

Anesthesia Applications in Experimental Neurological Disease Modeling

Deneysel Nörolojik Hastalık Modellemelerinde Anestezi Uygulamaları

Ayhan ÇETİNKAYA¹
 0000-0002-8212-7149
Hümeysra ÇELİK²
 0000-0002-3394-2438

¹Department of Physiology, Bolu
Abant İzzet Baysal University Faculty
of Medicine, Bolu, Türkiye

²Department of Physiology, Alanya
Alaaddin Keykubat University Faculty
of Medicine, Antalya, Türkiye

Corresponding Author Sorumlu Yazar

Ayhan ÇETİNKAYA
cetinkayaayhan@hotmail.com

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ABSTRACT

Neuroscience, which covers the molecular mechanisms employed by the brain to cause neurological diseases and how they can be treated, remains current as the subject of high-budget investigations to develop early diagnosis and new treatment modalities with multidisciplinary approaches. For this purpose, creating the appropriate model with the correct modality and anesthesia in neurological in vivo experimental models is the most important phase to obtain accurate results and ensure animal welfare. To manage anesthesia in rodents, knowing the physiological characteristics of anesthetics and their risks will strengthen experimental procedures. Among the injectable anesthetics, ketamine, xylazine, and pentobarbital are the most frequently preferred agents for general anesthesia in short surgical procedures. Isoflurane and sevoflurane are inhaler anesthetics that are administered through a vaporizer because they are liquids. Important advantages of inhaled anesthetics, such as rapid induction and rapid withdrawal, make inhaled anesthetics stand out in neuroscience studies. In this review, the properties of frequently used anesthetic agents in rodents, their usage methods, and which model they are preferred will be discussed. For this purpose, the selection of appropriate anesthetics in animal models such as epilepsy, Alzheimer's disease, ischemia-reperfusion injury, traumatic brain injury, ischemic stroke, experimental autoimmune encephalomyelitis, and ophthalmic surgical procedures and their side effects will be reviewed.

Keywords: Anesthesia; neuroscience; in vivo models.

ÖZ

Beynin hangi moleküler mekanizmalar kullanarak nörolojik hastalıkların oluştuğu ve nasıl tedavi edilebileceği kapsamındaki sinir bilim, multidisipliner yaklaşımlar ile erken tanı ve yeni tedavi geliştirmek üzere yüksek bütçeli araştırmaların konusu olarak güncelliğini korumaktadır. Bu amaçla nörolojik in vivo deneysel modellerde uygun modeli doğru metot ve anestezi ile oluşturmak doğru sonuçları elde etmenin ve hayvan refahını sağlamanın en önemli basamaklarıdır. Kemirgenlerde anesteziyi yönetmek adına anesteziklerin fizyolojik özelliklerini tanımak ve risklerine hakim olmak deneysel prosedürleri güçlendirecektir. Enjekte edilebilir anesteziklerden ketamina, ksilazin ve pentobarbital genel anestezi için kısa cerrahi prosedürlerde en sık tercih edilen ajanlardır. İzofluran ve sevofluran sıvı olduklarından bir vaporizer ile verilen inhaler anesteziklerdendir. İnhaler anesteziklerin hızlı indüksiyon ve hızlı çekilme gibi önemli avantajları sinirbilim çalışmalarında inhaler anestezikleri öne çıkarmaktadır. Bu derlemede kemirgenlerde sık kullanılan anestezik ajanların özellikleri, kullanım şekilleri ve hangi modelde tercih edildiğinden bahsedilecektir. Bu amaçla epilepsi, Alzheimer hastalığı, iskemi-reperfüzyon hasarı, travmatik beyin hasarı, iskemik inme, deneysel otoimmün ensefalomyelitis, oftalmik cerrahi prosedürler ve yan etkileri gibi hayvan modellerinde uygun anesteziklerin seçimi gözden geçirilecektir.

Anahtar kelimeler: Anestezi; sinirbilim; in vivo modeller.

INTRODUCTION

The brain is the most important organ in living things and remains largely unknown compared to the present century. How the brain works and remembers, what molecular mechanisms it employs to make decisions, how neurological diseases occur, and how they can be treated are the subjects of neuroscience (1). Recently, neuroscience has become a research subject not only in the field of medicine but also in many other branches of science such as engineering, chemistry, physics, computer science, philosophy, and psychology. This multidisciplinary approach reveals large-budget research for early diagnosis and new treatment modalities of neurodegenerative diseases, which are increasing in the entire world (2). For this purpose, experimental models in neurological in vivo and in vitro studies remain up-to-date.

Creating the appropriate model with the accurate method and choosing the appropriate anesthesia according to the model to be studied is a must in experimental research to obtain accurate results and ensure animal welfare (3). Managing anesthesia and analgesia in rodents is challenging because of their small size and varying sensitivity to anesthetics and analgesics. Also, the characteristics of the preoperative and postoperative periods may be overlooked if the characteristics of the anesthetic are not known because the focus is only on the experimental procedure to be performed. For this reason, knowing the physiological characteristics of various anesthetics and knowing their risks is important to strengthen experimental procedures (4).

This review aims to discuss the properties of frequently used anesthetic agents in rodents, their usage methods, and the selection of appropriate anesthetics in animal models for neurological diseases and their side effects.

INJECTABLE ANESTHETICS IN RODENTS

Mice and rats require higher doses of anesthetic to reach full anesthesia depth because of their higher metabolic rates than larger animals with lower metabolic rates. Although the duration of anesthesia in rodents is short, on average, 20-30 minutes, respiratory depression, hypothermia, and dehydration might occur because of high doses (5). Injectable anesthetics allow short surgical procedures to be performed by anesthetizing multiple mice or rats simultaneously or serially. They are easy to use without starving the animals because these anesthetics do not require special equipment (6). Whichever injectable anesthetic is used, it must be dosed according to the size of the animal (5). If a repeated dose is required, this should be approximately 10-25% of the initial dose, but it should be noted that this might increase the risk of mortality.

When observing the depth of general anesthesia in animals, when the tail is pinched, the animal's pawing movement in response or the movement of the head towards the stimulus as a positive response to the stimulus is evaluated to tell us that the anesthesia is not deep enough and it is too early to start the procedure.

As a benzodiazepine, pentobarbital provides sedation between 80-95 minutes in rats (40-50 mg/kg) and 10-300 minutes in mice (40-70 mg/kg) with intraperitoneal (IP) administration (7,8). It depresses the central nervous system by potentiating gamma aminobutyric acid (GABA) receptors (9).

The most commonly used agents are ketamine and xylazine in rats (40-90 mg/kg ketamine / 5-10 mg/kg xylazine), and in mice (60-100 mg/kg ketamine / 5-10 mg/kg xylazine). Ketamine and xylazine, which can be administered IP and intramuscularly (IM), provide 45-90 minutes of sedation in rats and 30-45 minutes of sedation in mice (7,8). Ketamine provides anesthetic effects by providing N-methyl-D-aspartate (NMDA) receptor antagonism and agonistic effects on GABA receptors (10). As an analgesic and muscle relaxant, xylazine has sedative effects as an alpha 2 receptor agonist (11). Ketamine and xylazine can be injected simultaneously in rats and mice, and this procedure is slightly different in rabbits (Table 1). First xylazine (5 mg/kg) injection is administered IM, and 10 minutes later ketamine (30 mg/kg) is administered IM in rabbits (12,13).

Table 1. Anesthetic administration for short surgical procedures

	Injectable anesthesia
Rats	40-90 mg/kg ketamine / 5-10 mg/kg xylazine, IP or IM
Mice	60-100 mg/kg ketamine / 5-10 mg/kg xylazine, IP or IM
Rabbits	30 mg/kg ketamine (after) / 5 mg/kg xylazine (before), IM

IP: intraperitoneal, IM: intramuscular

Locally administered bupivacaine (0.25% solution) and lidocaine (1% solution) are local anesthetics and can be used for short procedures in the incision area in mice and rats. Effect times can last from 1-15 minutes to 4-12 hours for bupivacaine, and can range from 1-5 minutes to 1.5-2 hours for lidocaine (7,8). They prevent ion transfer by stabilizing the neuron membrane and exert their effects by inhibiting voltage-gated sodium channels (14).

INHALED ANESTHETICS IN RODENTS

The most consistent anesthesia protocol is inhaler anesthesia for rodents (15). It is already known that inhalant agents activate GABA and glycine receptors while inhibiting the NMDA receptors. They prolong the duration of synaptic inhibition by increasing the response to endogenous GABA (16).

With its effects on multiple receptors, they allow almost 100% of animals to reach the desired surgical plane. Also, inhalant agents have low blood solubility, facilitating rapid drug uptake from the alveoli and effective distribution across the blood-brain barrier, which results in rapid anesthetic induction and rapid removal of inhalant agents from the central nervous system at the end of the procedure and the ability to fine-tune anesthetic depth during the procedure (17,18). Side effects of inhaled anesthetics include the use of excessive equipment to create the appropriate depth of anesthesia. Volatile anesthetics (nitrous oxide, halothane, isoflurane, desflurane, and sevoflurane) are liquid at room temperature and require the use of vaporizers for inhalational use (18).

Isoflurane is the most commonly used inhaled anesthetic. An inhaler effect of 1-3% must be applied with a mixture of air and oxygen or 100% oxygen (18).

Sevoflurane can be used at 3% concentration with 6 lt/min of 100% oxygen or 50% nitric oxide and 50% oxygen (19). Sedation is first provided with an inhaler anesthetic drug in a box in inhaler anesthetic drug administration, then it is removed from the box and the same drug is administered with a mask (20). Since the blood gas solubility coefficient of sevoflurane is lower than isoflurane, anesthesia induction and recovery from anesthesia are faster than isoflurane (21,22).

ANESTHETICS USED IN NEUROSCIENCE STUDIES

In animal models created in neuroscience studies, choosing the appropriate anesthetic is as important as animal modeling. If the anesthetic used is not appropriate, cerebral hypoperfusion because of developing hypotension affects cognitive functions (23,24) independent of the disease, causing unexpected effects on the experimental results (25). The fact that anesthesia is good for the disease model created is also a dilemma. In such a case, the anesthetic agent overshadows the effects of the test substance or treatment method itself and for this reason, choosing the appropriate anesthetic with therapeutic and minimized harmful effects must be considered the most appropriate approach. Also, depending on the experimental setup, the effects of the test substance can be combined with the anesthesia method, which has positive effects. In this review, information will be shared about the selection of appropriate anesthetics in neuroscience animal models.

Epilepsy

Epilepsy is a chronic brain disease caused by high synchronization of abnormal neuronal discharges (26) and can be affected by the anesthesia protocol and cause seizure interference. For this reason, understanding the pro- and anticonvulsant characteristics of the drugs employed for anesthesia in epilepsy studies must aim to minimize the risks of intra- and postoperative seizure activity (27).

Isoflurane or ketamine/xylazine can be used to provide intraoperative and postoperative analgesia in epilepsy studies in which stereotaxic examination will be performed primarily (28,29). Also, jump block can be prevented by applying 1% lidocaine to the periosteum before drilling the skull (30). Since isoflurane has no channel activity, it is known as a good agent for creating an epilepsy model and is recommended in intrahippocampal seizure models induced by kainic acid or penicillin (29). Also, the ketamine/xylazine combination does not seem very appropriate to use because it might delay the onset of seizures due to its blocking effect on NMDA receptors (29). Here, the approach must be chosen according to the experimental design.

Alzheimer's Disease

Alzheimer's disease (AD) is the most common neurodegenerative disease causing cognitive decline with the onset of progressive dementia with pathological symptoms such as senile plaque and neurofibrillary tangle formation in the brain (31). According to the studies on the information that inhaled anesthetics increase neuroinflammation in Alzheimer's patients (32), it has been observed that sevoflurane increases neurotoxicity in mice in transgenic AD (33). Also, isoflurane was found to increase amyloid pathology in AD model mice (34).

However, isoflurane anesthesia was shown to be safe in AD created in the Tg2576 mice model and did not affect experimental results by not interacting with vital parameters (35).

Ischemia-Reperfusion Injury

Ischemic stroke refers to the necrosis of the brain tissue because of insufficient cerebral blood flow (36). Although reperfusion following ischemia improves the situation, cerebral reperfusion might increase brain tissue damage by worsening oxidative stress and protein damage (37). Ischemia-reperfusion injury has extremely high mortality and morbidity rates (38) and is a model in which the test substance and treatment modality are intensively studied experimentally.

It is already known that ketamine and barbiturates have neuroprotective effects and might interfere with investigating the effect of the test substance in stroke models (39). Pentobarbital anesthesia was shown to be superior to inhaled anesthetics in cerebral ischemia-reperfusion injury because it has been shown to reduce the infarct area (40). In a study that compared isoflurane and barbiturates (thiopental), it was shown that isoflurane reduced cerebral perfusion, and the ameliorative effects of barbiturate use on ischemia-reperfusion injury were reported (41). It was shown that pentobarbital anesthesia reduces the infarct area in cerebral ischemia-reperfusion injury more than inhaled anesthetics (40). For this reason, the choice of inhalation anesthesia seems more appropriate to reveal the effects of the test substance in the induction of experimental stroke models more clearly (42).

Experimental Autoimmune Encephalomyelitis

Modeled on multiple sclerosis, experimental autoimmune encephalomyelitis (EAE) is a disease in which myelin damage occurs based on T cell-mediated neuroinflammation. Also, since (R)-ketamine was shown to improve the clinical score of EAE by reducing the pathological findings in the spinal cord in the EAE model, it does not seem appropriate to use ketamine as an anesthetic in the EAE model to demonstrate the effects of the test agent (43). Although an EAE study reported that sevoflurane, which is an inhaled anesthetic, suppressed neuroinflammation by reducing T-cell functions (44), it was reported that isoflurane is generally preferred in the EAE model (43).

Traumatic Brain Injury

Traumatic brain injury affects approximately 50 million people worldwide every year with a high morbidity and constitutes a large portion of young adult deaths worldwide (45). In a study conducted on rats, 50 mg/kg ketamine and 0.5 mg/kg chlorpromazine, an antipsychotic, were used to induce traumatic brain injury (46). It has been shown that the narcotic analgesic fentanyl, an anesthetic used in the management of traumatic brain injury, causes more hyperglycolysis and death of hippocampal CA1 neurons than the inhaled sedative agent isoflurane (47). In a study comparing inhaled isoflurane (5%) and fentanyl, it was emphasized that isoflurane has neuroprotective effects and this should not be overlooked when evaluating the test substance (48). In a study comparing propofol and isoflurane with hypothermia in traumatic brain injury in rats, it was observed that isoflurane reduced cerebral perfusion pressure and intracranial pressure more than propofol, and it was emphasized that propofol should be

preferred (49). It has also been observed that pentobarbital injection (50 mg/kg) IP is preferred when inducing traumatic brain injury (50).

Ischemic Stroke

It is a clinical condition characterized by sudden onset of focal neurological deficits in a vascular region of the brain, retina, or medulla spinalis, resulting from underlying cerebrovascular pathologies. It is a cause of high morbidity and mortality (51). Research on anesthetic drugs in ischemic stroke is difficult because there are many comorbidities depending on the patient's history. For this reason, experimental ischemic stroke models are important for anesthetic drug research (52). Isoflurane, a volatile anesthetic, is frequently preferred because it provides neuroprotection against excitotoxicity, ease of use, and rapid recovery (53,54). However, these results caused by volatile anesthetics used in experimental studies probably lead to great confusion in the investigation of stroke mechanisms and consequences (52). Among injectable anesthetics, ketamine/xylazine, 80/20 mg/kg, (55) and pentobarbital, 30 mg/kg, (56,57) are frequently used. The therapeutic effects of ketamine (58) on ischemia should not be forgotten when evaluating the results.

Ophthalmic Surgery

In models where ophthalmic surgical procedures will be performed, ketamine/xylazine anesthesia is applied during intravitreal implant injection (59). However, it should not be forgotten that ketamine/xylazine anesthesia may increase intraocular pressure (60) and cause hyperopia (61). It is also known that IP injections cause corneal scarring (62). Ketamine-methodimine is used for enucleation (63). Isoflurane anesthesia is used to perform retroorbital injection into the venous sinuses (64).

CONCLUSION

Failure to choose appropriate anesthetic drugs, failure to determine the depth of anesthesia, and surgical intervention at an inappropriate time might affect the experimental results and cause unexpected results. For this reason, during the experimental planning phase, which anesthetic must be selected as well as the information of the test substance must be considered. In this respect, it must not be overlooked that the choice of the most appropriate anesthetic might vary depending on the subject of the study. Future studies to be conducted to determine the appropriate anesthetic will make it easier for clinicians to reach more accurate experimental results.

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