
Available online 16 February 2016

Keywords:
Ketamine
Zebrafish
Acetyl L-carnitine
Dopamine
Tyrosine hydroxylase
MAO

ABSTRACT

Ketamine, a noncompetitive *N*-methyl-D-aspartic acid (NMDA) receptor antagonist is commonly used as a pediatric anesthetic. We have previously shown that acetyl L-carnitine (ALCAR) prevents ketamine toxicity in zebrafish embryos. In mammals, ketamine is known to modulate the dopaminergic system. NMDA receptor antagonists are considered as promising anti-depressants, but the exact mechanism of their function is unclear. Here, we measured the levels of dopamine (DA) and its metabolites, 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the zebrafish embryos exposed to ketamine in the presence and absence of 0.5 mM ALCAR. Ketamine, at lower doses (0.1–0.3 mM), did not produce significant changes in DA, DOPAC or HVA levels in 52 h post-fertilization embryos treated for 24 h. In these embryos, tyrosine hydroxylase (TH) mRNA expression remained unchanged. However, 2 mM ketamine (internal embryo exposure levels equivalent to human anesthetic plasma concentration) significantly reduced DA level and TH mRNA indicating that DA synthesis was adversely affected. In the presence or absence of 2 mM ketamine, ALCAR showed similar effects on DA level and TH mRNA, but increased DOPAC level compared to control. ALCAR reversed 2 mM ketamine-induced reduction in HVA levels. With ALCAR alone, the expression of genes encoding the DA metabolizing enzymes, MAO (monoamine oxidase) and catechol-*O*-methyltransferase (COMT), was not affected. However, ketamine altered MAO mRNA expression, except at the 0.1 mM dose. COMT transcripts were reduced in the 2 mM ketamine-treated group. These distinct effects of ketamine and ALCAR on the DA system may shed some light on the mechanism on how ketamine can work as an anti-depressant, especially at sub-anesthetic doses that do not affect DA metabolism and suppress MAO gene expression.

Published by Elsevier Inc.

1. Introduction

The pediatric general anesthetic, ketamine, is a noncompetitive antagonist of the *N*-methyl-D-aspartic acid (NMDA) receptor (Kohrs and Durieux, 1998). Although recognized as a dissociative anesthetic (Corssen et al., 1968), ketamine also has analgesic and amnesic effects and its potential anti-depressant properties are also being investigated (Aroni et al., 2009; Berman et al., 2000; Niesters et al., 2012; Rowland, 2005). Ketamine is listed as a Schedule III controlled substance in the USA, due to its potential for abuse (Morgan et al., 2010; Rowland, 2005). A number of studies on rodents and nonhuman primates have shown that treatment with high doses of ketamine or exposure for long durations during susceptible periods of development (the brain growth spurt) can induce abnormally high levels of neuroapoptosis (Haberny et al., 2002; Ikonomidou et al., 1999; Lahti et al., 2001; Larsen et al., 1998; Malhotra et al., 1997; Maxwell et al., 2006; Slikker et al., 2007; Wang et al., 2006).

Acetyl L-carnitine (ALCAR) belongs to the family of carnitines, a group of naturally occurring compounds that is essential for β-oxidation of fatty acids in mitochondria during ATP generation (Bieber, 1988). ALCAR effectively prevents mitochondrial injury resulting from oxidative damage (Palacios et al., 2011). It is also suggested that the carnitines have neuroprotective effects on conditions associated with mitochondrial dysfunction and oxidative stress and possibly in neurodegenerative disorders such as Parkinson's disease (Beal, 2003). During early developmental stages, co-administration of ALCAR significantly diminished reactive oxygen species (ROS) generation and provided significant protection of neurons from ketamine-induced neurodegeneration in rats (Liu et al., 2013). Additionally, ALCAR has been shown to protect neurons from inhalation anesthetic-induced neurotoxicity in rat cortical neurons (Zou et al., 2008). In zebrafish embryos, ALCAR offers protection against ketamine-induced cardiotoxicity and neurotoxicity (Cuevas et al., 2013; Kanungo et al., 2012). ALCAR reversed the effects of 2 mM ketamine not only by restoring serotonin (5-HT) and its metabolite (5-hydroxyindoleacetic acid or

Abbreviations: COMT, catechol-*O*-methyl transferase; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; MAO, monoamine oxidase; 3-MT, 3-methoxytyramine.

* Corresponding author at: Division of Neurotoxicology, National Center for Toxicological Research, U.S. Food and Drug Administration, 3900 NCTR Road, Jefferson, AR 72079, USA.

E-mail address: jyotshnabala.kanungo@fda.hhs.gov (J. Kanungo).

http://dx.doi.org/10.1016/j.ntt.2016.02.004
0892-0362/Published by Elsevier Inc.

5-HIAA) levels but also 5-HT turnover rate to control levels in the zebrafish embryos (Robinson et al., 2015).

In the dopamine (DA) biosynthesis process, conversion of L-tyrosine to L-DOPA by tyrosine hydroxylase (TH) is the rate-limiting step (Kuhn et al., 1999). DOPA is subsequently converted to DA by L-aromatic amino acid decarboxylase. Released DA is metabolized to 3,4-dihydroxyphenyl-acetic acid (DOPAC) by intra-neuronal monoamine oxidase (MAO) after reuptake by the nerve terminal and is also converted to 4-hydroxy-3-methoxyphenylacetic acid (HVA), probably at an extra-neuronal site through the sequential action of catechol-*O*-methyltransferase (COMT) and MAO (Meiser et al., 2013). A number of studies suggest that ketamine not only affects the glutamate and GABA system (Boczek et al., 2015; Catena-Dell'Osso et al., 2013) but also may influence the DA system (Li et al., 2015; Owolabi et al., 2008; Tan et al., 2012) in a way that could contribute to the disruption of normal development. Ketamine reportedly exhibits a strong affinity for the D2 dopaminergic receptors indicating that the DA system could be modulated by ketamine (Kapur and Seeman, 2001). The prefrontal dopaminergic system, responsible for working memory and executive function, appears to be adversely affected by chronic ketamine exposure (Narendran et al., 2005). In young monkeys anesthetized during the brain growth spurt, ketamine has been shown to cause long-lasting deficits in several cognitive functions (Paule et al., 2011). Pharmacological evidence suggests that NMDAR dysfunction may cause dopaminergic dysfunction in schizophrenia (Rujescu et al., 2006; Stefani and Moghaddam, 2005) and ketamine has been shown to induce DA release in lateral prefrontal and anterior cingulate cortex in healthy men (Aalto et al., 2005). However, little is known about the precise mechanism of ketamine's effects on the DA system.

In MPTP-treated monkeys, ALCAR provided behavioral protection from MPTP, but it also altered DA mechanisms since HVA values were about 30% of those of normal monkeys in the caudate nucleus and about 60% in the substantia nigra (Bodis-Wollner et al., 1991). A week-long regimen of ALCAR treatment produced a steady increase in DA and serotonin (5-HT) output in the nucleus accumbens shell in rats (Tolu et al., 2002) and striatal infusion of ALCAR increased DA efflux with no changes in the efflux of DOPAC or HVA (Harsing et al., 1992). A 4-day treatment with ALCAR elevated GABA levels in rat substantia nigra but not striatal DA (Fariello et al., 1988). ALCAR reportedly attenuated age-related changes in dopaminergic systems in rats (Sershen et al., 1991). Since ALCAR did not show any normalizing effects on catecholamine concentrations, TH, neurofilament and glial fibrillary acid protein, it has been suggested that ALCAR's protective role may be related to energy metabolism and not to direct effects on the dopaminergic systems (Pettegrew et al., 2000; Santarelli et al., 1995; Steffen et al., 1995). Chronic administration of ALCAR has been shown to lead to enhanced DA release in the striatum (Sershen et al., 1991).

In addition to its small size, prolific reproductive capacity, and easy maintenance, the zebrafish possesses the typical complexity of vertebrate systems and therefore has been used in several areas of research with the prospect of extrapolating findings to mammals including humans (Briggs, 2002; Kanungo et al., 2014; Lantz-McPeak et al., 2015; Parng et al., 2002). Since its early use, emphasis has been given to characterizing molecules within the zebrafish nervous system, a difficult task to undertake in mammals (Key and Devine, 2003). Zebrafish embryos/larvae have a functional DA system and they accordingly respond to a number of drugs including drugs of abuse (Buske and Gerlai, 2011; Kung et al., 2015; Levin et al., 2011; Suen et al., 2013). Furthermore, zebrafish has been used to study the antidepressant-like effects of MK-801, an NMDA receptor antagonist (da Silva et al., 2015). Our earlier studies demonstrated that exposure of embryos to 2 mM ketamine resulted in an internal exposure of ~8 μM (Trickler et al., 2014). This concentration is comparable to the lower end of the human anesthetic blood levels (2.2 μg/ml). Since ketamine is lately being used as a fast-acting anti-depressant (Blier, 2013), in the present study, in order to gain insight into the mechanism of ketamine's effect on the DA system, we examined whether ketamine and ALCAR evoke a response in the DA system in the zebrafish embryos in terms of DA biosynthesis, metabolism, and change the expression of in TH, MAO and COMT genes.

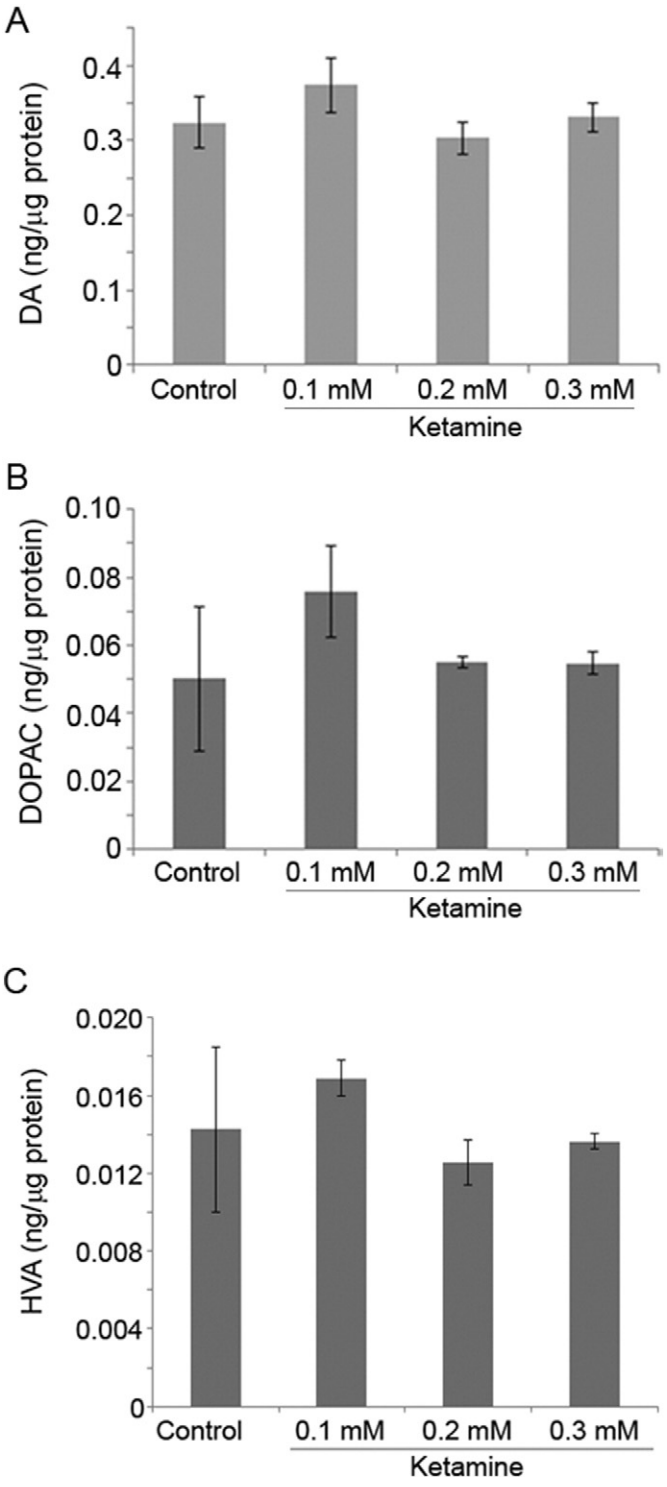

Fig. 1. Effects of low doses of ketamine on DA, DOPAC and HVA levels in zebrafish embryos. Embryos at 52 hpf were treated with 0.1–0.3 mM ketamine. Twenty four hours post exposure (76 hpf) embryos were processed for DA (A) as well as its metabolites, DOPAC (B) and HVA (C) measurements using HPLC/EC. Values are expressed in ng/μg protein in embryo extracts (means ± SDs). One-way ANOVA with post-hoc analyses were used to assess statistical significance in variation in DA, DOPAC and HVA levels between the experimental groups as well as with controls.