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)
Why Anesthetize an Animal

1 or more reasons:

- Avoid pain (loss of sensation)
- Immobilize (muscle relaxation)
- Avoid distress (loss of consciousness)
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

Pentobarbital 60 mg/kg
Inhalant Anesthetics - Advantages

Usually suitable as sole agents to reach ‘balanced’ anesthesia

• Loss of sensation
• Loss of consciousness
• Muscle relaxation
Inhalant Anesthetics - Advantages

- Precise control over anesthetic depth
- Rapid recovery
- 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
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

- 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 Metabolite¹

- Methoxyflurane: 50%
- Halothane: 20-25%
- Sevoflurane: 2-5%
- Enflurane: 2.4%
- Isoflurane: 0.17%
- Desflurane: 0.02-0.2%

¹Humans
Recovery times (min) in rats after 1 hour of anesthesia - Eger and Johnson, 1987
• Integrated digital vaporizer
• Pre-warmed isoflurane gas
• Tissue oxygen-hemoglobin concentration (SpO$_2$)
• Heart rate
• Respiration rate
• Ventilator
Somnosuite
6 lbs
Warm gas

Typical Anesthesia System
30+ lbs
Cold gas
The Savings

**Somnosuite vs Traditional**

**Isoflurane**

- Somnosuite: 1 bottle
- Traditional: 38 bottles

**Waste Gas Canister**

- Somnosuite: 9 canisters
- Traditional: 325 canisters

**Savings**

- Isoflurane Savings: 1:38
- Waste Gas Canister Savings: 1:36

*Reference:
Time: 8 hours
Concentration: 2% isoflurane
Subject: 30 g mice
Delivery: Nose cone*
Physiosuite (Kent Scientific)

- Homeothermic (warms rodent at exact temp through far infrared warming that goes beyond cutaneous warming by heating deep into rodent’s body with temp feedback)
- Pulse Oximeter and Heart Rate
- Automatic Ventilator (ventilate animals from 3g to 500g, enter weight and press run)
- End Tidal CO₂ Monitor
Open Drop

Pathology cassette with isoflurane or sevoflurane 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 soaked with anesthetic
Open Drop

- 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>
</thead>
<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>
</tr>
</tbody>
</table>

Replenish isoflurane every 3 mice
Injectable Anesthetics
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

- For cocktail doses refer to http://vpr.utsa.edu/files/larc/RodentSurgeryApplicationhandouts.pdf
Avertin

- Combination of 2,2,2 tribromomethanol & tertiary amyl alcohol (amylene 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
- Respiratory & cardiopulmonary functions minimally affected, including blood pressure due to effects on catecholamine release
- Good analgesia for surgery in rodents
Urethane - Disadvantages

- Peritoneal effusion & hemolysis
- Mutagen/carcinogen
- Readily absorbed through skin
  - pre-neoplastic changes in skin
  - targets multiple organs
  - suppresses bone marrow
  - readily crosses the placenta
  - 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
Resources

Presentations, References & Useful Notes

http://research.utsa.edu/research-funding/laboratory-animal-resources-center/training/
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