Pharmacological Reports
2005, 57, 467–474
ISSN 1734-1140

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Tricaine (MS-222) is a safe anesthetic compound compared to benzocaine and pentobarbital to induce anesthesia in leopard frogs (*Rana pipiens*)

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Abstract:
Tricaine (MS-222) is used commonly for sedation, immobilization, and anesthesia of poikilothermic animals. The anesthetic efficacy of different concentrations of MS-222 was compared to benzocaine and pentobarbital on the physiological changes, heart rate and ECG (electrocardiogram) parameters in the leopard frog, *Rana pipiens*. Loss of righting reflex (RR), loss of pain response (NR = nociceptor response) and recovery time were measured. Heart rate and ECG parameters were also tested before and during anesthesia. The time to loss of RR and NR decreased while recovery time markedly increased with the increasing concentration of MS-222. Benzocaine at 200 mg/l induced a rapid anesthesia, but all frogs needed resuscitation. Pentobarbital at 300 mg/l induced a slow anesthesia, however, all of the frogs also needed resuscitation. All anesthetics at the mentioned concentrations decreased heart rate significantly as well as altered the ECG parameters. All anesthetics prolonged the Q–T interval, and MS-222 at 800 mg/l and benzocaine at 200 mg/l were the most effective anesthetic concentrations in increasing the Q–T interval. Frogs anesthetized by benzocaine and pentobarbital and high concentrations of MS-222 required resuscitation due to hypoxia. Pentobarbital and benzocaine seem to be very effective compounds, but their safety margins are narrow because of ventilatory failure. Therefore, MS-222 at a concentration of 200 mg/l or less is highly recommended for leopard frogs because prolonged recovery, high mortality rate and significant ECG changes are observed with higher concentrations of MS-222.

Key words:
Tricaine, MS-222, safe anesthesia, leopard frog, righting reflex, pentobarbital, benzocaine, heart rate and ECG (electrocardiogram)

Abbreviations: ECG – electrocardiogram, NR – nociceptor response, RR – righting reflex

Introduction

Anesthesia is used to produce immobilization, skeletal muscle relaxation and relief from pain. An ideal anesthetic drug should induce anesthesia smoothly and rapidly and allow rapid recovery as soon as administration is ceased. The drug also should have a wide margin of safety and be devoid of adverse effects. Tricaine methanesulfonate (MS-222, tricaine), a water soluble local anesthetic, is used commonly for sedation, immobilization, and anesthesia of poikilothermic animals [11, 13, 15] and has been accepted as a common anesthetic for use in the cold-blooded animals. MS-222 was developed by Merck as a sulfonated analogue of benzocaine with high solubility in

water. It is more than 250 times as soluble in water as benzocaine [9].

Anesthetics target specific binding sites on Na^+ channels [2] and other ligand-gated ion channels [4]. Local anesthetics enter the nerve cell membrane and, in the ionic form, physically block the sodium channel and inhibit nerve conduction. The main advantage of MS-222 as an anesthetic is its short duration of action and rapid metabolism [9]. There are many reports describing the use of MS-222 for anesthetizing poikilothermic animals because it is a safe agent for immersion anesthesia even though the other anesthetics such as ether, ethanol, thiopental, halothane, isoflurane, barbiturates also could be used.

Amphibians could be anesthetized easily by immersion methods with MS-222, because amphibian skin is extremely permeable and water is absorbed across the skin rather than ingested [11]. Solutions of MS-222 are acidic, due to methanesulfonic acid formation, and under these conditions, it will be mostly ionized in the non-absorbable acidic form. The low pH of these solutions may cause stress in animals. In unbuffered solutions, the time required for induction of anesthesia is increased and duration of anesthesia is decreased [6]. However, in solutions buffered with sodium bicarbonate, more rapid onset of anesthesia occurs because raising the pH causes the meta-amino group of MS-222 to be less ionized, allowing more rapid diffusion across lipid membranes [6].

MS-222 has been administered as an injectable agent also. Letcher determined the effective injectable dose for leopard frogs (*Rana pipiens*) and bullfrogs (*Rana catesbeiana*) [10]. Leopard frogs required 100–250 mg/kg of MS-222 intracoelomically, and bullfrogs were anesthetized with 250–400 mg/kg of MS-222 intracoelomically. Because MS-222 desensitizes serosal tissues, Letcher also concluded the injection to be relatively painless.

Benzocaine has been used in a similar manner to MS-222. Benzocaine is similar to MS-222 as an anesthetic although it is less water soluble and more potent than MS-222. Larvae can be anesthetized with 50 mg/l, and adult frogs and salamanders require 200–300 mg/l. Benzocaine before its administration is dissolved in ethanol to make it more soluble in water [5, 11].

Cardiovascular and respiratory systems of both poikilotherms and mammals are susceptible to the depressant action of anesthetic agents. Local anesthetics strongly change myocardial activity in mammals by reducing the electrical excitability of the heart, the rate of electrical conduction throughout the heart, and the force of ventricular contraction [3, 17]. As an anesthetic, MS-222 may also cause cardiodepression, cardiac arrest and respiratory arrest [11, 12]. Wayson et al. compared the effects of MS-222 administered at concentrations from 10^{-2} to 3×10^{-3} M between bullfrog atria and guinea-pig atria and concluded that tricaine methanesulfonate had negative inotropic and chronotropic effects [18]. In another study, 1/1000 solution of MS-222 used as immersion anesthesia increased the heart rate in intact toad (*Bufo marinus*) [16]. MS-222 also had a significant effect on the respiratory motor output producing both excitation and inhibition of breathing under normal concentrations [8].

This study, therefore, was aimed at determining the dose at which peripheral effects on the cardiovascular system occur and what the nature of these peripheral effects is. Accordingly, the anesthetic efficacy of different concentrations of MS-222 was compared to benzocaine and pentobarbital on the physiological changes, heart rate and ECG parameters in the leopard frog, *Rana pipiens*.

Materials and Methods

Animals

All experiments were carried out on frogs of the species *Rana pipiens* (leopard frogs) with a body mass ranging from 40 to 60 g, and frogs were adapted to 22°C before experiments. *Rana pipiens* of both sexes were purchased from a commercial source (Charles Sullivan, Nashville, TN). The institutional animal care committee of Ohio State University approved the experimental protocol. Loss of righting reflex (RR) and pain perception (NR), ECG parameters and heart rate were tested in response to MS-222, benzocaine and pentobarbital. Experiments were performed in late summer and autumn. Frogs were held in two eighty-liter glass aquaria in groups of 12 and were fed live crickets every other day. Care was taken with the animals to avoid exposure to compounds known to influence drug-metabolizing enzyme activity, such as chlorinated tap water [18]. The water in the aquaria was changed every other day. Animals were randomly grouped and each test was performed with 6 frogs per drug concentration.

Drugs and anesthetic solutions

MS-222 (Finquel-Argent Labs), benzocaine and pentobarbital (Sigma, USA) were obtained from commercial sources. MS-222 solution was prepared in the four different concentrations (100, 200, 400 and 800 mg/l) for each test group and buffered with 1 M $NaHCO_3$ to pH 7.0 [5]. Benzocaine was dissolved in ethyl alcohol (final concentration 0.1%), and the pH of benzocaine and pentobarbital solutions also were adjusted to 7.0 by buffering them with 1 M $NaHCO_3$ and 1 M HCl, respectively. Two liters of buffered MS-222, benzocaine and pentobarbital solutions were prepared and divided into six 2-liter beakers, each containing 300 ml of drug solution.

The ECG, heart rate and loss of RR and NR

A cover like rubber mesh was prepared containing 4 holes for the frog's limbs. In order to minimize movement, frogs were partially suspended in the rubber mesh jacket with their limbs just touching the supporting surface. The ECG was recorded prior to and after exposure to each drug concentration. The three standard limb leads I, II, III, were recorded from these connections on the electrocardiograph (Cardiofax, Nihon Koden). After the control ECG was recorded, frog was put in a beaker containing anesthetizing drugs. A glass support was placed at the bottom of the beaker to allow the frog to stay on it, and frogs did not submerge because the head and nostrils of the frog were out of anesthetizing solution. Frogs were tested for the loss of RR at every 10 min. For testing RR, the frogs were removed from the beaker, placed on their dorsum on a wet paper towel on a flat surface and observed to see if they righted themselves within 1 min. The time at the loss of was recorded for each frog. For testing nociceptor response (NR), a gentle pinch was applied to the frog's foot and the loss of withdrawal response was considered as loss of NR [7]. The frogs were placed back in the beakers, and allowed to stay there for a total of 4 h. ECG recording was made at the end of anesthesia. The following ECG parameters were measured: P–R interval – the time from atrial activation to the ventricular activation, QRS complex – the time required for impulse conduction along the ventricles, Q–T Interval – the duration of ventricular activation through ventricular repolarization.

ECG patterns were analyzed by measuring the duration of the QRS complex, and the P–R and Q–T intervals. Alterations in the pattern, such as premature beats and prolonged ECG intervals, were also noted. Control ECG intervals were estimated as the average of values measured from each frog on the last trace made before exposure to an anesthetic. Heart rates were determined by counting the number of QRS spikes in 10 s and expressed as beats per min. Heart rates were measured and recorded before and after the loss of RR. Next heart rate measurements were made at the end of each hour of drug exposure for a total of 4 h to monitor the changes in the heart rate in response to drugs. After the last ECG (4 h) was recorded, frogs were removed from beakers and put on wet paper towels in order to determine the recovery from anesthesia. The fresh water was added every 20 min to keep them moist. The time for recovery was recorded and frogs were returned to aquarium.

Statistical analysis

GraphPad Prism software was used for the statistical analysis. Two-way ANOVA (analysis of variance) with Tukey *post test* was used, and differences were considered significant if $p < 0.05$.

Results

RR, NR, recovery time

Frogs were exposed to MS-222 at concentrations ranging from 100 to 800 mg/l, benzocaine at a concentration of 200 mg/l and pentobarbital at a concentration of 300 mg/l for 4 h. Frogs demonstrated observable effects from loss of RR to unresponsiveness to intense stimulation. Figure 1 shows the effect of four concentrations of MS-222 on the RR, NR and recovery time. As can be seen in Figure 1, the recovery time increased whereas the time to loss of RR and NR decreased in a concentration-dependent manner. Frogs were completely immobilized at about 50 min of anesthesia, and all of them rapidly gained their spontaneous respiratory activities at 50 min, when 100 mg/l of MS-222 was used (Fig. 1). At 200 mg/l of MS-222, frogs lost their consciousness at 30 min and recovered from anesthesia at 130 min ($p < 0.05$). At 400 mg/l of MS-222, anesthesia was very fast, but recovery time markedly increased up to 230 min ($p <$

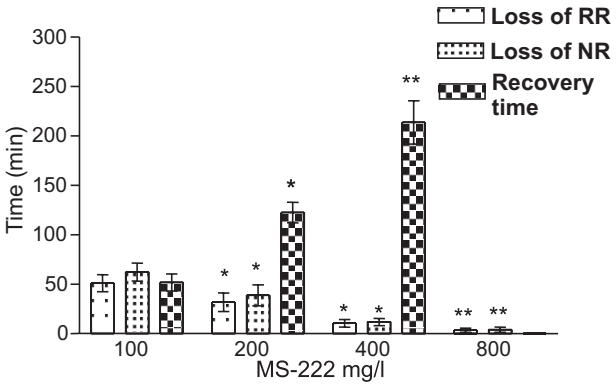

Fig. 1. The effects of MS-222 on the righting reflex (RR), pain perception (nociceptor response – NR) and recovery time. Frogs were exposed to MS-222 at concentrations ranging from 100 to 800 mg/l for 4 h. Frogs demonstrated observable effects from loss of RR to unresponsiveness to intense stimulation. Values were presented as means ± SEM. * $p < 0.05$; ** $p < 0.01$

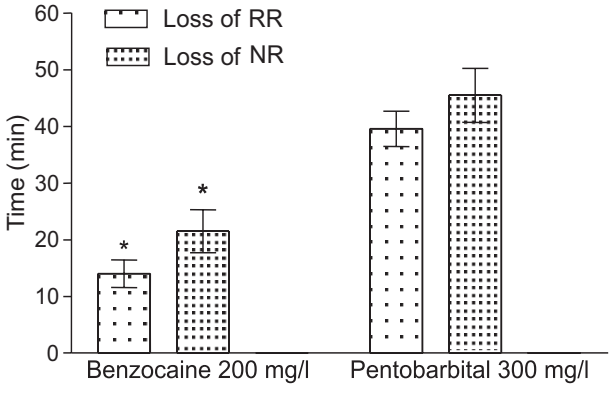

Fig. 2. The effects of benzocaine and pentobarbital on the righting reflex (RR), pain perception (nociceptor response – NR). Frogs were exposed to benzocaine at a concentration of 200 mg/l and pentobarbital at a concentration of 300 mg/l. Frogs demonstrated observable effects from loss of RR to unresponsiveness to intense stimulation. Please note that recovery time was not indicated here because frogs needed resuscitation. Values were presented as means ± SEM. * $p < 0.05$

0.01). All frogs receiving 800 mg/l of MS-222 were deeply and rapidly anesthetized and became apneic because the time required to loss of RR and NR was very short ($p < 0.01$) compared to other three concentrations of MS-222, and all frogs required resuscitation (Fig. 1). This data suggest that 200 mg/l of MS-222 concentration is necessary for a safe and short anesthesia because the strength of anesthesia and recovery from the anesthesia significantly increases when high concentrations of MS-222 are used.

Only one benzocaine concentration, 200 mg/l, was used to anesthetize the animals. As can be seen in Figure 2, frogs lost their RR at 15 min whereas they did not lose the feeling of pain until 23rd min of anesthesia ($p < 0.05$). Respiration decreased after 2 h and stopped, and all of the frogs were resuscitated (Fig. 2). With the exception of the recovery time, benzocaine induced an anesthesia similar to anesthesia caused by 400 mg/l of MS-222 (Fig. 1). This data indicate that benzocaine at this concentration is an effective compound to induce a short-term anesthesia because frogs may need to be revived if they are exposed to benzocaine for longer periods.

Pentobarbital at the concentration of 300 mg/l induced an anesthesia similar to 200 mg/l of MS222 (Fig. 2). Frogs lost their RR at 40th min of anesthesia and did not give any response to stimulation by 43rd min of anesthesia. However, all of the frogs used were removed from the beaker and resuscitated, because they developed a ventilatory failure. Again, with the exception of recovery time, pentobarbital also induced an anesthesia almost similar to one induced by 200 mg/l of MS-222 (Fig. 1). This data suggest that safety margin of pentobarbital at this concentration is narrow, and care must be taken when using this compound for the induction of anesthesia due to respiratory problems.

The effects of 200 mg/l of MS-222, 300 mg/l of pentobarbital and 200 mg/l of benzocaine on the RR, NR and recovery time were indicated in Figure 3.

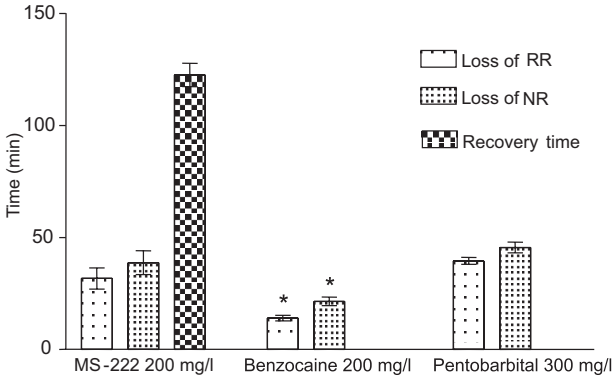

Fig. 3. The effects of 200 mg/l of MS-222, 200 mg/l of benzocaine and 300 mg/l pentobarbital on the righting reflex (RR), pain perception (nociceptor response – NR) and recovery time. Frogs demonstrated observable effects from loss of RR to unresponsiveness to intense stimulation. Please note that recovery time was not indicated here for benzocaine and pentobarbital because frogs needed resuscitation. Values were presented as means ± SEM. * $p < 0.05$

Tab. 1. The effects of anesthetics on the heart rate. Heart rates were determined by counting the number of QRS spikes in 10 s and expressed as beats per minute. Heart rates were measured and recorded before and after the loss of RR. Next heart rates measurements were made at the end of each hour of drug exposure for a total of 4 h. Values were presented as means ± SEM. * $p < 0.05$; ** $p < 0.01$

Drugs (mg/l)	Heart Rate, before the loss of RR	Heart Rate, at the loss of RR	Heart Rate, at 1st hour of anesthesia	Heart Rate, at 2nd hour of anesthesia	Heart Rate, at 3rd hour of anesthesia	Heart Rate, at 4th hour of anesthesia
100 mg/l MS-222	72 ± 3.5	66 ± 2.1	65 ± 4.6	62 ± 4.1	61 ± 3.3	60 ± 3.8
200 mg/l MS-222	77 ± 2.4	70 ± 3.3	65 ± 1.9	59 ± 2.4 *	52 ± 2.1 *	48 ± 4.2 *
400 mg/l MS-222	82 ± 2.3	71 ± 2.8	60 ± 3.5 *	48 ± 1.3 *	48 ± 2.3 *	44 ± 3.2 **
800 mg/l MS-222	84 ± 1.3	82 ± 3.5	48 ± 4.8 *	38 ± 2.2 **	32 ± 1.6 **	17 ± 3.1 **
200 mg/l Benzocaine	76 ± 2.9	63 ± 3.1	45 ± 1.3 *	37 ± 2.7 **	32 ± 1.4 **	24 ± 3.6 **
300mg/l Pentobarbital	75 ± 2.3	64 ± 2.9	55 ± 4.6 *	43 ± 2.5 *	34 ± 4.1 **	30 ± 4.2 **

Benzocaine significantly induced a faster anesthesia than both MS-222 and pentobarbital because frogs lost their RR and NR at 15 and 23rd min of anesthesia ($p < 0.05$). However, all of the frogs anesthetized by

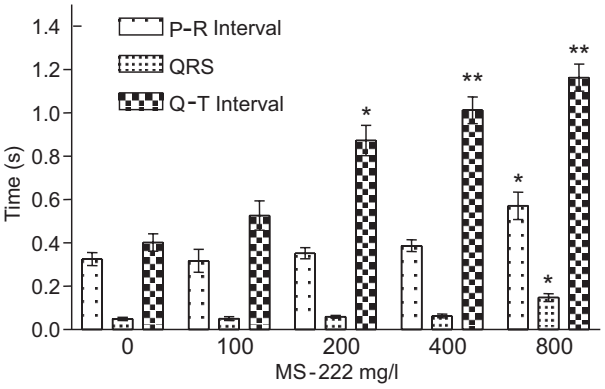

Fig. 4. The effects of MS 222 on the ECG parameters at the fourth hour of anesthesia. The ECG was recorded prior to and after exposure to each drug concentration. The three standard limb leads I, II, III, were recorded from these connections on the electrocardiograph. ECG patterns were analyzed by measuring the duration of the QRS complex, and the P–R and Q–T intervals. Values were presented as means ± SEM. * $p < 0.05$; ** $p < 0.01$

both benzocaine and pentobarbital needed resuscitation due to significant reductions in respiration at the 2nd h of anesthesia (Fig. 3). Consequently, this data clearly indicate that 200 mg/l of MS-222 is an effective anesthetic because at this concentration of MS-222, not only all of the frogs were immobilized easily but also they recovered without any ventilatory failure.

Heart rates

Table 1 displays the changes in the heart rate for each drug concentration. There was no significant change in the heart rates when animals lost their RR. In every treatment group, the mean heart rate was the lowest at the fourth hour of anesthesia. Heart rates decreased significantly ($p < 0.05$) in a dose dependent manner at 200, 400 and 800 mg/l of MS-222 (Tab. 1). High concentrations of MS-222 markedly decreased the heart rate during 4 h of the immobilization, and the lowest heart rate was recorded at the end of fourth hour of anesthesia caused by 800 mg/l of MS-222 ($p < 0.01$). Benzocaine and pentobarbital also markedly decreased the mean heart rates during 4 h of anesthesia

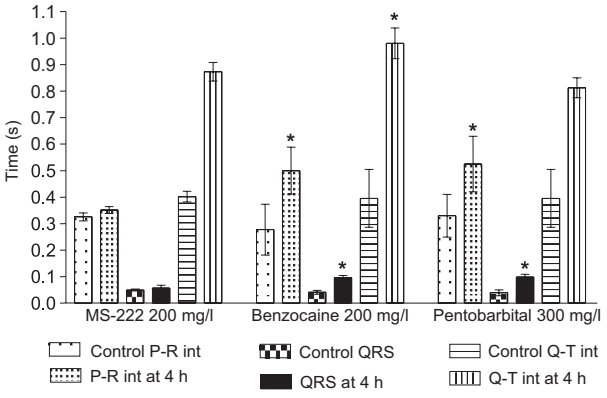

Fig. 5. The effects of benzocaine and pentobarbital on the ECG parameters at the fourth hour of anesthesia. The ECG was recorded prior to and after exposure to each drug concentration. The three standard limb leads I, II, III, were recorded from these connections on the electrocardiograph. ECG patterns were analyzed by measuring the duration of the QRS complex, and the P–R and Q–T intervals. Values were presented as means ± SEM. * $p < 0.05$; ** $p < 0.01$

from 76 2.9 to 24 3.6 beats/min and 75 2.3 to 30 4.2 beats/min, respectively ($p < 0.01$). When the duration of anesthesia was increased, the heart rates were decreased proportionally (Tab. 1). For the short-term immobilizations, the anesthetics did not cause important changes in the heart rates. These findings indicate that high concentrations of MS-222 may remarkably reduce heart rates causing possible respiratory problems for longer periods of anesthesia, and that both benzocaine and pentobarbital concentrations reduce heart rate as well.

ECG intervals

All anesthetics significantly changed the ECG parameters. At the fourth hour of anesthesia, MS-222 markedly increased P–R interval and QRS complex only at the concentration of 800 mg/l ($p < 0.05$) and Q–T interval at 200, 400 and 800 mg/l concentrations ($p < 0.01$) (Fig. 4). The lowest concentration of MS-222 did not significantly change the ECG parameters. Benzocaine and pentobarbital also significantly increased mean P–R interval, QRS complex and Q–T interval ($p < 0.05$) (Fig. 5). MS-222 at 800 mg/l and benzocaine at 200 mg/l were the most effective anesthetic concentrations in increasing the Q–T interval (Fig. 5). Compared to MS-222 at 200 mg/l, benzocaine caused significant increases in all ECG parameters whereas pentobarbital markedly increased only P–R interval and QRS complex ($p < 0.05$). The most crucial increase among the ECG parameters in response to all anesthetics used in this study was observed in the Q–T interval because it almost doubled at the mentioned concentrations of anesthetics. This data, therefore, clearly indicate that MS-222 at 200 mg/l causes an effective anesthesia because at this concentration of MS-222, all ECG parameters were not critically increased and all of the frogs recovered safely from anesthesia.

Discussion

MS-222 has a mechanism of action similar to other local anesthetics, which works through stabilization of cellular membranes, by inhibiting transient increases in sodium ion permeability, thereby decreasing excitability and blocking impulse conduction in excitable tissues. Increasing systemic levels of local anesthetics first block small unmyelinated fibers causing loss of sensation, then larger fibers with loss of motor function followed by CNS depression, and suppression of myocardial excitability [4, 11].

MS-222 exerts a particular pharmacological action on poikilotherms. When a frog is placed into a solution of MS-222, at a concentration that will generate anesthesia, the drug concentration in its plasma increases until a plateau is reached, resulting in a steady state between plasma and anesthetizing solution [18]. As soon as a steady state is maintained, there is no net transfer of drug between solution and plasma water because both of these aqueous phases contain equal concentrations of freely diffusible drug [7].

In this study, 200 mg/l of MS-222 generated light surgical anesthesia with loss of RR and absent or much decreased responses to pain after about 40 min of exposure. The higher concentrations of MS-222 induced a more rapid onset of anesthesia and prolonged the time required to recover. Even though the time required for loss of RR and pain perception largely declined to 8 and 10 min, respectively, when frogs were exposed to 400 mg/l of MS-222, the recovery time increased up to 225 min. In our protocol, the frogs remained in contact with the anesthetizing solutions for 4 h. In routine use, this exposure time would likely be much shorter. Had the frogs been removed shortly after loss of RR, recovery from even the 800 mg/l of MS-222 may have been expected. It appears that concentrations of MS-222 greater than 200 mg/l should be avoided for deep anesthesia because of high mortality rates at high exposure levels. If short induction times are necessary requiring high anesthetic concentrations, then exposure times must also remain short. The maximum safe concentration in buffered solutions of MS-222 in our study was 200 mg/l.

Due to the significance of cutaneous ventilation in poikilotherms, the loss of spontaneous respiration in anesthetized amphibians does not correspond to the similar situation in mammals [5, 11]. In this study, loss of respiration was likely associated with deep surgical anesthesia [8], because pulmonary respiration was greatly reduced or absent during the anesthesia. For instance, at 400 and 800 mg/l of MS-222, respiration slowed rapidly and ceased after the third hour of anesthesia and all frogs receiving 800 mg/l of MS-222 required resuscitation. Frogs receiving 100 and 200 mg/l of MS-222 did not cease to respire and recovered. It appears that the respiratory arrest is not an acute lethal

event at the low MS-222 concentrations because extensive gas exchange occurs through the skin. However, the high MS-222 concentrations must have reduced the skin respiration, possibly through reduced perfusion at low heart rates. This, along with reduced pulmonary exchange probably caused severe hypoxia [15] because in our study all frogs receiving high concentrations of MS-222 were resuscitated.

Benzocaine is less water soluble and more potent than MS-222 although it seems to be qualitatively similar to MS-222 [6]. Benzocaine, at the concentration of 200 mg/l, induced a rapid onset of immobilization similar to one induced by 400 mg/l of MS-222, because all the frogs lost their RR and NR at the first 15 and 20 min of exposure, respectively. However, frogs did not seem to recover from the anesthesia and all of them were resuscitated. The reason of the fast induction of anesthesia and necessity of resuscitation should be attributed to benzocaine's high lipid solubility that enhances the passing of drugs through the lipid bilayer, including the CNS.

Pentobarbital caused anesthesia similar to 400 mg/l of MS-222. However, all frogs exposed to pentobarbital also required resuscitation. Pentobarbital is absorbed very well from the skin, because it is a weak acid as well as a highly lipid soluble. Furthermore, pentobarbital redistributes to the other body tissues, including skeletal muscle and adipose tissue [5]. These sites slowly release the drug from the body and may account for the toxic effect of pentobarbital that prolongs induction time and recovery.

Although the primary pharmacological action of local anesthetics is the inhibition of the excitation-conduction process in peripheral nerves, any excitable membrane such as those that exist in the heart will be affected if local anesthetics achieve a sufficient tissue concentration [17]. Local anesthetics exert a generalized action on excitable tissues by antagonizing the membrane's ability to propagate action potentials because almost all local anesthetics strongly alter the cardiac muscle performance by reducing the electrical excitability of the heart [17]. Local anesthetics bind to the fast type Na^+ channels and prevent them from opening, decrease membrane depolarization and action potential formation in the cardiovascular and nervous system [4]. Progressive increases in the concentration of local anesthetics result in the interruption of transmission of autonomic, sensory and motor neural impulses and, hence, produce autonomic nervous system blockade, sensory anesthesia and skeletal muscle paralysis [3].

MS-222's direct action on the amphibian heart is depressant although unbuffered MS-222 solutions may cause tachycardia [7]. In this study, MS-222 suppressed the heart rate by inducing bradycardia. The lowest MS-222 concentration, 100 mg/l, did not significantly reduce the heart rate. The statistically significant changes in the heart rate have been observed at 200 mg/l of MS-222 at the second hour of anesthesia ($p < 0.05$). High MS-222 concentrations, 400 and 800 mg/l, strongly depressed the heart rate and respiration at the first hour of anesthesia. Likewise, benzocaine and pentobarbital suppressed the heart rate at the first hour of anesthesia and heart rate was significantly reduced at the fourth hour of anesthesia. This indicates that the cardiovascular system is particularly sensitive to pharmacological effects of anesthetics used in this study due to its rich autonomic innervations and the unique properties of myocardial cells. In our study, negative chronotropic effects of different concentrations of anesthetics on the heart rate may be attributed to direct actions of those anesthetics on the cardiac membrane by either decreasing the maximum rate of depolarization in the ventricle muscle or reducing the availability of some of the channels such as Na^+ and Ca^{++}, because the primary effect of anesthetics on the heart is to block the ability of the myocardial cells to conduct the impulses [7, 14].

Normal sequence of events in myocardial stimulation may be altered by anesthetics [17]. In this study, all anesthetics depressed the heart and markedly changed the ECG parameters such as P–R interval, QRS complex and Q–T interval by prolonging their time. In our ECG records, benzocaine, pentobarbital and only the highest concentration of MS-222 significantly prolonged the QRS complex, indicating a reduced ventricular conduction velocity. This is a typical response to the drugs with sodium channel blocking properties [3]. High MS-222 concentrations, benzocaine and pentobarbital also caused significant Q–T prolongation. In the ECG record, the T wave represents the combined electrical vectors of the entire terminal repolarization phase, and Q–T interval corresponds to the time taken for all the ventricular cells to be repolarized after being stimulated. The prolonged Q–T intervals in frogs exposed to high concentrations of anesthetics could have resulted from a decline in the heart rate attributed to the hypoxia, because the T-wave and Q–T interval are important parameters of

ECG for determining myocardial hypoxia caused by anesthetics [12, 17].

From this point of view, our study suggests that the altered heart rate and ECG parameters that result from exposure to MS-222, benzocaine and pentobarbital may probably be attributed to the following two factors: a) direct effects of anesthetics on the cardiovascular and respiratory systems by modulating cardiac membrane channels, and b) indirect effects mediated by the release of neurotransmitters [12, 14]. It seems that the excessive concentrations of anesthetics depress breathing, decrease ventilation and result in hypoxia, which will change cardiac functions unless it is corrected [1, 12] because severe hypoxia caused by ineffective pulmonary ventilation, inadequate supply of oxygen and impaired organ perfusion produces a deep depression of myocardial contractility. In addition, increased blood carbon dioxide that is going to increase the cardiac depression produced by hypoxia suppresses the myocardial contraction. The present study shows that MS-222 at 200 mg/l is very well tolerated by frogs because the Q–T interval, a marker of myocardial hypoxia, was not critically increased and all of the frogs recovered safely from anesthesia.

In conclusion, the present study shows that MS-222, benzocaine and pentobarbital have direct effects on the physiological state and electrical properties of frog heart by inducing an anesthesia in a dose-dependent manner. Respiratory depression and changes in the ECG parameters occur when high concentration of MS-222 is used. Furthermore, although both pentobarbital and benzocaine at mentioned concentrations seem to be very effective compounds to induce significant increases in the ECG parameters, their safety margins are very narrow because of ventilatory failure. Therefore, it is highly recommended that low MS-222 concentrations (around 200 mg/l) should be used to immobilize leopard frogs because prolonged recovery of spontaneous respiratory activity, high mortality rate and significant ECG changes might be observed with higher concentrations of MS-222.

References:

1. Andersen JB, Hedrick MS, Wang T: Cardiovascular responses to hypoxia and anemia in the toad *Bufo marinus*. J Exp Biol, 2003, 206, 857–865.
2. Bai CX, Glaaser IW, Sawanobori T, Sunami A: Involvement of local anesthetic binding sites on IVS6 of sodium channels in fast and slow inactivation. Neurosci Lett, 2003, 337, 41–45.
3. Biebuyck JF: Mechanisms for cardiac dysrythmias during anesthesia. Anesthesiology, 1990, 72, 347–374.
4. Butterworth JF, Strichartz GR: Molecular mechanism of local anesthesia: a review. Anesthesiolgy, 1990, 72, 711–734.
5. Crawshaw GJ: Amphibian medicine. In: Current Veterinary Therapy. XI. Small Animal Practice. Ed. Kirk RW, Bonagura JD, Saunders, Philadelphia, 1992, 257–269.
6. Downes H: Tricaine anesthesia in Amphibia: a review. Bull Assoc Reptilian Amphibian, 1995, 5, 11–16.
7. Downes H, Kienle EA, Pederson C: Metamorphosis and the steady state anesthetic concentrations of tricaine, benzocaine and ethanol. Comp Biochem Physiol, 1994, 107C, 95–103.
8. Hedrick MS, Winmill RE: Excitatory and inhibitory effects of tricaine (MS-222) on fictive breathing in the isolated bullfrog brainstem. Am J Physiol Regul Integr Comp Physiol, 2003, 284, R405–R412.
9. Herr VD, Sonnenburg DC, Courogan PM, Filamengo SA, Downes H: Muscle weakness during tricaine anesthesia. Comp Biochem Physiol, 1995, 110C, 289–296.
10. Letcher J: Intracoelomic use of tricaine methanesulfonate for anesthesia of bullfrogs (*Rana catesbeina*) and leopard frags (*Rana pipiens*). Zoo Biol, 1992, 11, 243–251.
11. Machin KL: Amphibian pain and analgesia. J Zoo Wild Med, 1999, 30, 2–10.
12. Martinez M, Hedrick MS: Cardiorespiratory control in paralyzed, decerebrate toads (*Bufo marinus*): influence of anesthesia and postsurgical recovery time. Comp Biochem Physiol, 2002, 132A, S27–S36.
13. Palmer LM, Mensinger AF: Effect of the anesthetic tricaine (MS-222) on nerve activity in the anterior lateral line of the Oyster Toadfish, *Opsanus tau.* J Neurophysiol, 2004, 92, 1034–1041.
14. Rusy BF, Komai H: Anesthetic depression of myocardial contractility. Anesthesiology, 1987, 67, 745–766.
15. Sladky KK, Swanson CR, Stoskopf MK, Loomis MR, Lewbart GA: Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu (*Piaractus brachypomus*). Am J Vet Res, 2001, 62, 337–342.
16. Smith DG: Sympathetic cardiac stimulation in *Bufo marinus* under MS-222 anesthesia. A J Physiol, 1974, 226, 367–369.
17. Surawicz B: Electrophysiologic Basis of ECG and Cardiac Arrhythimias. Williams and Wilkins Co., Baltimore, 1995.
18. Wayson KA, Downes H, Lynn RK, Gerber N: Studies on the comparative pharmacology and selective toxicity of tricaine methanesulfonate: metabolism as a basis of the selective toxicity in poikilotherms. J Pharmacol Exp Ther, 1976, 198, 695–707.

Received:
November 16, 2004; in revised form: May 12, 2005.