

Anesthetic Techniques for Laboratory Animals

Strict maintenance of anesthesia levels, good pre and post-operative care and asepsis during surgical procedures are essential for both experimental success and humane care of experimental animals.

1. Anesthesia

Anesthesia is defined as the absence of sensitivity to stimuli. It is a reversible loss of sensation.

Safe anesthesia = careful selection of the drugs to be administered and diligent monitoring of the patient

Appropriate monitoring allows one to anticipate complications and correct them before disastrous conditions occur.

A. Indications for anesthesia

to perform humane surgery  
for restraint  
to allow euthanasia  
to control convulsions

B. Types of anesthesia

i local--inject lidocaine/procaine beneath the skin or a short distance from the operative site; it requires sedation of the animal and aseptic technique

regional--nerve blocks, spinal anesthesia (epidural); these techniques require good knowledge of anatomy, skill and strict adherence to aseptic technique

general--pain sensation is abolished by producing unconsciousness in the patient; it is accomplished via the injection or inhalation of drugs

C. General considerations in choosing anesthetic agents

i) species of animal

size differences--mouse versus cow

species differences in drug responses--very important in veterinary medicine (for example, Rompun dosage for a cow is 1/10th the dosage for a dog)

surgical site

duration of the procedure--it is very difficult to use injectable anesthetic agents successfully when the procedure is over several hours in duration

type of experiment performed--physiologic studies may require the use of specific agents

- v) post-operative fate of the animal--recovery versus non-recovery procedure; alpha-chloralose and urethane should not be used for recovery surgeries

health and physiologic status of the animal--pregnancy or pre-existing diseases should be considered prior to choosing anesthetic agents

#### D. Stages of anesthesia

- i) Stage 1: stage of narcosis, sedation and analgesia; standing animals are uncoordinated

Stage 2: stage of involuntary excitement; good anesthetic methods provide quick passage through this stage

Stage 3: stage of surgical anesthesia; this stage is divided into 4 planes

Plane 1: earliest stage of surgical anesthesia  
Plane 2: level desired for most surgery  
Plane 3: respiratory muscle paralysis begins  
Plane 4: stage of anesthetic overdosage; respiration and heart function are depressed

Stage 4: paralysis of respiratory center in the brain and death

#### E. Monitoring the depth of anesthesia

- i) attempts at purposeful movement--the animal is very light and is inadequately anesthetized

heart rate and respiration rate--if heart rate and respiration rate increase when surgical stimulation occurs, the animal is feeling pain and is inadequately anesthetized

peripheral perfusion--the color of the oral mucous membranes should be monitored during anesthesia and surgery. Capillary refill time can be determined by pressing on the gums and counting the length of time it takes the blanched tissue to return to normal color. Normal capillary refill time is less than 2 seconds.

Pale mucous membranes indicate that hemorrhage or shock is occurring. Blue mucous membranes indicate that hypoxia (lack of oxygen) is present.

- v) maintenance of a patent airway--it is essential that airway occlusion be prevented during surgery. Small rodents should be positioned with the head and neck extended in a straight line. Larger species should have an endotracheal tube inserted into the trachea. Position of the head should be monitored. Rabbits and pigs do not breathe well when lying on their backs, and they require careful monitoring when placed in this position.

#### F Pre-anesthetic evaluation

- i The general health status of an animal or group of animals should always be ascertained prior to induction of anesthesia. The purpose of a pre-anesthetic evaluation is to identify any conditions present in the animal which may complicate anesthesia or surgery.

- ii) It is recommended that most animals be fasted prior to the induction of anesthesia in order to minimize the possibility that vomiting will occur during induction and to decrease abdominal distention, which can compromise respiratory function when excessive. The recommended lengths for fasting animals are:

rodents: no fasting required (most species cannot vomit)  
 rabbits: no fasting required (cannot vomit)  
 dog: overnight (approximately 12 hours)  
 cat: overnight  
 pig: overnight  
 primate: overnight  
 sheep: 24-48 hours

Dogs, cats, pigs, primates and sheep should be routinely fasted prior to anesthesia, unless specifically prohibited by the experimental design.

#### G. Anesthetic drug selection

- i) Single drug versus balanced technique

single drug: only one type of drug used

balanced technique: use a combination of one or several pre-anesthetic drugs, followed by a general anesthetic

- ii advantages of balanced technique

maximize beneficial actions of several drugs while minimizing potential toxicity of general anesthetic agents

for example, if a phenothiazine tranquilizer is used as a preanesthetic, the amount of barbiturate required to induce general anesthesia is reduced by 50%

In general, the use of balanced technique is preferable to single injection regimens. Balanced technique provides an anxiety and pain-free induction and recovery for the animal. Post-operative recovery is much smoother and often much quicker when balanced technique is used. Many general anesthetic agents provide little or no pain relief once the animal regains consciousness.

### iii) disadvantages of balanced technique

requires more time to give multiple injections than a single injection

The common physiologic and pharmacologic effects of all drugs administered must be known, and these effects must not be incompatible with the goals of the research.

## H Preanesthetic drugs

### i anticholinergic drugs--given to decrease salivation and prevent slowing of the heart rate

drug name: atropine sulfate, glycopyrrolate

indications for use: give prior to inhalation agents, Innovar-Vet, ketamine and barbiturates

contraindications for use: rapid heart rate

phenothiazine tranquilizers--given to prevent vomiting to calm the animal and stabilize the heart

drug name: acepromazine, promazine

indications for use: given prior to inhalation agents, fractious animals

contraindications for use: heart disease, low blood pressure, anemia, seizures

thiazine derivatives--given to produce sedation, muscle relaxation, analgesia, and to induce vomiting (all cats and most dogs)

drug name: xylazine (Rompun)

indications for use: frequently combined with ketamine for injectable general anesthesia

contraindications for use: pregnancy (it will induce abortion), do not combine with other tranquilizers

reversal agents: yohimbine (complete reversal)  
dopram (will reverse respiratory depression)

## I. Injectable anesthetic drugs

### i) Dissociative anesthetics

drug names: ketamine, tiletamine

indications for use: pre-anesthetic immobilization, frequently combined with either rompun or acepromazine for general anesthesia

If given alone, ketamine produces muscle rigidity, salivation and increased respiration and heart rate. Analgesia is reasonable for skeletal muscles, but poor for abdominal or thoracic procedures. Swallowing and coughing reflexes are maintained after administration. Cats and dogs will sometimes have seizures or exhibit bizarre behavior (ie., throwing themselves against the wall of the cage) after being given this drug.

contraindications for use: liver and kidney disease, epilepsy

duration of anesthesia: 45-60 minutes for combinations of rompun + ketamine

### ii Barbiturates--These drugs are used to control convulsions and are used as the sole anesthetic agent, or for induction of general anesthesia prior to maintenance with anesthetic gasses.

drug names: thiamylal and pentothal (ult. -short acting)  
pentobarbital sodium (short acting)

indications for use: general anesthesia

contraindications for use: respiratory or cardiac disease

duration of anesthesia: 15-20 minutes with ultra-short acting, 1-2 hours with pentobarbital

If additional dosages must be administered, the animal will require a much longer postoperative recovery period. It takes a dog 6-24 hours to recover from a single bolus injection of pentobarbital.

Pentobarbital produces poor analgesia. Rough postoperative recoveries are common. Shivering, vocalization, whining and paddling are seen. Animals must be carefully monitored following the administration of this drug to prevent hypoxia induced by shivering and respiratory depression.

Tolerance develops rapidly with repeated administration because these drugs alter liver function.

reversal agent: none

These drugs are formulated for intravenous injection only. They will cause tissue necrosis if injected outside the vascular system. If inadvertent perivascular injection occurs, the area should be infiltrated with large volumes of saline and lidocaine.

#### J. Inhalation anesthetic agents

##### i) advantages of inhalation anesthesia

precise control of anesthetic depth/ controlled reversal  
if intubated, airway patency is established and oxygen is present

some agents are not metabolized in the body and are excreted unchanged via the lungs

some agents provide analgesia in the postoperative period (methoxyflurane)

##### ii) disadvantages of inhalation anesthesia

requires more equipment and training to administer

it generally takes more time to induce anesthesia

personnel must be protected from exposure to anesthetic gas fumes

##### iii) methods of administration

clear jars--can be glass or plastic. They can be used for induction of small rodents, followed by placement of a nosecone over the face for maintenance of anesthetic

level. Volatile anesthetic is placed on a cotton ball that is inserted at the base of the nosecone

facemask attached to an anesthetic machine-used on animals that are not intubated

endotracheal tube and attachment to anesthetic machine

iv) selected inhalation agents

ether: safe for rats and mice; do not use in rabbits or guinea pigs because it irritates the respiratory mucosa; flammable; always use in ventilated hood, store open containers only in explosion-proof refrigerator

methoxyflurane: good for small rodents; can be safely used in jars; protect personnel from waste gas

isoflurane: minimal depressant effects on heart and respiration; usually administered using an anesthetic machine; very quick recovery

carbon dioxide: 100% CO<sub>2</sub> is used for euthanasia; it is usually diluted 50:50 with oxygen for anesthesia of small rodents; very quick recovery

nitrous oxide: will not produce surgical anesthesia if used alone; usually used in combination with other inhalation agents; animal must be on oxygen for several minutes after nitrous oxide is turned off in order to ; event hypoxia

K. Miscellaneous anesthetic agents

Avertin (tribromoethanol)--good anesthetic agent for small rodents, but it will cause the formation of fatal intestinal adhesions if administered more than one time. It should only be used once.

Urethane--is a carcinogen. It should be used for non-survival procedures only. Personnel must be protected from exposure.

Chloralose--is a convulsant. It should be used for non-survival procedures only.

Chloroform--is a carcinogen and is very toxic for mice. It should never be used for anesthesia

## L. References

Bennett, B.T. Essentials for Animal Research: A Primer for Research Personnel, National Agricultural Library, April 1990, Chapter 4.

Flecknell, P.A. Laboratory Animal Anesthesia, An Introduction for Research Workers and Technicians. Academic Press, London, 1987.

- M. The following drug dosage tables were obtained from Laboratory Animal Anesthesia, P. A. Flecknell, Academic Press, Inc., 1987.

### Drug Dose Rates

#### A. Mouse, Rat, Hamster and Gerbil (drugs listed in alphabetical order)

Drug	Mouse	Rat	Hamster	Gerbil
Alphaxalone/ alphadolone	10-15 mg/kg i/v ***	10-12 mg/kg i/v ***	150 mg/kg i/p **/*** 1	80-120 mg/kg i/p **/*** 1
Aspirin	120 mg/kg per os 4 hourly	100 mg/kg per os 4 hourly		
Atropine	0.04 mg/kg s/c,i/m	0.05 mg/kg s/c,i/m	0.04 mg/kg s/c,i/m	0.04 mg/kg s/c,i/m
Buprenorphine	2 mg/kg s/c 12 hourly	0.1-0.5 mg/kg s/c,i/v 8-12 hourly	—	
Codeine	60-90 mg/kg per os, 20 mg/kg s/c 4 hourly	60 mg/kg s/c 4 hourly		
Doxapram	5-10 mg/kg i/v	5-10 mg/kg i/v	5-10 mg/kg i/v	5-10 mg/kg i/v
Hypnorm (Fentanyl + fluanisone)	0.01 ml/30 g i/p */**	0.4 ml/kg i/m or i/p **	1 ml/kg i/m or i/p **	0.5-1 ml/kg i/m or i/p **
Hypnorm + diazepam	0.01 ml/30 g i/p 5 mg/kg i/p ***	0.3 ml/kg i/m 2.5 mg/kg i/p	1 ml/kg i/m 5 mg/kg i/p	0.3 ml/kg 5 mg/kg i/p
Hypnorm + midazolam (2 parts water for injection, 1 part Hypnorm and 1 part midazolam (5 mg/ml) (see text)	10 ml/kg i/p ***	2.7 ml/kg i/p ***	4 ml/kg i/p ***	8 ml/kg i/p ***

Drug	Mouse	Rat	Hamster	Gerbil
Immobilon SA (Etorphine + methotrimeprazin- e)		0.05 ml/100 g i/m ***		
Immobilon SA + midazolam (1 part Immobilon, 1 part midazolam (5 mg/ml))	***	0.05 ml/100 g i/m		
Inactin		80 mg/kg i/p **/***		
Ketamine	200 mg/kg i/m **	100 mg/kg i/m **	200 mg/kg i/p **	200 mg/kg i/m **
Ketamine + acepromazine	100 mg/kg i/m 2.5 mg/kg i/m **/***	75 mg/kg i/m 2.5 mg/kg i/m **/***	150 mg/kg i/m 5 mg/kg i/m **/***	75 mg/kg i/m 3 mg/kg i/m **/***
Ketamine + diazepam	200 mg/kg i/m 5 mg/kg i/p **/***	— —	— —	50 mg/kg i/m 5 mg/kg i/p **/***
Ketamine + xylazine	200 mg/kg i/m 10 mg/kg i/p **/*** !	90 mg/kg i/m 10 mg/kg i/m **/***	200 mg/kg i/p 10 mg/kg i/p ***	50 mg/kg i/m 2 mg/kg i/m **
Methohexitone	6 mg/kg i/v	7-10 mg/kg i/v ***		
Metomidate + Fentanyl	60 mg/kg s/c 0.06 mg/kg s/c ***			50 mg/kg s/c 0.05 mg/kg s/c ***
Morphine	10 mg/kg s/c 2-4 hourly	10 mg/kg s/c 2-4 hourly		
Pentobarbitone	40 mg/kg i/p i/p **/*** !	40 mg/kg i/p i/p **/*** !	50-90 mg/kg **/***	60-80 mg/kg **/***
Phenacetin	200 mg/kg per os 4 hourly	100 mg/kg per os 4 hourly		
Pethidine	20 mg/kg s/c or i/m 2-3 hourly	20 mg/kg s/c or i/m 2-3 hourly		
Thiopentone	30-40 mg/kg i/v ***	30 mg/kg i/v		
Tribromoethanol	125 mg/kg i/p (0.25%) **/*** !	300 mg/kg i/p *** !		250-300 mg/kg i/p (1.25%) *** !
Urethane		1000 mg/kg i/p ***		

- \* = sedation
- \*\* = immobilisation
- \*\*\* = anaesthesia
- ! = high mortality possible

B. Guinea Pig, Rabbit, Dog and Cat

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Drug	Guinea Pig	Rabbit	Dog	Cat
Acepromazine		5 mg/kg i/m	0.1-0.25 mg/kg i/m •	0.1-0.25 mg/kg i/m •
Alphaxolone/ alphadolone	40 mg/kg i/p ••	6-9 mg/kg i/v ••• 1		9-12 mg/kg i/v ••• 12-18 mg/kg i/m ••/•••
Aspirin			10 mg/kg per os 6-8 hourly	
Atropine	0.05 mg/kg s/c,i/m	0.2 mg/kg i/m	0.05 mg/kg s/c,i/m	0.05 mg/kg s/c,i/m
Buprenorphine	0.05 mg/kg s/c 8-12 hourly	0.02-0.05 mg/kg s/c or i/v 8-12 hourly	0.01-0.02 mg/kg i/m,s/c,i/v 12 hourly	0.005-0.01 mg/kg s/c or i/v 12 hourly
Chloralose (1% soln.)		80-100 mg/kg i/v ••/•••	80-110 mg/kg i/v ••/•••	80-90 mg/kg i/v ••/•••
Codeine			0.25-0.5 mg/kg- per os with paracetamol 6 hourly	
Doxapram	5 mg/kg i/v	2.5 mg/kg i/v	2.5 mg/kg i/v	2.5 mg/kg i/v
Hypnorm (fentanyl/ fluanisone)	1 ml/kg i/m	0.5 ml/kg i/m	0.1-0.2 ml/kg i/m ••	
Hypnorm + diazepam	1 ml/kg i/m 2.5 mg/kg i/p	0.3 ml/kg i/m 2 mg/kg i/p or i/v •••		
Hypnorm + midazolam (2 parts water + 1 part Hypnorm + 1 part midazolam (5 mg/ml)	8 ml/kg i/p •••	0.3 ml/kg i/m + 2 mg/kg i/v or i/p •••		
Immobilon SA (etorphine/ methotrimeprazine)		0.025-0.05 ml/kg i/m ••/•••	(0.5 ml/4 kg i/m) ••/•••	
Ketamine	100-200 mg/kg i/m ••	50 mg/kg i/m	—	20 mg/kg i/m
Ketamine + acepromazine	125 mg/kg i/m 5 mg/kg i/m ••/•••	75 mg/kg i/m 5 mg/kg i/m •••		
Ketamine + diazepam	100 mg/kg i/m 5 mg/kg i/m ••/•••	25 mg/kg i/m 5 mg/kg i/m ••		

## APPENDIX 2

Drug	Guinea Pig	Rabbit	Dog	Cat
Ketamine + xylazine	40 mg/kg i/m 5 mg/kg s/c **/***	10 mg/kg i/v 3 mg/kg i/v ***	5 mg/kg i/v 1-2 mg/kg i/v,i/m ***	15 mg/kg i/m 1 mg/kg s/c, i/m ***
Methohexitone	31 mg/kg i/p **	10 mg/kg i/v ***	4-8 mg/kg i/v ***	4-8 mg/kg i/v ***
Morphine	10 mg/kg s/c or i/m 4 hourly	5 mg/kg s/c or i/m 2-4 hourly	0.5-5 mg/kg s/c or i/m 2-4 hourly	0.1 mg/kg s/c 4 hourly
Naloxone	0.01-0.1 mg/kg i/p,i/v	0.01-0.1 mg/kg i/m,i/v	0.01-0.05 mg/kg i/m,i/v	0.01-0.05 mg/kg i/m,i/v
Paracetamol	—	—	10-20 mg/kg plus codeine 6 hourly	
Pentazocine		10-20 mg/kg s/c or i/m 4 hourly	2 mg/kg i/m 4 hourly	8 mg/kg i/p ? 4 hourly
Pentobarbitone	37 mg/kg i/p **/***	45 mg/kg i/v *** !	20-30 mg/kg i/v ***	25 mg/kg i/v
Pethidine	20 mg/kg s/c or i/m 2-3 hourly	10 mg/kg s/c or i/m 2-3 hourly	10 mg/kg s/c or i/m 2-3 hourly	10 mg/kg s/c or i/m 2 hourly
Propofol			5-7.5 mg/kg i/v ***	7.5 mg/kg i/v ***
Thiopentone		30 mg/kg i/v	10-20 mg/kg i/v ***	10-15 mg/kg i/v ***
Urethane	1500 mg/kg i/v or i/p **/***	1000 mg/kg i/v or i/p **/***	1000 mg/kg i/v **/***	1250 mg/kg i/v **/***
Xylazine		3 mg/kg i/m	1-2 mg/kg i/m */**	1-3 mg/kg i/m,s/c */**

- \* = sedation
- \*\* = immobilisation
- \*\*\* = anaesthesia
- ! = high mortality possible

Drug	Primates	Pig	Sheep and Goats	Ferret
Acepromazine	0.2 mg/kg i/m •	0.2 mg/kg i/m •	0.05-0.1 mg/kg i/m •	0.2 mg/kg i/m •
Alphaxalone/ alphadolone	12-18 mg/kg i/m **/*** 10-12 mg/kg i/v***	6 mg/kg i/m ** then 2 mg/kg i/v ***	2.2 mg/kg i/v (adult) 6 mg/kg i/v (lamb) ***	12-15 mg/kg i/m **/*** 8-12 mg/kg i/v ***
Aspirin	20 mg/kg per os 6-8 hourly			
Atropine	0.05 mg/kg i/m	0.05 mg/kg i/m	0.05 mg/kg i/m	0.05 mg/kg i/m
Azaperone	—	5 mg/kg i/m •	—	—
Buprenorphine	0.01 mg/kg i/m or i/v 12 hourly	0.1 mg/kg i/m 12 hourly	0.005 mg/kg i/m 4-6 hourly	
Diazepam	1 mg/kg i/m	1-2 mg/kg i/m •	2 mg/kg i/m 1 mg/kg i/v •	2 mg/kg i/m
Doxapram	2 mg/kg i/v	1-2 mg/kg i/v	2 mg/kg i/v	1-2 mg/kg i/v
Hypnorm (Fentanyl/ fluanisone)	0.3 ml/kg i/m ••	—		0.5 ml/kg i/m
Immobilon LA (Etorphine/ acepromazine)		0.5 ml/50 kg ••/*** 1	0.5 ml/50 kg i/m (>30 kg) ••/***	
Immobilon SA (Etorphine/ methotrimeprazine)			0.5 ml/4 kg (<30 kg) ••/***	
Ketamine	5-25 mg/kg i/m •/***	10 mg/kg i/m	20 mg/kg i/m	20-30 mg/kg i/m ••
Ketamine + diazepam	15 mg/kg i/m 1 mg/kg i/m ••/***	10 mg/kg i/m 2 mg/kg i/m ••	4 mg/kg i/v 2 mg/kg i/m or 1 mg/kg i/v ***	25 mg/kg i/m 2 mg/kg i/m ***
Ketamine + xylazine	10 mg/kg i/m 0.5 mg/kg i/m ••/•••	5 mg/kg i/m 1 mg/kg i/m •/••	4 mg/kg i/v 1 mg/kg 0.05 mg/kg (goat) •/••	25 mg/kg i/m 1-2 mg/kg i/m •••
Pentobarbitone	5-15 mg/kg i/v •••	30 mg/kg i/v ••/***	30 mg/kg i/v •••	25-30 mg/kg i/v •••
Pethidine	2-4 mg/kg i/m 3-4 hourly	2 mg/kg i/m 4 hourly	up to 200 mg i/m total dose 4 hourly	
Thiopentone	15-20 mg/kg i/v ••/***	6-9 mg/kg i/v •••	15 mg/kg i/v •••	
Xylazine			1 mg/kg i/m (sheep) 0.05 mg/kg i/m (goat) •	

- = sedation
- = immobilisation
- = anaesthesia
- 1 = high mortality possible