Current anesthetic protocols used in obtaining leporidae experimental models: a review

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Abstract
Rodents and lagomorphs were used as laboratory animals since experiments concerning the effect of different substances were done on animals. They have the advantage of early sexual maturity, short pregnancy period and a high rate of fecundity, all leading to an increased rate of multiplication. All these features and easy growing in lab conditions make them the most common laboratory animals. From all common used laboratory rodents and lagomorphs; the rabbit has the advantage of having the biggest size.

In most of the cases, rabbits should be anaesthetized for biological sampling. Therefore, the anesthetic protocols must not change in any way the samples or influence the results. So, depending on the aim of the experiment, a specific anesthetic protocol must be chosen.

This review summarizes and compares different anesthetic protocols, used in rabbits for different experiments. Thus, the researchers will now have a set of anesthetic protocols in order to achieve the desired level of anesthesia and unaffected experimental results. Each protocol produces a certain degree of anesthesia and analgesia and different physiological effects on the experimental models.

Key words: rabbit, anesthesia, laboratory medicine

Three principles must be followed to obtain suitable anesthesia:
1. animal must be restrained in order to obtain total immobility
2. an optimal degree of unconsciousness should be achieved for the animal in order not be psychologically traumatized.
3. obtaining an optimal analgesia so that values of respiratory rate, cardiac rate, pH blood do not undergo physiological changes in response to some painful stimuli.( B. Alderton [1], R. Borkowski & al [2], P.A. Flecknell [3])

Drug substances used in anesthesia
In this review, there are presented only anesthetic drug substances used in the protocols described below.
Anticholinergic substances
To offset the parasympathomimetic effects of certain anesthetic protocols, it is useful an anticholinergic drug such as atropine, at a dose of 0.005 mg / kg (SQ or IM), or glycopyrrolate, at a dose of 0.1 mg/kg (SQ or IM). (S.L. Cantwell & al [4]).
Minor tranquilizers
Minor tranquilizers are used in obtaining light or moderate sedation. Diazepam and midazolam are used at a same dose of 0.5-2 mg/kg (IV, IM or IP). ( G. Neiger-Aeschbacher [5]).
Major tranquilizers
Major tranquilizers have a strong sedative effect and some of them may also have an analgesic effect on patients. Major tranquilizers used in rabbits are acepromazine and alpha 2 agonists (like xylazine and medetomidine).
The protocols, described in this review, will refer only at alpha 2 agonists. 

XYLAZINE - has a light to moderate sedation effect and some analgesia. It is used at a dose of 2-5 mg/kg (SQ or IM). (S.M. Difilippo & al [6]).

MEDETOMIDINE - has a stronger sedative effect than xylazine and also an analgesic effect. It is used at a dose of 0.1-0.5 mg/kg (IM or SQ). (S.M. Difilippo & al [6], L.J. Hellebrekers & al [7]).

Alpha 2 agonists have the advantage of drug antidotes that can be administered after the anesthetic protocol. Atipamezole, which is a specific antagonist, can be administered to rabbits at a dose of 0.1-1 mg/kg (IM, IP, SQ or IV). Yohimbine, which is a non-specific antagonist, can be administered depending on the way of inoculation at a dose of 0.5 mg/kg IM or 0.2 mg/kg IV. ([8], [9])

Opioids

The use of opioids remains a major part of analgesic therapy, particularly in treatment of moderate to acute, postsurgical or traumatic pain. Opioids exert their effect by inhibiting transmission of nociceptive stimulation in the dorsal horn of the spinal cord, activating descending inhibitory pathways, inhibiting supraspinal afferents and causing a decrease in the release of neurotransmitters in the spinal cord. Opioids commonly used in rabbits include butorphanol, buprenorphine, hydromorphone, oxymorphone, and fentanyl. (P.A. Henry & al [10], C. Yardimci & al [11]).

FENTANYL is a very short-acting, potent pure μ-agonist with analgesic effects similar to morphine. The effects of fentanyl last less than 30 minutes after a single intravenous injection. It can also be administered by a transdermal delivery system (fentanyl patch). Rabbits receiving fentanyl have a severely decreased appetite, and management of ileus induced by fentanyl is challenging. Although some authors do not recommend the use of fentanyl in rabbits, it can be administered at a dose of 0.22 mg/kg IM with sedative and analgesic effects, having itself a restraining effect, enough for minor surgical procedures. (P.L. Foley & al [12], C.H. Weinstein & al [13])

Barbiturate

Barbiturates are drugs that act as central nervous system depressant and, by this property; they produce a wide spectrum of effects, from mild sedation to total anesthesia. They are also effective as anxiolytic, hypnotic, and anticonvulsants. They have addiction potential, both physical and psychological. Barbiturates are used in general anesthesia, as well as for epilepsy. Barbiturates are derivatives of barbituric acid. (A.J. Robinson & al [14])

PENTOBARBITAL is part of different anesthetic protocols in rabbit, at a dose of 30-45 mg/kg IV, for immobilization and light to medium anesthesia effects, for 20-30 minutes.

Injectable anesthetics

PROPOFOL is commonly used in veterinary medicine as a short-acting, intravenously administered hypnotic agent. Its uses include the induction and maintenance of general anesthesia, sedation for mechanically ventilated animals and procedural sedation. Propofol is not considered an analgesic, so opioids such as fentanyl may be combined with propofol to reduce pain. For rabbits it is used at a dose of 10 mg/kg IV for reaching a light anesthesia which last 5-10 minutes.

KETAMINE is known primarily for its sedative properties. Ketamine is used often in rabbits as a premedication. Recently, administration of a ketamine CRI has been used to augment intraoperative and postoperative analgesia. Ketamine has been shown to act as an analgesic by antagonism of the excitatory N-methyl-D-aspartat receptors in the CNS. N-methyl-D-aspartat stimulation has been shown to mediate central sensitization of the CNS. This activity of ketamine has not been confirmed in rabbits, suggesting that ketamine alone is not an acceptable analgesic in most instances. However, a ketamine CRI may allow a lower
dosage of an opioid or other analgesic to be administered. In rabbits, it is used at a dose of 25-50 mg/kg IM, producing moderate to heavy sedation and mild analgesia. (L. Lang-Lazdunski & al [15]).

URETHANE is a crystalline soluble powder. It is used in experimental surgery as long-term narcotic (6-8 h). Urethane is less toxic, well tolerated and non-irritating. It is used as 10% or 20% solution intravenous or intraperitoneal at a dose of 1-1.5 g/kg, without premedication for laborious surgeries. It produces dangerous hypothermia.

Inhalant anesthetics

Although rabbits are difficult to intubate, a suitable anesthesia, achieving all three basic principles, is based on maintaining anesthesia with inhaled anesthetics. (J.C.Smith & al [16], H.S. Tran & al [17], S.G. Worthley & al [18])

ISOFLURANE is used in rabbits at a dose of 3.5-5% for induction and 2-3.5% for maintenance. Isoflurane is the most recommended inhalant anesthetic in rabbits, mainly with xylazine/ketamine induction. (P.A. Flecknell & al [19], P. Hedenqvist & al [20])

Local anesthetics

Local anesthetics such as lidocaine and bupivacaine are also commonly used in veterinary practice. In rabbits, they can be used topically, via direct infiltration into soft tissue containing nerve endings, intravenously or epidural. Care must be taken to avoid administration of toxic dosages of these drugs when they are used to treat small animals like rabbits. During subcutaneous administration of a local anesthetic, one should always aspirate to ensure that the drug will not be accidentally administered intravenously. (A. Topal & al [21])

BUPIVACAINE is used in rabbits at a maximum dose of 1.5 mg/kg SQ. When used epidural, bupivacaine may lead to motor weakness in the hind limbs for up to 12 hours post injection. This motor weakness may lead to increased postoperative morbidity.

LIDOCAINE is used in rabbits at the same maximum dose as bupivacaine, 1.5 mg/kg SQ. For abdominal procedures, as a line block before surgical incision, a 2.5-in, 22 gauge spinal needle with the stylet removed is used to infiltrate the subcutaneous tissue with lidocaine.

Anesthetic protocols

Xylazine, ketamine and isoflurane

To achieve this protocol an anesthetic machine is required. Isoflurane may be administered by cuffed (Murphy Eye) or uncuffed (Cole type) endotracheal tubes or by a pediatric laryngeal mask airway. Animals are sedated with intramuscular ketamine (50 mg/kg) and xylazine (10 mg/kg)[20]. After 5 minutes, the larynx is numbed with lidocaine, an intubation device positioned, and anesthesia maintained with isoflurane (2%) in oxygen (1 liter/min), (S.M. Difilippo & al [6]). This general anesthetic protocol may be used in any surgery procedure for implantation and histological samples.

Fentanyl and isoflurane

Induction is performed using inhalation anesthesia with isoflurane in 3% concentration. Then gradually reduce its dose, but gradually increasing the dose of fentanyl (5, 10, 20, to 40 μg/kg) IV at an interval of 120 minutes. (P.A. Flecknell & al [19], P.L. Foley & al [12]). Is a safe anesthesia with a very good analgesia.

Medetomidine, midazolam, atropine and propofol

The protocol uses medetomidine at a dosage of 0.2 mg/kg, midazolam (0.5 mg/kg) and atropine 0.5 mg/kg IM for induction of anesthesia. For maintenance of general anesthesia it is used propofol infusion at a rate of 0.5 mg/kg/min which is effective with few side effects on
the cardiopulmonary system for 30 minutes. However, slight hypotension, hypercapnia and respiratory acidosis are associated with infusion of this anesthetic. The recovery is smooth. So this is a safe method to induce and maintain general anesthesia enabling short-term surgical procedures in healthy animals. (S.M. Difilippo & al [6], D. Rozanska & al [22])

For this protocol, no intubation kit is needed, it does not change the cardio-pulmonary parameters and it is a safe method for short surgery (e.g. for experimental implants). (D. Rozanska & al [22])

**Pentobarbital, urethane, and chloralose**

This protocol uses pentobarbital at a dose of 30 mg/kg, urethane (800 mg/kg), $\alpha$-chloralose (40 mg/kg), or a mixture of $\alpha$-chloralose (40 mg/kg) and urethane (0.4 g/kg). The purpose of this protocol is to determine the effects of different anesthetics on the arterial baro-reflex performance, the open-loop characteristic of the carotid sinus reflex. The response range and the slope parameters under $\alpha$-chloralose anesthesia were significantly smaller than those obtained under the other anesthetics. The $\alpha$-chloralose weakens the reflex control of arterial pressure in rabbit. (N. Ishikawa & al [23])

**Long-term epidural anesthesia**

Long-term administration of local anesthetic is made through a catheter implanted via a non-traumatic route in the lumbar epidural space between the apex coccyis-sacri and the first vertebra coccygea after 1 cm incision was made in the skin at the root of the tail. The ligamentum was punctured to allow the catheter to slide gently 10 cm into the epidural space and avoid subperiosteal soft tissue dissection. Lidocaine is used as 1 or 2% solution, and bupivacaine as 0.25-0.5% solution at a 48 hours interval. (Y. Komoda & al [24], J.M. Malinovsky & al [25])

The effects do not consist in neurologic disturbance, discomfort, infection or weight loss. Complete motor block is observed during a mean of 27, 43, and 51 minutes with increasing doses of lidocaine. Repeated injections of local anesthetics produce consistent duration of motor block. (S. Kramer & al [26])

### The use of certain anesthetic protocols depending on the experiment

**Xylazine-ketamine- isoflurane protocol**

This is a general anesthesia protocol used for obtaining experimental models, both implantation and collecting of biomaterials, looking for tissue response when the organism needs them (e.g. different collagen matrix, metallic implants, etc.). (S.M. Difilippo & al [6])

It does not influence histological aspects of the tissue, it is affected only the anesthetics inoculation site.

**Fentanyl and isoflurane protocol**

This protocol is very easy to be done, it is used an isofurane induction and fentanyl analgesia. Because the substances come into organisms by airways and intravenous path , this protocol has the advantage that it does not change the histological tissues inoculated with anesthetics. (P.A. Flecknell & al [19], P.L. Foley & al [12], M. Hayashida & al [27])

The protocol may be used any time is needed a good and long analgesia (e.g. bone marrow sampling, ophthalmic experiments). (W. Park [28])

**Medetomidine-midazolam-atropine- propofol protocol**

Unlike other anesthetic protocols, this does not use inhaled anesthetics. This means it has some advantages:

1. it does not require special equipment and qualified personnel for intubation;
2. the lab has classic features, without inhalation anesthesia machine
3. the airways are kept free for taken experimental surgeries on them;
4. protocol does not alter the cardiopulmonary parameters and therefore do not affect experiments in which there is interest in these parameters (e.g. cardiac or nervous stimulation by intra-cardiac or intrarachidian inoculation).
5. it is useful in short-term experimental surgeries (inoculations, sampling, biopsy, punctures).
6. it is a safe anesthetic method which is not life-threatening.

So this protocol is very useful in experimental surgeries when oral, pharyngeal, laryngeal and tracheal regions should be free of masks or endotracheal tubes (S.M. Difilippo & al [6], G. Alfredo [29], P. Hedenqvist & al [30])

**Pentobarbital-urethane-chloralose protocol**

This protocol is very useful especially when there is an interest in carotid sinus reflex. It weakens the reflex control of arterial pressure in rabbits. (Z. Baturaite & al [31], N. Ishikawa & al [23], A.G. Neiger [32])

**Epidural chronic anesthesia protocol**

It is used for obtaining experimental models in which it is needed to block only the hindquarters continuously on a long period, or many times, and a general anesthesia is not wanted. (S.J. Matthew & al [33])

At recommended dosage, this protocol does not produce any change in physiological parameters, and therefore will not change the results of different experimental models.( A. Peukert & al [34])

**Conclusions**

1. The anesthetic protocol should be chosen depending on the used experiment to obtain reliable date, unaffected by the anesthesia.
2. Some anesthetic protocols can be applied only on experimental models, when rabbits will then be euthanized, because they produce handicap or life threatening effects.
3. Depending on the degree of analgesia produced by certain anesthetics, there can be applied maneuvers with a certain level of pain.
4. The chosen anesthetic protocol for experimental model has to meet the requirements of the local ethics committee.

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