Effect of prenatal ketamine exposure: A focused literature review

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Abstract: Ketamine is a dissociative anesthetic and antidepressant with several biological targets. Among the many targets, ketamine, notably, has an antagonistic effect on molecular N-methyl-D-aspartate (NMDA) receptors and has been identified as a non-competitive inhibitor of these receptors. Although ketamine has a wide range of therapeutic uses in the clinical setting, it is often used recreationally due to its psychoactive and analgesic effects. Regardless of the indication, prenatal exposure to ketamine has been widely investigated. The misuse of this drug, particularly in pregnant women, has been a point of concern. The neurotoxic potential of ketamine positions it as a danger to a developing fetus. Furthermore, the ability of ketamine to cross the blood-placental barrier poses a threat to the health and maturation processes of the neonate. This paper reviewed published work that explores the mechanisms through which prenatal ketamine exposure can cause altered neurodevelopment, neurobehavior, and the physiological consequences that follow. By exploring investigations using multiple different subjects such as: rodents, non-human primates, and human subjects, this paper develops a full picture of the existing data to generate a strong foundation for improved and informed public health policies.

Keywords: N-methyl-D-aspartate (NMDA) receptors; prenatal exposure; neurotoxic

Abbreviation

CYP: Cytochrome P450

DNA: deoxyribonucleic acid

$GABA_AR$: γ-Aminobutyric acid receptors

iPSC: Induced pluripotent stem cell

IV: Intravenous

LDH: Lactate dehydrogenase

NMDA: *N*-methyl-D-aspartate

NMDAR: *N*-methyl-D-aspartate receptors

PCP: Phencyclidine

PFC: Prefrontal cortex

PSD-95: Postsynaptic density protein 95

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RNA: ribonucleic acid

RT-PCR: Reverse transcription polymerase chain reaction

SAMHSA: Substance Abuse and Mental Health Services Administration

SY-38: Anti-Synaptophysin antibody

TEDS: Treatment Episode Data Set

TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling

1. Introduction

With the increase in substance misuse rates, monitoring drug misuse in pregnant women has become a prominent health concern in America. Approximately 90% of drug-misusing women are of reproductive age (1). In addition, Treatment Episode Data Set (TEDS), which is published by the Substance Abuse and Mental Health Services Administration (SAMHSA), reports approximately 4 in 100 women seeking treatment for substance misuse are pregnant (2). Misusing drugs during pregnancy can cause detrimental effects for the mother and fetus.

Ketamine is one of the many drugs that is highly misused, which is denoted by its Schedule III drug classification. Ketamine was derived from phencyclidine (PCP) with the intention of lowering the psychiatric effects of PCP. However, ketamine has still been linked to producing dissociative effects in individuals with the potential for misuse (3). Despite the occurrence of negative side effects, it has been considered a strong alternative to PCP because of its short half-life and ability to avoid respiratory impairment (3,4). Therefore, ketamine is traditionally used in medicine as a dissociative analgesic due to its hypnotic and anti-inflammatory effects, as well as its antidepressant activity via the serotonergic pathway (3,5,6). One dosage of ketamine can produce a rapid physiologic response (7). These effects make it a popular drug to be misused recreationally, especially due to its psychotropic effects of euphoria and hallucinations, but also as a pain management medication (2,8). Given its dissociative effects on cognition in fully developed individuals, the effects of ketamine on fetal development are of growing concern due to its potential neurotoxicity, which can lead to developmental defects in the fetal brain. Therefore, it is important to investigate these effects further. This review focused on the neurodevelopmental effects of offspring whose mother was exposed to ketamine while pregnant.

2. Methods

The following databases were used to acquire references to conduct this literature review: National Library of Medicine and Google Scholar. References were chosen based on if they were peer reviewed and deemed reliable and relevant. No exclusion factors were applied such as publication date and journal. The following key phrases were used during the database search: ketamine usage in rodents, ketamine usage in primates, ketamine misuse, clinical ketamine, prenatal ketamine, substance misuse.

2.1. Ketamine

Commercial ketamine is most commonly distributed as a racemic mixture of S(+) and R(-) ketamine (9). S(+) ketamine was discovered to possess four times the affinity of R(-) ketamine for the N-methyl-D-aspartate (NMDA) receptors, and also has the ability to bind to mu and kappa opioid receptors, which makes its anesthetic potency three times greater than the racemic mixture (9). The R(-) enantiomer is more effective in facilitating airway smooth muscle relaxation. Once ingested, the liver metabolizes ketamine to its active form: norketamine. Ketamine is both water and lipid soluble

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with various routes of administration, including intravenous, intramuscular, oral, rectal, subcutaneous, epidural, and transnasal (9).

2.2. Relationship Between Ketamine and NMDA Receptors

As research on ketamine-induced neurotoxicity continues, substantial evidence has emerged to suggest the NMDA receptor is involved. Ketamine is a non-competitive open-channel blocker of NMDA receptors, which binds to an electrically deep site within the ion channel and prevents the flow of ions (7,10). This is due to its antagonistic abilities at the NMDA receptors throughout the central nervous system (9). NMDA receptors are believed to be involved in cognition following numerous significant results demonstrating how the antagonism of NMDA receptors resulted in disrupted learning, immediate recall, and long-term retention (11). Drugs can act as NMDA antagonists leading to widespread apoptosis in mouse brains (12,13). Neuroapoptosis is one of the most detrimental effects of ketamine neurotoxicity, as it directly contributes to developmental and cognitive issues. Neuroapoptosis is believed to be dose- and time-dependent to ketamine (14). These factors can play important roles in determining appropriate administration dosing and timing to pregnant mothers.

2.3. Consequences of Ketamine Use and Misuse

The evaluation of the effects of ketamine on memory in healthy volunteers was analyzed (11). Participants were administered ketamine through an intravenous (IV) drip and asked to complete attention, recall, and recognition tasks at structured time points. The results demonstrated that ketamine produced significant decreases in performance of all three tasks (11). Another similar investigation identified similar results where different doses of ketamine caused immediate and delayed paragraph recall scores in healthy adult males (15). Given ketamine's effects on cognition in fully developed individuals, it gives cause for concern regarding how it affects less developed brains.

When ingested by a pregnant woman, ketamine can cross the placenta and enter fetal circulation. This ability of ketamine has been observed in both non-human animal models as well as humans (16,17). When administered intravenously, ketamine has been located in the umbilical cord blood as early as one minute and 37 seconds after injection (8).

3. Ketamine Exposure in Rodents

3.1. Neurological Effects of Ketamine Exposure

A great variety of neurotoxic effects associated with ketamine exposure have been observed using rodent models. A variety of studies have documented the neurotoxicity which results from prenatal exposure to ketamine (13,8,18). After injecting a subanesthetic dose of ketamine in the pregnant rats, a large increase in positive cells was observed within terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) stained samples, therefore confirming the degeneration of neurons and apoptosis within the developing brain (8,18). Moreover, the offspring of the exposed maternal rodents exhibited a greater loss of neurons within the prefrontal cortex laminae compared to that of the control group (8). Neuronal loss in the prefrontal cortex (PFC) is detrimental due to the connections the PFC holds with other brain structures such as the amygdala, thalamus, and hippocampus (8,19,20). Damage to these structures contributes to many cognitive and neurobehavioral problems. Vital functions such as memory and the ability to retain information were affected. (8,18,19,21,22,23,24,25,26). Additional findings revealed that prenatal exposure caused the inhibition of the NMDA receptor, ultimately lowering the number of GABAnergic interneurons within the PFC of offspring (18,27). The differences in impact caused by administering a low or high ketamine dose has been closely monitored. After administering a low prenatal dose of approximately three mg/kg, detriments to learning capabilities were observed. On the contrary, administering a

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larger dose of 50 mg/kg to postnatal rat pups caused a number of neurobehavioral deficits (8,18,13,28,29). Additionally, more of the effects of ketamine exposure on the prefrontal cortex was assessed. This led to an altered number of proteins within the prefrontal cortex (13,24,30). The expression of proteins such as NR2B, NR2A, Anti-Synaptophysin antibody (SY-38), and postsynaptic density protein 95 (PSD-95) were all altered in the rat offspring of mothers who were given a subanesthetic dose of ketamine via injection between postnatal day zero and postnatal day 30 (13). This prenatal exposure affected essential neurological functions, synaptic regulation, structural aspects of the synapse, and neurotransmitter migration (31,32,33).

3.2. Neurobehavioral Effects of Ketamine Exposure

Behavioral deficits such as difficulty learning, poor memory, and impaired motor skills are all results of postnatal exposure to ketamine (18,34). After administering a 50 mg/kg subcutaneous injection to a group of postnatal rat pups, it was apparent that learning and memory were compromised (34). In hopes of identifying the types of neurobehavioral deficits that arose from prenatal exposure, a number of tests such as the Morris water maze and open field test was conducted (18,34,35). Another piece of literature examined the neurobehavioral impact of ketamine exposure prenatally. After separating maternal rats into their respective control and experimental groups, one group of rats was injected with an approximate dose of 144.2 mg/kg of ketamine (22). Prenatal ketamine exposure caused impairment associated with spatial learning and memory while also enhancing anxiety in rat pups (18,35,22). The anxious behavior in rats was further explored by exposing a group of maternal rats to ketamine during the second trimester during which the brain begins developing and taking on essential roles (35). The effects of the ketamine were then tested through a locomotor activity test which showed that the offspring of exposed rats were more immobile and avoided the center zone of the open field compared to the control group (18,35).

3.3. Prenatal Ketamine Exposure Effects on Specification of Mouse Embryonic Stem Cells

As broad as the effects of ketamine exposure can be in prenatal stages, one significant consequence that has been investigated is the impact on primary germ cell layer specification. The mechanism of ketamine has been determined to be via NMDA receptors which serve as a receptor for glutamate (36). Due to this information and the knowledge of ketamine's blockade of NMDA receptors, researchers sought to develop a more thorough understanding of the long-term neurological effects of ketamine on fetal brains. Previous inquiries about this receptor have looked into the effects of alcohol consumption on the fetal brain via the NMDAR pathway and have uncovered the risk of apoptotic neurodegeneration due to NMDAR antagonism in the rat forebrain (37). Such impacts have been observed as reduced brain mass and neurobehavioral deficits in developing mice (37). With $GABA_Ar$ being a known regulator of embryonic stem cell proliferation, the search for the correlation between ketamine and specification was sparked (38).To further the scope of understanding, studies have specifically indicated that such effects are due to NMDAR antagonism or activation of γ-Aminobutyric acid receptors ($GABA_A$) (37).

Mouse models were used to explore the specificity of embryonic stem cells within the primary germ layer post-ketamine exposure (39). Prior to testing the effects of ketamine exposure, 50 mg/mL ketamine hydrochloride was administered to these mice. The steps of processing and analysis were carefully crafted to ensure the results were reflective of ketamine's effects. One essential part of the process included the induced growth and spinal motor differentiation of embryoid bodies, which were generated from mouse embryonic stem cells (39). Prior research has indicated the role of retinoic acid signaling in the development and neuronal differentiation of the spinal cord (40). The culturing of mouse embryoid bodies with 0.01 M retinoic acid allowed for the induction of the cell specifications toward spinal motor neuron identities. Through flow cytometry, immunohistochemistry, confocal immunofluorescence imaging, induced pluripotent stem cell (iPSC) generation, embryonic motor

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neuron and ribonucleic acid (RNA) sequencing, microarray analysis, and RT-PCR, the effects of prenatal ketamine on spinal cord specification was observed. Ultimately, ketamine exposure during early neuronal development, which caused NMDAR antagonism, resulted in the impairment of mouse embryonic stem cell specification into neuronal, neuronal progenitor, neuroectodermal cells, and mesoendodermal cell lineages (39).

3.4. Consequences of Stem Cell Proliferation Impairments

The effects of early ketamine exposure on stem cell specification have been linked with a variety of abnormalities and syndromes in humans. This form of prenatal NMDAR antagonism causes disturbances in the specification has resulted in spontaneous abortion of fetuses, and complications in fetal forebrain development (41,42). Beyond the deleterious effects aforementioned, NMDAR antagonism has been associated with deficits in memory and sensorimotor gating that persist until adulthood, learning impairments, and schizophrenia-like symptoms seen in both human and rodent models (43,44).

4. Prenatal Ketamine Exposure in Monkeys

4.1. Neurobehavioral Effects of Ketamine Exposure

In humans, embryonic exposure to ketamine through pregnancy can create a great threat to brain development (45). Exposure to the neurotoxicity of ketamine has a greater effect on a developing brain than a mature adult brain (8,46,47). This threat starts during the last trimester of gestation all the way until three years of age. When ketamine is exposed to the developing brain during this period, it can likely cause long-term brain dysfunctions and possibly lead to neurobehavioral impairment (8,46,48).The exposure of prenatal ketamine in animals can lead to many abnormal behaviors such as: disinhibition and hyperactive behavior, cognitive impairments, social withdrawal, anxiety, depression, and aggressive-like behaviors (8,49). Non-human primates have been previously used to resemble the human neurobehavioral effect of prenatal ketamine exposure. One investigation using rhesus monkeys observed cognitive function tasks, such as: learning, motivation, color discrimination, and short-term memory. They observed these tasks at the age of three using both monkeys that were prenatally exposed to ketamine and those that were not exposed to ketamine. Ketamine exposure consisted of an initial 20 mg/kg intramuscular injection followed by continuous infusion (20-50 mg/kg/hr) to maintain a light anesthesia for 24 hours. This was administered to monkeys that were 5 and 6 days post natal. The data collected suggested that long-term and possibly permanent behavioral effects are plausible when prenatal developing brains are exposed to ketamine (8,48,50,51). After a prolonged amount of time exposed to ketamine prenatally (5 to 24 hours), significant neuronal damage on the immature brain during early developmental stages can occur(52,53,54,55,56). These reports have shown a greater effect of prenatal ketamine exposure in fetal brains compared to neonatal brains. Ketamine exposure showed a greater loss of neuronal cells in fetal brains than those in neonatal brains (54). Additionally, prenatal exposure ot ketamine can result in apoptotic and necrotic features in these monkeys (52). When high doses of ketamine are exposed in monkeys and humans, it causes lactate dehydrogenase (LDH) to be released, and therefore causes necrotic tissue. As a result of the necrosis, early exposure to ketamine in humans will more likely cause greater damage compared to that of rodents (57,58).

4.2. Physiologic Effects of Ketamine Exposure

The physiologic function in rhesus monkeys was observed following prenatal exposure to ketamine. The physiological parameters used during this study included percent oxygen saturation, exhaled carbon dioxide, body temperature, heart rate, blood pressure, glucose, and hematocrit. Ketamine exposure included a bolus dose of 60 mg/kg via an intramuscular injection, followed by a

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ketamine infusion at a rate of 60-90 mg/kg/h (10 mg/mL diluted with saline). Data collected indicated that heart rate, respiratory rate, and blood pressure were all reduced in monkeys that had prenatal exposure to ketamine. Expired CO_2 concentration was increased in these exposed monkeys compared to control infants. Body temperature and blood glucose were not affected by the prenatal ketamine exposure (45).

4.3. Neurotoxic Effects in Fetal Cynomolgus Monkeys

Genetic analysis has determined that cynomolgus monkeys demonstrate a high level of similarity in cytochromes P450 (CYPs) cDNA and amino acid sequences with humans (59). Since CYPs play essential roles in ketamine metabolism and clearance, cynomolgus monkeys have been identified as effective models for pharmacological experimentation (60). Due to the physiologic similarity, cynomolgus monkeys were used to compare the effects of dexmedetomidine and ketamine on the fetal brain (61). Twenty pregnant cynomolgus monkeys were divided into multiple groups at gestational day 120. One group received no treatment and was the control. The second group received intramuscular doses of ketamine (20 mg/kg) followed by a twelve-hour infusion (20 to 50 mg/kg/hr). The third group and fourth groups received dexmedetomidine infusions (30 μg/kg/hr), which is ten times the human equivalent dosage. Six hours following the infusion administering, Cesarean sections were formed on the mothers in order to extract the fetuses. Analysis of the brains revealed substantial neurodegeneration and apoptosis in layers I and II of the prefrontal cortexes of all of the subjects in the ketamine-exposed group (61).

5. Prenatal Ketamine Exposure in Humans

5.1. Late Pregnancy Ketamine Exposure

While ketamine is most notably used as an anesthetic, it is often used in a recreational manner in certain parts of the world. Particularly in Asia, ketamine is a commonly misused drug in both the male and female populations (62). With this issue plaguing the continent, researchers aimed to determine whether the misuse of ketamine by pregnant mothers affected the health of the fetuses (63). Suspected pregnant women using ketamine were further questioned. The self report aspect led to some questionable results. For example, one mother reported that she ceased ketamine misuse early in the pregnancy. This statement was refuted after the newborn child's hair was sampled for ketamine and came back positive. Also of importance, the child was born at 2,250 grams which is considered to be a low birth weight (63,64). Lastly, the neonate presented with hypotonicity in addition to moderate cerebral dysfunction (63). This case was identified to be an instance of late pregnancy ketamine exposure based on the levels of ketamine identified in the hair samples. The study did not note taking any measures to rule out any other substance abuse besides the hair sample testing that screened for amphetamines, ketamine, and opiates. Although this analysis linked prenatal ketamine exposure to postnatal impairments, it happens to be one of the few prenatal ketamine exposure investigations using humans. Therefore, the effect of late pregnancy exposure of ketamine should be further explored using human models.

6. Public Health Relevance

6.1. Ketamine Misuse

The misuse of ketamine is directly related to cognitive impairment, disorders of mood, and dissociative symptoms. When it comes to misuse, ketamine is administered in a variety of ways. These routes include: intravenous injection, nasal inhalation, combined smoking with other substances such as marijuana or tobacco, and oral consumption of beverages (65). Intravenous injection brings about the quickest hallucinogenic response within seconds to minutes, whereas the

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oral route will take anywhere from five to thirty minutes (65). The self administration and rapid physiological response make this drug susceptible for misuse among the general population (66). The adverse effects that coincide with misusing ketamine have been explained by the increased dosage used recreationally compared to the clinical dosage (67). Additionally, ketamine has been proven to significantly change the structure and network function within the substantia nigra, ventral tegmental area, and posterior cingulate cortex. All of these regions have been directly linked to the pathophysiology of addiction (66). Furthermore, research has shown its ability to produce anxiety-like symptoms (45). This is also another risk factor, as ketamine increases the vulnerability of an individual to misuse this drug. During the state of abstinence, exposures producing anxiety-like symptoms promote the craving for relapse (68). The doubling effect of misuse and the production of anxiety in individuals makes them that much more susceptible to ketamine misuse (45,68).

6.2. Drug Education

School-based drug education programs have been a common route of misuse prevention in communities. These programs have proven to be effective in terms of the reduction and prevention of cigarette, drug, alcohol, and tobacco usage (69,70,71). Within the scope of ketamine misuse, the effectiveness of outpatient treatment programs for substance misuse that was based on motivational enhancement principles was further investigated in Taiwan (72). Ketamine-using youth were the subjects of interest and the results demonstrated a decrease in substance cravings and an improvement in family well-being (72). The findings offered a promising approach to substance misuse treatment and rehabilitation.

7. Conclusion

Examination of the existing literature contextualizes the deleterious effects of prenatal ketamine on neurodevelopment and cognition. Evidently, the misuse of ketamine is an issue of public concern. The neurological consequences of fetal exposure to ketamine are extensive and can even be fatal. Rodent models have been used to demonstrate that ketamine can lead to the degeneration of neurons and neuronal loss, leading to significant neurobehavioral deficits. Additionally, using rhesus monkeys revealed that exposing a developing brain to ketamine can lead to long-term deficits and abnormal behavior such as hyperactivity, aggression, and anxiety. The data regarding the human response to prenatal ketamine exposure that exists stands to corroborate the concerns identified in non-human animal literature. With the consequences being so debilitating and long-term, the need for public health policy and information distribution is apparent. Educating the public as well as the medical community on the risks associated with ketamine usage during the perinatal period would allow for more informed decisions. The present review of literature establishes the severity of the neurological and cognitive impact that ketamine exposure can have on a developing fetus. This relationship requires further investigation regarding the potential treatment options for fetuses that acquire deficits due to prenatal ketamine exposure.

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