Pain Perception and Anaesthesia in Research Frogs

Sarah Annie GUÉNETTE, Marie-Chantal GIROUX, and Pascal VACHON

Department of Veterinary Biomedicine, Faculty of Veterinary Medicine, University of Montreal, 3200 Sicotte, St-Hyacinthe Quebec, Canada

Abstract: Frogs possess pain receptors and pathways that support processing and perception of noxious stimuli however the level of organization is less well structured compared to mammals. It was long believed that the experience of pain was limited to 'higher' phylums of the animal kingdom. However, it is now commonly accepted that amphibians possess neuro-anatomical pathways conductive of a complete nociceptive experience. *Xenopus laevis* frogs have been one of the most popular aquatic research models for developmental studies and genetic research. These frogs have been extensively used in research for their eggs, that can be collected following hormonal stimulation either naturally or by surgical intervention. Many anaesthetics have been used in amphibians such as bath solutions of MS-222, benzocaine and eugenol as well as systemic injections of ketamine or tiletamine, barbiturates, propofol and gas administrations of methoxyflurane, halothane and isoflurane. Most of these anaesthetic drugs produce variability in depth and duration of anaesthesia. MS-222 appears to be one of the most reliable anaesthetics. This review will focus on the evidence of pain perception in frogs and will compare the effectiveness and limitations of different anaesthetics used in *Xenopus laevis* frogs.

**Keys words:** anesthesia, benzocaine, eugenol, frogs, MS-222

Introduction

*Xenopus laevis* and *Rana pipiens* are the main amphibian species used in research however recent developments with *Xenopus* frogs have made this species one of the most used amphibians in research [35]. These frogs have been one of the most popular aquatic research models for developmental studies and genetic research. Since minor and major surgical procedures are performed in these animals more need to be known about pain perception, analgesia and anaesthesia. This paper will focus on the pain mechanism and anaesthesia in frogs with a focus on *Xenopus laevis* frogs whenever possible.

Pain perception in anurans

Nociception is defined as the transmission of pain from a peripheral receptor, usually an unmyelinated nerve ending, to the central nervous system. Brain processing is the final process where pain perception occurs. Frogs have nociceptors in superficial and deep layers of the skin [11, 20, 37, 42] that transduce mechanical and chemical noxious stimuli [1, 52]. Anurans have both myelinated and unmyelinated afferent fibers that compose the peripheral sensory nervous system however small slowly conducting fibers transmit the majority of noxious stimuli [1, 21]. These fibers, identified as Aδ and C, are similar to those found in mammals in relation to their physiological characteristics. Frogs therefore have both fast and well localized pain perception, related to large diameter fibers (Aδ fibers), as well as diffuse pain (C fibers). The spinal cord differs little from other vertebrates [10] however pathways relaying infor-
formation from the spinothalamic tract to the cortex remain largely unknown [34]. Ascending spinal cord projections reach the brainstem and the thalamus. Thalamocortical connections, as in mammals, terminate in non-olfactory telencephalon [25, 47] however projections appear diffuse and poorly organized compared to mammals. These anatomical findings suggest that nociception is poorly represented in the brain and that central neural circuits are mainly related to spinal and long loop reflexes to the brain stem and thalamus [7, 22, 33, 34]. Nevertheless the projections to the telencephalon suggest that frogs perceive pain and that proper anaesthetics should be used whenever minor or major surgical procedures are used.

Many anaesthetics depress the activity of central nervous system neurons. This is done by either increasing the activity of GABAergic inhibitory neurons, GABA being the main inhibitory neurotransmitter of the CNS, or decreasing glutamatergic activity, glutamate being the most important excitatory neurotransmitter in the CNS. GABAergic immunoreactive cells have been shown across all areas of the central nervous system of anurans [23]. However across different species of anurans, moderate to dense GABAergic cell populations can be found in the CNS, which may explain the variability of anaesthesia depth observed following the administration of anaesthetics with GABAergic mechanisms.

Anurans therefore do possess pain receptors and pathways that support control processing and perception of noxious stimuli; however, the level of organization is less well structured compared to mammals. It was long believed that the experience of pain was limited to ‘higher’ phylums of the animal kingdom. However, it is now commonly accepted that amphibians possess neuroanatomical pathways conductive of a complete nociceptive experience [9, 36, 40, 45]. Whether or not there is conscious nociception and suffering, similar to that presumed of other mammals, carefully planned analgesia and anaesthesia should prevent recruitment and sensitization of pain fibers, and hence promote healthy healing of existing or induced wounds, or other sources of pain [20].

## Anesthesia in Anurans

### Commonly used anesthetics

**Tricaine methanesulfonate (MS-222):** Tricaine methanesulfonate is a safe water soluble acid salt [5] present as an un-ionized form that has been successfully used to immobilize and anaesthetize amphibians [8, 44]. When dissolved in water, at concentrations required for anaesthesia, the solution has a pH of approximately 3. For this reason a buffered solution is prepared (1 g of tricaine methanesulfonate in 1 l of water with 25 ml of 0.5 M Na2HPO4 to yield a 0.1% solution). However since water hardness, alkalinity and pH vary by source, a solution can also be prepared by titrating to neutral pH with NaHCO3. Induction time is approximately 30 min and the animal should be removed from the induction solution and moved to fresh water upon the attainment of desired anaesthetic level. Fifteen mininutes following an immersion bath of 15 min (1 and 2 g/l), electroencephalographic activity is strongly depressed in *Xenopus leavis* frogs [29] which suggests that MS-222 crosses the blood brain barrier.

Erythema of light color skin areas, such as the ventrum, occurs during MS-222 anaesthetic induction. If complications arise, frogs should be rinsed with well oxygenated clean water. It has been suggested that in order to prevent severe hypoxia, hypercarbia and acidosis that occur during apnea induced by anaesthesia, 100% oxygen bubbling into the anaesthetic solution is recommended however experiments have shown that following a bath immersion with MS-222, cardiovascular parameters (cardiac frequency and oxygen saturation) are largely unaffected for 2h in *Xenopus leavis* frogs [28, 29].

Tricaine methanesulfonate will also anaesthetize frogs when administered intracoelomically, and via the dorsal lymph sacs [8, 30] however no histopathology was performed in these studies to evaluate possible toxicity.

**Benzocaine:** Variable concentrations of benzocaine have been used to achieve surgical anaesthesia in amphibians [4, 46, 51]. The solution can be prepared either from Orajel® or benzocaine powder dissolved first in ethanol. Another method is to apply benzocaine cream (0.1 ml/10 g; Orajel®) topically [2, 5]. Benzocaine will immobilize frogs and anaesthetize most animals for approximately 15–60 min. It doesn’t cause any deaths and appears relatively safe in amphibians. No studies have evaluated the effects of this drug on the CNS or on the cardiovascular parameters. Local anaesthetic not only cause sensory desensitization but also induce motor block at high doses [39], and their use would therefore be questionable since a paresis or paralytic effect is possible. Therefore it would appear necessary to evaluate the CNS depressant effect of benzocaine before this drug
can be further used even if the drug is highly soluble and should cross the blood brain barrier [5].

**Eugenol (clove oil extract):** Clove oil can be used to anaesthetize *Rana pipiens* frogs [15, 27]. At a concentration of 315 mg/l, anaesthesia level can be attained in most animals. The active molecule of clove oil is eugenol (density 1.007 g/ml) and adult *Xenopus laevis* frogs can be anaesthetized using a concentration of 350 ml/l by placing the frog for 15 min in an immersion bath [18]. Eugenol has no significant effect on heart rate or oxygen saturation for 1 h. Surgical anaesthesia is seen for approximately 30 min. Complete recovery was observed within 1 to 2 h. Eugenol provides a very good level anaesthesia for minor and major procedures for approximately 30 min. No EEG studies have been performed with this drug however eugenol does cross the blood brain barrier [32] and is able to anaesthesia in small rodents [17]. However care must be taken since *Xenopus laevis* frogs of different body weights required different exposure times to a eugenol bath administration to produce 15 and 30 min of anaesthesia [13]. Other routes (patch application, intracoelomically, subcutaneously and intralymphatically) have not been successful to induce a surgical level of anaesthesia in *Xenopus laevis*. Care should be taken not to administer high concentrations drug topically since cutaneous necrosis may occur [38].

Since eugenol can be purchased in its near pure form from Sigma Aldrich, a controlled administration of a single anaesthetic substance can be obtained when compared to clove oil which may contain more than one active substance (such as methyl eugenol). Histopathology of selected tissues (heart, lungs, liver, kidneys, skin, and eyes) showed no evidence of lesions 24 h following a single drug administration however toxicity was observed mainly in the kidney following multiple daily administrations [14]. No such study has been performed with MS-222.

**Less commonly used anaesthetics**

**Anaesthetic gases:** Methoxyflurane [49], halothane [50], and isoflurane [41, 43, 44] can be used for induction and maintenance of anaesthesia of frogs. This is accomplished by bubbling the solution through water, intubation, or by direct application of anaesthetics to the skin. The use of intubation is limited due to the narrow glottis. Anaesthetic saturation of 2 to 5% promotes surgical anaesthesia within 20 min but it is highly variable. Methoxyflurane is applied by soaking cotton with 10 ml of methoxyflurane in a 1 l jar [49] and an exposure time of 5 min will provide approximately 40 min of surgical anaesthesia. However recovery time may last up to 7 h. Methoxyflurane is not readily available due to its adverse side effects in mammals. A 5% isoflurane saturation with a low oxygen flow will induce amphibians in less than 20 min and recovery will occur in less than 2 h. Different methods of isoflurane application or delivery have been used in amphibians [40, 42]. Direct topical application, an isoflurane bath (0.25 ml/125 ml water), topical application of isoflurane gel (3 ml isoflurane/3.5 ml water soluble gel), 5% isoflurane bubbled through water or in an anaesthetic chamber have all provided acceptable methods. Isoflurane has also been administered intracoelomically, subcutaneously and intramuscularly [41]. Use of isoflurane produces highly variable durations of surgical anaesthesia. Very little has been published on the use of halothane for amphibian anaesthesia and it could be used in a similar fashion to isoflurane [51]. In the authors’ experience, isoflurane provides variable depth and duration of anaesthesia and both (isoflurane and halothane) would not be considered as a first choice for amphibian anaesthesia.

**Injectable drugs**

**Propofol:** Propofol is a short acting intravenous drug used in mammals. Propofol is prepared in a similar way as for eugenol. To obtain the most homogenous suspension, thorough mixing prior to administration is performed. In frogs (10 mg/kg), sedation is rapidly obtained but not surgical anaesthesia [27]. It has also been reported that intraceolomic administrations (30 mg/kg) may provide adequate anaesthesia [48] however this result has been seen in only one *Pelodryas caerula* frog. An immersion propofol bath (75 ml/l) will provide sedation and chemical restraint however anaesthesia duration and depth is highly variable [19].

**Ketamine and tiletamine:** Ketamine hydrochloride has been used with very variable results in amphibians. Intramuscular ketamine (70–100 mg/kg) can be used for minor procedures however recovery may take as long as 12 to 18 h [12]. Even with high doses of ketamine, animals remain sensitive to pain [16]. Tiletamine administered at 20 mg/kg in *Rana pippens* does provide analgesia however recovery may take up to 26 h [31]. Since results are variable between amphibian species [31], an evaluation of the anaesthetic potential of Telazol® in
Xenopus frogs would be required before it can be properly used. Neither ketamine nor tiletamine are recommended in Xenopus leavis frogs as first choice anaesthetics. Barbiturates: Pentobarbital administered at 30–60 mg/kg intracoelomically will provide a level of surgical anaesthesia [24]. Responses are variable between species and caution should be used if this anaesthetic is used for anaesthesia [51].

In summary, tricaine methanesulfonate and eugenol will provide good safe anaesthesia in amphibians. Benzocaine is also an option but further studies are required that will establish a safe dose to attain anaesthesia in all animals. For all anaesthetics discussed in this section, no studies of the toxicity with repeated administrations have been published. Therefore caution is of the utmost importance if daily administrations are required.

### Monitoring Anurans during Anaesthesia

Monitoring the patient is the basis of a successful surgery or any other kind of anaesthetic procedure. The level of anaesthesia, cardiovascular efficiency, respiratory frequency, and general functioning of the organs all need to be assessed before and during anaesthesia, as well as during recovery from anaesthesia. As previously mentioned, abdominal (coelomic) and guttural respiratory movements cease during anaesthesia in amphibians, and cutaneous absorption of oxygen becomes the main breathing pathway. Since peripheral pulses are rarely appreciable in amphibians, direct observation of the beating of the heart can instead be conveniently performed at the xiphoid process of the sternum, when the animal is positioned in dorsal recumbency. Monitoring of the heart rate is crucial in the monitoring of anaesthesia. In addition to direct observation, ECG (electrocardiogram), ultrasounds (B-mode) or the use of a Doppler with flow detector [51] are all suitable techniques to monitor heart rate. However, general concerns with skin fragility have been formulated in regards to the traumatic effects the commonly used metal tweezers during ECG recording in amphibians. Two options are available to circumvent this problem. First, adjusting the metal clips of the ECG onto hypodermic needles inserted at selected points provides a quick and easy technique to avoid contact with the skin. This simple technique can be used with any type or size of amphibian. Secondly, the single probe of oesophageal ECG provides a safe and even more practi-
Frogs should not be immersed in water during the recovery phase which may lead to drowning [17]. Return to normal breathing will also be accelerated if ambient air, rather than pure oxygen, is used for oxygenation during this phase since respiratory movements will cease under hyperoxic conditions in amphibians [51]. Allowing a certain degree of hypercapnia to occur during wakeup will also further stimulate a prompter return to normal respiratory movements.

Since metabolic rate during recovery from anaesthesia is so closely related to body temperature, one might intuitively be tempted to increase ambient temperature to accelerate awakening however this is not desirable. Amphibians must be allowed to recover quietly at room temperature ranging between 21 and 24°C [15]. Any attempt to increase the metabolic rate will also increase the oxygen demand of the animal, and may overcome the oxygenation capacity of the skin [6]. Most importantly post anaesthetic monitoring of amphibians should be maintained until full recovery.

Conclusions

Frogs possess pain receptors and pathways that support processing and perception of noxious stimuli however further studies are required to identify neurotransmitters implicated and pain pathways to target better analgesics and anaesthetics. The review of literature supports the use of 3 anaesthetics in frogs that are effective for minor and major surgical procedures in frogs which are MS-222, benzocaine and eugenol. Further work is required to better characterize the effectiveness and limitations of these drugs when using different routes of administration.

References

37. P.196, 415-429. [Medline] [CrossRef]


