Commonly Used Laboratory Anaesthetics

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1 Introduction

Anaesthetics are the drug that induces temporary state of unconsciousness, loss of senses in order to carry out surgical procedures. Anaesthetics are used during tests and surgical operations to prevent pain and discomfort and enable a wide range of medical procedures to be performed on patients. Analgesia is the absence of pain in response to stimulation that would normally be painful. An analgesic drugs act at the level of the central nervous system or at the peripheral site of inflammation to diminish or block pain signals.

At cellular level, the main function of anaesthetics is to inhibit synaptic transmission by reducing transmitter release and inhibition of transmitter action or reduction of the excitability of the post-synaptic cell or by blocking axonal conduction. For a drug to be useful as an anaesthetic, it should be readily controllable, so that induction and recovery are rapid, allowing the level of anaesthesia to be adjusted as required during the course of operation. Anaesthetic drugs are usually given in combination with analgesics or sedatives in order to achieve full anaesthesia. For anaesthetic drugs, the duration of action has not been provided. Duration of anaesthesia is influenced by the drugs used, strain, age, sex, body weight, procedure performed and the amount of stimulus during the procedure

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2 Classification of Anaesthetics

Anaesthetics are broadly classified into two categories: local and general anaesthetics depending on goals and type of medical intervention (Fig. 1). The anaesthetics are widely being used for carrying out surgical procedures in experimental animals. This chapter discusses the general pharmacology of anaesthetics and their use in laboratory animals.

2.1 General Anaesthetics

General anaesthetics (GA) are used to render patients unaware of and unresponsive to painful stimulation during surgical procedures for almost 170 years. Prior to their discovery, surgery was a traumatic and barbaric affair, yet today, it is accepted as a routine and essential part of modern medicine. They are given systemically and exert their main effects on central nervous system. These drugs produce reversible loss of all sensation and consciousness associated with immobilization, analgesic, amnesic effect. GA acts primarily by depressing synaptic transmission. The main target for action of GA appears to be thalamic nuclei or reticular activating system (RAS), hypothalamus for memory related affects or blocking of muscular reflexes at spinal level. As the concentration of GA is increased, all the brain functions are affected including motor control, reflex activity, respiratory depression and autonomic regulation. Therefore, doses should be carefully monitored and adjusted within therapeutic levels by the anaesthetist before using it for surgical procedures on animals.

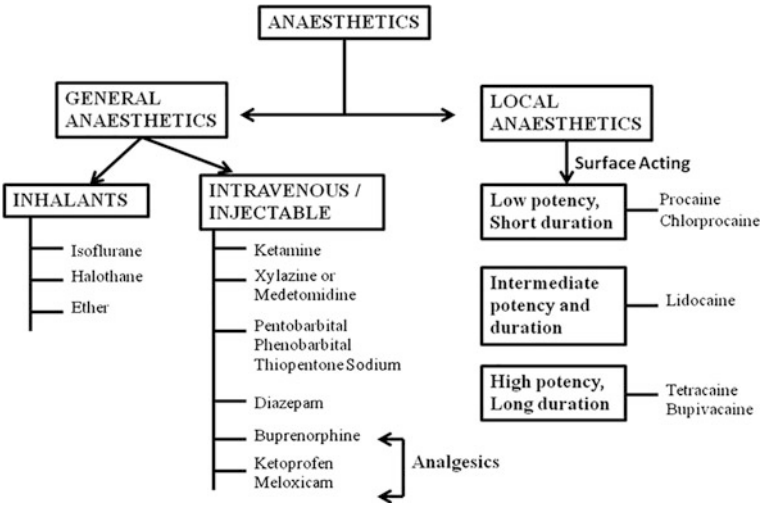

Fig. 1 Classification of commonly used laboratory anaesthetics

2.1.1 Mechanism of Action

In general, some GA may selectively inhibit excitatory ion channels (NMDA type of glutamate receptor which gates Ca^{+2} ion channel (for ex: N20, ketamine). Whereas, few of them potentiate the action of inhibitory transmitter GABA to open Cl^{-} ion channels (barbiturates, BZDs, inhalational) (Fig. 2).

Lipid Theory

Overton and Meyer suggested that general anaesthetics act on the plasma membrane and exert their action. This is supported by proof that the potency of the drug has immediate, affirmative association with the lipid solubility of the blood. The mechanism of action was recommended to be enhanced fluidity of the membrane. The elucidation of the Overton and Meyer finding has been challenged and discredited. In 1978, a workgroup processed conformational model pathological situations which alter lipid membrane composition could decrease nervous response to anaesthetics.

Ion Channels

General anaesthetics inhibit excitatory functions of some CNS receptors such as glutamate or 5-HT receptors. Some general anaesthetics also excite inhibitory receptors, notably $GABA_A$ receptors and TREK (Temperature sensitive,

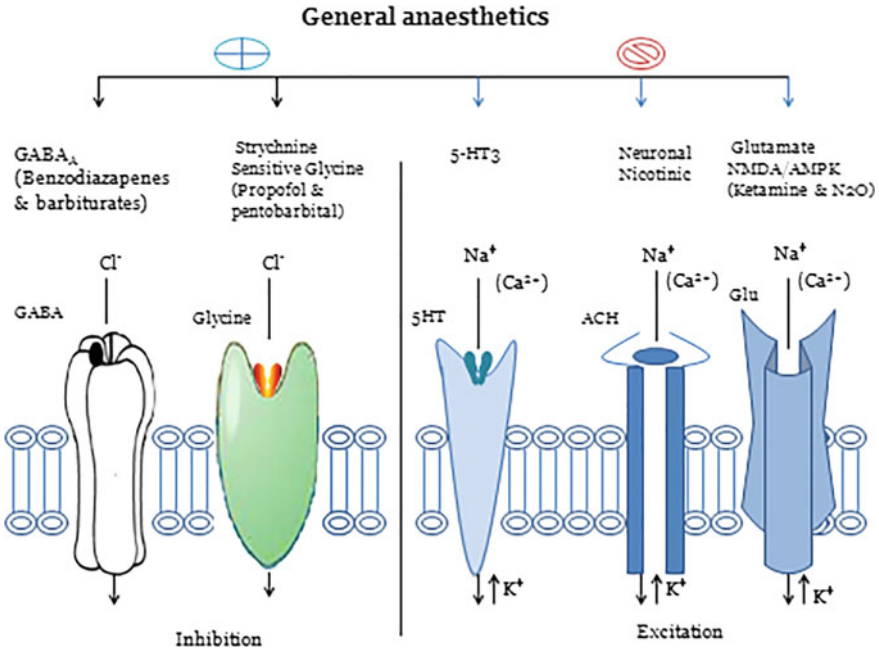

Fig. 2 Mechanism of action of general anaesthetics

osmosensitive and mechanogated K^+ channel). General anaesthetics may decrease transmitter release pre-synaptically or decrease excitability of post-synaptic neuron.

The exact mechanism of action of general anaesthetics is not known. The most accepted mechanisms are:

- Inhaled and some intravenous anaesthetics bind to specific sites on GABA receptor chloride channels and activate these receptors and increase the inhibitory neurotransmission which leads to CNS depression.
- Inhalational anaesthetics also enhance the sensitivity of glycine—gated chloride channels to glycine. These glycine receptors bring about inhibitory neurotransmission in the brainstem.
- Some anaesthetics like ketamine and nitrous oxide bind to and inhibit the N-methyl D-aspartate (NMDA) receptors.
- Inhalational and intravenous agents act at multiple sites in the nervous system and depress the neuronal activity at many sites in the brain.
- Some GA may activate the potassium channels in the brain leading to hyperpolarization of the membranes and thereby inhibitory effect. This in addition to their effects on the $GABA_B$ receptors.

2.1.2 Classification of General Anaesthetics

General Anaesthetics are broadly classified into two categories:

Intravenous and inhalational anaesthetics

Inhalational Anaesthetics

Inhalational anaesthetics are in the form of gases or volatile liquids and most preferred method in terms of safety and efficacy. It is easy to maintain and adjust the anaesthetic depth in case of inhalational anaesthetics. Because the exhalation, a process by which anaesthetics are eliminated from the blood and with less dependence on drug metabolism to eradicate the drug from the body, there is less possibility for drug-induced toxicity and allowing rapid induction and smooth recovery.

The disadvantages to inhalant anaesthesia are the intricacy and cost of the equipment required for administering the anaesthesia and possible hazards to personnel involved. As all inhalant drugs are volatile liquids, they should be stored in seperate rooms because the vapours are either flammable or toxic to inhale over extended period of time.

- Most inhalational anaesthetics that are widely used are ether, chloroform, cyclopropane which has been now replaced in developed countries particularly by halothane, enflurane, nitrous oxide (N_2O) and isoflurane.
- Inhalant anaesthetics offer a secure, consistent, reversible and reproducible means of depiction rodents unconscious in order to perform surgeries and other

complex or potentially painful procedures. Inhalant anaesthesia of small rodents is commonly maintained and makes use of face masks or nose cones.

Ether (Diethyl Ether):

- Ether is a highly volatile liquid and a potent anaesthetic which produces good analgesia and marked muscle relaxation.
- However, it is currently not employed for surgical procedures because of its unpleasant and inflammable properties.
- Low cost and easy dispensing by open drop method makes it use still in animal laboratories by inexperienced personnel.

Cyclopropane:

- It is a colourless gas with sweet odour and taste, available as liquid under pressure. It produces analgesia without loss of consciousness in 1–2% concentration, in 6–8% it produces loss of consciousness while 20–25% is required to produce surgical anaesthesia.
- It has low blood solubility. The induction and recovery are rapid and smooth. Blood pressure and cardiac contractility are well maintained with cyclopropane even on prolonged administration.
- Muscle relaxant activity is fairly good. Because of its highly inflammable and explosive nature, the close circuit has to be used to conserve the drug and to keep its concentration in the operating room low.
- Cyclopropane also sensitizes the myocardium to adrenaline and may produce a variety of cardiac irregularities such as tachycardia and fibrillation.

Chloroform:

- Chloroform is a commonly used laboratory inhalational anaesthetic.
- It is a colourless, volatile, liquid derivative of trichloromethane with an ether-like odour.
- One possible mechanism of action for chloroform is that it increases movement of potassium ion through certain types of potassium channels in nerve cells.
- Due to its highly inflammable and toxicity characteristics, it has been withdrawn from the market for surgical procedures and is no more used.
- Chances of cardiac disarrhythmia.

Halothane:

- It is widely used inhalational anaesthetic as it is non-explosive, non-irritant; induction and recovery are relatively fast.

- However, it is not a good analgesic or muscle relaxant which limits its use for surgical procedures.
- It is highly potent and can easily produce respiratory and CVS failure partly due to myocardial depression and vasodilation, so precise control of administered concentration is important. Two rare but serious adverse reactions associated with halothane are hepatotoxicity and malignant hyperthermia.

Enflurane:

- Halogenated anaesthetic is similar to halothane but less soluble in blood and, less metabolized, therefore less risk of toxicity.
- Enflurane cause faster induction and recovery compared to halothane probably due to its ability to cause less accumulation of fat.
- High doses are associated with risk of epileptic seizures.

Isoflurane:

- Isoflurane is the first choice of anaesthetic used for animal restraint or surgical procedures in laboratory animal species. Isoflurane is delivered via a nose cone and inhaled in rodents or provided through an intratracheal tube in larger species. Maintenance anaesthesia is typically between 1.5 and 3% isoflurane. Induction of anaesthesia with gas is typically achieved with <2 min exposure to 3–5% isoflurane.
- It is similar to enflurane, but lacks epileptogenic property.
- It is more potent and more volatile and less soluble in blood.

Advantages: It has better adjustment of depth of anaesthesia and low toxicity.

Disadvantages: It is of high cost and is a respiratory irritant.

Desflurane:

Desflurane is a congener of isoflurane. It has all the advantages of isoflurane. In addition, it has low solubility in blood and tissues because of which it rapidly attains therapeutic concentrations in the alveoli. Therefore, induction and recovery are very rapid and smooth.

Disadvantage:

- It is pungent which may induce coughing and sometimes laryngospasm. It is therefore used for maintenance of anaesthetia and is not preferred for induction.
- Because of low volatility, a special vaporizer is required for administration.
- It can cause transient sympathetic stimulation and tachycardia.

Sevoflurane:

Sevoflurane is the latest introduction to inhalational anaesthetics. It has the benefits of desflurane but is not pungent. It is a good bronchodilator and has rapid

and smooth induction and recovery because of low solubility in blood and tissues. This also makes it suitable for day—care surgeries.

Disavantages:

- Sevoflurane is chemically unstable and is degraded by carbon dioxide absorbents (soda lime) to a metabolite that can cause nephrotoxicity. Postoperative restlessness is avoided by premedication with midazolam.
- It undergoes biotransformation (about 3%) in the liver to release fluoride ions which can cause nephrotoxicity.
- It can precipitate malignant hyperthermia in genetically susceptible individuals.

Nitrous Oxide (N_2O):

Nitrous oxide (N_2O) was discovered in 1793 by the English scientist Joseph Priestley, who also discovered oxygen (O_2). In 1799, Sir Humphrey Davy administered N_2O to visitors at the Pneumatic Institute and gave it for the first time the term "laughing gas". He astutely noted the analgesic effects of the gas and even predicted its application in suppression of pain during surgical procedures.

- Nitrous oxide is given usually as an adjuvant to other anaesthetic due to its low potency, negligible toxicity, rapid induction and recovery and good analgesic properties.
- It may pose risk of bone marrow depression with long-term administration and accumulation in gaseous activities.

Newer anaesthetic:

Xenon:

Xenon is an inert gas that has properties very close to an ideal anaesthetic. It is available in selected centres in some countries.

Advantages:

- Rapid induction, insoluble in blood and tissues.
- Rapid recovery.
- Potent anaesthetic.
- No effect on hepatic, renal or pulmonary function.
- Not metabolized in the body.

Disadvantages:

Xenon cannot be manufactured but can only be extracted from air which makes it very expensive and largely unaffordable. If this problem is taken care of, xenon will top the list of anaesthetics.

Intravenous Anaesthetics

These are drugs which on i.v. injection produces rapid unconsciousness in about 20 s, as soon as drug reaches the brain from its sight of injection. At the same time, larger number of animals is maintained under anaesthesia. Repeated injections and constant rate infusions (CRI) can be helpful for maintaining anaesthesia for longer period. When using parenteral anaesthetics, it is important to consider accurate dosing with correct multidrug use ratios, storage conditions and feasibility of immediate use following reconstitution. It is critical to weigh each animal accurately prior to administration of a calculated dose of anaesthesia to avoid either over-dosing or under-dosing.

Barbiturates:

- The principal effect of a barbiturate is depression of the CNS by hindrance with passage of impulses to the cerebral cortex. Barbiturates act directly on CNS neurons in a manner similar to that of the inhibitory transmitter GABA, therefore acts as GABA-A agonists. Barbiturates are considered to be good anaesthetic agents but with less analgesic and sedative effect.
- Phenobarbital sodium is used for prolonged experiments.
- Pentobarbital sodium (Nembutal), the most commonly used drug of this class that causes rapid and long-acting anaesthetic action (6% dissolved in 10% alcohol). In prolonged anaesthesia, the drug has also been shown to cause respiratory depression and impaired myocardial contractility.
- Methohexital and Thiopental are considered short and ultra-short acting anaesthetics and are more commonly used as induction agents in large animal species.
- Sodium pentobarbital (Nembutal) and sodium thiopental (Pentothal) are currently the two most commonly used barbiturates. The duration of action of pentobarbital is considerably longer than that of thiopental.

Advantages: Rapid anaesthetic onset provides a prolonged duration of surgical anaesthesia.

Disadvantages: Prolonged recovery time; inadequate analgesic properties; extremely expensive; narrow margin of safety; produces respiratory depression at higher dosages; non-rodent species may experience a distressful anaesthetic recovery.

Thiopentane sodium:

- It is an only ultra-short acting barbiturate used for animal surgical operations. On i.v. injection it causes unconsciousness within 20 s that lasts for 20 min due to its high-lipid solubility. It is highly soluble in water, so it should be prepared freshly before injection.
- It is widely used as an induction agent for routine purposes.
- It is not a good analgesic and is a weak muscle relaxant. It is used as a sole anaesthetic for short operations that is not painful.
- Risk of CVS and respiratory depression

Ketamine:

- Ketamine is a dissociative anaesthesia characterized by profound analgesia, immobility, amnesia with light sleep and feeling of dissociation from one's own body and the surroundings. The target area for ketamine action is in the cortical and subcortical areas, not in the reticular activating system.
- It inhibits the entry of Ca^{+2} ions by blocking NMDA type of excitatory glutamate receptor; onset of action is 2–5 min.
- Ketamine is the most commonly used injectable anaesthetic used in a variety of species. However, ketamine used as the sole anaesthetic is not recommended. In most cases, ketamine is used in combination with other injectable agents such as α-2 agonists (xylazine or medetomidine) or benzodiazepines to reduce or eliminate many of the less desirable side effects if used alone.

Advantages: Ketamine has a wide margin of safety in most species; residual analgesic effect following anaesthetic recovery. In combination with other drugs, it can provide a surgical plane of anaesthesia for about one-half hour.

Disadvantages: Ketamine alone does not provide muscle relaxation and muscle spasms may be observed.

Ketamine combinations:

(A) **Ketamine + Xylazine**: A single syringe must be used for the administration of both drugs after mixed well to produce a deep level of sedation. This combination is the most common injectable anaesthetic used in rodent species.

- Advantages of ketamine/alpha2-agonist combinations are that they may produce short-term anaesthesia and also shows good analgesia, and the healing can be hasten by reversing the alpha2-agonist with atipamezole or yohimbine. Whereas, in some cases, the combination can cause profound cardiac depression.

(B) **Ketamine + Xylazine + Acepromazine**: These drugs can be mixed well before administration because the drugs are incompatible and once mixed, will decrease over time. The acepromazine is added to the ketamine/xylazine mixture consequences in deeper and/or longer plane of anaesthesia in small rodents, especially rats, and probably in mice.

(C) **Ketamine + Diazepam**: Before administration of these drugs, it can be mixed well in a single syringe. Advantages of ketamine and diazepam have advantage as compared to ketamine/xylazine combinations include it shows limited cardiovascular effects like minimal hypotension. However, in rodents, ketamine/diazepam is only combination which provides light anaesthesia so it may be suitable for chemical restraint. This combination is favoured for non-painful procedures and imaging since it is safer than the ketamine/alpha2-agonist combinations.

Alpha2-agonists (Xylazine or Medetomidine):

The alpha2-agonists (xylazine or medetomidine) are hypnotic analgesics. It is able to produce sufficient depth of anaesthesia for even minor surgical procedures. But xylazine and ketamine combinations produce good analgesics effect that may be useful during surgery.

Benzodiazepines:

It includes diazepam, midazolam and zolazepam. All drugs of this class have anti-anxiety and anticonvulsant drugs with excellent muscle relaxation activity. They have negligible cardiovascular and respiratory effects. Sedation is minimal in most species, except for swine and non-human primates with no analgesic effect. The primary use of these drugs in anaesthesia is in combination with other drugs. Ketamine—diazepam, midazolam—narcotic combinations can be very useful for induction of general anaesthesia and for short procedures.

Chloralose:

- It is a mixture of chloral and glucose prepared by heating equal parts of anhydrous glucose and chloral, when both α-chloralose (active form) and β-chloralose (inactive form) are formed.
- Chloralose is the active form (α-chloralose) freely soluble in hot water, alcohol and ethanol and slightly soluble in cold water. It is prepared by heating 1% solution by boiling in 0.9% NaCl (saline) or in distilled water, and administered i.v. or i.p. at a temperature of 30–40 °C before the chloralose comes out of the solution. Duration of action for α-chloralose is 8–10 h.
- It is suitable only for acute experiments, usually in dogs or cats and induce consistant depth of anaesthesia. The respiration and CVS depression does not occur at optimum doses.
- Main problem is associated with low-water solubility of chloralose but can make up 10% solution in PEG. It is not a suitable anaesthetic for rabbits since they get narcotized, large volume needed and may produce convulsions on slight stimulation.

Urethane (Ethyl Carbamate):

- Readily soluble in water (25%) giving a neutral solution.
- It is suitable only for acute experiments since it has delayed toxic effect on liver and can cause agranulocytosis and pulmonary adenomata. It produces little or no effect on nerve transmission and little reflex depression.
- It is suitable for rabbits or rats; duration of anaesthesia is 3–4 h or more. Mice develop an exceptionally high incidence of lung tumour regardless of route of administration.

Paraldehyde:

- It has a wide range of safety because it depresses only the cerebrum and not the medullary centres.
- On i.v. injection it produces cardiac dilation and pulmonary congestion and oedema.
- Bilateral carotid occlusion produces poor pressor response or even a depressor response.

Magnesium Sulphate:

- A 20% magnesium sulphate solution 5 ml/kg i.v. produces anaesthesia for about an hour. Its principal use is in producing euthanasia.

Chloral hydrate:

- Chloral hydrate (trichloroacetaldehyde monohydrate) has been used in physiological experiments in laboratory animals (particularly rodents) because of their hypnotic property. Chloral hydrate is generally considered to be a good hypnotic but a poor analgesic even at anaesthetic doses. For beginning/continuation of surgical anaesthesia (and for a smoother initiation/recovery period), it must be commonly administered with some other anaesthetic or tranquilizing agent.
- It is widely used in physiological experiments because at hypnotic doses, the drug has minimal effects on cardiorespiratory function. Larger doses may cause dangerous respiratory and thermoregulatory depression, cardiac arrhythmias and severe hypotension. At hypnotic doses, the drug has minimal effects on cardiorespiratory function.

Analgesics:

Improved pain management is the main objective in the use of experimental animals. Analgesics are those agents which decrease or relieve pain without loss of consciousness during surgical procedures. The distress, suffering and pain in laboratory animals is an ethical duty for all individuals who work with these species in biomedical research. Systemic and/or local analgesics may also cut the anaesthetic necessity and have a preventive effect on pain perception which continues into the period of recovery. Analgesic administration should be given both pre-operatively and post-operatively for sufficient pain relief in rodents.

In experimental animal species, two most commonly used analgesics are opioids and nonsteroidal anti-inflammatory drugs (NSAIDs). But type of experimental model in the study is also very important for the choice of drug as anaesthetics (Fig. 2).

Opioids (Buprenorphine):

- Opioids drugs are firstly bind with three different receptors [mu (μ), kappa (κ), and delta (δ)] as agonists, partial agonists or antagonists and produce their effect. The expression of these receptors is mainly within the brain and spinal cord.

Advantages: It is a potent analgesia; during surgery, simultaneous administration opioids can lower the dose of barbiturate as general anaesthetic; receptor mediated mechanism in the brain and spinal cord; long history of use in research; the effect is antagonised by naloxone.

Disadvantages: Highly probable for human abuse and addiction; duration of action is very short; repetitive use may consequence in tolerance development.

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs):

- Carprofen, Meloxicam, Ketoprofen, Ibuprofen, Acetaminophen.
- All classes of drugs belong to this group which shows inhibitory activity of the cyclooxygenase (COX) enzyme. The COX enzyme assists the production of Prostoglandin G2 (PGG2) which stimulates the range of enzymatic processes that are involved in normal physiological processes and production of Prostoglandin E2 (PGE2). PGE2 particularly plays an important role to produce pain in the periphery and within the central nervous system. Thus, inhibition of COX blocks the action of PGE2 that is effective in managing the pain, discomfort in periphery and within the CNS. COX-1 and COX-2 are the main two forms of the COX enzyme. Additionally, COX inhibitors are categorized as non-selective COX inhibitors and selective COX-2 inhibitors. This peculiarity has been made because COX-2 inhibition is supposed to be chief mechanism of NSAID to give analgesia and anti-inflammatory action even though this "consensus" is still under debate.
- Blockage of both COX-1 and COX-2 by NSAIDs is important in peri-operative pain practice.
  In the peri-operative situation, coxibs give an additional advantage over NSAIDs by avoiding the production of platelet thromboxane and clotting.

Advantages: Newer drugs include carprofen; meloxicam shows an analgesic activity for prolonged time; it reveals analgesic quality that equals to some opioids.

Disadvantages: Caution for inflammation models, infectious disease and coagulation research because of anti-inflammatory properties; COX-1 has side effects such as gastrointestinal complications, prolonged coagulation times and changes in kidney function with non-COX-2 selective forms.

2.2 Local Anaesthetics

Local anaesthetics (LA) are drugs that block nerve conduction when applied locally to nerve tissue in appropriate concentrations. Their action is completely reversible. They act on every type of nerve fibre and can cause both sensory and motor paralysis in the innervated area. They act on axons, cell body, dendrites, synapses and other excitable membranes that utilize sodium channels as the primary means of action potential generation.

Lidocaine, Bupivacaine, Proparacaine:

- Local anaesthetics particularly bind with voltage-gated Na + channel in the nerve cell membrane which blocks nerve impulses. Sufficient concentration of local anaesthetics is able to inhibit the conduction of nerves when applied locally.
- Routes of administration of local anaesthetic are either topical to mucus membranes of nose, eye, etc. or directly injected into tissues and around nerve bundles. Local anaesthetics would be considered as an additional analgesic to opioid and NSAID analgesics and is administred before starting surgery.
- The two most commonly used local anaesthetics in veterinary patients are **lidocaine** (xylocaine or novocaine) and **bupivacaine** (marcaine or sensocaine). Lidocaine has 1–2 min onset and 1½–2 h of duration of action. Bupivacaine shows slower onset (5–10 min) and a much longer duration of action (4–12 h, site dependent)**.**

Advantages: Administration of local anaesthetics can provide a good adjunct to general anaesthesia for pain relief in pre and intra-operatively procedure.

Disadvantages: Avoid intramuscular and intravenous administration because both routes reach systemic circulation very rapidly. Signs and symptoms of overdose or systemic toxicity include CVS effects, seizures and death.

3 Anaesthetics Used Alone in Laboratory

Anaesthetics	Species	Dosage (per kg of body weight)	Route of administration
Isoflurane	Mice, rats	Induction—3–4%	Inhalation, nose cone method
	Guinea pigs, rabbits	Maintenance dose–1–2%	
Pentobarbital	Mice	35 mg	I.V
		50–90 mg	I.P
	Rats	30–40 mg	I.V

(continued)

(continued)

Anaesthetics	Species	Dosage (per kg of body weight)	Route of administration
		30–60 mg	I.P
	Guinea pigs	30–50 mg	I.P
	Rabbits	50–60 mg	I.V
Alpha-chloralose	Mice	114 of 5% solution	I.P
	Rats	31–65 mg	I.P
	Rabbits	80–100 mg	I.V.
Urethane (ethyl carbamate)	Rats	1000–1500 mg	I.P
	Guinea pigs	1.5 g	I.P
	Rabbits	1000 mg	I.V, I.P
Chloral hydrate	Mice	370–400 mg	I.P
	Rats	300–450 mg	I.P
	Guinea pigs	400–600 mg	I.P
Buprenorphine	Mice	2 mg	I.P, S.C, P.O
	Rats	0.01–0.05 mg	I.P, S.C, P.O
	Guinea pigs	0.05 mg	S.C, I.M, I.P
	Rabbits	0.01–0.05 mg	S.C, I.M, I.V
Ketoprofen	Mice	2 mg	I.P, S.C, P.O
	Rats	0.01–0.05 mg	I.P, S.C, P.O
	Guinea pigs	0.05 mg	S.C, I.M, I.P
	Rabbits	0.01–0.05 mg	S.C, I.M, I.V
Meloxicam	Mice	1–2 mg	I.P, S.C
	Rats	1–2 mg	I.P, S.C, P.O
	Guinea pigs	0.5 mg	S.C, P.O
	Rabbits	0.3 mg	S.C
Lidocaine	Mice, rats, guinea pigs, rabbits	2–4 mg	Topical (surface), S.C, intra-incisional
Bupivacaine	Mice, rats, guinea pigs, rabbits	1–2 mg	Topical (surface), S.C,
Thiopentone sodium	Mice, rats	80–100 mg	I.P
Diazepam	Mice, rats, guinea pigs, rabbits	2.5–5 mg	I.P

4 Anaesthetics Used in Combination in Laboratory

Anaesthetics	Species	Dosage (per kg of body weight)	Route of administration
Ketamine + Xylazine	Mice	80–100 mg + 7.5–16 mg	I.P
	Rats	40–80 mg + 5–10 mg	I.P, I.M
	Guinea pigs	40 mg + 5 mg	I.P
	Rabbits	25–35 mg + 5 mg	I.M
Ketamine + Xylazine + Acepromazine	Mice	80– 100 mg + 20 mg + 3 mg	I.P
	Rats	75–90 mg + 5– 10 mg + 1–2 mg	I.P
Chloralose + Pentobarbitone	Rats	100 mg + 30 mg	I.P
	Guinea pigs	31–65 mg	
	Rabbits	100 mg + 30 mg	I.P.
Chloralose + Urethane	Rabbits	60–72 g + 1–1.2 g	I.V
Pentobarbitone Sodium + Urethane	Rats	400–500 mg + 5–20 mg	I.P
	Guinea pigs	1.5 gm + 6 mg	I.P
	Rabbits	700 mg + 40 mg	I.P

5 Ethical Statement

All institutional guidelines, national guidelines, state and local laws and regulations with professional standards for the care and use of laboratory animals should be followed. Studies involving animals must state that the institutional animal ethical committee has approved the protocol. For authors using experimental animals, a statement should be made that the animals' care is in accordance with institutional guidelines and animals used have been treated humanely and with regard for the alleviation of suffering. Researchers should treat animals as sentient and must consider their proper care and use and the avoidance or minimization of discomfort, distress or pain as imperatives. Animal experiments should be designed only after due consideration of animal health. It should be ensured that all researchers who are using animals have received instruction in research methods and in the care, maintenance and handling of the species being used. All the surgical procedures should be performed under appropriate anaesthesia and follow only those procedures which avoid infection and minimize pain during and after surgery.