KETAMINE Nomination

TABLE OF CONTENTS

KETAMINE

1.0 BASIS OF NOMINATION

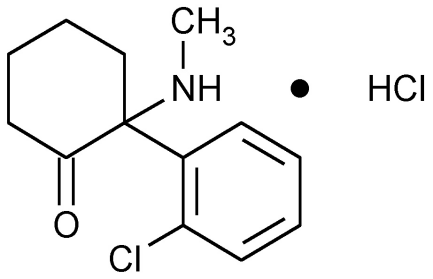
Ketamine, a noncompetitive NMDA receptor blocker, has been used extensively off - label as a pediatric anesthetic for surgical procedures in infants and toddlers. Recently, Olney and coworkers have demonstrated severe widespread apoptotic degeneration throughout the rapidly developing brain of the 7-day-old rat after ketamine administration. Recent research at FDA has confirmed and extended Olney's observations. These findings are cause for concern with respect to ketamine use in children. The issue of whether the neurotoxicity found in this animal model (rat) has scientific and regulatory relevance for the pediatric use of ketamine relies heavily upon confirmation of these findings that may be obtained from the conduct of an appropriate study in non-human primates.

2.0 BACKGROUND INFORMATION

The issue of potential ketamine neurotoxicity in children surfaced as a result of FDA's reluctance to approve an NIH pediatric clinical trial using this compound because of its documented neurotoxic effects in young rats (published in several papers over the last ten years by Olney and co-workers). This resulted in the formation of an FDA-wide Expert Working Group that met several times in the Spring of 2001 in conjunction with the CDER/Office of Pharmaceutical Sciences' Rapid Response Team. The Rapid Response Team issued two reports that articulated the known 'state of the art' or knowledge base at that time and formulated a strategy for dealing with the problem that resulted in pre-clinical studies to replicate and further evaluate Olney's findings. These initial Rapid Response Reports are attached (Appendix A and B) and provide considerable background information. In conclusion from the first Rapid Response Report, ketamine does have significant undesirable effects in the developing rat brain. These effects to alter apoptosis are not the classical Olney lesion (vacuole formation) seen in adult rats but the consequences may be far more serious, as a larger proportion of the brain's eventual complement of neurons may be compromised. Based on the information in the literature, another rat study is not necessary because a number have already been performed. The extant data in primates are very poor and inconclusive. The concern with respect to ketamine exposure in children is most likely not due to bouts of acute, single dose exposures, but rather to bouts of repeated doses over a relatively short period of time. If for example a second or multiple doses were applied to an infant to extend the duration of anesthesia, this may raise some concerns. Additionally, there is a longer period of development of the primate brain compared to the rat, and it is difficult to match the exact age at which a particular neurodevelopmental stage commences across species.

In summary, it is considered that a nonhuman primate study would be most appropriate to determine if the significant apoptosis seen in the rat exposed to ketamine during development is a potential concern for the developing human.

3.0 CHEMICAL PROPERTIES



3.1 Chemical Identification

Substance Identification:

Name of Substance: KETAMINE HYDROCHLORIDE

CAS Registry Number: 1867-66-9

Synonyms:
(+-)-Ketamine Hydrochloride
2-(O-chlorophenyl)-2-(methylamino) cyclohexanone hydrochloride
CI 581
CL 369
CN-52, 372-2
Cyclohexanone, 2-(2-chlorophenyl)-2-(methylamino)-, hydrochloride, (+-)-
Cyclohexanone, 2-(O-chlorophenyl)-2-(methylamino)-, hydrochloride
Ketaject
Ketalar
Ketamine
Ketanest
Ketaset
Ketavet
Ketolar
Vetalar

Molecular Formula: C13-H16-Cl-N-O.Cl-H

RTECS Number: NIOSH/GW1400000

Ketamine Hydrochloride – Identification:
C13H16ClNO·HCl 274.19
Cyclohexanone, 2-(2-chlorophenyl)-2-(methylamino)-, hydrochloride.
(±)-2-(o-Chlorophenyl)-2-(methylamino) cyclohexanone hydrochloride [1867-66-9].

» Ketamine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of C13H16ClNO·HCl.

Clarity and color of solution: Dissolve 1 g in 5 mL of water: the solution is clear and colorless

A. Infrared Absorption: Do not dry specimens.

B. Acid solvent: The ultraviolet absorption spectrum of a 1 in 3000 solution in 0.1 N hydrochloric acid exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Ketamine Hydrochloride RS, concomitantly measured, and the respective absorptivities, at the wavelengths of maximum absorbance at about 269 and 276 nm do not differ by more than 3.0%.

C. Basic solvent: The ultraviolet absorption spectrum of a 1 in 1250 solution in 0.01 N sodium hydroxide, in a 1 in 20 mixture of water and methanol, exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Ketamine Hydrochloride RS, concomitantly measured, and the respective absorptivities, at the wavelength of maximum absorbance at about 302 nm do not differ by more than 3.0%.

pH: Between 3.5 and 4.1, in a solution (1 in 10).

Residue on ignition: Not more than 0.1%.

Heavy metals, Method I: 0.002%.

Chromatographic purity: Buffer, mobile phase, system suitability solution, standard preparation, and Chromatographic system: Proceed as directed in the Assay.

3.2 Physico – Chemical Properties

Ketamine Hydrochloride: White, crystalline powder, having a slight, characteristic odor. Freely soluble in water and in methanol; soluble in alcohol; sparingly soluble in chloroform. (USP-24-NF19 Suppl)

Clarity and color of solution: Dissolve 1 g in 5 mL of water: the solution is clear and colorless

Color/Form: White crystals; colorless crystals.

Melting Point: 262-263 DEG C

Molecular Weight: 274.21

pH: Soln Acid to Litmus

Solubilities:

In Water: 20 g/100ml; 1 G in 14 ml Alcohol, 60 ml Chloroform, 60 ml Abs. Alcohol

Other Chemical/Physical Properties:

Log Kow = 2.18 /Ketamine

SAFETY AND HANDLING

Hazardous Reactions:

Decomposition: When heated to decomposition it emits very toxic fumes of /hydrogen chloride/ and /nitrogen oxide/.

Other Safety and Handling:

Storage Conditions: Store below 40 deg C (104 deg F), preferably between 15 and 30 deg C (59 and 86 deg F), unless otherwise specified by manufacturer. Protect from light and heat. Protect from freezing. /Ketamine hydrochloride injection USP/

3.3 Purity and Commercial Availability

Pharmaceutical Preparation

Ketamine Hydrochloride Injection
» Ketamine Hydrochloride Injection is a sterile solution of Ketamine Hydrochloride in Water for Injection. It contains an amount of ketamine hydrochloride ($C_{13}H_{16}ClNO \cdot HCl$) equivalent to not less than 95.0 percent and not more than 105.0 percent of the labeled amount of ketamine ($C_{13}H_{16}ClNO$).

Packaging and storage: Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light and heat.

Identification:

A. The ultraviolet absorption spectrum, measured in the region between 250 and 350 nm, of a dilution of Injection in 0.01 N methanolic sodium hydroxide containing ketamine hydrochloride equivalent to about 800 mg of ketamine per mL, exhibits maxima and minima at the same wavelengths as that of a similar preparation of USP Ketamine Hydrochloride RS, concomitantly measured.

B. The ultraviolet absorption spectrum of the solution employed for measurement of absorbance of the assay solution, prepared as directed in the Assay, exhibits maxima and minima at the same wavelengths as that of the Standard solution, prepared as directed in the Assay.

Bacterial endotoxins: It contains not more than 0.4 USP Endotoxin Unit per mg of ketamine hydrochloride.

pH: between 3.5 and 5.5.

Other requirements: It meets the requirements under Injections.

Assay: Transfer an accurately measured volume of Injection, equivalent to about 500 mg of ketamine hydrochloride, to a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 20.0 mL of this solution to a 125-mL separator, add 3 mL of 0.1 N sodium hydroxide, and extract with three 15-mLportions of chloroform. Collect the chloroform extracts in a second 125-mL separator, and extract with three 30-mL portions of 0.1 N sulfuric acid, collecting the acid extracts in a 200-mL volumetric flask. Dilute with 0.1 N sulfuric acid (saturated with chloroform) to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Ketamine Hydrochloride RS in the same medium having a known concentration of about 250 mg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 269 nm, with a suitable spectrophotometer, using 0.1 N sulfuric acid (saturated with chloroform) as the blank. Calculate the quantity, in mg, of ketamine ($C_{13}H_{16}ClNO$) in each mL of the Injection taken by the formula: (237.73 / 274.19) (2C / V) (A_U / A_S), in which 237.73 and 274.19 are the molecular weights of ketamine and ketamine hydrochloride, respectively, C is the concentration, in mg per mL, of USP Ketamine Hydrochloride RS in the Standard solution, V is the volume, in mL, of Injection taken, and A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively.

Ketamine
Ketalar®

NOTE: Ketamine is a schedule C-III controlled substance.

Description: Ketamine is a nonbarbiturate, dissociative anesthetic used parenterally to provide anesthesia for short diagnostic and surgical procedures and to supplement low-potency anesthetics such as nitrous oxide. It is also used as an inducing agent, as an adjunct to supplement local and regional anesthesia. Ketamine can be used concomitantly with muscle relaxants without complication because it does not provide muscle relaxation. Ketamine is a fairly short-acting anesthetic that provides a profound, rapid, dissociative anesthesia and a short recovery time. It is a desirable agent in patients who are hypotensive. The patient's airway remains intact due to maintenance of pharyngeal and laryngeal reflexes. Ketamine efficacy is offset by the frequent occurrence of dysphoria.

Mechanism of Action: Although the exact mechanism of action is not known, ketamine appears to be an agonist at CNS muscarinic acetylcholine-receptors and opiate-receptors. Ketamine depresses the thalamoneocortical pathways involved in pain perception and has been shown to suppress spinal cord activity. Ketamine's dysphoric adverse reactions may be a result of interactions with the sigma opiate receptor. Clinical effects observed following ketamine administration include a "dissociative anesthesia," increased blood pressure, and minimal respiratory depression. Ketamine has no effects on pharyngeal or laryngeal reflexes.

Pharmacokinetics: Ketamine is administered parenterally. Following IM injection, it is rapidly absorbed. Ketamine readily crosses the placenta and is rapidly distributed into the brain and other highly perfused tissues. Studies in animals reveal that ketamine is highly concentrated in the lung, body fat, and liver. The alpha phase of ketamine distribution lasts about 45 minutes, with a half-life of 10-15 minutes. The first phase corresponds clinically to the anesthetic effect of the drug. When administered intravenously, a sensation of dissociation occurs in 15 seconds, and anesthesia occurs within 30 seconds (in 3-4 minutes for IM route). The anesthetic effects are terminated by a combination of redistribution and hepatic biotransformation to an active metabolite, which is about as active as ketamine in reducing halothane MAC requirements. The beta phase half-life of ketamine is about 2-3 hours. Metabolites are excreted renally (90%) and fecally (5%), with 4% of an administered dose excreted unchanged in urine. Anesthesia lasts 5 to 10 minutes for IV administration and 12-25 minutes for IM administration.

[Revised 5/18/2001]

Product Identification:
Ketalar® Injection

Monarch Pharmaceuticals

Available as ketamine hydrochloride equivalent to 10 mg of ketamine base per ml in 20ml vials; ketamine hydrochloride equivalent to 50 mg of ketamine base per ml in 10 ml vials; or ketamine hydrochloride equivalent to 100 mg of ketamine base per ml in 5 ml vials.
50 mg/ml
vial
red cap

4.0 PRODUCTION PROCESSES AND ANALYSIS

HSDB - Hazardous Substances Data Bank

Manufacturing/Use Information

Methods of Manufacturing:

Product resulting from grignard reaction involving o-chlorobenzonitrile & bromocyclopentane is treated in presence of strong alkali to form epoxy cmpd. Reaction of this with methylamine yields imine which rearranges on heating in presence of hcl.

Formulations/Preparations: Ketamine hydrochloride is supplied as an acidic soln for iv or im use in ampuls containing 10 or 50 mg of ketamine base per ml.

Manufacturers: Warner-Lambert Co, Hq, 201 Tabor Rd, Morris Plains, NJ 07950, (201) 540-2000; Parke-Davis, division; Specialty Chemicals, 188 Howard Ave, Holland, MI 49423

5.0 PRODUCTION AND IMPORT VOLUMES

Figures for production volume and marketed units of ketamine are currently unavailable and may not be relevant since this compound is not an industrial chemical or an environmental toxicant. A more relevant measure, from a risk analysis point of view, is the yearly number of children treated with ketamine in the emergency department (ED) as well as those undergoing elective surgery. Pena and Krause (Pediatrics 34: 483, 1999) report that during the period of August, 1997 and July, 1998 over 220 children were treated with ketamine in the ED of a single large urban pediatric teaching hospital (Children's Hospital, Harvard Medical School). If these figures are expanded or projected for all of the treatment centers across the United States, the yearly numbers of children exposed could by conservative estimates easily exceed 20,000 – 30,000. The figure is very much larger when worldwide use is estimated.

6.0 USES

Human: Used alone and in combination with other agents as an anesthetic for short-term pediatric and adult surgical procedures.

Veterinary: Ketamine has not been approved for use in animals intended for human consumption. although ketamine has been approved by the FDA for use only in the cat and subhuman primates, it has been used in a number of other species. Ketamine-HCl and xylazine-HCl used in combination were effective in immobilizing captive & wild black bears. Dosages used were 4.5 to 9 mg/kg (ketamine) with 2 to 4.5 mg/kg (xylazine). Induction times for small bears (approx 25 kg) were shorter than for larger bears.

Major Uses: Medication in Veterinary Medicine.

7.0 ENVIRONMENTAL OCCURRENCE - NONE

8.0 HUMAN EXPOSURE: None except possibly during manufacture and as a result deliberate drug administration

9.0 REGULATORY STATUS: Ketamine is an FDA drug approved for use as an anesthetic in adults. It's patent is about to expire (March 2002) at which time generic preparations will be eligible for consideration.

10.0 CLINICAL PHARMACOLOGY

Indications:

- general anesthesia induction
- general anesthesia maintenance
- sedation induction

Dosage:

Parenteral Administration

- Ketamine is administered intramuscularly or intravenously.
- To prevent vomiting and aspiration, administer ketamine on an empty stomach.
- Do not mix ketamine and diazepam in the same syringe.
- Visually inspect parenteral products for particulate matter and discoloration prior to administration whenever solution and container permit.

Intramuscular injection:

- No dilution necessary.
- Inject into a large muscle mass. Aspirate prior to injection to avoid injection into a blood vessel.

Direct IV injection:

- Dilute the desired dose of the 100-mg/ml concentration with equal parts of sterile water for injection, NS, or D_5W.
- Inject intravenously over 60 seconds. More rapid injection can cause respiratory depression, apnea, or hypotension. Monitor heart rate, respiratory rate, and blood pressure during IV use.

Continuous IV infusion:

- Dilute 10 ml of the 50-mg/ml injection or 5 ml of the 100-mg/ml injection in 500 ml of NS or D_5W and mix well. The resultant infusion solution should contain 1 mg/ml of ketamine. If fluid restriction is necessary, 250 ml of diluent may be used to give a concentration of 2 mg/ml.
- Infuse intravenously at a rate of 1-2 mg/minute. Titrate rate based on patient response. The development of tonic-clonic movements during ketamine anesthesia does not necessitate a dosage increase. Monitor heart rate, respiratory rate, and blood pressure during IV use.

For general anesthesia induction:

Intravenous dosage:

- Adults: 1-4.5 mg/kg IV, slowly over 60 seconds. Approximately 2 mg/kg will produce 5—10 minutes of surgical anesthesia.
- Children: 0.5-2 mg/kg IV. Do not exceed 0.5 mg/kg/minute or administer any dose faster than over 60 seconds. Usual induction doses are 1-2 mg/kg IV and will produce 5-10 minutes of surgical anesthesia.

Intramuscular dosage:

- Adults: 6.5-13 mg/kg IM. Approximately 10 mg/kg will produce 12-25 minutes of surgical anesthesia.
- Children: 3-7 mg/kg IM will produce 12-25 minutes of surgical anesthesia.

For general anesthesia maintenance:

Parenteral dosage:

- Adults and children: 0.5-4.5 mg/kg IV slowly over 60 seconds or IM, repeated as needed.

For pre-procedure **sedation induction** † (minor procedures):

Oral dosage:
- Children: 6-10 mg/kg PO (mixed in cola or other beverage) given 30 minutes before procedure.

Intravenous dosage:
- Children: Usual dosage is 0.5-1 mg/kg IV (range: 0.5-2 mg/kg). Do not exceed 0.5 mg/kg/min or administer any dose faster than over 60 seconds.

Patients with renal impairment:
Specific guidelines for dosage adjustments in renal impairment are not available; it appears that no dosage adjustments are needed.

†non-FDA-approved indication

PHARMACOKINETICS

Absorption, Distribution and Excretion

1. Drug is eliminated via kidneys.

2. Animal studies indicate that ketamine hydrochloride is rapidly absorbed after parenteral administration and rapidly distributed to all body tissues. Relatively high concentrations are found in body fat, liver, lung, and brain; lower concn in heart, skeletal muscle, and blood plasma. Placental transfer has been shown to occur in dogs and monkeys.

3. Placental transfer of ketamine occurred after iv doses to women and the levels of anesthetic in cord blood equaled, or exceeded, those in plasma within 1.5 min of dosing...the apparent volume of distribution is 3.3 l/kg, and the clearance rate is 1.3 l/min.

4. After IV injection of 0.25 & 0.125 mg/kg to 5 volunteers, ketamine-hcl disposition was rapid and fitted a 2-compartmental model with disposition half-times during fast & slow phases of 16 & 180 min; clearance was 17.7 ml/min/kg. Pharmacokinetics values were independent of dose. The short period of analgesia (5-10 min at 0.25 mg/kg & less than 5 min at 0.125 mg/kg) corresponded to a plasma concn of more than 100 ng/ml. absorption was rapid & extensive after im injection (0.5 mg/kg) & concn were above 100 ng/ml within 10 min. oral admin (0.5 mg/kg) produced low concn & extent of absorption was small.

5. Renal elimination accounts for 90% of the total, with about 4% as unchanged ketamine. Fecal elimination accounts for up to 5%. /Ketamine/

BIOLOGICAL HALF-LIFE

1. 2.5 to 4 hours

2. The distribution half-life is approximately 7 to 11 minutes and the elimination half-life is approximately 2 to 3 hours. /Ketamine/

METABOLISM/METABOLITES

1. Biotransformation of ketamine in rhesus monkeys and in man involves oxidative n-demethylation, hydroxylation of the cyclohexanone ring, and dehydration of the hydroxylated metabolites to give the cyclohexanone derivative.

2. In 5 volunteers concentrations of norketamine and dehydronorketamine were higher after oral than after im admin, suggesting that extensive first-past metabolism occurred.

3. The pharmacokinetics of ketamine-hcl in the cat was described by a 2-compartment open model. The n-dealkylated amine metabolite of ketamine-hcl was detected in plasma of all cats and peak levels which were 0.27 to 0.38 times ketamine-hcl level were reached between 5 and 20 min after injection.

Interactions with:
- Antidepressants
- Articaine; epinephrine
- Barbiturates
- Benzodiazepines
- General anesthetics
- Levodopa
- Local anesthetics
- Opiate agonists
- Phenothiazines
- Sedating h1-blockers
- Skeletal muscle relaxants
- Thyroid hormones

Concurrent use of ketamine with CNS depressants can potentiate their CNS depression and increases the risk of developing respiratory depression. Commonly used CNS depressants include ethanol, general anesthetics, local anesthetics, antidepressants, sedating H_1-blockers, opiate agonists, skeletal muscle relaxants, phenothiazines, barbiturates, and benzodiazepines. Concurrent use of halogenated general anesthetics, such as enflurane, halothane, methoxyflurane, and isoflurane, can lengthen the elimination half-life of ketamine and delay recovery. Patients taking thyroid hormones have an increased risk of developing hypertension and tachycardia when given ketamine.

Concomitant use of antihypertensive agents and ketamine increases the risk of developing hypotension. Prior to general anesthesia, carbidopa; levodopa may be continued as long as the

patient is permitted to take oral medication. If levodopa-based therapy is interrupted temporarily, the patient should be observed for signs of neuroleptic malignant syndrome, and the usual dosage should be administered as soon as the patient is able to take oral medication. The use of articaine; epinephrine during or following treatment with general anesthetics has been associated with the development of cardiac arrhythmias, and should be avoided if possible.

Drugs that have significant interactions with **ketamine**:
- Amobarbital
- Atropine; Hyoscyamine; Phenobarbital; Scopolamine
- Butabarbital
- Desiccated Thyroid
- Enfluorane
- Etomidate
- Halothane
- Isoflurane
- Levothyroxine
- Lyothyronine
- Liotrix
- Methoxyflurane
- Pentobarbital
- Phenobarbital
- Primidone
- Secobarbital

Adverse Reactions:
- Anorexia
- Anxiety
- Apnea
- Delirium
- Diplopia
- Dysphoria
- Hallucinations
- Hypertension
- Hypotension
- Insomnia
- Laryngospasm
- Maculopapular rash
- Nausea/vomiting
- Nightmares
- Nystagmus
- Psychological dependence
- Psychosis
- Respiratory depression
- Sinus bradcardia

- Sinus tachycardia
- Tolerance
- Withdrawal

Cardiovascular side effects of ketamine use include (in order of decreasing frequency): hypertension and sinus tachycardia, hypotension and sinus bradycardia, and other cardiac arrhythmias (rare). Respiratory depression and apnea have been reported and are more likely to occur following rapid administration or high doses of ketamine. Laryngospasm and other forms of airway obstruction are rare but can occur.

Nausea/vomiting, injection site reaction, maculopapular rash (morbilliform), and anorexia have also been reported, although they are rare adverse reactions to ketamine use. In patients receiving ketamine, CNS and psychological adverse effects can occur in as many as 12% of patients and include visual hallucinations, nightmares or illusions, and post-anesthesia emergence delirium (often consisting of dissociative or floating sensations). These reactions occur more frequently in patients between 15 and 45 years of age and typically last only a few hours, although some patients may experience flashbacks several weeks postoperatively. Outpatients should not be released until recovery is complete and should be accompanied by a caregiver. Less frequent or rare CNS side effects include diplopia and nystagmus. Ketamine has been reported being used as a drug of abuse leading psychological dependence. Reports suggest ketamine produces a variety of symptoms including, but not limited to, anxiety, dysphoria, disorientation, insomnia, flashbacks, hallucinations, and psychosis. Ketamine physical dependence and tolerance are possible following prolonged administration. A withdrawal syndrome with psychotic features has been described following discontinuation of long-term ketamine use. Tonic/colonic movements, which can resemble seizures, have occurred with ketamine use and are believed to result from enhanced skeletal muscle tone.

Contraindications:
- Alcoholism
- Cardiac disease
- Driving or operating machinery
- Ethanol intoxication
- Head trauma
- Heart failure
- Hypertension
- Increased intraocular pressure
- Intracranial bleeding
- Intracranial mass
- Intravenous administration
- Myocardial infarction
- Ocular trauma
- Psychosis
- Schizophrenia
- Stroke

- Substance abuse
- Thyrotoxicosis

Ketamine is contraindicated in patients with hypertension, cardiac disease, myocardial infarction, congestive heart failure, stroke, head trauma or intracranial mass, or intracranial bleeding, or in other patients in whom a significant rise in blood pressure would be hazardous. Similarly, ketamine is relatively contraindicated in patients with increased intracranial pressure, space-occupying intracranial lesions, or CNS hemorrhage because it has been reported to increase the CSF pressure. Outpatient surgery patients should not be released until recovery is complete and should be accompanied by a caregiver. Patients should be instructed to avoid driving or operating machinery until they have recovered from the effects of the anesthesia. Ketamine must be administered slowly over at least 60 seconds; more rapid intravenous administration can result in respiratory depression, apnea, and enhanced pressor response. Use with caution in patients with ocular trauma resulting in open globe injury or increased intraocular pressure because ketamine can further increase intraocular pressure. Ketamine is relatively contraindicated in patients with psychiatric disorders, such as schizophrenia or acute psychosis, and in patients with alcoholism or acute ethanol intoxication. Use ketamine with caution in patients with a history of thyrotoxicosis because they are at an increased risk of developing tachycardia and hypertension.

Use with caution during surgical procedures of the pharynx, larynx, or trachea because ketamine increases salivary and tracheal-bronchial secretions and usually does not suppress pharyngeal and laryngeal reflexes. Substance abuse of ketamine has been reported. A variety of symptoms including physical dependence, tolerance, and a withdrawal syndrome have been reported with long-term ketamine use (see Adverse Reactions). Therefore, ketamine should be prescribed and administered with caution.

11.0 TOXICOLOGICAL DATA

11.1 General Toxicology

Human Toxicity Excerpts

Studies on effects of ketamine on fetus when used during delivery indicate that doses greater than 2 mg/kg are likely to cause fetal depression.

Non-Human Toxicity Excerpts

1. Excessive salivation and tachycardia may occur 10-60 min after admin to cats. causes convulsions and barking fits in dogs, which do not occur when it is added to other psychotropic drugs.

2. Ketamine-HCl admin IM @ dose of 120 mg/kg into rats on gestation days 9-13 was not toxic or teratogenic to fetuses examined on day 21 of pregnancy.

3. Adverse reactions reported have included emesis, salivation, vocalization, erratic and prolonged recovery, dyspnea, spastic jerking movements, convulsions, muscular tremors,

hypertonicity, opisthotonos and cardiac arrest. Ketamine-hcl admin altered the hemogram of rhesus monkeys when compared to physiological restraint for venipuncture. Alterations were decreases in leukocyte count, total plasma proteins, and hematocrit. The decrease in leukocyte count was due primarily to a decr in lymphocytes with a smaller decr in neutrophils.

4. 75 mg/kg ketalar admin ip in rats of both sexes showed relationship between increased age and decreased duration of sleeping time for both sexes for 1st 3 wk of age, assoc with increased amount of its cyclohexanone oxidn metabolite. Males had greater ability to produce metabolite.

Non-Human Toxicity Values

1. LD50 Mouse IP 224 mg/kg /Ketamine/

2. LD50 Rat IP 229 mg/kg /Ketamine/

11.2 NEUROTOXICOLOGY

The discovery that NMDA receptor antagonists cause dose-dependent reversible or irreversible degeneration in cerebrocortical neurons in adult rats was made over ten years ago (Olney et al., 1989), and has since been referred to as the "Olney lesion". At that time there was no distinction between the type of effects observed in the adult rat 4 hours after exposure and those seen in the developing brain. The presumption was that the latter was much less sensitive (Farber et al., 1995). This was later shown to be incorrect because it requires 16-24 hrs for the developing brain to manifest profound apoptotic changes, and this process is entirely different and distinct from alterations that are the result of excitotoxicity (Ishimaru et al., 1999).

In a recent report, Olney and Coworkers (Ikonomidou et al., 1999) made the following statement: "Programmed cell death (apoptosis) occurs during normal development of the central nervous system, however the mechanisms that determine which neurons will succumb are poorly understood. Blockade of N-methyl-D-aspartate (NMDA) glutamate receptors for only a few hours during the late fetal or early neonatal life triggered widespread apoptotic neurodegeneration in the developing rat brain, suggesting that the excitatory neurotransmitter glutamate, acting at NMDA receptors, controls neuronal survival. These findings may have relevance to human neurodevelopmental disorders…."

Olney et al., (2000) proposed that a variety of agents including several NMDA antagonists: "have the potential to delete large numbers of neurons from the developing brain by a newly discovered mechanism involving the interference in the action of neurotransmitters (glutamate and gamma amino butyric acid, GABA) at NMDA and $GABA_A$ receptors during the synaptogenesis period, also known as the brain growth-spurt period. Transient interference (lasting $\geq$ 4 hrs) in the activity of these transmitters during the synaptogenesis period (the last trimester of pregnancy and the first several years after birth in humans) causes millions of developing neurons to commit suicide (die by apoptosis)."

These very recent findings are the first to describe and establish the significant difference of the so-called "Olney lesion" involving brain neurodegeneration and vacuolation seen in adult rats following high doses of NMDA receptor antagonists, with the pathology consisting of apoptosis in multiple brain regions observed in neonates. Olney found that apoptosis was greatest in rats administered ketamine 7 days after birth (PND –7). This coincides with the greatest period of hypersensitivity of NMDA receptors. Blockade of these receptors during this period resulted in deletion of large numbers of neuronal cells by significantly increasing the density of apoptotic neurons in various brain regions. The increases in apoptotic cell density varied from 3 to 39 fold depending on the NMDA blocker administered and the area of the brain measured. These changes were dose-dependent, neuron specific and did not occur when blockers of other receptor types (Ca^{++} channel, muscarinic, non-NMDA glutamatergic or dopaminergic) were administered. PND-7 represents the age when the rat forebrain is most vulnerable to the apoptotic effects of the NMDA antagonists indicating the dependence of this cell type on glutamatergic input for survival. This period also corresponds to the greatest expression of NR1 as well as the peak of the brain growth-spurt. The data indicate that the NMDA receptor must be blocked for at least 4-6 hrs at a threshold dose of 0.25 mg/kg (+) MK-801. This phenomenon is stereospecific in that the same dose of (-) MK-801 showed a much weaker effect.

Recently, within the last year, a ketamine study was conducted by FDA that replicated and confirmed the findings of Olney et al. The administration of ketamine to PND-7 rats at a dose of 20 mg/kg, subcutaneously, 7 times at 90 minute intervals and sacrificed at 24 hrs later produced widespread neurodegeneration of apoptotic origin throughout the brain of treated animals. The brains were smaller than controls in proportion to the severity of the lesions. The animal experiments and brain perfusions were performed in the laboratory of Dr. Neil Grundberg at USUHS by Hirsch Davis and Scott Pine of FDA/CDER. The histological preparation, analysis and report was conducted and prepared by Dr. Robert Switzer, Director of NeuroSciences Associates.

CONCLUSION from APPENDIX A

In addition, Drs. Andrew Scallet and Larry Schmued of NCTR, Division of Neurotoxicology conducted a separate pathology study on sections that had been prepared by Dr. Switzer. They made similar observations of extensive neurodegeneration in all parts the brain having prepared silver, fluorojade, GFAP, Caspase (rhodamine) and DAPI stained sections. The also conducted a stereology study, in which they counted the numbers of degenerating cells in various parts of the brain of treated and control rats (see **CONCLUSION FROM Appendix B).**

Blood samples were obtained from all treatment groups one minute after injection, plasma prepared, and ketamine determine in each of these samples by Dr. Pat Faustino of the Division of Product Quality/OPS CDER. The results of these analyses indicated levels of ketamine in rats that were similar (within 2 fold or less) as those found in children receiving an equivalent dose. (data not presented).

Recently the FDA Expert Working Group completed a review article entitled: "Ontogeny of the N-methyl-D-Aspartate (NMDA) Receptor System and Susceptibility to Neurotoxicity." A draft (preprint) of this document is to be found in Appendix C.

12.0 CONCLUSIONS

The FDA confirmation, of Olney's findings of widespread neural degeneration throughout the brain in PND-7 rats at doses used in children during pediatric surgical procedures, makes it imperative that these studies be extended in an appropriate non-human primate model. The rat model used takes advantage of the period of greatest growth and proliferation of the brain in this species, however its applicability to human neurotoxicology remains problematic. The non-human primate by virtue of its genetic similarity to humans, its pattern of brain development and closest to human level of encephalization and brain organization is the most logical choice as a surrogate for a human clinical trial.

A non-human primate study should be designed to explore the vulnerability of the monkey brain to ketamine during its period of most rapid development and conducted in a way that optimizes its analogy to patterns of pediatric exposure to this drug.

REFERENCES

1. Olney JW, Labruyere J, Price MT (1989) Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 244:1360-1363
2. Farber NB, Wozniak DF, Price MT, Labruyere J, Huss J, St.Peter H, Olney JW (1995) Age-specific neurotoxicity in the rat associated with NMDA receptor blockade: Potential relevance to schizophrenia. Biol.Psychiatry 38:788-796.
3. Ishimaru MJ., Ikonomidou C., Tenkova TI., Der TC., Dikranian, K.,Sasma MA, and Olney JW. (1999) Distinguishing excitotoxic from apoptotic neurodegeneration in the developing rat brain. J. Comp. Neurol. 408: 461-475.
4. Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vockler J, Dikranian K, Tenkova TI, Stefovska V, Turski L, Olney JW (1999) Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 283: 70-74.
5. Olney, J.W., Farber, N.B, Woznizk, D.F., Jevtovik-Todorovik, V. and Ikonomidou, C. (2000). Environmental agents that have the potential to trigger massive apoptotic neurodegeneration in the developing brain. Environmental Health Perspectives 108: (Suppl. 3) 383-388.

APPENDIX A

CDER/OPS – Rapid Response Report #102 – February 2001

REGULATORY BACKGROUND

Ketamine is being used off -label as an anesthetic in children. Ketamine is a noncompetitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist. NMDA antagonists have been shown to induce neuropathological effects in adult rats, beginning with formation of intracellular vacuoles followed by neuronal cell death in distinct brain regions. In young rats developmental neurotoxicity has also been reported. An important question is, could ketamine have similar effects in pediatric patients?

KETAMINE

Ketamine is a nonbarbiturate, dissociative anesthetic used to provide anesthesia for short diagnostic and surgical procedures. It may also be used as an inducing agent, to supplement low-potency anesthetics such as nitrous oxide, and as a supplement to regional anesthesia. It is short acting and provides rapid dissociative anesthesia followed by a short recovery time. It received FDA approval in 1970 for use as an anesthetic. Studies in animals reveal that ketamine is highly concentrated in the lung, body fat and liver (1). Upon IM injection, the anesthetic effect lasts approximately 45 minutes with a half-life of 15-30 min. After intravenous administration it takes approximately 15 seconds for dissociation to occur and anesthesia is accomplished within 30 seconds, but the duration of anesthesia is shorter than that obtained after IM injection. Termination of the anesthetic effect is due to redistribution and hepatic biotransformation. This process has an approximate half-life of 2-3 hours.

There are numerous adverse reactions associated with ketamine (1). Psychological and other adverse CNS effects can occur in as many as 12% of patients. These effects range from visual hallucinations, nightmares or illusion, and post-anesthesia delirium. These reactions occur most frequently in patients between 15-45 years and generally last only a few hours post anesthesia. Some patients have reported experiencing flashbacks several weeks afterwards.

Ketamine is a *noncompetitive* inhibitor of the NMDA glutamate receptor (2) and it exhibits stereospecific action. The dextro-S (+) form is three- to four-fold more potent that the levo-R (-) form. The dextro-form is more rapidly cleared, while the levo-form is considered to produce a higher rate of emergence reactions (PCP-like psychotomimetic action) and agitated behavior. These undesirable effects are more common in adults than children, in women more than in men, and generally only at higher doses of ketamine (3). The short pharmacological half-life of this compound is related to its low affinity for the NMDA receptor.

In adult swine, it has been shown that some of the neurotoxic effects of ketamine are not observed in the absence of preservative (4): neurotoxic effects were only observed with the combined treatment of ketamine with preservatives.

KETAMINE USE IN PEDIATRICS

In a 1994 workshop on the evaluation of neurotoxicity associated with NMDA receptor antagonists, then Director of the Division of Neuropharmacological Products, Dr. Paul Leber, warned that "NMDA antagonists pose a potential threat to the developing brain" and that treatment with NMDA antagonists "is especially relevant to [their] use…in children" (5).

Recently, there has been a resurgence in the use of ketamine for emergency-department procedures requiring anesthesia (6). Administration protocols for ketamine include the nasal, oral, rectal, intramuscular and intravenous routes. IM and IV are the most common routes employed (6). In numerous epidemiological studies, it has been concluded that ketamine can be used safely for anesthesia in infants and children (5,6,7,8,9). However, numerous adverse events have been associated with ketamine's use including emesis, agitation during recovery, apnea, respiratory depression, and laryngospasm (7,8). In the case of inadvertent ketamine overdoses in children, adverse effects relating to respiratory difficulties were reported for some, but there were no persistent neurological findings (10).

NMDA RECEPTOR ACTIVITIES IN RATS

Olney lesion. The noncompetitive NMDA receptor antagonists have been investigated for their potential as neuroprotective therapeutic agents. However, there have been considerable concerns as to their potential psychotomimetic and memory-impairing side effects (11). In 1989, Olney and colleagues (12) reported that PCP, MK-801 and related NMDA antagonists induced a neurotoxic response in the posterior cingulate and retrosplenial cortex of the adult rat. Mitochondria and endoplasmic reticulum are acutely transformed into large intracytoplasmic vacuoles. Mitochondria disappear from the cytoplasm (12). Relatively, low doses of NMDA antagonists induced this response but it was found to be reversible over 24 hours. In a similar manner, the glial marker, glial fibrillary acidic protein (GFAP), generally considered to be a marker of neurotoxicity, exhibited a transient dose-dependent increase (13). Higher doses or longer durations of treatment lead to neuronal cell death and to the involvement of neurons in other neocortical and limbic regions (11, 14, 15). In addition, extensive gliosis is visible as late as 14 days after MK-801 administration (16).

Behavioral effects. NMDA receptor antagonists have been shown to have an impact on learning and memory functions. Higher doses of NMDA antagonists given acutely induce increased locomotion in rats rendering them incapable of normal behavioral functioning (17). The acute effects of lower doses are minimal. Significant changes in memory and learning functions are observable with chronic treatment of rats with lower doses of NMDA antagonists (17). The types of effects seen depend on the dose and the behavioral paradigm used. Results are often complicated but changes in normal behavioral functioning have been demonstrated. Mice may be more sensitive than rats (11).

Developmental Effects. In 1994, Dr. Leber reported, "NMDA antagonists pose a potential threat to the developing brain" (3). He continues that this is not necessarily due to the toxic nature of these compounds, but possibly due to their ability to interfere with the process of neuronal connectivity which is very active during early stages of brain development. In 1994, Dr.

Constantine-Paton warned that disturbing NMDA receptor function could "severely disrupt" the wiring of neuronal networks (18). However, she recognized that there was insufficient information at the time to know with any certainty. In an older study by Olney and colleagues (19), using vacuoles as markers of neurotoxicity, infant animals were shown to be insensitive to MK-801 neurotoxicity. Onset of vacuole formation was seen in 45-day-old rats. However, in a recent study using apoptosis of neurons in the developing brain as a biomarker of neurotoxicity caused by NMDA receptor antagonists, significant increases in apoptosis were noted in subjects as young as 7 days (20). This study suggested that the NMDA receptor was acting as a critical component of neuronal survival during development. In addition, the increased apoptosis was found in multiple brain regions, and thus was not limited to the posterior cingulate and retrosplenial cortices as reported in the original studies in adult animals. Thus, use of an alternative biomarker for neurotoxicity demonstrated an entirely different result from that reported earlier. These observations suggest that the intracellular vacuoles induced by NMDA antagonists might not be appropriate biomarkers for neurotoxicity in the developing brain.

DEVELOPMENTAL EFFECTS OF KETAMINE IN RATS

In the first paper reporting the Olney lesion (12), a single high dose of ketamine was shown to induce vacuole formation. Thus, it was placed in the same class as other competitive and noncompetitive NMDA antagonists. Numerous studies since have corroborated these ketamine findings. A higher acute dose of ketamine is necessary to induce these effects, possibly due to its short half-life. In the discussion by Constantine-Paton, it was also suggested that NMDA receptor antagonists might affect neuronal wiring (18). Recent studies by numerous groups have shown that ketamine does affect neuronal functioning in the developing brain of the rat. The work of Olney and colleagues in1999 (20) shows significant apoptosis in multiple brain regions after multiple injections of ketamine in 7 day old rats. In addition, there are significant decreases in polysialylated neural cell adhesion molecules and postsynaptic densities during striatal development after single exposures in day 20 animals (21). These findings may be due to the increased neuronal cell loss due to the ketamine treatment. These studies provide strong evidence that ketamine may have profound effects on neuronal development in the developing rat brain, hence, supporting the concerns of Drs. Constantine-Paton and Leber.

EFFECTS OF KETAMINE IN PRIMATES VS. RATS

In the 1994 NIMH-organized workshop on NMDA antagonist actions, considerable concerns were expressed on the potentially hazardous effects of these agents on the developing brain. The majority of cited studies were, however, performed in the rat. All discussants strongly supported the extension of these studies to other animal models such as nonhuman primates. There is considerable information on the ontogeny of glutamate receptors in humans (22). It is generally considered that NMDA receptor binding sites are present in the human fetal brain by gestational day 115, increase until gestational days 140-150 and then decrease slightly by gestational days 168-182 (see attached review, Haberny et al.). The localization of NMDA receptors in monkey cortices is similar to that in human (23). In, contrast, the distribution of NMDA receptors is different in rats and monkeys (24). In earlier studies by Corey-Slechta and colleagues (17), it was shown that there were differences between rats and monkeys with respect to the selective nature of NMDA receptor complex antagonists on the learning process.

In 2000, it was shown using Positron Emission Tomography (PET), that ketamine rapidly distributes throughout the monkey brain in a number of different regions (25). In addition, ketamine is shown to interfere with dopamine receptor binding in the monkey brain (26). Studies by Paule and colleagues (27) demonstrated differential effects of the NMDA antagonists, MK-801 and PCP, on learning tasks in adult non-human primates. These studies suggested that MK-801 could be expected to have an effect on learning behavior in humans. In earlier reports on ketamine there is conflicting evidence as to its effects on learning and other behaviors in monkeys (28,29). While the behavioral tests used in these nonhuman primate studies have not been consistent across studies, all studies have indicated an ability of NMDA antagonists to disrupt important brain functions. Additionally, there are studies on the effects of NMDA antagonists on non-human primates that have not been published. These generally suggest that there are significant neurotoxic actions of NMDA antagonists. Studies in non-human primates are sparse and limited making conclusions difficult or tenuous. No studies focused on the effect of ketamine or related NMDA antagonists on apoptosis during development could be found for nonhuman primates.

CONCLUSIONS

Ketamine does have significant undesirable effects in the developing rat brain. These effects to alter apoptosis are not the classical Olney lesion (vacuole formation) seen in adult rats but the consequences may be far more serious, as a larger proportion of the brain's eventual complement of neurons may be compromised. Based on the information in the literature, another rat study is not necessary because a number have already been performed. The extant data in primates are very poor and inconclusive. The concern with respect to ketamine exposure in children is most likely not due to bouts of acute, single dose exposures, but rather to bouts of repeated doses over a relatively short period of time. If for example a second or multiple doses were applied to an infant to extend the duration of anesthesia, this may raise some concerns. Additionally, there is a longer period of development of the primate brain compared to the rat, and it is difficult to match the exact age at which a particular neurodevelopmental stage commences across species.

In summary, it is considered that a nonhuman primate study would be most appropriate to determine if the significant apoptosis seen in the rat exposed to ketamine during development is a potential concern for the developing human.

REFERENCES

1. Ketamine. (2001) Clinical Pharmacology Online.
2. Bergmann SA (1999) Ketamine: Review of its pharmacology and its use in pediatric anesthesia. Anesth.Prog. 46:10-20.
3. White PF, Ham J, Way, WL (1980) Pharmacology of Ketamine isomers in surgical patients. Anesthesiology 52:231.
4. Errando CL, Sifre C, Moliner S, Valia JC, Gimeno O, Mingues A, Biol P (1999) Subarachnoid Ketamine in swine – pathological findings after repeated doses: acute toxicity study. Reg.Anesth.Pain Med. 24:146-152.
5. Leber P (1994) Introduction. Psychopharm. Bull. 30:527-532.

6. McCarty EC, Mencio GA, Walker LA, Green NE (2000) Ketamine sedation for the reduction of children's fractures in the emergency department. J.Bone Joint Surg. 82A: 912-918.
7. Green SM, Rothrock SG, Lynch EL, Ho M, Harris T, Hestdalen R, Hopkins GA, Garrett W, Westcott K (1998) Intramuscular Ketamine for pediatric sedation in the emergency department: safety profile. Ann.Emerg.Med. 31: 688-697.
8. Green SM, Kupperman N, Rothrock SG, Hummel CB, Ho M (2000) Predictors of adverse events with intramuscular Ketamine sedation in children. Ann. Emerg.Med. 35:35-42.
9. Green SM, Rothrock SG, Harris T, Hopkins GA, Garrett W, Sherwin T (1998) Intravenous Ketamine for pediatric sedation in the emergency department: safety profile with 156 cases. Acad.Emerg.Med. 5:971-976.
10. Green SM, Clark R, Hostetler MA, Cohen MD, Carlson D, Rothrock SG (1999) Inadvertent Ketamine overdose in children: clinical manifestations and outcome. Ann.Emerg.Med. 34:492-497.
11. Olney JW (1994) Neurotoxicity of NMDA receptor antagonists: an overview. Psychopharm.Bull. 30:533-540.
12. Olney JW, Labruyere J, Price MT (1989) Pathological changes induced n cerebrocortical neurons by phencyclidine and related drugs. Science 244:1360-1363.
13. O.Callaghan JP (1994) Biochemical analysis of glial fibrillary acidic protein as a quantitative approach to neurotoxicity assessment: Advantages, disadvantages and application to the assessment of NMDA receptor antagonist-induced neurotoxicity. Psychopharmacol.Bull. 30:549-554.
14. Ellison G, Switzer RC (1993) Dissimilar patterns of degeneration in brain following four different addictive stimulants. Neuroreport 5:17-20.
15. Horvath Z, Buzsaki G. (1993) MK-801-induced neuronal damage in normal rats. Soc.Neurosci.Abstr. 19:354.
16. Fix AS (1994) Pathological effects of MK-801 in the rat posterior cingulate/retrosplenial cortex. Psychopharmacol. Bull. 30:541-547.
17. Cory-Slechta DA (1994) The impact of NMDA receptor antagonists on learning and memory functions. Psychopharmacol. Bull. 30:601-612.
18. Constantine-Paton M (1994) Effects of NMDA receptor antagonists on the developing brain. Psychopharmacol.Bull. 30:561-556.
19. Farber NB, Wozniak DF, Price MT, Labruyere J, Huss J, St.Peter H, Olney JW (1995) Age-specific neurotoxicity in the rat associated with NMDA receptor blockade: Potential relevance to schizophrenia. Biol.Psychiatry 38:788-796.
20. Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vockler J, Dikranian K, Tenkova TI, Stefovska V, Turski L, Olney JW (1999) Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 283: 70-74.
21. Butler AK, Uryu K, Rougon G, Chesselet MF (1999) N-methyl-D-aspartate receptor blockade affects polysialylated neural cell adhesion molecule expression and synaptic density during striatal development. Neuroscience 89: 1169-1181.
22. Johnston MV (1994) Developmental aspects of NMDA receptor agonists and antagonists in the central nervous system. Psychopharmacol.Bull. 30:567-575.
23. Huntley GW, Vickers JC, Morrison JH (1997) Quantitative localization of NMDAR1 receptor subunit immunoreactivity in inferotemporal and prefrontal association cortices of monkey and human. Brain Res. 749:245-262.

24. Meoni P, Bunemann BH, Trist DG, Bowery NG (1998) N-terminal splice variants of the NMDAR1 glutamate receptor subunit: differential expression in human and monkey brain. Neurosci. Lett. 249:45-48.
25. Tsukada H, Harada N, Nishiyama S, Ohba H, Sato K, Fukumoto D, Kakiuchi T (2000) Ketamine decreased striatal [(11)C] raclopride binding with no alterations in static dopamine concentrations in the striatal extracellular fluid in the monkey brain: multiparametric PET studies combined with microdialysis. Synapse 37:95-103.
26. Hartvig P, Valtysson J, Antoni G, Westerberg G, Langstrom B, Ratti-Moberg E, Oye I (1994) Brain kinetics of ®- and (S)-[N-methyl-11C]ketamine in the rhesus monkey studied by positron emission tomography (PET). Nucl.Med.Biol. 21:927-934.
27. Paule M (1994) Acute behavioral toxicity of MK-801 and phencyclidine: effects on rhesus monkey performance in an operant test battery. Psychopharmacol.Bull. 30:613-621.
28. France CP, Moerschbaecher JM, Woods JH (1991) MK-801 and related compounds in monkeys: discriminative stimulus effects and effects on a conditional discrimination. J.Pharmacol.Exp.Ther. 257:727-734.
29. Thompson DM, Winsauer PH, Mastropaolo J (1987) Effects of phencyclidine, ketamine and MDMA on complex operant behavior in monkeys. Pharmacol.Biochem.Behav. 26:401-405

APPENDIX B

CDER/OPS – Rapid Response Report #105 – April 2001

REGULATORY BACKGOUND

An important issue relates to whether and how plasma levels of ketamine relate to the duration of its anesthetic effect. A secondary issue is whether plasma ketamine levels are comparable or relate to brain levels of ketamine.

INTRODUCTION

Ketamine is used as a dissociative anesthetic for numerous applications. It can be given by a number of different routes of administration. Ketamine is considered to be a very useful anesthetic in pediatrics as it has a short half-life and is reported to have relatively few observable adverse effects. It can rapidly cross the blood brain barrier since it is highly lipophilic. In addition, it can be used in conjunction with other surgical medications such as midazolam, atropine, propofol, fentanyl, and others (1-4). Routes of administration include IM, IV, rectal, oral, and nasal. The dose depends on the route of administration as well as the desired anesthetic response. The maximal dose in humans is 10 mg/kg, with doses in the 1-3 mg/kg range used for IM or IV administration.

Generally, ketamine is used as an anesthetic which upon wearing off exhibits effective analgesic properties (5). Subanesthetic doses of ketamine have been found to induce psychosis in humans and animals (6-8). The subanesthetic effects do not appear to have long-term behavioral consequences.

The major metabolite of ketamine is norketamine and this can be measured in plasma (9). Biotransformation of ketamine occurs predominantly in the liver. Ketamine is more rapidly metabolized in children than in adults and norketamine is an active metabolite with one-third the anesthetic potency of ketamine.

PLASMA LEVELS

Plasma levels of ketamine have been measured in both adults and children. In children, the drug reaches a maximal plasma concentrations in less than10 minutes, depending on the route of administration, and then rapidly declines (9-11). After an additional 10-15 minutes, the rate of decline decreases. 30 - 60 minutes after administration levels are approximately 50% of their highest concentration. For IV injections, peak plasma concentrations occur within one minute, followed by a 15-minute redistribution period (9). The elimination half-life is 2.17 hr and the redistribution half-life is 4.68 min (10). The PK properties of ketamine are relatively similar for all routes of administration. However, the route and process of administration has a significant effect on the maximal plasma concentration attained. When ketamine is coadministered with other anesthetic agents, the redistribution phase is significantly extended (12). The effect on the elimination phase is also extended but to a lesser degree (13). In adults, norketamine levels show

a linear increase over 15-20 minutes and then remain relatively stable for 60 minutes (14). At 60 minutes post ketamine treatment, norketamine and ketamine plasma levels are similar (15).

BRAIN LEVELS

Using Positron Emission Tomography (PET), PK studies have been made on ketamine uptake in the brain of humans, monkeys, rats and mice (14-16). These studies used sub-anesthetic doses of ketamine. It has generally been found across species that the PK properties of the brain are similar to those for plasma. In humans, baboons and mice, labeled ketamine was rapidly taken up and reached peak concentrations within 5 minutes. The concentrations of ketamine were found to be similar in both the plasma and the brain. The drug is relatively uniformly distributed through different brain regions at similar concentrations to those found in the plasma. There may be distinct differences in concentrations in specific brain regions depending on the species but this relates to redistribution of the drug and not to its elimination. In order to determine that the administered material is bound to receptors, it would be necessary to perform appropriate pharmacological tests in exposed subjects. The available data support the notion that ketamine is rapidly taken up into the brain and it's various regions and then eliminated in a manner that parallels that seen for plasma levels.

ANESTHESIA

The majority of pediatric clinical studies involving ketamine usually have some other agent associated with its administration. Anesthesia is usually induced using drug cocktails. As indicated above, lower doses of ketamine (0.02-0.63 mg/kg/min for 60 min) can induce psychosis but not anesthesia (6-8) in adults. The relation between steady state plasma concentrations of ketamine and its pharmacological effects is highly linear between 50 and 200 ng/ml (7). In pediatric procedures, the intention is to allow patients to reach recovery to a degree suitable for emergency department discharge 30 to 120 min after termination of the procedure (10). In a typical study of more than 100 children aged between 14 months to 13 years, ketamine (4 mg/kg) plus atropine (0.01 mg/kg) was administered IM. And if anesthesia was inadequate, a repeat ketamine dose (2 to 4 mg/kg IM) was administered. Maximal effects of ketamine were generally noted within 5-10 min after injection. Dissociation typically persisted for 15 to 30 minutes corresponding to the completion of the redistribution phase (10). The mean time from injection until recovery criteria for discharge was 82 minutes, with a range of 30 to 175 minutes (17). Thus, the recovery from anesthesia tends to correlate with the end of the initial redistribution phase of ketamine. While the recovery from anesthesia is quite rapid, there is a prolonged analgesic effect with ketamine.

RECEPTOR OCCUPANCY

The binding of ketamine to the NMDA receptor is quite specific. MK-801 competes competitively with it (18). The kinetic data supports the rapid association rate of the drug from the receptor. Binding occurs in most brain regions (19) which would correlate with the distribution pattern seen using PET.

In a recent conversation with Dr. J. Olney, whose laboratory produced the results showing the ketamine effects in juvenile rats, he indicated that the apoptotic effect was dependent upon duration of receptor occupancy. It did not correlate to dose, plasma levels or anesthetic state. The dosing regimen that was used for juvenile rats resulted in the animals being in a continual anesthetic state. He indicated that a single high dose of ketamine did not induce the apoptosis in the juvenile rat. In further discussion, he indicated that GABA agonists could also induce the same effect. Often, ketamine is given in conjunction with GABA agonists (20). The GABA agonist would block the release of glutamate. Thus, the overall effect of these two pharmaceuticals is to cause a lack of excitability of glutamatergic cells. Olney's conclusion would suggest that it is the prolonged inexcitability of these cells that results in the noted increases in apoptosis.

CONCLUSION

Blood and brain levels of ketamine correlate well. The biphasic plasma and brain concentration curves correlate with anesthesia and recovery. The redistribution phase of ketamine upon correlates with duration of anesthesia. Norketamine levels remain constant for extended periods of time. The apoptotic response in juvenile rats appears to be related to extended ketamine exposure at levels not typically seen during pediatric use.

REFERENCES

1. J.A. Giovannitti Jr. (1994) Regimens for Pediatric Sedation. Compend.Contin.Educ.Dent. 14:1002-1014.
2. B. Golianu, E.J.Krane, K.S.Galloway, M.Yaster (2000) Pediatric acute pain management. Ped.Clin.Nth.Amer. 47:559-587.
3. A.M.Broennle, D.E.Cohen (1993) Pediatric anesthesia and sedation. Curr.Opin.Pediatrics 5:310-314.
4. A.D.Slonim, F.P.Ognibene (1998) Sedation for pediatric procedures, using ketamine and midazolam, in a primarily adult intensive care unit: a retrospective evaluation. Crit. Care Med. 26:1900-1904.
5. K.W.Hirlinger, W.Dick (19984) Intramuscular ketamine analgesia in emergency patients. II. Clinical study of traumatized patients. Anesthetist. 33:272-275.
6. A.Breier, A.K.Malhotra, D.A.Pinals, N.I.Weisenfeld, D.Pickar (1997) Association of ketamine-induced psychosis with focal activation of the prefrontal cortex in healthy volunteers. Am.J.Psychiatry 154:805-811.
7. T.A.Bowdle, A.D.Radant, D.S.Cowley, E.D.Kharasch, R.J.Strassman, P.P.Roy-Byrne (1998) Psychedelic effects of ketamine in healthy volunteers. Relationship to steady-state plasma concentrations. Anesthesiol. 88:82-88.
8. J.W. Newcomer, N.B. Farber, V. Jevtovic-Todorovic, G. Selke, A.K. Melson, T. Hershey, S. Craft, J.W. Olney (1999) Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. Neuropsychopharmacology 20:106-18.
9. S.M.Green, N.E.Johnson (1990) Ketamine sedation for pediatric procedures: Part 2, review and implications. Annal.Emerg.Med. 19:1033-1046.
10. S.A.Bergman (1999) Ketamine: Review of its pharmacology and its use in anesthesia. Anesth.Prog. 46:10-22.

11. I.S.Grant, W.S.Nimmo, L.R.McNicol, J.A.Clements (1983) Br.J.Anaesth. 55:1107-1111.
12. T.Kudo, M.Kudo, F.Kimura, H.Ishihara, A.Matsuki (1992) Pharmacokinetics of ketamine and pentazocine during total intravenous anesthesia with droperidol, pentazocine and ketamine. Masui 41:1772-1776.
13. P.Hartvig, E.Larsson, P.O.Joachimsson (1993) Postoperative analgesia and sedation following pediatric cardiac surgery using a constant infusion of ketamine. J.Cardiothorac.Vasc.Anesth. 7:148-153.
14. P.Hartvig, J.Valtysson, K.-J.Lindner, J.Kristensen, R.Karlsten, L.L.Gustafsson, J.Persson, J.O.Svensson, I.Oye, G.Antoni, G.Westerberg, B.Langstrom. (1995) Central nervous system effects of subdissociative doses of (S)-ketamine are related to plasma and brain concentrations measured with positron emission tomography in healthy volunteers. Clin.Pharm.Therap.58: 165-173.
15. E. Kumlien, P.Hartvig, S.Valind, I.Oye, J.Tedroff, B.Langstrom (1999) NMDA-receptor activity visualized with (S)-[N-methyl-^{11}C] ketamine and positron emission tomography in patients with medial temporal lobe epilepsy. Epilepsia 40:30-37.
16. C.Y.Shiue, S.Vallabhahosula, A.P.Wolf, S.L.Dewey, J.S.Fowler, D.J.Schlyer, C.D.Arnett, Y.-G.Zhou (1997) Carbon-11 labeled ketamine – synthesis, distribution in mice and PET studies in baboons. Nucl.Med.Biol. 24:145-150.
17. S.M.Green, R.Nakamura, N.E.Johnson (1990) Ketamine sedation for pediatric procedures: Part 1, a prospective series. Annal.Emerg.Med. 19: 1024-1032.
18. R.H.Porter, J.T. Greenamyre (1995) Regional variations in the pharmacology of NMDA receptor channel blockers: implications for therapeutic potential. J.Neurochem. 64:614-623.
19. I.Bresink, W.Danysz, C.G.Parsons, E.Mutschler (1995) Different binding affinities of NMDA receptor channel blockers in various brain regions – indication of NMDA receptor heterogeneity. Neuropharm. 34:533-540.
20. J.W. Olney, N.B.Farber, D.F. Wozniak, V. Jevtovic-Todorovic, C. Ikonomidou (2000) Environmental agents that have the potential to trigger massive apoptotic neurodegeneration in the developing brain. Environ Health Perspect 108: 383-388.

APPENDIX C

Ontogeny of the N-methyl-D-Aspartate (NMDA) Receptor System and Susceptibility to Neurotoxicity

Kathleen A. Haberny, Merle G. Paule, Andrew C. Scallet, Frank D. Sistare, David S. Lester[1], Joseph P. Hanig and William Slikker, Jr., CDER/FDA, Rockville, MD and NCTR/FDA, Jefferson, AR

1 Present address: Pharmacia Corporation, 100 Route 206 North, Peapack, NJ 07977.

Introduction

The N-Methyl-D-Aspartate (NMDA) receptor system is associated with many of the primary functions and developmental mechanisms of the nervous system. Memory is one such primary function. Long-lasting changes in the excitability of several associated neurons as a result of repeated release of L-glutamate and activation of the NMDA receptor are associated with the phenomenon of long-term potentiation (LTP) (Kato et al., 1999). The involvement of the glutamate receptor system and LTP is strongly linked to new learning and memory in animal models (Scheetz and Constantine-Paton, 1994). In regards to brain development, the glutamate receptor system has been implicated as a major signaling pathway for neuronal migration. As developing inputs increase in strength and number, postsynaptic Ca^{++} ion influx through glutamate activated NMDA receptors increases and this Ca^{++} ion influx is postulated to trigger changes in neuronal metabolism and gene expression (Scheetz and Constantine-Paton, 1994).

Along with these central roles as "brain sculptor" and "memory maker," excessive activation or disruption of the NMDA receptor system also has the potential to mediate cellular damage. Over-stimulation of this controlling receptor system can result in cell death. Via a cascade of events, excess glutamate release and receptor interaction can result in even further exaggerated glutamate release and receptor stimulation. Described by Choi (1988) as the spiral of death, this mechanism of cell death involves the influx of Ca^{++}, and other ions with water. Subsequent cellular swelling and release of degradative enzymes results in cell death. Many neurotoxicants, including the excitotoxicants kainate and domoate, are thought to produce their adverse effects by over- stimulating the fully mature glutamate receptor system.

During development in the rat, especially during postnatal days 7-14, the central nervous system (CNS) exhibits enhanced susceptibility to the toxic effects of modulation of the NMDA receptor system. This enhanced susceptibility has been suggested to derive from the increased expression of specific NMDA receptor subunits (Miyamoto et al., 2001). Because of the critical role of the NMDA receptor system in brain development, antagonism of this system can have profound, long-lasting and detrimental effects (Behar et al., 1999). If stimulation of glutamate release reinforces neuronal connections, then blockade of that stimulation by NMDA antagonists may result in fewer or nonfunctional connections. Selected anticonvulsants and dissociative anesthetics are reported to produce their toxicity on the developing nervous system via antagonism of the NMDA receptor system (Ikonomidou et al., 1999 and 2001; Popke et al., 2001a). The developmental toxicity of several agents including methylmercury, lead and ethanol (Miyamoto et al., 2001; Kumari and Ticku, 1998; Guilarte, 1997; Guilarte and McGlothan, 1998; Ikonomidou et al., 2000) is also thought to result from interaction with the NMDA receptor system.

NMDA Receptor System Function and Anatomy

The NMDA receptor has been widely investigated in recent years as a target for the pharmacological management of pain and a variety of neurological disorders, its function in normal central nervous system (CNS) activity and development, and its role in the development of CNS abnormalities and degeneration. Abnormalities in glutamate transmission, particularly involving excessive or insufficient activation of NMDA receptors, have been implicated in aberrations of normal CNS development (McDonald and Johnston, 1990), in the development of epilepsy, and in the neurodegeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis. Excitotoxic neuronal death observed after head injury, ischemic events, hypoxia and hypoglycemia have also been attributed to excessive NMDA receptor activation (Choi, 1988).

The NMDA receptor is one of three pharmacologically distinct subtypes of ionotropic receptor channels that are sensitive to the endogenous excitatory amino acid, L-glutamate. The non-

NMDA glutamate receptor subtypes are pharmacologically sensitive to α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate. As described in the review by Dingledine et al., (1999) NMDA, AMPA and kainate receptor subunits are encoded by at least six gene families as defined by sequence homology. There are three families for NMDA receptors, two for kainate and one for AMPA. NMDA receptors play a role in nearly all excitatory synaptic transmission, and in activity-dependent synaptic plasticity underlying learning and memory, and pre- and postnatal CNS development, including brain cell differentiation, axonal growth and degeneration of unused neurons (Lalonde and Joyal, 1993).

NMDA receptors are distributed ubiquitously throughout the CNS. Most NMDA receptors are located on postsynaptic dendrites and dendritic spines in membranal structures named postsynaptic density (PSD, Reviewed under Sheng, 2001). However, NMDA receptors have also been found on cortical astrocytes, and presynaptically, as autoreceptors and heteroreceptors (Reviewed under Conti, 1997).

NMDA receptors consist of multiple components that recognize and bind glutamate, coagonist, modulatory molecules (such as glycine and polyamines), dissociative anesthetics (such as phencyclidine and ketamine), redox agents, steroids, histamine, zinc, and Mg^{++}, and a transmembrane channel that is selective for specific cations (i.e., Na^{+}, Ca^{++}, K^{+}) with particular permeability to Ca^{++} (Reviewed under McBain and Mayer, 1994; Danysz and Parsons, 1998). The Mg^{++} binding site within the cation channel blocks ion permeability in a voltage-dependent and use-dependent manner.

There is evidence that the NMDA receptor can exist in two different functional states depending on the level of maturity of the rat, based on pharmacological response to the non-competitive NMDA receptor antagonist dizocilpine (MK-801, Sircar, 2000). The functional states involving different channel conductances, mean open times, and sensitivities to Mg^{++} blockade appear to reflect differential combinations of NMDA receptor subunits (Reviewed under Feldmeyer and Cull-Candy, 1996).

Molecular cloning technology has identified multiple tetrameric, and heteromeric subunits of the NMDA receptor including NR1 (zeta), NR2A-D (epsilon), and NR3 forms (Reviewed under McBain and Mayer, 1994; Danysz and Parsons, 1998, Dingledine et al., 1999, Cull-Candy et al., 2001; see Danysz and Parsons, 1998 and Dingledine et al., 1999 for diagrams of the NMDA receptor complex). The receptor appears to have three transmembrane domains plus a cytoplasm-facing re-entrant membrane loop. This membrane loop is postulated to control important aspects of ion channel function (Dingledine et al., 1999). The subunits are expressed in varying combinations throughout the CNS, and are responsible for conferring distinct pharmacological properties and functional diversity to the receptors. There are eight known isoforms of the NR1 subunits (Sugihara *et al*., 1992; Hollmann *et al*., 1993), the glycine recognition sites that are likely present in all NMDA receptors throughout the CNS. The NR2A-NR2D subunits are the glutamate recognition sites found in varying combinations with NR1 (Reviewed under Stone, 1993; Conti, 1997). In the adult, the NR2A subunit appears predominantly in the forebrain, hippocampus, and cerebellum, and the NR2B subunit is highly represented in the olfactory tubercle, hippocampus, olfactory bulb and cerebral cortex, with intermediate expression in the striatum and midbrain. The adult cerebellum has the greatest concentration of the NR2C subunit (Stone, 1993), and the NR2D subunit is expressed weakly in the adult thalamus, brainstem, olfactory bulb, and spinal cord (Monyer et al., 1994; Watanabe et al., 1994a).

Functional NMDA receptors require the presence of the NR1 subunit (Lynch et al., 1994) in addition to variable combinations of the NR2 subunits. The two isoforms of the NR3 subunit may play a modulatory role in NMDA receptor function, reducing channel open time and conductance (Das et al., 1998, Perez-Otano et al., 2001). The differential distribution of the various combinations of NMDA receptor subunits throughout the CNS confers diversity of pharmacological sensitivity to different agonists and antagonists (Reviewed under Monaghan et al., 1998).

Ontogeny

The NMDA receptor system has been shown to play a major role in the normal development of the CNS. CNS development occurs through many stages of neurogenesis, migration, proliferation and death of neurons, axonal outgrowth, and synapse formation and elimination. Glutamate was identified as one of the more than 36 trophic factors involved in modulating each of these phases of neuronal differentiation. The results of recent studies suggest that sustained alteration of NMDA receptor activation during critical periods of development may have deleterious effects on normal CNS development and function (Ikonomidou et al., 1999 and 2001).

During early cortical development, NMDA and other amino acid receptors (e.g., GABA) are found on cycling neuroblasts prior to development into functioning neurons (Dammerman and Kriegstein, 2000). However, the NMDA receptor does not appear to be functionally mature in the rodent until several weeks after birth. For example, a recent study by Zhu and Barr (2001) showed that pretreatment with MK801 failed to attenuate the development of morphine dependence and the behavioral signs of acute opiate withdrawal in 7 day-old rat pups. However, it did reduce the development of morphine dependence in 14-day -and 21-day-old pups and in adult rats, and decreased the expression of morphine withdrawal in 14-day-old pups.

Cortical neurogenesis and neuronal migration is complete by the first week of postnatal life in the rat. Thereafter, neocortical development proceeds by progressive and variable strengthening or elimination of synapses and neuronal death. Animal studies have demonstrated specific patterns of transient increases and decreases in the expression of NMDA receptors and receptor subunits, and glutamate regulation of prenatal and postnatal CNS development via NMDA receptor activity in many regions, including cerebellum (Burgoyne et al., 1993), visual cortex (Quinlan et al., 1999 a&b), superior colliculus (Simon et al., 1992), forebrain (Watanabe et al., 1992), hippocampus (Guilarte and McGlothan, 1998), striatum (Hurst et al., 2001), and neostriatum (Colwell et al., 1998). In the neonatal rat striatum, NMDA receptor maturation occurred later than kainate and AMPA receptor expression (Colwell et al., 1998; Nansen et al., 2000).

A study by Rao et al. (1997) using *in vitro* receptor autoradiography showed increased global and NMDA-receptor specific glutamate binding from birth to postnatal day 9, and decreased global, but not NMDA-receptor specific, binding thereafter to postnatal day 30 in the rat nucleus tractus solitarii and ventrolateral medulla (brainstem regions involved in the regulation of several autonomic functions including breathing, blood pressure regulation and swallowing). In the rat neostriatum, NMDA-receptor responses, measured using infrared videomicroscopy and whole-cell patch clamp analysis, were absent at age 3 days, showed increasing response strength at age 7 days to a maximum at age 14 days, and then an attenuation in response strength on days 21 and 28 (Colwell *et al*., 1998). The results of that study showed increased MK-801 binding in rat neostriatal tissue homogenates from postnatal days 3 to 7 and 14 to 21, a peak at postnatal day 28, and then a decline in binding thereafter to adult levels by postnatal day 60, with no changes in MK-801 binding affinity throughout the study.

In the fetal human brain, NMDA receptor binding sites were demonstrated in the hippocampus, thalamus, and subthalamic nucleus by gestational day 115 (Lee and Choi, 1992). The numbers of ionotropic glutamate receptor binding sites in those regions increased until gestational days 140-150, and then decreased in number by gestation days 168 or 182.

Examination of NMDA receptor subunit mRNA expression and binding sites has revealed differential expression at varying stages of development in different regions of the CNS (Reviewed under Scheetz and Constantine-Paton, 1994; Vallano, 1998; Dunah et al., 1999; Cull-Candy et al., 2001; Ritter et al., 2001). High levels of NMDA receptor activity (Hestrin, 1992), and synaptic plasticity (Lund and Lund, 1976) were observed in the superior colliculus shortly after birth, and declined over the subsequent two weeks of neonatal life in rats. It is during that time that the expression of the NR1 subunits showed an increase from postnatal day 6 to a peak at day 19, and then declined thereafter to levels observed in adult rats (Hofer et al., 1994; as reviewed in Scheetz and Constantine-Paton, 1994). In contrast, expression of the NR2B subunit decreased during the early neonatal weeks from its highest observed level at birth in rodents (Watanabe et al., 1992; Hofer et al., 1994). The pattern was different in the rat striatum than in other brain regions, showing low levels of NMDA receptor responses in the early postnatal

period and increases in NMDA receptor sensitivity in the second and third postnatal weeks (Hurst et al., 2001).

In the rat cerebellum, the NR1 subunit is expressed in fetal, postnatal and adult stages, the NR2B subunit is expressed early in the postnatal period and decreases as the animal matures, and the NR2A subunit increases to its highest levels in the adult (Takahashi et al., 1996). After postnatal day 10, the expression of the NR2C subunit also increases to high levels in the adult rat cerebellar granule cells (Watanabe et a*l*., 1994b). The NR2D subunit is expressed in Purkinje cells for the first 8 days of postnatal life (Akazawa et a*l*., 1994).

Autoradiography and *in situ* hybridization demonstrated the presence of NMDA receptor subunit mRNA in human fetal brains obtained at gestational ages 57-140 days, with transient increases in gestation weeks 11, 13, and 19 (Ritter *et al*., 2001). The predominant subunits represented in the human fetal brains were the NR1, NR2B and NR2D types similar to observations in neonatal rat brain (Ritter et al., 2001). The NR2A and NR2C subunits were detected in human fetal cortex, but were not expressed until after birth in the rodent (Watanabe et al., 1992). The high level of expression of the NR2D subunit in prenatal and neonatal brain compared to that in adult CNS suggests a strong role in brain development (Reviewed under Dunah et al., 1999). NR2A subunit mRNA was found in most regions of human neonatal brain, gradually replacing the NR2B function throughout the CNS, followed by an increase in NR2C receptor subunits, particularly in the cerebellum (see review by Cull-Candy et a*l*., 2001; Monyer et a*l*., 1994; Akazawa et al., 1994).

CNS development can be modified by sustained exposure to specific NMDA-receptor agonists and antagonists, and by factors such as sensory deprivation and isolation, chronic pain, and maternal separation during critical periods of CNS development. Neonatal rats administered the NMDA receptor antagonists 2-amino-5-phosphonovalerate (AP5) and MK-801 during the first two weeks of life developed abnormal axonal arborization in the retinal connections to the superior colliculus, interfering with normal visual responses (Simon et al., 1992). Stress-induced circulating glucocorticoids can alter normal hippocampal neurogenesis indirectly by interaction with NMDA-receptor dependent activity (Gould and Tanapat, 1999). It has been hypothesized

that aberrant behavior in adult rats subjected to sensory isolation, chronic pain or maternal separation during critical periods of CNS development as neonates, resulted from excessive or insufficient NMDA receptor activity and subsequent increased or decreased apoptosis in specific areas of the developing brain (Anand and Scalzo, 2000).

Considerably more is known about the timing and sequence of NMDA receptor subunit maturation in the rodent than in the human. The general pattern of NMDA subunit expression appears to be similar in rats and humans, with ubiquitous NR1 subunit expression throughout development and adulthood, and high levels of NR2B and NR2D early in development that decrease while NR2A and NR2C subunit expression increases into adulthood. The NR3A subunit expression is initiated around the time of birth in rodents. Observed interspecies differences in subunit expression reflect varying temporal patterns related to CNS maturation. The maturity of rat CNS at postnatal days 6-9 was suggested to correspond with that in human infants at birth when full-term, based, in part, on measures of thermal pain thresholds, peak NMDA receptor density, and peak rates of brain growth and synaptogenesis (Anand and Scalzo, 2000).

Behavioral Effects of NMDA receptor Blockade

Given the important role of the NMDA receptor systems in both normal development and learning processes, the functional consequences of exposure to agents that affect these systems during development are of great interest. While little is known about the ontogeny of specific NMDA receptors in nonhuman primate species, it is logical to presume that they continue to play a critical role throughout development and in learning processes in these animals. Data from human studies also support the presumption that NMDA receptor populations continue to evolve with age since there are significant differences in amino acid binding sites across development from the neonatal period into old age (Court et al., 1993; D'Souza et al., 1992; Johnson et al., 1993; Slater et al., 1993). Recent studies in juvenile rhesus monkeys (Popke et al., 2001a, b) examined the effects of chronic (18 months) daily exposure to remacemide or MK-801 (dizocilpine) on the acquisition of several cognitive function tasks designed to model learning, color and position discrimination, short-term memory, and motivation.

Remacemide acts as a relatively low-affinity, non-competitive antagonist of NMDA receptors and as a relatively high-affinity blocker of fast sodium channels. MK-801 is the classical high-affinity, selective, noncompetitive NMDA receptor antagonist. Treatment during this protracted period of development was thought to provide sufficient exposure to allow for the assessment of effects of NMDA receptor blockade during continuing brain maturation. It is unknown whether NMDA receptor blockade during this stage of development in the nonhuman primate will cause either the classical 'Olney lesion' (see below) or cause changes in the pattern of apoptosis as noted in neonatal rats (see also below) or neither. Low or high doses of both drugs were administered orally and daily for 18 months to separate groups of young monkeys beginning at about 9 months of age. Their ability to learn how to perform several complex tasks was measured throughout treatment. The dosing and testing procedures that were utilized served to minimize the role that acute drug effects played in the results, and enabled analyses to focus on the long-term effects of chronic treatment. Because both compounds are known to inhibit the function of NMDA receptors, it was hypothesized that both remacemide and MK-801 would disrupt the acquisition of behaviors.

The data showed that chronic developmental exposure to a relatively high dose of remacemide (50 mg/kg/day) delayed acquisition of simple discriminations (e.g., color and position discrimination; Popke et al., 2001b) and the acquisition of tasks which require new learning. This latter effect was both striking and long lasting: affected subjects showed no evidence of recovery after exposure was either decreased or eliminated for six months (Popke et al., 2001a). Alternatively, a lower dose of remacemide (20 mg/kg/day) had no discernible effects on these same tasks, and there was no effect of either dose of remacemide on motivation (Popke et al., 2001a) or short-term memory (Popke et al., 2001b). There were also no effects of either dose of remacemide on clinical chemistry, hematology, or ophthalmic parameters, or on general comportment; thus, the noted behavioral effects appeared targeted to very specific aspects of brain function. Chronic treatment with MK-801 manifest only as a delay in the acquisition of simple discriminations and this effect was only noted at the high dose (1.0 mg/kg/day; Popke et al., 2001b). Given the differential effects of these drugs and their somewhat different mechanisms of action, it is likely that the long-lasting effects of remacemide on learning resulted

either from its ancillary activity at fast sodium channels or from its ability to block NMDA receptors and sodium channels concurrently.

While a role for sodium channel blockade in the effects of remacemide seems likely, it is also possible that the effects of remacemide resulted in part from the action of its primary metabolite. Shortly after oral administration, remacemide is desglycinated to an active metabolite, which has an even greater affinity for the NMDA receptor than does remacemide (Palmer et al., 1992). Thus, although the time course of MK-801 and remacemide in blood may be similar (Hucker et al., 1983; Vezzani et al., 1989), the persistence of the active remacemide metabolite (up to 24 hours after high-dose administration) may result in a somewhat longer inactivation of NMDA receptors. This, in turn, may have resulted in a longer duration of NMDA receptor blockade (and a more prolonged blocking of the laying down of memory) after remacemide treatment than after MK-801 treatment.

Although the effects of remacemide are indeed noteworthy, so is the fact that chronic treatment with MK-801 had minimal effects. Previous experiments in monkeys indicate that acute treatment with MK-801 can have pronounced effects on the performance of the same behavioral tasks in adults (Buffalo et al., 1994; Paule, 1994). Yet, in the Popke studies, daily administration of MK-801 (1.0 mg/kg) during development starting at 9 months of age was largely without effect. This appears remarkable given the substantial literature suggesting that the excitatory amino acids, and NMDA receptors in particular, play important roles regulating neuronal survival, axonal and dendritic structure, synaptogenesis and plasticity (McDonald and Johnston, 1993). Developmental observations in humans indicate that marked differences exist with respect to excitatory amino acid binding sites from the neonatal period through the 10th decade of life (Court et al., 1993; D'Souza et al., 1992; Johnson et al., 1993; Slater et al., 1993). These observations suggest that the infant brain is differentially sensitive to agents that affect NMDA receptor function relative to the adult (D'Souza et al., 1992). This may help to explain why the juveniles in the monkey study were relatively insensitive to the effects of MK-801. It is important to emphasize that this experiment did not include subjects that began treatment and testing as adults. As a result, it is impossible to discern whether the results reported here reflect a

specific effect of these NMDA receptor antagonists during development or whether a similar pattern of results would emerge in adult animals that were chronically exposed in this way.

Another explanation for the lack of pronounced effects of chronic MK-801 treatment is that subjects could have become tolerant to its cognitive-behavioral effects. Hasselink et al. (1999) reported that acute injections of MK-801 resulted in impaired passive avoidance performance in rats, but these effects disappeared after 14 days of chronic treatment. In the monkey experiment, MK-801 produced significant increases in response rates on a motivation task that emerged about 5 months after treatment began, but this effect disappeared within the next 5 months. In ongoing rodent studies (Paule et al., unpublished), however, it is clear that chronic treatment with the same dose of MK-801 (1.0 mg/kg/day, per os) during development (from weaning well into adulthood) stunts growth, profoundly disrupts the ability of subjects to learn simple discriminations and decreases their ability to demonstrate new learning. These findings in the rat are in stark contrast to those observed in the monkey study where little effect of chronic exposure to MK-801 was observed and suggest that there may be dramatic species differences with respect to the effects of chronic NMDA receptor blockade and brain function.

In summary, the results of the monkey study suggest that chronic developmental exposure to high doses of remacemide has pronounced effects on learning whereas similar exposure to MK-801 does not. These effects occurred in the absence of reductions in motivation or reductions in the ability of subjects to perform the motor requirements of the tasks. The effects of remacemide on learning persisted for months, even after treatment ceased, suggesting an enduring effect of blocking NMDA receptors and fast sodium channels.

Neuroanatomical Effects of NMDA Blockade

The discovery that NMDA receptor antagonists such as MK-801 cause dose-dependent reversible or irreversible degeneration in cerebrocortical neurons in adult rats was made over ten years ago (Olney et al., 1989), and has since been referred to as the “Olney lesion”. The localization of the lesion was restricted to a very specific region of the retrosplenial cortex, just distal to the transition zone between the hippocampal subiculum and the cortex. The findings

indicate that the adult neurotoxic effect occurs when an NMDA antagonist blocks the endogenous glutamatergic excitation of an inhibitory innervation of the retrosplenial cortex. When disinhibited, the retrosplenial cortex becomes sensitive enough to sustain "excitotoxic" damage from its excitatory glutamatergic and cholinergic input (Olney at al., 1995; Olney et. al., 1997).

This adult type neurotoxicity cannot be induced in the developing brain until the animals reach almost full adult age (Farber et al., 1995). However, If NMDA receptors are blocked during a specific period in neonatal life (first two weeks postnatally in the rat), it leads to massive apoptotic (not excitotoxic) neurodegeneration, due not to excitotoxic over stimulation of neurons but to deprivation of stimulation. There is a period between the first two postnatal weeks and adolescence during which blockade of NMDA receptors in the rat has not been shown to produce either apoptosis or disinhibition-mediated excitotoxicity (Ishimaru et al., 1999; Ikonomidou et al., 1999 and 2001).

In a recent report, Olney and Coworkers (Ikonomidou et al., 1999) made the following statement:

"Programmed cell death (apoptosis) occurs during normal development of the central nervous system, however the mechanisms that determine which neurons will succumb are poorly understood. Blockade of N-methyl-D-aspartate (NMDA) glutamate receptors for only a few hours during the late fetal or early neonatal life triggered widespread apoptotic neurodegeneration in the developing rat brain, suggesting that the excitatory neurotransmitter glutamate, acting at NMDA receptors, controls neuronal survival. These findings may have relevance to human neurodevelopmental disorders…."

In 2000, they proposed (Olney et al., 2000) that a variety of agents including several NMDA antagonists:

" …have the potential to delete large numbers of neurons from the developing brain by a newly discovered mechanism involving the interference in the action of neurotransmitters

(glutamate and gamma amino butyric acid, GABA) at NMDA and $GABA_A$ receptors during the synaptogenesis period, also known as the brain growth-spurt period. Transient interference (lasting $\geq$ 4 hrs) in the activity of these transmitters during the synaptogenesis period (the last trimester of pregnancy and the first several years after birth in humans) causes millions of developing neurons to commit suicide (die by apoptosis)."

These very recent findings are the first to describe and establish the significant difference of the so-called "Olney lesion" involving brain neurodegeneration and vacuolation seen in adult rats following high doses of NMDA receptor antagonists, with the pathology consisting of apoptosis in multiple brain regions observed in neonates (PND-7 in the rat). PND-7 coincides with the greatest period of hypersensitivity of NMDA receptors and blockade of these receptors during this period results in deletion of large numbers of neuronal cells and significantly diminished cell density in various brain regions. The decrements in cell density vary from 3 to 39 fold depending on the NMDA blocker administered and the area of the brain measured. These changes were dose-dependent, neuron specific and did not occur when blockers of other receptor types (Ca^{++} channel, muscarinic, non-NMDA glutamatergic or dopaminergic) were administered. PND-7 represents the age when the rat forebrain is most vulnerable to the apoptotic effects of the NMDA antagonists indicating the dependence of this cell type on glutamatergic input for survival. This period also corresponds to the greatest expression of NR1 as well as the peak of the brain growth-spurt. The data indicated that the NMDA receptor must be blocked for at least 4 – 6 hrs at a threshold dose of 0.25 mg/kg (+) MK-801. This phenomenon is stereospecific in that the same dose of (-) MK-801 showed a much weaker effect.

Of course, other subcellular mechanisms, probably ones associated with apoptosis, may be involved with the mechanism of the developmental neurotoxicity produced by NMDA antagonists or the adult neurotoxicity produced by NMDA agonists. For example, important pathways in the initiation of apoptosis involve the production of excessive oxidative stress within the mitochondria, secondary to the operation of the tricarboxylic acid (TCA) cycle (Benzi *et al.*, 1991; Bondy and Lee, 1993). If not compensated for by sufficient free-radical scavengers, the release of cytochrome-c (Luetjens *et al.*, 2000) through the mitochondrial membrane then leads to activation of cytoplasmic caspases (Glazner *et al.*, 2000; Tenneti and Lipton, 2000) and

ultimately to apoptosis. Thus any toxicants or environmental conditions, whether indirectly activating the NMDA receptor through afferents from another transmitter system or bypassing the NMDA receptor to act intracellularly, that promote oxidative phosphorylation through the TCA cycle would lead to apoptosis. Moreover, other agents besides NMDA receptor antagonists, particularly those that block the actions of growth factors and steroid hormones (Toran-Allerand, 1996), would be expected to cause apoptosis.

Something old, something new: additional evidence regarding the induction of apoptosis by NMDA-antagonists.

Although the mechanisms were not fully elucidated, Nobel Prize winning research by David Hubel and Torsten Wiesel (Hubel and Wiesel, 1970; Wiesel 1999) established that visual experience during critical periods of development was necessary for the normal anatomical and functional development of the visual system. When deprived of light stimulation, normally preserved neural pathways atrophied and aberrant pathways were maintained. Glutamate is widely considered to be a major and ubiquitous excitatory neurotransmitter (Brann, 1995; Hertz et al., 1999; Trudeau and Castellucci, , 1993).

Therefore, it is not surprising that the visual system plasticity explored by Hubel and Wiesel in response to light deprivation, may have its roots in reduced glutamate neurotransmission. In fact, evidence for NMDA receptor involvement in long term potentiation (Kato et al., 1999; Youssef et al., 2000; Altmann et al., 2001) and neuronal plasticity of the visual system (Philpot et al., 2001; Bear and Rittenhouse, 1999; Quinlan *et al.*, 1999 a&b; Udin and Grant, 1999) has been reported.

Thus NMDA receptor antagonists may block neurotransmission mediated by glutamate, just as deprivation of light prevents the propagation of glutamate-driven action potentials in visual system pathways. The original observations that CNS apoptosis is associated with agents such as MK-801 or ketamine (Ikonomidou et al., 1999) are consistent with a mechanism involving blockade of NMDA receptor mediated neurotransmission. More recently, Ikonomidou , Olney and colleagues have replicated and expanded their observations to include nitrous oxide,

isoflurane, propofol, midazolam, halothane, barbiturates, benzodiazepines, and ethanol as suspect apoptotic agents either alone or in combination when administered to neonatal rodents (Pohl et al., 1999; Ikonomidou et al., 2001). Several other independent groups of investigators have also reported that MK-801 increases apoptosis either *in vivo* (Hsu et al., 2000), in motor neurons of a chick embryo preparation (Llado et al., 1999), or in cultured neurons (Terro et al., 2000).

Questions left unanswered

The neuropathology induced by glutamate agonists on various species of adult animals is well documented. The histopathologic structural changes identified by Olney and coworkers are identifiable by the neuronal intracellular vacuoles, resulting in a phenomenon known as "vacuolation." As discussed previously, recent reports from Olney's group have described the action of some of these glutamate antagonists in neonatal rats, 7-10 days of age. The significant damage on neuronal cytoarchitecture is different in the neonatal versus adult models, perhaps because in the developing rat, neurons are migrating and connections are being formed. This apoptosis that is triggered by exposure of the developing brain at a critical period to NMDA receptor antagonists has the potential to disrupt vast developmental and proliferative processes that result in serious decrements in neuron numbers and synaptic density, as well as producing profound functional and behavioral deficits (Pohl et al., 1999; Ikonomidou et al., 1999 and 2001; Popke et al., 2001 a, b).

These observations require further laboratory evidence and support in order to establish the relevance of the observations of Olney and coworkers to drug-induced human neurodevelopmental concerns. Extension of the observations of Olney in the developing rat is a logical first step. Toxicokinetic information derived from rodent studies would be very useful for further investigations in other animal species. It is necessary to investigate the relevance of these findings in other animal species, most notably, nonhuman primates, where neuronal cytoarchitecture and development are significantly different than the rodent but more like the human. Such studies are significantly more complicated to perform than small animal studies, where issues such as timing, dosing, nutrition, temperature, and maternal nurturing may all have

significant impact on the experimental outcome. The data indicate that for the rat, a critical relationship exists between dose, duration, and the developmental timing of exposures to NMDA receptor antagonists. The 7-10 day age window appears to be critical for even brief exposures of NMDA-receptor antagonists to result in CNS damage in the rat. Whether a similar critical window of susceptibility exists in primates has not been established but should be explored. As suggested by Anand and Scalzo (2000) the developmental maturity of the NMDA receptor system in the primate brain at birth may most closely mimic that of the 7-10 day old rat. Whether the observed neuropathology in the 7-10 day old rat results in irreversible behavioral alterations that may have a logical corollary in the primate, should also be investigated. The accumulation of such data could provide pivotal information for assessing the potential public health risk associated with the use of agents known to interact with the NMDA receptor system.

References

Akazawa C, Shigemoto R, Bessho Y, Nakanishi S, Mizuno N. 1994. Differential expression of five N-methyl-D-aspartate receptor subunit mRNAs in the cerebellum of developing and adult rats. J. Comp. Neurol., **347**:150-160.

Altmann, L., Mundy, W. R., Ward, T. R., Fastabend, A., and Lilienthal, H. 2001. Developmental exposure of rats to a reconstituted PCB mixture or aroclor 1254: effects on long-term potentiation and [3H]MK-801 binding in occipital cortex and hippocampus. Toxicological Sciences **61**:321-330.

Anand KJS, Scalzo FM. 2000. Can adverse neonatal experiences alter brain development and subsequent behavior? Biol. Neonate, **77**:69-82.

Bear, M. F., Rittenhouse, C. D. 1999. Molecular basis for induction of ocular dominance plasticity. Journal of Neurobiology **41**:83-91.

Behar, TN., Scott, CA., Greene, LC., Wen, X., Smith, SV., Maric, D., Liu, Q-Y., Colton, CA. and Baker lJ. 1999. Glutamate acting at NMDA receptors stimulates embryonic cortical neuronal migration. **19**:4449-4461.

Benzi, G., Curti, D., Pastoris, O., Marzatico, F., Villa, R. F., and Dagani, F. (1991). Sequential damage in mitochondrial complexes by peroxidative Stress. Neurochemical Research **16**:1295-1302.

Bondy, S. C., and Lee, D. K. (1993). Oxidative stress induced by glutamate receptor agonists. Brain Research **610**: 229-233.

Brann, DW. 1995. Glutamate: a major excitatory transmitter in neuroendocrine regulation. [Review]. Neuroendocrinology. **61**(3):213-225.

Buffalo, E. A.; Gillam, M.P.; Allen, R.R.; Paule, M.G. 1994. Acute behavioral effects of MK-801 in rhesus monkeys: Assessment using an operant test battery. Pharmacol. Biochem. And Behav., **48**(4): 935-940.

Burgoyne RD, Graham ME, Cambray-Deakin M. 1993. Neurotrophic effects of NMDA receptor activation on development of cerebellar granule cells. J. Neurocytol., **22**(9):689-695.

Choi DW. 1988. Glutamate neurotoxicity and diseases of the nervous system. Neuron, **1**:623-34.

Colwell CS, Cepeda C, Crawford C, Levine MS. 1998. Postnatal development of glutamate receptor-mediated responses in the neostriatum. Dev. Neurosci., **20**(2-3):154-163.

Conti F. 1997. Localization of NMDA receptors in the cerebral cortex: a schematic overview. Brazilian J. Med. Biol. Res., **30**:555-560.

Court, J.A.; Perry, E.K.; Johnson, M.; Piggott, M.A.; Kerwin, J.A.; Perry, R.H.; Ince, P.G. 1993. Regional patterns of cholinergic and glutamate activity in the developing and aging human brain. Devel. Brain Res., **71**:73-82.

Cull-Candy S, Brickley S, Farrant M. 2001. NMDA receptor subunits: diversity, development and disease. Cur. Opin. Neurobiol., **11**:327-335.

Dammerman RS, Kriegstein AR. 2000. Transient actions of neurotransmitters during neocortical development. Epilepsia, **41(8):**1080-1081.

Danysz W, Parsons CG. 1998. Glycine and N-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. Pharm. Rev., **50(4):**597-664.

Das S, Rothe YF, Premkumar LS, Takasu M, Crandall JE, Dikkes P, Conner DA, Rayudu PV, Cheung W, Chen HS, Lipton SA, Nakanishi N. 1998. Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. Nature, **393**:377-381.

Dingledine R, Borges K, Bowie D, Traynelis SF. 1999. The glutamate receptor ion channels. Pharmacol. Rev., **51**:7-61.

D'Souza, S.W.; McConnell, S.E.; Slater, P.; Barson, A.J. 1992. N-methyl-D-aspartate binding sites in neonatal and adult brain. Lancet**, 339**(8803):1240.

Dunah AW, Yasuda RP, Luo J, Wang Y, Prybylowski KL, Wolfe BB. 1999. Biochemical studies of the structure and function of the N-methyl-d-aspartate subtype of glutamate receptors. Mol. Neurobiol, **19(2):**151-179.

Farber NB, Wozniak DF, Price MT, Labruyere J, Huss J, St.Peter H, Olney JW 1995. Age-specific neurotoxicity in the rat associated with NMDA receptor blockade: Potential relevance to schizophrenia. Biol.Psychiatry, **38**:788-796.

Feldmeyer D, Cull-Candy S. 1996. Functional consequences of changes in NMDA receptor subunit expression during development. J. Neurocytol., **25**:857-867.

Glazner, G. W., Chan, S. L., Lu, C., and Mattson, M. P. 2000. Caspase-mediated degradation of AMPA receptor subunits: a mechanism for preventing excitotoxic necrosis and ensuring apoptosis. Journal of Neuroscience **20**:3641-3649.

Gould E, Tanapat P. 1999. Stress and hippocampal neurogenesis. Biol. Psychiatry, **46**(11):1472-1479.

Guilarte T R. 1997. Glutamatergic system and developmental lead neurotoxicity. Neurotoxicology. **18:**665-672.

Guilarte TR, McGlothan JL. 1998. Hippocampal NMDA receptor mRNA undergoes subunit specific changes during developmental lead exposure. Brain Res., **790**:98-107.

Hasselink, M.B.; Smolders, H.; De Boer, A.G.; Breimer, D.D.; Danysz, W. 1999. Modifications of the behavioral profile of non-competitive NMDA receptor antagonists, memantine, amantadine, and (+) MK-801 after chronic administration. Behav. Pharmacol., **10**(1):85-98.

Hertz, L., R. Dringen, et al. 1999. Astrocytes: glutamate producers for neurons. Journal of Neuroscience Research, **57**(4):417-28.

Hestrin S. 1992. Developmental regulation of NMDA receptor-mediated synaptic currents at a central synapse. Nature, **357**:686-689.

Hofer M, Prusky GT, Constantine-Paton M. 1994. Regulation of NMDA receptor mRNA during visual map formation and after receptor blockade. J. Neurochem,. **62**(6):2300-2307.

Hollmann M, Jordan LM, Schmidt, BJ. 1993. Zinc potentiates agonist-induced currents at certain splice variants of the NMDA receptor. Neuron, **10**:943-954.

Hsu C, Hsieh YL, Yang RC, Hsu HK. 2000. Blockage of N-methyl-D-aspartate receptors decreases testosterone levels and enhances postnatal neuronal apoptosis in the preoptic area of male rats. Neuroendocrinology, **71**(5): 301-307.

Hubel, D. H. and T. N. Wiesel 1970. The period of susceptibility to the physiological effects of unilateral eye closure in kittens. Journal of Physiolog, **206**(2): 419-36.

*Hucker, H.B.; Hutt, J.E.; White, S.D.; Arison, B.H.; and Zacchei, A.G. 1983. Disposition and metabolism of (+)-5-methyl-10, 11-dihydro-5H-dibenzo[a, d,]cyclohepten-5, 10-imine in rats, dogs and monkeys. Drug Metab. Dispos., **11(1):** 54-58.*

Hurst RS, Cepeda C, Shumate LW, Levine MS. 2001. Delayed postnatal development of NMDA receptor function in medium-sized neurons of the rat striatum. Dev. Neurosci., **23:**122-134.

Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vockler J, Dikranian K, Tenkova TI, Stefovska V, Turski L, Olney JW. 1999. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science, **283:**70-74.

Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, Genz K, Price MT, Stefovska V, Horster F, Tenkova T, Dikranian K, Olney JW. 2000. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. Science, **287**:947-948.

Ikonomidou, C., Bittigau P., Koch, C., Genz, K., Hoerster, F., Felderhoff-Mueser, U., Tenkova, T., Dikranian, K., and Olney, J. W. 2001 . Neurotransmitters and apoptosis in the developing brain. Biochemical Pharmacology, **62**(4): 401-5.

Ishimaru MJ., Ikonomidou C., Tenkova TI., Der TC., Dikranian, K.,Sasma MA, and Olney JW. 1999. Distinquishing excitotoxic from apoptotic neuro-degeneration in the developing rat brain. J. Comp. Neurol., **408**: 461-475.

Johnson, M., Perry, E.K., Ince, P.G., Shaw, P.J. and Perry, R.H. 1993. Autoradiographic comparison of the distribution of [3H]MK801 and [3H]CNQX in the human cerebellum during development and aging. Brain Res., **615**:259-266.

Kato, K., Li, S. T., and Zorumski, C. F. 1999. Modulation of long-term potentiation induction in the hippocampus by N-methyl-D-aspartate-mediated presynaptic inhibition. Neuroscience **92**:1261-1272.

Kumari, M. and Ticku, K. 1998. Ethanol and Regulation of the NMDA Receptor Subunits in fetal cortical neuron. J. of Neurochem., **70**:1467-1473.

Lalonde R, Joyal CC. 1993. Effects of ketamine and l-glutamic acid diethyl ester on spatial and nonspatial learning tasks in rats. Pharmacol. Biochem. Beh., **44**:539-545.

Llado, J., Caldero J., Ribera, J., Tarabal, O., Oppenheim, R. W. and Esquerda, J. E. 1999. Opposing effects of excitatory amino acids on chick embryo spinal cord motoneurons: excitotoxic degeneration or prevention of programmed cell death. Journal of Neuroscience, **19**(24): 10803-12.

Lee H, Choi BH. 1992. Density and distribution of excitatory amino acid receptors in the developing human brain: a quantitative autoradiographic study. Exp. Neurol., **118**:284-290.

Lund RD, Lund JS. 1976. Plasticity in the developing visual system: the effects of retinal lesions made in young rats. J. Comp. Neurol., **169**:133-154.

Luetjens, C. M., Bui, N. T., Sengpiel, B., Munstermann, G., Poppe, M., Krohn, A. J., Bauerbach, E., Krieglstein, J., Prehn, J. H. 2000. Delayed mitochondrial dysfunction in excitotoxic neuron death: cytochrome c release and a secondary increase in superoxide production. Journal of Neuroscience **20**:5715-5723.

Lynch DR, Anegawa, NJ, Verdoorn T, Pritchett DB. 1994. N-methyl-D-aspartate receptors: different subunit requirements for binding of glutamate antagonists, glycine antagonists, and channel-blocking agents. Mol. Pharmacol., **45**:540-545.

McBain CK, Mayer ML. 1994. N-methyl-D-aspartic acid receptor structure and function. Physiol. Rev., **74**(3):723-760.

McDonald JW, Johnston MV. 1990. Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. Brain Res. Brain Res. Rev., 15:41-70.

McDonald, J.W.; Johnston, M.V. 1993. Excitatory amino acid neurotoxicity in the developing brain. NIDA Research Monograph, **133**:185-205.

Miyamoto, K., Nakanishi, H., Moriguchi, S., Fukuyama,N., Eto, K., Wakamiya, J., Murao, K., Arimura, K. and Osame, M. 2001. Involvement of enhanced sensitivity of N-methyl-D-aspartate receptors in vulnerability of developing cortical neurons to methylmercury neurotoxicity. Brain Res. **901**:252-258.

Monaghan DT, Andaloro VJ, Skifter DA. 1998. Molecular determinants of NMDA receptor pharmacological diversity. Prog. Brain Res., **116**:171-190.

Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH. 1994. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron, **12**:529-540.

Nansen EA, Jokel ES, Lobo MK, Micevych PE, Ariano MA, Levine M. 2000. Striatal ionotropic glutamate receptor ontogeny in the rat. Dev. Neurosci., **22**(4):329-340.

Olney JW, Farber NB. 1995. Glutamate receptor dysfunction and schizophrenia. Arch Gen Psychiatry, **52(12):**998-1007.

Olney, J.W., Farber, N.B, Woznizk, D.F., Jevtovik-Todorovik, V. and Ikonomidou, C. 2000. Environmental agents that have the potential to trigger massive apoptotic neurodegeneration in the developing brain. Environmental Health Perspectives, **108**: (Suppl. 3) 383-388.

Olney JW, Labruyere J, Price MT 1989. Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science, **244**:1360-1363.

Olney JW, Wozniak DF, Farber NB. 1997. Excitotoxic neurodegeneration in Alzheimer disease. New hypothesis and new therapeutic strategies. Arch Neurol, **54(10):**1234-40.

Palmer, G.C.; Murray, R.J.; Wilson, T.C.M.; Eisman, M.S.; Ray, R.K.; Griffith, R.C.; Napier, J.J. Fedorchuk, M.; Stagnitto, M.L.; Garsky, G.E. 1992. Biological profile of the metabolites and potential metabolites of the anticonvulsant remacemide. Epilepsy Res., **12**:9-20.

Paule, M.G. 1994. Acute behavioral toxicity of MK-801 and phencyclidine: Effects on rhesus monkey performance in an operant test battery. Psychopharmacol. Bull., **30**(4):613-621.

Perez-Otano I, Schulties CT, Contractor A, Lipton SA, Trimmer JS, Sucher NJ, Heinemann SF. 2001. Assembly with NR1 subunit is required for surface expression of NR3A-containing NMDA receptors. J. Neurosci., **21**:175-218.

Philpot, B. D., Sekhar, A. K., Shouval, H. Z., Bear, M. F. 2001. Visual experience and deprivation bidirectionally modify the composition and function of NMDA receptors in visual cortex. Neuron **29**:157-169.

Pohl, D., Bittigau, P., Ishimaru, M. J., Stadthaus, D., Hubner, C., Olney, J.W., Turski, L., and Ikonomidou, C. 1999. N-Methyl-D-aspartate antagonists and apoptotic cell death triggered by head trauma in developing rat brain. Proceedings of the National Academy of Sciences of the United States of America, **96**(5): 2508-13.

Popke, E.J., Allen, R.R., Pearson, E.C., Hammond, T.C., and Paule, M.G. 2001a. Differential effects of two NMDA receptor antagonists on cognitive-behavioral performance in young non-human primates I. Neurotox. Teratol., **23**:319-332.

Popke, E.J., Allen, R.R., Pearson, E.C., Hammond, T.C., and Paule, M.G. 2001b. Differential effects of two NMDA receptor antagonists on cognitive-behavioral performance in young non-human primates II. Neurotox. Teratol., **23**:333-347.

Quinlan EM, Olstein DH, Bear MF. 1999 a. Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. Proc. Natl. Acad. Sci. USA, **96**(22):12876-12880.

Quinlan, E. M., Philpot, B. D., Huganir, R. L., Bear, M. F. 1999 b. Rapid, experience-dependent expression of synaptic NMDA receptors in visual cortex in vivo. Nature Neuroscience **2**:352-357.

Rao H, Jean A, Kessler JP. 1997. Postnatal ontogeny of glutamate receptors in the rat nucleus tractus solitarii and ventrolateral medulla. J. Auton. Nerv. Sys., **65**(1):25-32.

Ritter LM, Unis AS, Meador-Woodruff JH. 2001. Ontogeny of ionotropic glutamate receptor expression in human fetal brain. Dev. Brain Res., **127**(2): 123-133.

Scheetz AJ, Constantine-Paton M. 1994. Modulation of NMDA receptor function: implications for vertebrate neural development. FASEB, **8**:745-752.

Sheng M. 2001. Molecular organization of the postsynaptic specialization. Proc. Natl. Acad. Sci. USA, **98**(13):7058-7061.

*Sircar, R. 2000. Developmental maturation of the N-methyl-D-aspartic acid receptor channel complex in postnatal rat brain. Int. J. Dev. Neurosci., **18**:121-131.*

Simon DK, Prusky GT, O'Leary DDM, Constantine-Paton M. 1992. N-methyl-D-aspartate receptor antagonists disrupt the formation of a mammalian neural map. Proc. Natl. Acad. Sci. USA, **89**:10593-10597.

Slater, P., McConnell, S.E., D'Souza, S.W., Barson, A.J. 1993. Postnatal changes in N-methyl-D-aspartate receptor binding and stimulation by glutamate and glycine of [3H]-MK-801 binding in human temporal cortex. Brit. J. Pharmacol., **108**(4):1143-1149.

Stone, TW. 1993. Subtypes of NMDA receptors. Gen. Pharmac., **24**(4):825-832.

Sugihara H, Morihoshi K, Ishii T, Masu M, Nakanishi S. 1992. Structure and properties of seven isoforms of the NMDA receptor generated by alternative splicing. Biochem. Biophys. Res. Comm., **185**:826-832.

Takahashi TD, Feldmeyer N, Suzuki K, Ono-Dera SG, Cull-Candy K, Sakimura K, Mishina M. 1996. Functional correlation of NMDA receptor subunits expression with the properties of single-channel and synaptic currents in the developing cerebellum. J. Neurosci., **16**:4376-4382.

Tenneti, L., Lipton, S. A. 2000. Involvement of activated caspase-3-like proteases in N-methyl-D-aspartate-induced apoptosis in cerebrocortical neurons. Journal of Neurochemistry **74**:134-142

Terro, F., Esclaire, F., Yardin, C., and Hugon, J. 2000. N-methyl-D-aspartate receptor blockade enhances neuronal apoptosis induced by serum deprivation. Neuroscience Letters, **278**(3):149-52.

Toran-Allerand, C. D. 1996. Mechanisms of estrogen action during neural development: mediation by interactions with the neurotrophins and their receptors? Journal of Steroid Biochemistry & Molecular Biology **56**:169-178.

Trudeau, L. E. and V. F. Castellucci 1993. Excitatory amino acid neurotransmission at sensory-motor and interneuronal synapses of Aplysia californica. Journal of Neurophysiology, **70**(3): 1221-1230.

Udin, SB., Grant S. 1999. Plasticity in the tectum of Xenopus laevis:binocular maps. Progress in Neurobiology **59(2):** 81-106.

Vallano ML. 1998. Developmental aspects of NMDA receptor function. Crit. Rev. Neurobiol., **12**(3):177-204.

Vezzani, A.; Serafini, M.A.; Stasi, S.; Caccia, I.; Conti, R.V.; Tridico, R.V.; Samanin, R. 1989. Kinetics of MK-801 and its effect on quinolitic acid-induced seizures and neurotoxicity in rats. J. Pharmacol. Exp. Ther., **249**(1): 278-283.

Watanabe M, Inoue Y, Sakimura K, Masayoshi M. 1992. Developmental changes in distribution of NMDA receptor channel subunit mRNAs. NeuroReport, **3**:1138-1140.

Watanabe M, Mishina M, Inoue Y. 1994a. Distinct distributions of five NMDA receptor channel subunit mRNAs in the brainstem. J. Comp. Neurol. **343**:520-531.

Watanabe M, Mishina M, Inoue Y. 1994b. Distinct spatiotemporal expressions of five NMDA receptor channel subunit mRNAs in the cerebellum. J. Compar. Neurol. **343**:513-519.

Wiesel, T. N. 1999. Early explorations of the development and plasticity of the visual cortex: A personal view. Journal of Neurobiology, **41**(1): 7-9.

Youssef, F., Stone, T. W., and Addae, J. I. 2000. Interactions of glutamate receptor agonists with long-term potentiation in the rat hippocampal slice. European Journal of Pharmacology **398**:349-359

Zhu H, Barr GA. 2001. Inhibition of morphine withdrawal by the NMDA receptor antagonist MK-801 in rat is age-dependent. Synapse **40**:282-293.

Allaben, William
From: Howard, Paul
Sent: Wednesday, February 13, 2002 3:00 PM
To: Allaben, William
Subject: FW: brief ketamine proposal

Proposed studies in developing nonhuman primates

Experiment #1: Assessment for nervous system apoptosis at three developmental stages using histological and immunocytochemical techniques: control and ketamine treatment conditions at each developmental stage. N = 4 time-mated pregnancies/neonates x 3 stages x 2 conditions = 24. All studies will include physiological monitoring and determination of plasma and tissue levels where possible.

Experiment #2: Behavioral assessment of infants exposed to ketamine during the developmental stage shown most sensitive in Experiment #1. N = 8 time-mated pregnancies/neonates x 2 conditions = 16. All studies will include physiological monitoring and determination of plasma and tissue levels where possible. A variety of spontaneous and operant behaviors will be assessed along with central nervous system imaging where possible until two years of age. Histological and immunocytochemical techniques will be used to confirm any anatomical effects.