Anesthesia Considerations in Rodent Biomedical Research

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Anesthesia Types in Rodents
- Injectable (e.g. ketamine cocktails)
- Volatile gas (e.g. isoflurane)
- Physical (e.g. hypothermia)

Injectable or Inhalation Anesthesia?
Injectable Anesthetics - Advantages

- Simple
- Cheap
- Minimal equipment
- Many routes (IM, SQ, IP, IV)

Injectable Anesthetics - Disadvantages

- Higher morbidity & mortality when compared to inhalants
- Easier to overdose
- Slow and variable absorption
- Longer recovery times
- Variable effects from a single dose
- Strain and gender differences

Mice Strain & Male/Female Sleep Times

<table>
<thead>
<tr>
<th>Strain</th>
<th>Male Sleep Time</th>
<th>Female Sleep Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE</td>
<td>60 minutes</td>
<td>80 minutes</td>
</tr>
<tr>
<td>AKR</td>
<td>70 minutes</td>
<td>90 minutes</td>
</tr>
<tr>
<td>C57BL</td>
<td>80 minutes</td>
<td>100 minutes</td>
</tr>
<tr>
<td>DBA/1</td>
<td>90 minutes</td>
<td>120 minutes</td>
</tr>
</tbody>
</table>
Inhalant Anesthetics - Advantages

- Usually suitable as sole agents to reach 'balanced' anesthesia
- Unconsciousness
- Absence of pain perception
- Blunted reflexes
- Immobility

Inhalant Anesthetics - Advantages

- Precise control over anesthetic depth
- Recovery from anesthesia is rapid
- Delivery of agents usually involves oxygen
- Greater consistency between strains and genders = experimental consistency
Inhalant Anesthetics - Disadvantages

- Anesthetic delivery system - $ but eventually cheaper
- Requires a scavenging system
- Requires yearly calibration (unless otherwise specified by manufacturer)

Volatile Anesthetics

- Ether
- Halothane
- Methoxyflurane
- Enflurane
- Isoflurane
- Desflurane
- Sevoflurane
Isoflurane

- Decreases rate of production of cerebrospinal fluid (CSF) while increasing the rate of its absorption
- Most commonly used gas anesthesia in rodents

Isoflurane - Advantages

- Rapid induction & recovery
- Can manipulate depth of anesthesia easily & rapidly
- Non-irritant, non-explosive & non-flammable
- Main advantage: Nearly 100% eliminated in exhaled air... minimal interference in drug metabolism
- Inexpensive... eventually

Isoflurane - Disadvantages

- Some respiratory depression but cardiovascular (CV) depression is minimal
- Pungent odor may lead to breath holding in rabbits, but does not appear to be a problem in other spp
- Decreases CSF production / increases CSF absorption - A factor in central nervous system (CNS) studies???
Sevoflurane - Advantages

- Becoming increasingly more popular
- Rapid induction & recovery
- Major advantage: Nonirritating to airways and well accepted, lending itself to mask or chamber inductions
- Becoming increasingly more popular in rodents

Sevoflurane - Disadvantages

- Undergoes slightly greater hepatic metabolism than isoflurane
- Cost has been higher than isoflurane... but price coming down now that it is off patent (generics)

Anesthetic Gas Recovery as Metabolite

- Methoxyflurane: 50%
- Halothane: 20-25%
- Sevoflurane: 2.5%
- Enflurane: 2.4%
- Isoflurane: 0.17%
- Desflurane: 0.02-0.2%

1Humans
Pre-warmed isoflurane gas
Tissue oxygen-hemoglobin concentration (SpO₂)
Heart rate
Respiration rate
Ventilator
Superior data
Warming gas

Somnosuite (Kent Scientific)

Return of righting reflex
Successful rotarod test

Desflurane
Sevoflurane
Isoflurane
Halothane

Recovery times (min) in rats after 1 hour of anesthesia - Eger and Johnson, 1987

Somnosuite (Kent Scientific)

6 lbs Warm gas
30+ lbs Cold gas
Open Drop

Pathology cassette with isoflurane soaked material

Centrifuge tube
Depth of anesthesia can be controlled by moving the nose cone closer or farther away from the nostrils

Wire or plastic grate

Absorbent material
Isoflurane soaked

Open Drop

- Although current practice is to vent out (e.g., fume hood), 2009 publication has shown this method may be safe on table top w/o scavenging, [http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2694708/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2694708/)
- Check with your Safety Department
- Used for short term anesthesia (vs. calibrated vaporizer)
- Prolonged use = deaths

Open Drop Formula

<table>
<thead>
<tr>
<th>Volume of liquid agent/1000 ml chamber volume</th>
<th>Approximate concentration of isoflurane</th>
</tr>
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<tbody>
<tr>
<td>0.05 ml</td>
<td>1%</td>
</tr>
<tr>
<td>0.1 ml</td>
<td>2%</td>
</tr>
<tr>
<td>0.2 ml</td>
<td>4%</td>
</tr>
<tr>
<td>0.3 ml</td>
<td>6%</td>
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Replenish isoflurane every 3 mice

Emory University
Pentobarbital - Advantages

- Placental transfer of barbiturates occurs rapidly. However, when used in proper induction doses, excessive depression of the fetus does not occur
- Administered IV or IP
- Neither hepato- or nephrotoxic

Pentobarbital - Disadvantages

- Pharmaceutical grade very pricey
  - High cost renders it “unavailable in pharm grade” ***
- Chemical grade use must be addressed & justified

*** NIH statement 2013
Pentobarbital - Disadvantages

- Prolonged recovery esp. if additional doses administered
- Severe CV & resp depression
  - Anesthetic & lethal doses close to each other... high mortality possible

Ketamine

- Exact mechanism of action not established
- Thought to be a specific antagonist of N-methyl-D-aspartate glutamate receptors (NMDA)
- "Cocaine-like" effect in that it inhibits uptake of catecholamines into postganglionic sympathetic nerves
- Cleared by hepatic metabolism
- Alone it is a poor anesthetic and analgesic in rodents (when used alone)... must be used with other agents ('cocktail')

Ketamine

- CV effects resemble sympathetic nervous stimulation - i.e. increased arterial BP, HR, CO, cardiac workload & myocardial oxygen consumption
- Such effects obtunded by prior or co-administration of tranquilizers or sedatives ('cocktail')
- Transient increase of norepinephrine & epinephrine in plasma
Ketamine - Advantages

- In hypovolemic patients, arterial BP is maintained with ketamine because of peripheral vasoconstriction
- No significant effect on hepatic or renal function
- Useful in ‘cocktail’ preps with sedatives & tranquilizers
- Increases myocardial contractility

Ketamine - Disadvantages

- Increased airway & salivary secretions in some species (mild in mice & rats)
- Induces epileptiform bursts in thalamus & limbic system, but w/o spread to cortical areas - may increase seizure threshold in rats & mice
- Some respiratory depression following anesthetic doses in rodents

Ketamine ‘Cocktails’

- Safer, more ‘balanced’ anesthesia than pentobarbital or ketamine alone
  - Ketamine/xylazine
  - Ketamine/dexmedetomidine
  - Ketamine/xylazine/acepromazine
  - Other combinations
Ketamine ‘Cocktail’ Reversal

- Antagonist atipamezole shortens recovery of ketamine/ xylazine & ketamine/dexmedetomidine
- Atipamezole (0.5-1 mg/kg SC, IP, IM, IV) partially reverses xylazine & dexmedetomidine (not ketamine)
- Early reversal (10-20 minutes after induction) associated with undesirable behavioral disturbances due to effects of ketamine

Ketamine ‘Cocktails’

- Use of yohimbine for reversal of ketamine/xylazine no longer recommended due to yohimbine’s reported side effects

Avertin

- Combination of 2,2,2 tribromethanol & tertiary amyl alcohol (amyline hydrate)
- Adequate anesthesia up to 30 min
- Repeat doses not recommended due to abdominal irritation & peritonitis reports
- Degrades in presence of heat or light - refrigerate, wrap in foil
- Non-pharmaceutical grade compound
Urethane

- Ethyl ester of carbamic acid

Urethane - Advantages

- IP Administration results in long-lasting unconsciousness of 6-10 hr
- Spinal reflexes, neural transmission and cardiopulmonary function minimally affected
- Good analgesia for surgery in rodents
- Stable CV & respiratory functions

Urethane - Disadvantages

- Mutagen/carcinogen
- Readily absorbed through skin
  - initiates pre-neoplastic changes in the skin
  - targets multiple organs
  - suppresses bone marrow
  - readily crosses the placenta
  - induces fetal tumor formation (in utero)
Urethane - Disadvantages

- Strict guidelines (gloves, mask, prepare in fume hood)
- Requires safety approval
- Non-pharmaceutical grade compound
- Not used for survival surgeries